Food Consumption of the Free-Living Aquatic Nematode Pelodera chitwoodi[1]

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Abstract: A Cartesian diver respirometer was used to measure O_2 uptake and respiratory quotients at 25 C. Respiratory quotients were about 0.70 in starved nematodes, and 0.80 in third-stage and adult nematodes that had fed on bacteria. The energy output as measured by O_2 uptake was inversely related to the concentration of bacteria in the medium, indicating reduction in feeding effort. Feeding bacteria to third-stage nematodes in divers quickly resulted in peak respiration rates averaging 6.4 nl $O_2/\mu g$ wet weight nematode per hour (Q_{O_2}) or six times the endogenous rate. In about 4 hr, the rates fell and then stabilized at a Q_{O_2} of 2.5. Adult males fed bacteria in divers had a peak Q_{O_2} of 2.8 or twice the starved rate. Adult females fed bacteria had a peak Q_{O_2} of 3.7. Starving adult males and third-stage larvae were estimated to lose 2.4% and 1.4%, respectively, of their body weight per day in the form of fat based on the caloric equivalent of oxygen used and a respiratory quotient of 0.70. The caloric content of the bacteria fed to nematodes in divers was determined. It was then calculated that both third-stage larvae and adult males ingested bacteria equivalent to 4.4×10^{-5} cal/μg wet weight nematode tissue per hour when feeding. Of the bacterial calories ingested, the larvae used 27% and adults 21% for respiration. It was estimated that males ingested 3.1×10^6 bacteria and females 10×10^6 bacteria during an 8-day life span. *Key Words:* respiration, calorimetry, Cartesian diver.

Received for publication 28 August 1970.

[1] Portion of a Ph.D. dissertation submitted by the senior author to the Department of Botany and Microbiology, Auburn University, Auburn, Alabama. This study was supported in part by the Water Resources Research Institute of Auburn University, and by the USDI Office of Water Resources Research as authorized under the Water Resources Research Act of 1964 (Project A-004-ALA).

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Free-living nematodes are found in nearly all fresh-water environments, and they significantly affect the microbial floras which decompose organic matter (6, 9, 12, 13, 14, 16). *Pelodera chitwoodi* (Bassen) Dougherty, a free-living bacteriophagus nematode, is widely distributed. It has been found in diseased *Sagittaria* corms (3), in manure piles in Germany (10), in municipal water supplies in various U.S. cities (6), in sewage drying beds in Texas (5) and it is a common nematode

inhabitant of muds in several grossly polluted streams in Alabama (E. Mercer, *unpublished*).

The objectives of this study were to obtain information concerning the ecological role of *P. chitwoodi* in terms of the caloric value of bacterial food consumed, respired and assimilated and to determine rates of utilization of energy reserves.

MATERIALS AND METHODS

Nematode: The *P. chitwoodi* population used in these experiments was isolated from sediment from Saugahatchee Creek, a highly polluted stream (textile mill waste and sewage) near Auburn, Alabama. Monaxenic cultures of the nematode were maintained on cultures of an unidentified gram-negative nonspore-forming, yellow, rod-shaped bacterium isolated from the same sediments. Nematodes were starved overnight and washed with 1:100 clorox solution for 10 min and rinsed in 10 ml sterile tap water before introduction into bacterial cultures. Nematodes were reared at 25 C in petri dishes containing Czapek-Dox broth and the bacterium.

Respirometry: A Cartesian diver respirometer was used to measure respiration rates and respiratory quotients under various experimental conditions by the procedures of Holter (11). The Cartesian diver was used because viable individuals could be selected which were the desired stage and size. Activity was observed during experiments at X20 magnification.

All experiments were run at 25 ± 0.01 C. Four to six replicate divers having volumes of 10 to 15 µl were used in each experiment. A diver without nematodes was used as a control. Oxygen uptake rates (Q_{O_2}) were expressed as nl per µg wet weight of nematodes per hour. Weights of individual nematodes were calculated using volume measurements and specific gravity (1). Specific gravity (1.072) was determined with a series of sucrose dilutions.

