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Modelling nematode life cycles using dynamic energy budgets

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Summary

1. To understand the life cycle of an organism, it is important to understand the underlying physiological mechanisms of their life histories. We here use the theory of dynamic energy budgets (DEB) to investigate the close relationships between growth, reproduction and respiration in nematodes.

2. Using a set of simplified equations based on DEB theory, we are able to accurately describe life-cycle data from the literature for the free-living bacterivorous nematodes *Caenorhabditis elegans*, *C. briggsae* and *Acrobeloides nanus*, under different temperature or food regimes.

3. Nematodes apparently differ from other animals, as the initial growth is slower than expected. We explain this phenomenon by food limitation in the larvae, which is supported by more detailed physiological studies.

4. Food density and temperature are shown to have predictable effects on the growth curves (temperature affects only growth rate, whereas food density also affects ultimate size), although the reproduction patterns reveal some deviations from model predictions.

5. The presented model integrates the different aspects of the life cycle into a single framework, and can be applied as such to interpret the effects of various stressors.

Key-words: *Acrobeloides nanus*, *Caenorhabditis briggsae*, *Caenorhabditis elegans*, life history

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Introduction

Nematodes or roundworms comprise phytophagous, bacterivorous, fungivorous and predatory species, which occupy an important position in soil food webs. It is generally recognized that they contribute importantly to soil ecosystem processes, especially the mineralization of carbon and nitrogen. Their contribution is direct through the consumption and processing of food, but also indirect through stimulating growth of soil microbes (e.g. Bouwman *et al*. 1994), or even stimulating plant growth by killing root-feeding insects (Preisser 2003). To gain further insight into the role of bacterivorous nematodes in soil food webs, it is important to unravel the underlying physiological mechanisms of their life histories. However, most bioenergetic or life-cycle studies in free-living nematodes were performed more than two decades ago, and these studies were more descriptive than mechanistic. Different end-points are described (e.g. growth, reproduction and survival), but these are treated as isolated processes (e.g. Sohlenius 1973; Byerly, Cassada & Russell 1976), or linked using descriptive allometric relations, e.g. relating body size to respiration (Klekowski, Schiemer & Duncan 1979; Schiemer 1982).

To increase our understanding of the life cycle, the various aspects should be integrated into a single framework. For example, reproduction generally starts at a certain body size, which means that any treatment that decreases growth automatically leads to a delay in reproduction, e.g. temperature (Byerly *et al*. 1976) and food density (Schiemer 1982). Furthermore, the organism converts food into offspring, and feeding rates depend on body size. We here use the theory of dynamic energy budgets (DEB) (Kooijman 2000; Nisbet *et al*. 2000; Kooijman 2001) to investigate the closely interrelated processes of feeding, growth and reproduction. This theory describes how individuals acquire and utilize energy, based on a set of simple rules for metabolic organization, treating the organism as a system with a closed mass and energy balance (Fig. 1). This model-based

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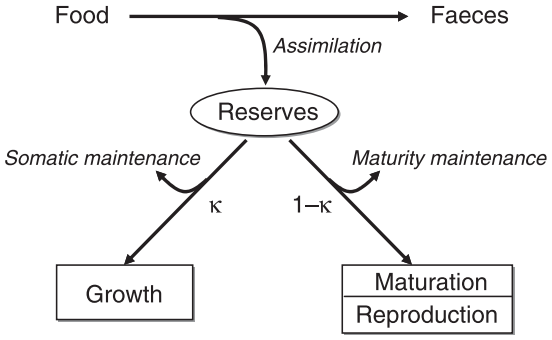

Fig. 1. Schematic representation of DEB. The organism is transferring food to faeces, and part of the energy is assimilated and contributes to the reserves. The reserves are distributed in a fixed fraction (κ) between somatic growth/maintenance and maturation/reproduction.

approach allows for an integrated interpretation of the effects of stressors on various aspects of the life cycle, and helps to identify where organisms follow different strategies.

To demonstrate the applicability of this approach, it is most interesting to look at responses under stress. Well-known environmental factors that influence aspects of the nematode life cycle are food availability and temperature. Therefore, we re-analyse data sets from the literature for the bacterivorous, soil-inhabiting species *Caenorhabditis briggsae*, *C. elegans* (Rhabditidae) and *Acrobeloides nanus* (Cephalobidae). These are cosmopolitan species and, depending on organic matter content and soil moisture, may occur in high densities (1000–10 000 individuals/kg soil). Like many bacterivorous nematode species, they can easily be reared under laboratory conditions in Petri dishes where a thin layer of nutrient agar is seeded with a lawn of bacteria (e.g. *Escherichia coli*). *C. elegans* is a facultative self-fertilizing hermaphrodite, which can also reproduce sexually. Males apparently occur at very low frequencies in natural populations (see Ward & Carrel 1979), and hermaphroditic reproduction is thus the most common, yielding a lifetime progeny of about 300 eggs per nematode. This species has a relatively short life cycle with a generation time (egg to egg-laying parent) of 5·5 days at 15 °C, and only 2·5 days at 25 °C. When the eggs hatch, the animals proceed through four larval stages, each of which ends in a moult. They live approximately 2 weeks at 20 °C. *C. briggsae* is very similar to *C. elegans*, in morphology and behaviour, although this species has a somewhat shorter development time and lower fecundity (Fodor *et al*. 1983). *A. nanus* has an average life span of 50–60 days, and is a parthenogenetic species, where the ovum develops into a new individual without fertilization. The reproductive stage lasts about 40 days (at 20 °C), during which they can produce a number of progeny ranging 400–500. They are normally reared on lawns of the soil bacterium *Acinetobacter johnsonii* but are not too selective, and can be grown on *E. coli* without problems.

