

# Using dynamic energy budget modeling to predict the influence of temperature and food density on the effect of Cu on earthworm mediated litter consumption

### P.H.F. Hobbelen\*, C.A.M. van Gestel

Department of Animal Ecology, Faculty of Earth and Life Sciences, Vrije Universiteit Amsterdam, De Boelelaan 1085, 1081 HV Amsterdam, The Netherlands

#### ARTICLE INFO

Article history: Received 27 July 2006 Received in revised form 21 October 2006 Accepted 3 November 2006 Published on line 4 December 2006

Keywords: Earthworms Lumbricus rubellus Energy budget model Temperature Food density Cu

#### ABSTRACT

The aim of this study was to predict the dependence on temperature and food density of effects of Cu on the litter consumption by the earthworm Lumbricus rubellus, using a dynamic energy budget model (DEB-model). As a measure of the effects of Cu on food consumption, EC50s (soil concentrations causing 50% reduction compared to a control) were predicted with the total amount of consumed litter from birth to the age of 3 years as an endpoint, for a sandy loam soil. All possible combinations of six temperature and six food density scenarios were used. Temperature was kept constant at 10, 15 and 20  $^\circ$ C or fluctuated around these temperatures with an amplitude of 5 °C. The effect of food density on food consumption was represented by a dimensionless functional response, varying between 0 (no food) and 1 (unlimited food), which was kept constant at 0.25, 0.5 or 0.75 or fluctuated around these values with an amplitude of 0.25. Assuming that the DEB-model is able to describe litter consumption by L. rubellus, model simulations predicted EC50s between 212 and 284 mg Cu/kg, depending on the scenarios used for the temperature and functional response. Increasing the temperature for constant temperature scenarios or the average temperature for fluctuating temperature scenarios from 10 to 20 °C resulted in a decrease of EC50s of 28–34 mg Cu/kg, depending on the scenario for the functional response. Comparing EC50s for temperature regimes with a seasonal fluctuation to those with a constant temperature, showed that fluctuation resulted in a small decrease of EC50s (1-7 mg Cu/kg), independent of the functional response scenario. If the functional response did not fluctuate, increasing its value had a negligible effect on EC50s for all temperature scenarios. However, if the functional response fluctuated, increasing its average value from 0.25 to 0.75 resulted in a decrease of EC50s of 29-53 mg Cu/kg, depending on the temperature scenario. The effect of fluctuation of the functional response itself resulted in an increase of EC50s between 6 and 62 mg Cu/kg compared to constant scenarios, depending on the average functional response and the temperature. Because not enough data could be found in the literature to reliably estimate all parameter values, effects of temperature and food density on EC50s as predicted by the DEB-model should be interpreted carefully. Keeping this in mind, this study shows that temperature and food density can affect the sensitivity of litter consumption by earthworms to Cu pollution and should be taken into account in the risk assessment of heavy metal pollution for the functioning of detritivores.

© 2006 Elsevier B.V. All rights reserved.

<sup>\*</sup> Corresponding author. Present address: University of Wisconsin-Madison, College of Agricultural and Life Sciences, Department of Wildlife Ecology, 218 Russell Labs, 1630 Linden Drive, Madison, WI 53706, USA. Tel.: +1 608 263 7595; fax: +1 608 262 6099.

E-mail address: hobbelen@wisc.edu (P.H.F. Hobbelen). 0304-3800/\$ – see front matter © 2006 Elsevier B.V. All rights reserved. doi:10.1016/j.ecolmodel.2006.11.003

#### 1. Introduction

Litter decomposition is an important ecosystem function, because it completes the element cycle in ecosystems and makes nutritional elements from litter available for uptake by plants again. Earthworms play an important role in the first step of the decomposition of litter (Brussaard, 1998). Toxicity tests have shown that heavy metal pollution negatively affects life-history characteristics of earthworms, such as growth, reproduction and survival (Spurgeon and Hopkin, 1999; Spurgeon et al., 2000; Ma, 1988). Negative effects of heavy metals on these characteristics will decrease earthworm populations in the field and, as a result, also litter consumption by earthworm populations will decrease. To be able to predict the decrease in litter consumption by earthworm populations it is necessary to know how the food consumption by individual earthworms is related to biomass and is influenced by heavy metal pollution and environmental factors such as temperature and food density. Not much data are available in the literature on the effects of heavy metals on food consumption by earthworms. Ma (1984) found that litter consumption by the earthworm L. rubellus was reduced by exposure to Cu. Data on the effect of temperature and food density on litter consumption are also scarce. Daniel (1991) reported an increase of food consumption by Lumbricus terrestris with temperature until an optimum was reached, followed by a rapid decline. Ma (1984) found that food consumption by L. rubellus declined slower after reaching the optimum temperature. The studies mentioned above were always conducted at constant temperatures and food densities. In reality, however, temperature and food density will fluctuate and possibly change the effects of metals on food consumption by earthworms.