Respiratory quotients (RQ) were determined using the "direct" method (19). The average Q_{O_2} of the divers having a KOH droplet (neck seal) was used in conjunction with data from CO_2 divers not containing KOH to calculate Q_{CO_2}. Average Q_{CO_2} divided by average Q_{O_2} gave the RQ. Techniques for the use of diver neck seals were adapted from Anfinsen and Claff (2) and Boell (4): 1.25 µl KOH were placed in the respiration chamber instead of in the diver neck. Nematodes were put in a 0.5-µl neck seal.

Utilization of stored energy: A unit of O_2 utilized in energy metabolism is equivalent to a definite caloric value. The range of the caloric equivalent of O_2 is not wide even when different types of food are metabolized (5). One nl of O_2 yields 4.7×10^{-6} cal when fats alone are utilized (RQ=0.7) and 5.0×10^{-6} cal when only carbohydrates are consumed (RQ = 1.0) (19). The difference between the two represents a range of about 6%. The maximum error is only 3% if either fat or carbohydrate is metabolized.

During starvation (RQ = 0.7) one nl O_2 is required to metabolize 5×10^{-4} µg fat. This figure was used to calculate fat reserve utilization rates.

Second-stage larvae were obtained directly from gravid females placed in divers. Females were removed after 12 hr leaving an average of 83 larvae in four divers (range 77 to 99 larvae).

Third-stage dauer larvae were taken from masses of such larvae formed in senescent agar cultures.

Adult nematodes and third-stage larvae used in respiration experiments were washed with 10 ml sterile tap water or modified Czapek-Dox broth (lacking sugar and yeast extract) on a 13 mm diam 8-µ or 14-µ pore diameter Millipore® filter mounted in a Swinney-type filter holder (Millipore Filter Corp., Bedford, Mass. 01730). Washed nematodes were placed in sterile tap water or modified Czapek-Dox broth. Ten nematodes were transferred aseptically to 1 µl fluid in each diver using a dental pulp canal file guided by a plastic jig.

Food requirements of growing nematodes: Bacteria used for feeding experiments were isolated from the monoxenic nematode cultures and inoculated into Czapek-Dox broth in test tubes which were rotated 24 hr at 25 C. Bacteria were washed three times by centrifugation, suspended in tap water sterilized by filtration, and allowed to "starve" for at least 2 hr to reduce respiration rates to a minimum.

In feeding experiments, 1 µl KOH was put in the respiration chamber. Washed bacteria suspended in 0.25 µl tap water were introduced as a side drop in the diver neck just above the respiration chamber. The 0.5 µl neck seal containing nematodes was placed above the side drop. Endogenous (starving) O_2 uptake was measured. Then, by increasing the manifold

pressure, the neck seal containing nematodes was pushed down until it merged with the side drop containing the substrate bacteria. Two divers, one containing bacteria but not nematodes and the other without bacteria or nematodes were used as controls.

The numbers of bacteria consumed by the nematodes were calculated from dilution plate counts of the original bacterial suspension and from 0.5 μl of the combined bacteria-nematode neck seal at the end of each experiment. The caloric value of ingested bacteria and the caloric equivalent indicated by the measured O_2 uptake and RQ were calculated and expressed as percent bacterial calories used for respiration and percent assimilated for growth and lost as waste.

Calorimetry: The caloric value of the bacteria was determined with a Parr adiabatic oxygen bomb calorimeter. Measurements were made during the active growth phase on two repetitions of bacteria grown in Czapek-Dox broth and three repetitions of bacteria reared on nutrient broth, each containing 0.3 gm dry bacteria. Numbers of bacteria in the cultures were determined by dilution plate counts. There was no significant difference in caloric value between bacteria grown in Czapek-Dox or nutrient broths. The average was 4878 cal/g ash-free dry weight or 1.59×10^{-9} cal per bacterium. The caloric equivalent of O_2 utilized by nematodes consuming bacteria was assumed to be that of the average mixed diet of animals, 4.83×10^{-6} cal/nl (RQ = 0.83) (17).