Methods

MODEL FOR GROWTH

A consequence of the DEB theory is that organisms grow according to a Von Bertalanffy growth curve, as long as the food density remains constant. The rationale underlying this specific curve is that food uptake is generally proportional to a surface area (squared length for isomorphs), whereas maintenance costs are proportional to volume (cubed length) (see Nisbet *et al*. 2000). When the organism grows, volume increases more rapidly than surface area, and at a certain body size, all the resources allocated to growth and somatic maintenance are used for the latter, and growth ceases (see Fig. 1). This conceptual view of growth also explains why growth rates and ultimate body size decrease at lower feeding rates.

To simplify the equations, we will work with scaled body length, i.e. length as fraction of the ultimate length at abundant food. Scaled lengths are denoted by a lower-case symbol (l), absolute length by upper case (L). As long as the organism does not change its shape, in practice, any length measure can be used (e.g. total body length, body width or length of a specific organ), as well as the third root of body weight (the 'volumetric length', which can be interpreted as a kind of length measure as long as the specific density of the organism is constant). At constant food levels, the DEB theory reduces to the Von Bertalanffy growth equation, giving scaled length as a function of time and food level (Kooijman & Bedaux 1996):

$$\frac{\mathrm{d}}{\mathrm{d}t} l = r_{\mathrm{B}}(f - l) \tag{1}$$

All symbols are explained in Table 1. The food level is included in equation 1 through the scaled ingestion rate (f), which is the ingestion rate as fraction of the maximum. Parameter f is linked to food density by the functional response. When f and the growth rate constant (r_{B}) are constants, equation 1 can be integrated:

$$l = f(1 - e^{-r_{\mathrm{B}}t}) + l_0 e^{-r_{\mathrm{B}}t} \tag{2}$$

The growth rate constant (r_{B}) has, within DEB theory, a special relationship with food level and maintenance costs (see equation 9). Changing f should thus have a predictable influence on the ultimate size (see equation 1; growth stops when l equals f), as well as on r_{B}. The r_{B} at limiting food levels relates to r_{B} at maximum food ($f = 1$) as:

$$r_{\mathrm{B}}(f) = r_{\mathrm{B}}(1)\frac{1 + g}{f + g} \tag{3}$$

where the energy investment ratio g is a dimensionless parameter, with the interpretation of the cost of new biomass relative to the maximum potentially available energy for growth and maintenance. For water fleas, g

Table 1. Explanation of symbols

Symbol	Description	Unit
E_C	Catabolic energy (proportionality in equation 8)	μJ
f	Scaled ingestion rate (fraction of maximum, 0–1)	–
g	Energy investment ratio (default for nematodes $g = 10$)	–
k_M	Maintenance rate coefficient	day^{-1}
l	Scaled body length (fraction of maximum at $f = 1$) ($L = l \times L_m$)	–
l_0	Scaled initial length, at the beginning of the experiment ($L_0 = l_0 \times L_m$)	–
l_f	Scaled length at which ingestion is half of maximum ($L_f = l_f \times L_m$)	–
l_p	Scaled length at puberty (start of investment in eggs) ($L_p = l_p \times L_m$)	–
L_m	Absolute maximum length	μm
p_C	Catabolic energy flux	μJ day^{-1}
r_B	Von Bertalanffy growth rate constant	day^{-1}
R	Reproduction rate per nematode	eggs day^{-1}
R_m	Maximum reproduction rate per nematode	eggs day^{-1}
R_+	Maximum number of eggs per nematode per lifetime	eggs
s_f	Stress level on ingestion, depending on body size (0–1)	–
T	Actual temperature	K
T_A	Arrhenius temperature	K
V	Body volume	μm^3

is probably close to 1 (Kooijman & Bedaux 1996), and because g should increase with decreasing body size (Kooijman 2000, p. 272), a value of 10 may be used as a first estimate for nematodes (these species differ roughly a factor of 10 in volumetric body length). The absolute value of this parameter is not very important: as can be seen from equation 3, model results will be insensitive to changes in g when its value is much larger than 1 (f is between 0 and 1). Note that equation 3 dictates that less food leads to a higher r_B. This perhaps counter-intuitive result is explained by the fact that the Von Bertalanffy rate constant is a model parameter and not a (relative) growth rate. Even when r_B increases (depending on the value of g in equation 3), the resulting growth rate (dL/dt) will be lower at low food levels, because the factor $f - l$ in equation 1 is always lower (for the same body size).

This equation produces a sigmoid curve of body weight or volume against time, but the change in body length strictly decreases in time, yielding the non-sigmoid curve of equation 2. The Von Bertalanffy curve provides an excellent fit on data for a broad range of organisms, from yeast to birds (Kooijman 2000, p. 276). However, the available literature reveals that nematodes grow differently; the initial growth is slower than expected from this model. As a result, growth curves for several free-living nematode species are sigmoid when body length is plotted, e.g. for *C. elegans* (Byerly *et al*. 1976), *C. briggsae* (Jantunen 1964; Schiemer 1982), *Aphelenchus avenae* (Fisher 1970), *A. nanus* (Sohlenius 1973), *Rhabditis terricola* and *Diplogaster nudicapitatus* (Sohlenius 1968). The growth of *C. elegans* appears to be continuous (Byerly *et al*. 1976), but in detail, there are discontinuities at the four moults (Sohlenius 1973; Wilson 1976; Knight *et al*. 2002). Apparently, growth is nearly linear during each larval stage, at a rate increasing with each moult. The resulting larval growth curve (before reaching the reproductive adult stage) is more or less exponential when length is plotted against time. An explanation for this behaviour is usually not given; researchers simply take a different, empirical, curve for growth (e.g. the Chapman–Richards model as used by Schiemer 1982).

For our model, we need to find a mechanism for this deviating behaviour; simply using another curve is not an option as this leads to inconsistencies in the DEB theory. For example, using an empirical model obstructs the possibility to include the food density in a mechanistic manner, as in equation 1. Starting from equation 1, it is possible to change the curve shape by taking r_B and/or f as variable instead of constant. However, r_B is a rather complex compound parameter (Kooijman 2000, p. 95), so changing it requires more radical changes in the model to guard the overall consistency. Varying f is not only less invading; it is also more realistic, as indicated by physiological studies. Details of the nematode natural history suggest that the ingestion rate depends in a particular way on body size. Knight *et al*. (2002) speculate that the size of the buccal cavity (i.e. the tube-like mouth cavity, opening up into the oesophagus or pharynx) limits the feeding rate, and thereby growth of the larval worms. The cross-sectional area of the buccal cavity grows only at moults, thereby dictating the feeding rate during each larval stage. This hypothesis is consistent with the observation that larvae after hatching have lower respiration per unit of body weight than older larvae and adults (Klekowski *et al*. 1979; Schiemer 1982): less food means less growth and thereby less respiration (see also equation 7). It must, however, be noted that Schiemer considered these lower respiration rates to be experimental artefacts. Further evidence is given by Avery & Shtonda (2003), who suggest that the size of the *C. elegans* pharynx is important for efficient feeding (food particles are most efficiently transported when they are small compared with the diameter of the pharynx). Feeding rates (observed as pumping movements of the pharynx), are also consistent with the hypothesis of feeding limitation, as small specimens of *C. elegans* have lower pumping rates than adults (O. Alda Álvarez, personal observations).

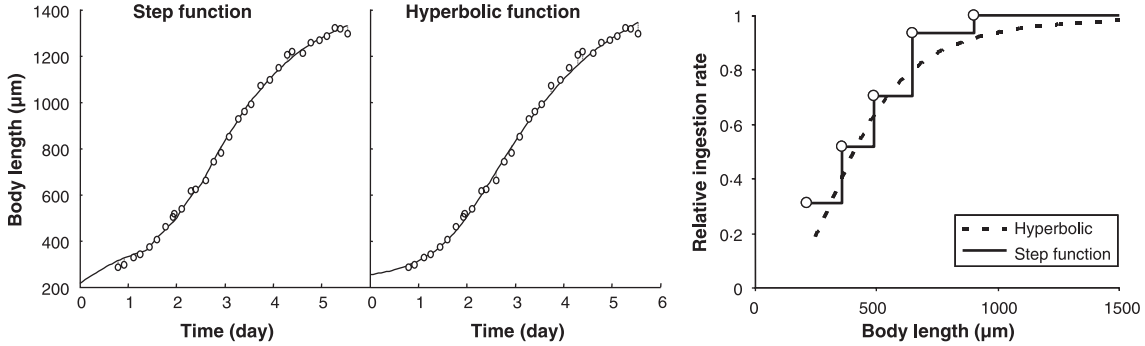

Fig. 2. Growth curve for the nematode *Caenorhabditis elegans* at 16 °C. The curve is a DEB growth model, assuming food limitation depending on body size. Two different types of limitation are fitted: a step function where the ingestion rate increases with each moult as a linear function of body length, and ingestion as a smooth hyperbolic function of body volume.