Amount of food required from hatching to death: The amount of food needed was estimated using the "growth formula", $Nt = Noe^{rt}$ (15), and using data indicating food ingested per unit body weight remained constant through the life cycle. The formula was used first to calculate the rate at which nematodes added weight from first-stage larvae to adults, then to determine the total amount of calories required during growth.

To determine rate of increase of weight, Nt = μg/adult nematode, No = μg/second-stage larva, r = rate of continuous growth, t = time from first stage to maturity and e = the base of the natural logarithm. After finding r, the calories ingested were calculated when No = calories ingested/μg per hr $\times$ weight of first-stage larva, and Nt = total calories ingested during growth from first stage larva to adult. Total calories were calculated for the time from maturity to death and converted to numbers of bacteria.

RESULTS AND DISCUSSION

Utilization of stored energy: Newly hatched second-stage larvae had an average QO_2 of 9.3 that declined to 3.1 in 22 hr and fell slowly to 2.5 in the subsequent 34-hr period (Fig. 1). The leveling of the respiration rate may indicate a metabolic adjustment to lack of food which conserves fat reserves.

Third-stage larvae in divers utilized about 1.4% (wet weight basis) stored food reserves per day. The rate of utilization of reserves was calculated on the basis of an endogenous

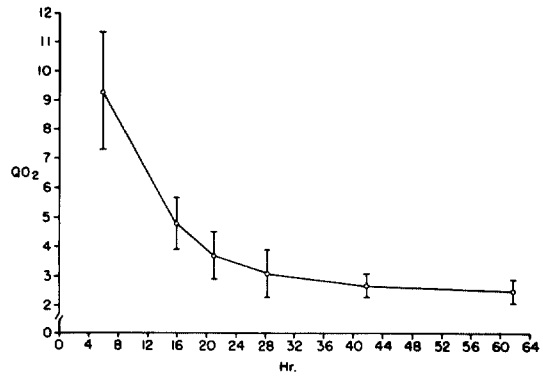

FIG. 1. Decline of respiration rates of newly hatched *Pelodera chitwoodi* second-stage larvae. Means and standard deviations of four Cartesian divers containing an average of 83 larvae per diver (range 77 to 99 larvae).

average QO_2 of 1.1, assuming that fats were being metabolized (Table 1). Calories expended per μg nematode per hr ranged from 2.8 to 7.5 $\times 10^{-6}$, averaging 5.2×10^{-6}. On a per nematode basis only 0.82×10^{-6} calories/hr were expended. The amount of fat utilized per hr averaged 5.4×10^{-4} μg fat/μg nematode or 0.9×10^{-4} μg fat/nematode.

The fat loss rate is about 10% of the body weight per week. The rate in divers was undoubtedly higher than the rate occurring in the inactive masses of dauer larvae found in senescent agar cultures because these lived a year or longer without food (but could not survive desiccation). They could not survive that long if they lost 10% of their weight each week. Nearly all larvae were dead after a week in sterile tap water, presumably because fat

TABLE 1. Respiration rates of starving third-stage larvae and adult males of *Pelodera chitwoodi* with calculated O_2 equivalents of fat and calories expended when RQ = 0.7. Ten nematodes per diver.

Rates per hour	Third-stage larvae[a] Range	Third-stage larvae[a] $\bar{X}$	Adult males[b] $\bar{X}$
nl O_2/µg nema	0.60-1.6	1.1	1.7
nl O_2/nema	0.08-0.21	0.17	3.2
cal/µg nema	2.8 -7.5 $\times 10^{-6}$	5.2 $\times 10^{-6}$	8 $\times 10^{-6}$
cal/nema	0.38-0.99 $\times 10^{-6}$	0.82 $\times 10^{-6}$	15 $\times 10^{-6}$
µg fat/µg nema	2.9 -7.8 $\times 10^{-4}$	5.4 $\times 10^{-4}$	8.4 $\times 10^{-4}$
µg fat/nema	0.4 -1 $\times 10^{-4}$	0.9 $\times 10^{-4}$	16 $\times 10^{-4}$
µg bact./µg nema	0.6 -1.6 $\times 10^{-3}$	1.1 $\times 10^{-3}$	1.7 $\times 10^{-3}$
µg bact./nema	0.80-0.21 $\times 10^{-3}$	0.17 $\times 10^{-3}$	3.2 $\times 10^{-3}$
bact./µg nema	1.9 -4.9 $\times 10^{3}$	3.4 $\times 10^{3}$	5.2 $\times 10^{3}$
bact./nema	240- 650	520	9900