Assuming feeding limitations for the larvae, the scaled ingestion rate (f) is not constant but increases with every moult in a stepwise manner. We may assume a linear relation between f and body length at the moult, up to a maximum of 1, representing *ad libitum* conditions. A relationship with body length is appropriate as it is probably the diameter of the pharynx that determines the feeding limitation, which is assumed to be proportional to the length of the whole body. Figure 2 shows that the nematode growth curve (data from Byerly *et al*. 1976) can be described by equation 1, assuming that f changes in a stepwise manner. In this case, the body lengths at the four moults have been determined experimentally, so only the slope of the ingestion rate with body length is estimated as additional parameter. Even though the buccal cavity grows discontinuously with moults, we nevertheless propose to use a hyperbolic function of body size. This has the practical advantage that it is continuous (which facilitates the numerical simulation of the differential equation for growth), costs only one additional parameter and does not require information on the body size at each moult. This model is therefore an acceptable simplification for most ecological applications. The effective ingestion rate of the nematodes is thus given by the ingestion rate (f) times a size-dependent stress factor, defined as:

$$s_f(l) = 1 - \left(1 + \frac{l_f^3}{l^3}\right)^{-1} \tag{4}$$

for some constant l_f, which can be interpreted as the scaled length at which the ingestion rate is half the maximum value. The stress factor is high for larvae and approaches zero for adults. The full growth model becomes:

$$\frac{\mathrm{d}}{\mathrm{d}t} l = r_B[(1 - s_f)f - l] \tag{5}$$

MODELS FOR REPRODUCTION AND RESPIRATION

DEB theory specifies that the reproduction rate is a function of body size (or scaled length l), as given by Kooijman & Bedaux (1996):

$$R(l) = \frac{R_m}{1 - l_p^3}\left(\frac{g + l}{g + (1 - s_f)f}(1 - s_f)fl^2 - l_p^3\right)$$

The scaled length (l) is given by solving equation 5, and the stress factor of equation 4 is also included here. This is a simplification of the DEB model, specifically intended for the analysis of toxicity data. Other symbols are explained in Table 1. DEB theory assumes that reproduction starts after a certain amount of energy has been invested in maturation, which can usually be simplified to a fixed size at first reproduction, l_p (Kooijman 2000, p. 111). When the organism is approaching its ultimate size, the reproduction rate will become constant in time (at R_m, as long as f equals 1). However, the data for *C. elegans* and *C. briggsae* clearly show that reproduction slows down and stops after a certain time point. This is related to the rather peculiar mode of self-fertilization in these species (Ward & Carrel 1979). The germ line tissues first form a limited number of sperm cells. After the last moult, the germ line starts to produce oocytes, but the sperm production is totally halted. The stored sperm cells are used with an efficiency of nearly 100%, and when this storage is exhausted, egg production ceases. The model is therefore expanded with a parameter governing the maximum number of eggs that can be produced (R_+; R is set to 0 when the cumulative reproduction exceeds this value).

Respiration is sometimes measured in experiments (e.g. as oxygen consumption), and reflects the catabolic use of energy by the organism (excluding contributions from assimilation). The respiration rate (proportional to the catabolic rate p_C) is made up from contributions of growth (the change in volume $\mathrm{d}V/\mathrm{d}t$) and maintenance (proportional to body volume V) (Kooijman 2000; p. 84):

$$p_C \propto \frac{\mathrm{d}V}{\mathrm{d}t} + k_M V \tag{7}$$

which, on scaled-length basis, is equivalent to:

$$p_C = E_C\left[3l^2 \frac{\mathrm{d}}{\mathrm{d}t} l + k_M l^3\right] \tag{8}$$

where k_M is the maintenance rate coefficient (ratio of costs for maintaining a unit of biomass to costs of producing it) and E_C is a proportionality constant with the dimension of energy. The logic of DEB dictates that the maintenance rate coefficient cannot be chosen independently from the growth rate constant, as these are connected through (Kooijman & Bedaux 1996):

$$k_M = r_B \frac{3(f + g)}{g} \tag{9}$$

From this equation, it is clear that g becomes unimportant for k_M when its value is large compared with f, as was also the case in equations 3 and 6.

Reproduction is not explicitly included in the respiration equation (equation 7) for two reasons. Most of the energy allocated to reproduction will be transferred into the eggs, and thereby does not contribute to respiration (only the overhead costs of this process will contribute). Furthermore, the energy mobilized from the reserves is allocated to growth/somatic maintenance on the one hand and reproduction/maturity maintenance on the other (see Fig. 1). One of the basic assumptions of DEB is that this allocation is made according to a fixed fraction (Kooijman 2000, p. 86). This implies that the energy used for the reproduction process is proportional to that used for growth and somatic maintenance, and thus that reproductive overheads are included in the proportionality constant of equation 7.

EFFECTS OF FOOD LIMITATION AND TEMPERATURE

Temperature and food density have, within DEB context, a predictable effect on growth, reproduction and respiration. Deviations from these predictions therefore require rethinking of the mechanisms behind the model. Food limitation decreases the amount of energy available for the organism, which is covered by decreasing the relative ingestion rate (f) in equations 5 and 6. Food limitation indirectly influences respiration by affecting body size in equation 7. A temperature increase is expected to increase physiological rates such as the growth and reproduction rate in ectotherms, because all molecular reactions will proceed faster at higher temperatures. Other parameters, such as the length at puberty and the maximum length, should not be affected. Maximum size depends on the ratio of two rates (the assimilation and maintenance rates), which can be expected to change in a similar way with temperature (otherwise the organism would face serious regulation problems). It was shown that the rate constants generally follow the relation proposed by Arrhenius (see Kooijman 2000, p. 53):

$$k(T) = k(T_{ref}) \exp\left(\frac{T_A}{T_{ref}} - \frac{T_A}{T}\right) \tag{10}$$

where the value of the rate constant k at a certain temperature T is related to k at an arbitrary reference temperature T_{ref}. In this relationship, T_A is the 'Arrhenius temperature', which can realistically be assumed to have the same value for all physiological processes (Kooijman 2000, p. 54). It is, however, frequently found that also the maximum body size decreases with increasing temperature for ectotherms (Van Voorhies 1996). This is not expected from DEB theory, but may represent indirect effects on resources. Feeding rates increase with temperature, and at higher temperatures, food supplies are more likely to become limited (Kooijman 2000, p. 58).

MODEL IMPLEMENTATION AND COMPARISON TO EXPERIMENTAL DATA

To test the model's performance, three data sets from the literature are used. The first data set is for *C. elegans*, grown at three different temperatures (16, 20 and 25 °C) (Byerly *et al*. 1976). Nematodes were grown in Petri dishes with agar medium, on a lawn of bacteria (*E. coli*). Body size was determined using an electronic counter. The data used in this study were measured from the graphs given in the original paper, and recalculated to μm. Egg-laying rates had been determined by counting young animals. We converted reported reproduction rates into cumulative number of offspring by fitting a cubic spline to the data points, and calculating the area under this curve. Using cumulative numbers facilitates the comparison with the model results.

The study by Sohlenius (1973) with *A. nanus* employs a similar set-up: exposing the nematodes to two temperatures (13 and 21 °C) in drops of agar with *E. coli*. Two different food concentrations were used, but the results were so similar between the different food regimes that the data sets were combined. Because the experiments started with eggs, the hatching times as given in the original paper were used as lag time. The third data set originates from Schiemer (1982), resulting from experiments with *C. briggsae* at three different food levels. The food medium was prepared using 'sloppy agar' and known densities of bacterial food (5×10^8, 5×10^9 and 5×10^{10} cells of *E. coli* ml^{-1}), and the animals were incubated on microscopic slides in wet chambers at 20 °C. Body weight was calculated by the original author from body length and width. The values available in the original paper are, however, not the measured data, but derived from model regressions on those results. Nevertheless, because the model fits in the original paper appear to describe the data accurately, they provide a reasonable estimate for the purpose of demonstrating model performance. Reproduction is reported as the weight of the eggs produced per unit body weight per 10 h. These data were first translated back to number of eggs per nematode per hour, and than to cumulative reproduction as explained above. This data set also contains values for respiration rates (measured as oxygen use). However, this author plots the original data, but only reports in a table the values that are based on the log-linear regression of the data. This is problematic as the author thereby ignored the values

Table 2. Estimated parameters (max. likelihood estimates with likelihood-based 95% confidence intervals) for the fits in Figs 3–5. Symbols explained in Table 1. nr. not relevant

	C. elegans (temperature)			*A. nanus* (temperature)		*C. briggsae** (food density, cells per ml)		
	Low 16 °C	Medium 20 °C	High 25 °C	Low 13 °C	High 21 °C	Low 5×10^8	Medium 5×10^9	High 5×10^{10}
L_0 (µm)		282 (276–286)		228 (224–238)			23·4 (22·3–23·8)	
L_f (µm)		444 (436–450)		273 (266–281)			31·3 (30·5–31·6)	
L_p (µm)	1150 (1130–1160)	1020 (1000–1040)	1190 (1170–1210)	519 (509–529)	490 (445–516)		64·0 (61·3–64·4)	
L_m (µm)		1540 (1500–1550)		630 (609–637)			96·9 (96·2–97·7)	
r_B (day^{-1})		0·923‡ (0·890–1·03)		0·805‡ (0·639–1·13)			0·888 (0·840–0·938)	
R_m (eggs day^{-1})		304‡ (286–321)		16·1‡ (13·0–26·1)			27·0 (22·5–29·4)	60·0 (52·6–64·5)
f (–)		1†		1†		0·844 (0·838–0·848)	0·914 (0·909–0·918)	1†
T_A (K)		6540 (6340–6740)		6150 (5300–7020)				
R_+ (eggs)		265 (259–271)	165 (157–173)	∞†	∞†		141 (140–148)	
g (–)		10†		10†			9·59 (3·26–∞)	
E_G (µJ)		n.r.		n.r.			433 (395–452)	

*For this species, length measures are given as volumetric length (cubic root of volume).
†Parameter is not estimated.
‡Parameter is affected by temperature, according to equation 10; value is at a reference temperature of 20 °C.

for the newly hatched nematodes, which respire less than expected from the regression. Schiemer considered these data to be artefacts, but as stated earlier, the young nematodes also grow less than expected. Therefore, it is likely that this decreased respiration reflects the food limitation experienced by the young larvae. We used the graphical data to estimate the data for the young worms (weight < 0·1 µg) separately.