[a]Three divers.
[b]One diver.

reserves were exhausted. The 10% of the body weight utilized during this time may have comprised most of the remaining reserve fat. Apparently the dauer state was broken and could not be re-established.

The metabolism of adult *P. chitwoodi* may not adjust as efficiently to starvation as that of second- and third-stage larvae.

The QO_2 of adult females removed from rapidly growing cultures averaged 2.8 after 4 hr (Table 2). The QO_2 during several hours of starvation was not measured because the presence of newly hatched larvae could give misleading data.

The QO_2 of adult males from declining cultures, measured 5 hr after removal from cultures, averaged 1.7; those from rapidly growing cultures averaged 2.3, or 35% higher (Table 1). During starvation, QO_2 of males from declining cultures dropped about 0.016/hr (Fig. 2B). The QO_2 of males from growing cultures decreased 0.013/hr, or 19% in the first 14 hr (Fig. 2A).

Fat consumption in starving adults was 1.4% of the body weight per day or about 17% of the

TABLE 2. Respiration rates (nl/µg wet weight) of starving adult and larval *Pelodera chitwoodi* taken from declining and rapidly growing cultures compared with nematodes fed bacteria in divers.

Treatments	Adults Female	Adults Male	Larvae Stage 2	Larvae Stage 3
Starved				
Declining culture				
$\bar{X}$ QO_2		1.7		1.1
SD		0.79		0.44
No. of divers		12		5
Growing culture				
$\bar{X}$ QO_2	2.8	2.3	9.3	
SD	0.67	1.1	2.03	
No. of divers	7	17	4	
Fed bacteria				
$\bar{X}$ QO_2	3.7	2.8		6.7
SD	1.2	0.72		5.1
No. of divers	9	9		9

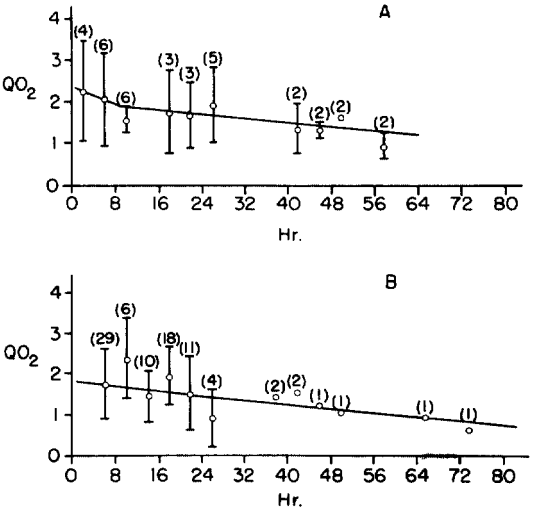

FIG. 2. Comparison of QO_2 means and standard deviations representing respiratory decline in starving *Pelodera chitwoodi* adult males from: A. Actively growing cultures, and B. Declining cultures. Numbers in parentheses give the number of replicate measurements (from separate divers) used to calculate $\bar{X}$ and s.

body weight per week (Table 1). The decline in respiration rates during starvation may result from lack of energy reserves, or it may signify a metabolic adjustment to a lower level which conserves energy reserves and increases longevity. Work done on *Meloidogyne javanica* by Van Gundy et al. (20) indicates that the metabolism of some nematodes adjusts to long-term starvation. Subsequent work by Cooper and Van Gundy (7) using a *Caenorhabditis* sp. indicated the QO_2 dropped after 48 hr unless the bathing fluid was changed. However, in my experiments there was twice as much bathing fluid per nematode.