The model (equations 3–6 and 8–10) is implemented in MatLab® 6·5 (The Mathworks Inc., Natick, MA, USA). And the set of differential equations is solved using an ODE solver. Data sets for different treatments and different end-points (growth, reproduction and respiration, when available) are fitted simultaneously because these data sets share common parameters. For example, the growth rate constant (r_B) depends on food level in a predictable way (equation 3), and affects body length, but through its effect on length, indirectly affects reproduction and respiration as well. Therefore, only one value of r_B is estimated to cover all food levels and all end-points (see Table 2). All model fitting is done on the basis of maximum likelihood estimation, approximated by a weighted least-squares approach (see also Jager *et al*. 2004). Data points receive weighting factors that decrease linearly with the average value (large values receive lower weights). The end result is comparable to likelihood estimation, assuming normally distributed errors with a variance increasing with the average. The different end-points (growth, reproduction and respiration) receive equal weight in the analysis. Confidence intervals are generated using profile likelihoods (see, e.g., Meeker & Escobar 1995).

Results

EFFECTS OF TEMPERATURE

The growth and reproduction data for *C. elegans* at three temperatures largely corresponds to the model predictions (Fig. 3). Temperature affects the rate constants, as predicted by the Arrhenius relationship (equation 10). This implies that, together with the Arrhenius temperature, we need only one growth rate constant and one reproduction rate for all temperatures (Table 2). However, at the highest temperature, there is some misfit as the animals do not quite reach the maximum length predicted by the model. Even though a decrease in temperature leads to a delay in reproduction, as expected, the length at puberty (L_p) is not exactly constant (this is confirmed by direct measurements of Byerly *et al*. 1976). There is no clear pattern with temperature, but the differences are not very large. The total number of eggs produced during the lifetime (R_+) is the same at 16 and 20 °C, but is much lower at the highest test temperature. Apparently, egg production is more sensitive to high temperature than is body growth, as was also concluded by the original authors. Nevertheless, the fit of the model is very good, using only 11 parameters to describe six curves simultaneously, an average of fewer than two parameters per curve.

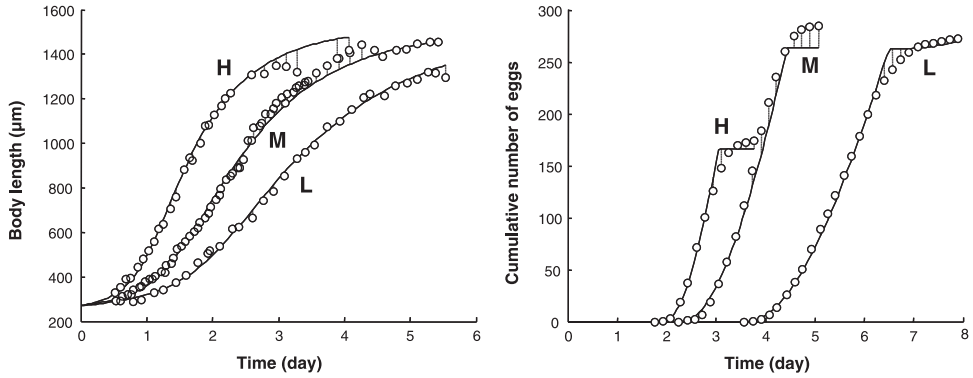

Fig. 3. Data and model fits for the effects of temperature (high, medium, low) on the growth and reproduction of *Caenorhabditis elegans*. Parameter estimates are given in Table 2.

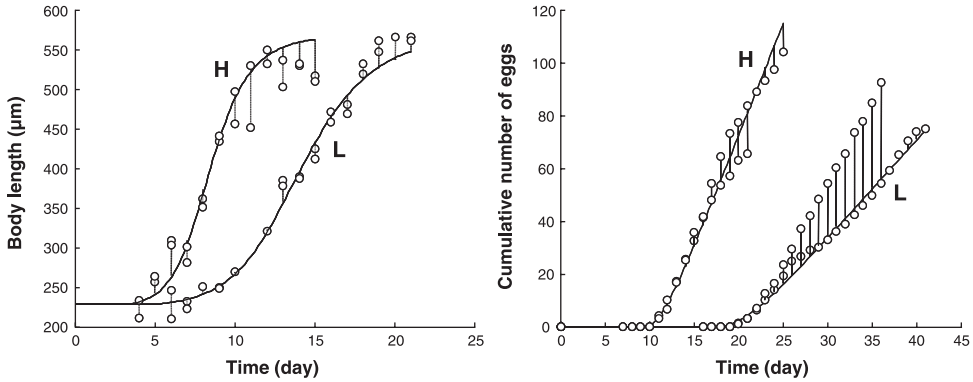

Fig. 4. Data and model fits for the effects of temperature (high, low) on the growth and reproduction of *Acrobeloides nanus*. Parameter estimates are given in Table 2.

The growth data for *A. nanus* are also well described by the model (Fig. 4, Table 2), although the data for 21 °C show an unexpected decrease when approaching the maximum length. However, the author observed a change in shape for these animals (these adults were somewhat thinner than at 13 °C). A change in shape hampers the use of the total body length as a representative length measure of the organism, the volumetric body length (cubic root of body volume) does not suffer as much. The reproduction data are reasonably well described by the model, but here, several deviations from model predictions arise. Firstly, a different length at puberty (L_p) is needed at both temperatures; the animals at the high temperature start egg production at a somewhat smaller body size. Secondly, the reproduction pattern at the low temperature differs between both feeding levels, whereas there is no difference between the growth curves. The use of the Arrhenius relationship favours the lower reproduction rate of the low food experiment, but direct effects of temperature on the reproduction rate itself cannot be ruled out. For these model fits, eight parameters are estimated for four curves, an average of two parameters per curve.

EFFECTS OF FOOD LIMITATION

The model can describe the data for *C. briggsae* at three food levels simultaneously (Fig. 5, Table 2), using the same parameters for all food levels. The only parameter that changes with all treatments is the scaled maximum ingestion rate (f). Despite the fact that the difference in bacterial density is a factor of 10 between the treatments, the estimate of f changes only little. This shows that the experiment was probably conducted in the saturating part of the functional response. The model fits for reproduction are good, but although the same L_p can be applied, we cannot use one maximum reproduction rate for all food levels. It should be noted that food level is included in equation 6, and therefore, a decrease in the actual reproduction rate at lower food levels should not involve a change in R_m. Rather, we predicted that the difference in reproduction pattern with food level should be explained from the different growth pattern (and thus different body length as function of time) and the decreased ingestion rate (f). For low and medium food level, one estimate for R_m can be used, but the estimated reproduction rate in the high food treatment is nearly two times higher. The respiration data in Fig. 5 are well predicted by the growth patterns (see equation 7), using only two additional parameters (g and the proportionality E_C, see Table 2). As predicted, model results are insensitive to changes in g when its value is relatively high, and the default value of 10 (Table 1) results in an only slightly poorer fit than the best estimate. The model fit requires 12 parameters for nine curves, which is much fewer than two parameters per curve. Compared with the *C. elegans* study, *C. briggsae* has nearly the same growth rate, but much lower fecundity (expressed as reproduction

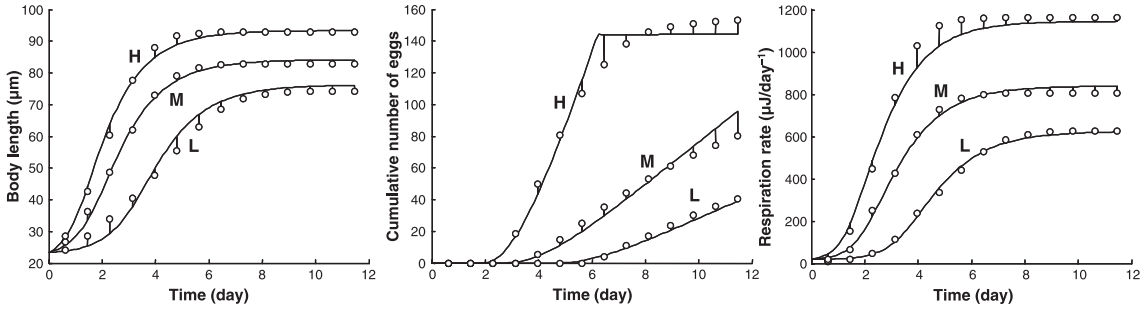

Fig. 5. Data and model fits for the effects of food density (high, medium, low) on the growth, reproduction and oxygen consumption of *Caenorhabditis briggsae*. Parameter estimates are given in Table 2. Length is given as volumetric length (cubic root of volume).

rate, as well as total number of eggs). This difference may partly relate to genetic defects that seem to have accumulated in *C. briggsae* strains (Fodor *et al*. 1983).