Minimum food requirements: If the starving respiration rates are the "basal" rates of adult males and larvae, the amount of food required to maintain energy reserves at a minimum survival level may be estimated (Table 1). For dauer larvae this is 1.1×10^{-3} μg dry weight bacteria/μg wet weight nematode. The rate is slightly higher in adult males with 1.7×10^{-3} μg bacteria/μg nematode required per hr. Larvae require an average of 3400 and adults 5200 bacteria/μg nematode per hr for respiration alone. This calculates to about 9/min/nematode for third-stage larvae and 165/min/nematode for adult males.

The average weight of adult males (1.63 μg) is about 12 times that of third-stage larvae (0.13 μg) but the minimal number of bacteria required by a male per unit time to survive is about 19 times that required by a larvae. If starving adults have higher "basal" metabolic rates than dauer larvae, the concentration of bacteria in the medium would become critical for adults sooner than for larvae. This was observed in the nematode cultures when declining numbers of bacteria were associated first with fewer adults, then later a decline in numbers of larvae.

Food requirements of growing nematodes: "Fasting" third-stage dauer larvae had an endogenous QO_2 of 1.1. After feeding on bacteria for 6 hr the QO_2 was 9.3 or 8.3 times the endogenous rate (Fig. 3). The QO_2 multiplied by the caloric equivalent per nl O_2 (4.83×10^{-6} cal/nl O_2 with an RQ of 0.82) gave the calories expended per μg nematode per hr. The initial endogenous caloric output was 7.7×10^{-6} cal/μg/hr. The output with bacteria added minus the endogenous rate was 37×10^{-6} cal/μg/hr. Whether metabolism of endogenous reserves continued at the same rate after bacteria were added was unknown.

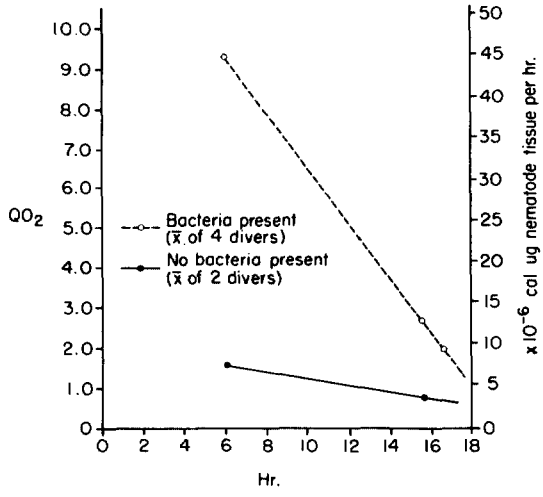

FIG. 3. Respiration rates and calories expended by third-stage dauer larvae of *Pelodera chitwoodi* with and without bacteria present. Ten larvae per diver.

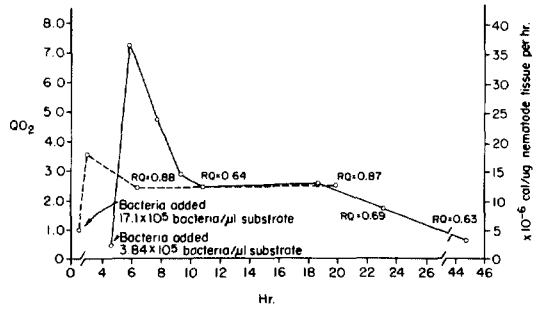

FIG. 4. Respiration rates, RQ values and calories expended by *Pelodera chitwoodi* third-stage dauer larvae fed washed bacteria in Cartesian divers. Two concentrations of bacteria, data points are means of two divers. Ten larvae per diver.

Probably the energy from bacteria was utilized preferentially.

Feeding of dauer larvae in divers resulted in 3.5 to 11-fold increase in respiration rates (Fig. 4). QO_2 peaks ranged from 3.5 to 9.3 with an average of 6.7; about six times greater than the starved rate (Table 1). The increases were reflected in the caloric expenditure and equivalent numbers of bacteria ingested. Peak rates were reached within 30 min after adding bacteria. Because of the small weight of larvae in divers, total O_2 uptake was too small to measure in less than 30 min.

The energy output of newly fed dauer larvae was inversely related to the concentration of food. Peak QO_2 of dauer larvae fed 3.8×10^5

TABLE 3. Peak respiration rates after feeding third-stage larvae and adult males of *Pelodera chitwoodi* with calculated O_2 equivalents of calories expended and bacteria when RQ = 0.8. Ten nematodes per diver.

Rates per hour	Third-stage dauer larvae[a]		Adult males[b]	
	Range	$\bar{X}$	Range	$\bar{X}$
nl O_2/μg nema	3.5 - 9.3	6.7	2.8- 2.8	2.8
nl O_2/nema	0.69- 1.2	1.0	3.6- 5.3	4.5
cal/μg nema	17 -45 $\times 10^{-6}$	32 $\times 10^{-6}$	14 -14 $\times 10^{-6}$	14 $\times 10^{-6}$
cal/nema	3.2 - 5.8 $\times 10^{-6}$	4.8 $\times 10^{-6}$	17 -26 $\times 10^{-6}$	23 $\times 10^{-6}$
μg bact./μg nema	3.5 - 9.2 $\times 10^{-3}$	6.7 $\times 10^{-3}$	2.8- 2.8 $\times 10^{-3}$	2.8 $\times 10^{-3}$
μg bact./nema	0.66- 1.2 $\times 10^{-3}$	1.0 $\times 10^{-3}$	3.6- 5.3 $\times 10^{-3}$	4.5 $\times 10^{-3}$
bact./μg nema	9.8 - 2.8 $\times 10^{3}$	21 $\times 10^{3}$	8.6- 8.6 $\times 10^{3}$	8.6 $\times 10^{3}$
bact./nema	1.9 - 3.7 $\times 10^{3}$	3.1 $\times 10^{3}$	11 -16 $\times 10^{3}$	14 $\times 10^{3}$

[a]Three divers.
[b]Two divers.

bacteria/μl substrate were higher than for those fed 17.1 $\times 10^5$ bacteria/μl (Fig. 4). Feeding effort, as measured by the amount of O_2 respired, decreased as the food supply concentration increased. Adding more concentrated food resulted in higher rates of food intake indicating that feeding became more efficient because increasing amounts of food were ingested while O_2 uptake remained the same.

Adult males also exhibited an increase in respiration when bacteria were added. The peak QO_2 was 2.8 or about twice the starved rate (RQ = 0.70). Adult females fed bacteria had QO_2 of 3.7 or 32% higher than the rate without food and about 32% higher than the peak rate for males. The difference was probably caused by larvae developing in the females. Caloric expenditure per μg in starved adults was about 50% higher than in starved third-stage larvae. After feeding, peak rates in larvae were 32 $\times 10^{-6}$ cal/μg/hr while the rate for adults was 56% lower, 14 $\times 10^{-6}$ cal/μg/hr (Table 3).

The dry weight of bacteria necessary to maintain the peak respiration rates was 6.7 $\times 10^{-3}$ μg bacteria/μg nematode/hr for larvae and 2.8 $\times 10^{-3}$ μg bacteria/μg adult male nematode (Table 3).

These rates are equivalent to 42 bacteria/larva/min. Recall that the numbers of bacteria/nematode required for endogenous respiration was 19 times greater for adults than for larvae. However, peak rates of adults required only four times as many bacteria per nematode. The greater caloric expenditure in larvae is expected as respiration per unit body weight of active organisms generally increases as the size of the organism decreases (21).

The rapid increase in O_2 uptake following addition of bacteria may be a behavioral reaction to the food stimulus. More likely, as indicated by increased RQ's, bacteria were utilized for energy. The experiment with dauer larvae having RQ's which indicated fat metabolism throughout the experiment had the lowest concentration of bacteria of any of the divers in feeding experiments, 3.84 $\times 10^5$ bacteria/μl substrate (Fig. 4). After bacteria were added the peak respiration rates were probably due to nematodes and not to bacterial respiration because: (i) given the same number of nematodes but different concentrations of bacteria, the nematodes with the fewest bacteria had the highest respiration rates, (ii) third-stage nematodes were starved and utilizing fats, thus the amounts of unoxidized nematode wastes utilizable for bacterial respiration were probably very small; and (iii) third-stage nematodes were placed in the diver with a volume of substrate some 500 times greater than the combined volume of the nematodes, thus diluting unoxidized wastes.