Discussion

In this paper, we present a set of simple model equations, based on DEB theory, that is able to accurately describe the life-cycle data for the free-living bacterivorous nematodes *C. elegans*, *C. briggsae* and *A. nanus*. The model requires only a few parameters, which all have a direct physiological interpretation, in contrast with many descriptive models. These parameters are themselves compound parameters; build up from parameters with a more direct interpretation in terms of energy (Kooijman 2000; e.g. for r_B: p. 95). However, to obtain these underlying parameters, more detailed experimental work is needed. With the presented simplifications, we can work with standard life-cycle endpoints (without the need to measure energies directly) and obtain model parameters with a simple interpretation, while still having the benefit of an underlying theory that ensures consistency with regard to energy allocation and mass flows. The DEB theory provides a reference situation with basic rules that apply to all forms of life. However, every species will have its own peculiarities that may require additional assumptions on top of the general framework. The need for additional assumptions depends on the purpose of the study; e.g. for a very detailed description of the nematode growth curve, the step function as shown in Fig. 2 is needed, whereas the hyperbolic function will suffice in most other cases.

Using the DEB model as a reference, we recognize that nematodes have a different growth pattern than many other organisms; the initial growth is slower than expected. Similar patterns have been observed in birds, although the mechanism behind those patterns was a lower body temperature in the juvenile birds (Kooijman 2000; p. 258). We explain the phenomenon in nematodes by food limitation, imposed by the size of the buccal cavity, an assumption that is supported by more detailed studies (Knight *et al*. 2002; Avery & Shtonda 2003). The current analysis supports the feeding-limitation hypothesis because it provides a good description of the data for all three species, coupled to the fact that the estimate for L_f does not depend on temperature or food density. However, other and more complicated mechanisms could also be involved, such as a size-dependent change in resource allocation. This alternative has not been pursued because it requires much more radical changes to the DEB model (and is therefore less realistic), and because there are no physiological studies supporting such a mechanism.

Nematode reproduction generally follows the shape predicted by the model. However, the cessation of reproduction in *C. elegans* and *C. briggsae* is more gradual than the sudden stop that is implemented in the model. This could either mean that the egg production rate of individual nematodes slows down when the sperm storage is almost depleted, or that not every individual worm stops reproducing at the exact same time.

The effects of food limitation and temperature are shown to have highly predictable effects on the growth curves. Temperature only affects the Von Bertalanffy growth rate according to the Arrhenius relationship (equation 10), whereas food limitation affects both the growth rate and the ultimate size in a specific manner (equations 3 and 5). Values for T_A are on the lower part of the range of values observed for other organisms (5500–15 000) (Kooijman 2000, p. 56), which means that the physiological rates do not depend heavily on temperature compared with other species. The maximum size is not clearly affected by temperature, although there are some deviations from model predictions at the high temperatures. For *C. elegans* as well as *A. nanus*, this was accompanied by a deviating shape of the growth curve, thereby indicating experimental artefacts. For *A. nanus*, the original authors observed a change in shape for the older animals at the high test temperature, but for *C. elegans* we need a different explanation (the counter signal is based primarily on body volume). In view of the decreased total reproductive output, we can speculate on a direct adverse effect of high temperature on the physiology later in life, or an indirect effect through food availability or food quality.

Effects on the growth curve immediately translate into a delay in the start of egg production. The reproduction curves, however, deviate in some respects from the model predictions. For *C. elegans*, the size for the start of reproductive investment various slightly with

temperature, although the reproduction rate follows the Arrhenius predictions. For *A. nanus*, the start of egg production occurs at a smaller size at higher temperatures, and the reproduction rate is only accurate in describing one of the two data sets at the lowest temperature. For *C. briggsae* at different food levels, reproduction does start at the same body size, but the reproduction rate at high food is twice the rate at medium and low food levels. It is possible that these effects are caused by changes in using the energy allocated to reproduction, although this would also have led to deviations in the growth curve, which were not observed. Deviations in the reproduction rate from the model predictions may also be caused by a difference in efficiency of using the allocated energy, or a change in the energy content per egg, as is known for *Daphnia* under stress, e.g. toxic stress (Bodar *et al*. 1988) or food limitation (Enserink, De la Haye & Maas 1993). The number of eggs produced may therefore not accurately reflect the energy allocated. Direct effects of the stressors on the reproductive machinery, however, cannot be ruled out. Respiration rates at different food levels were also well predicted by the model, including the low values for the newly hatched larvae. The predictability of respiration rates implies that the apparent change in reproduction rate at the various food levels is not accompanied by a different respiration pattern. These data support the conclusion by Houthoofd *et al*. (2002) that metabolic rate (per unit body weight) is not reduced in adults of food-restricted *C. elegans*. As shown in equation 7, the respiration rate for adults is dominated by maintenance costs which are assumed to scale with body volume. However, in juveniles, respiration should be lower under limited food because the maintenance requirements are the same per unit of body volume, but the growth rate is less.

The presented model is useful in ecological studies as it integrates the different aspects of the life cycle (growth, reproduction and respiration) into a single framework. In this way, the model can be applied to interpret the effects of various stressors on the life cycle, such as food and temperature (this study), but also senescence and toxicants (Jager *et al*. 2004).

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