Amount of food required from hatching to death: In two experiments both third-stage larvae and adult males ingested 4.5 $\times 10^{-5}$ bacterial cal/μg nematode tissue/hr. The males utilized 21% and larvae 38% of the calories for respiration. Larvae were exposed to 17.1 $\times 10^5$ bacteria/μl substrate and the QO_2 was 3.5 (Fig. 4). When the concentration of bacteria was lower, the respiration rate for these larvae was higher. When the QO_2 was 6.4, the amount of bacteria used for respiration was 69% of 45 $\times 10^{-5}$ bacterial cal ingested per μg nematode, implying a greater proportion of energy is expended for feeding. Feeding effort

TABLE 4. Estimated calories and equivalent numbers of bacteria ingested by *Pelodera chitwoodi* males and females from hatching to maturity and hatching to death.[a] See text for explanation of method used.

	Hatching to maturity (5.5 days)		Hatching to death (8 days)	
	Calories	Bacteria	Calories	Bacteria
Males	0.7×10^{-3}	0.47×10^{6}	5.0×10^{-3}	3.1×10^{6}
Females	2.3×10^{-3}	1.4×10^{6}	16×10^{-3}	10×10^{6}

[a]Average wet weights of males and females at maturity were 1.6 μg and 5.3 μg, respectively.

apparently increases until a critical food concentration is reached. Then the respiration rate drops to a low level until adequate food is again available or death from starvation occurs.

The number of bacterial calories utilized during the life cycle was estimated. Assuming all stages ingested 4.5×10^{-5} cal/μg of nematodes/hr and growth could occur, males ingested about 8.1×10^{6} bacteria and females about 10×10^{6} bacteria during an 8-day life span (Table 4). These numbers are equivalent to 5.0×10^{-3} cal and 16×10^{-3} cal, respectively. One-seventh of these amounts was required for growth from egg to maturity (5.5 days).

The rates for females (Table 4) do not include calculations of the energy needed to produce eggs. However, the calculated increase in weight of gravid females is the result mostly of increased body width caused by developing young. Thus the calculation of calories required may indicate the actual caloric intake of gravid females.

Possible ecological role of aquatic nematodes: Decomposer micrometazoans utilize only one-fourth to one-third of the caloric value of their food for respiration (13). Engelmann (8) found that orbatid soil mites used only 19% of ingested calories for respiration. Macfayden (12) calculated that nematodes respire an amount of food equivalent to about 3% of the energy of organic matter entering the soil. He pointed out that an increase in rate of breakdown of organic matter increased the amount of nutrients available to plant and animal members of a community. Free-living nematodes may affect energy flow in soil communities out of proportion to their respiration rates since they are present in large numbers (10^{6} to $10^{7}/m^{2}$) and feed on bacteria (12). Hinshelwood (9) showed that bacterial colonies become senescent after a phase of active growth due to lack of food and/or accumulation of metabolites. Senescent colonies of bacteria are "bottle-necks" in which energy utilization is slow and mineral nutrients are unavailable to other organisms. Masses of bacteria surrounding particles of organic matter may inhibit growth of the colony as the distance from the particle to the outermost bacteria increases. Metabolites could accumulate. This is also true in aquatic sediments. As organic matter in a lake decomposes, the proportion of living to dead bacteria declines (18). Nematodes may contribute to the decomposition of organic matter in polluted aquatic environments by maintaining rapid bacteria growth. The activity of nematodes is threefold: mechanical "stirring" of bacterial colonies and organic matter, transport of bacteria to undecomposed organic matter, and removal of part of the bacterial populations. Colonies remain in an active growth phase increasing energy flow and mineralization.

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