It is also possible that metals, temperature and food density interact and that litter consumption depends on the combination of these three variables. Temperature could influence the uptake and elimination rate (Janssen and Bergema, 1991) and the detoxification of toxicants and therefore affect toxicity. Donker et al. (1998) suggested that temperature affects the physiological interaction of Zn with energy metabolism. It can also be reasoned that food density influences the energy available for elimination and detoxification processes.

The aim of this study was to investigate if the effect of Cu on litter consumption by *L. rubellus* is influenced by temperature and food density. To do this, simulations were performed with a dynamic energy budget model for a number of scenarios with constant or seasonally fluctuating temperature and food density. As a measure for the effects of Cu on food consumption, EC50s were predicted with the total amount of litter consumed from birth to the age of 3 years as the endpoint. EC50s were defined as soil concentrations at which the performance of earthworms was decreased 50% compared to the control.

#### 2. Materials and methods

#### 2.1. Structure of the dynamic energy budget model

The dynamic energy budget (DEB) model used in this study was developed and described in detail by Kooijman (2000). The model can also describe mass fluxes, but this study focused



Fig. 1 – Structure of the dynamic energy budget model developed by Kooijman (2000).

on energy fluxes. Fig. 1 shows the structure of the model. Assimilated energy flows into an energy reserve. The energy flow out of the reserve is divided over somatic maintenance and growth on one side and maintenance of maturity and development (juveniles) or reproduction (adults) on the other, using a dimensionless constant  $\kappa$ . The energy flow to maintenance of maturity is needed to in order to maintain a certain state of development. The reasons for assuming the existance of this energy flow are described in Kooijman (2000). The model uses two state variables: the volume of the structural body mass, V, and the energy density, [E]. [E] is the energy per unit of volume of the structural body mass V. Structural body mass is defined as the body mass without energy reserves. The DEB-model distinguishes reserves from structure, because organisms with an equal weight (structure) can have different reserves, reserves do not require maintenance and structure is made from reserves. The energy density is defined as the amount of energy stored in reserves, divided by the volume of the structural body mass. In this study, it is assumed that shapes of organisms do not change during growth (isomorphism). If the length of an organism equals L and the shape of an organism is determined by the dimensionless coefficient  $\delta_m$ , then the surface area equals  $(\delta_m L)^2$  and the volume  $(\delta_m L)^3$ .

#### 2.2. Differential equations

#### 2.2.1. Uptake of energy

The ingestion rate at different food densities (functional response) and assimilation efficiency should be known to determine the assimilation rate of earthworms. In Kooijman (2000) the ingestion rate is assumed to be proportional to  $(t_s + t_h)^{-1}$ , in which  $t_s$  is the time spent searching for a food item and t<sub>h</sub> is the handling time per food item. When F stands for the searching rate, X for the food density and when  $\dot{J}_{\rm Xm}$  is the maximum ingestion rate, then it is assumed that  $t_s = (FX)^{-1}$ and  $t_h = (\dot{j}_{Xm})^{-1}$ . So the ingestion rate  $\dot{j}_X$  can be expressed as  $\dot{J}_X = \dot{J}_{Xm}X/(X_K + X)$  in which  $X_K = \dot{J}_{Xm}/F$ . The DEB-model assumes that at a constant food density the maximum ingestion rate is proportional to the surface area of an organism, so  $\dot{J}_{Xm} = \{\dot{J}_{Xm}\}V^{2/3}$  in which  $\{\dot{J}_{Xm}\}$  is the maximum surface-areaspecific ingestion rate (amount of ingested food per unit of time and per unit of surface area). Substitution in the equation for  $\dot{J}_X$  and using  $f = X/(X_K + X)$  results in the following equation for the ingestion rate:

$$\dot{J}_{\rm x} = \{\dot{J}_{\rm Xm}\}fV^{2/3} \tag{1}$$

In this equation, *f* is the dimensionless functional response  $(0 \le f \le 1)$ , which relates ingestion rate to food density.

*L. rubellus* is an epigeic species and spends time on the soil surface, eating litter as well as in the soil, feeding on organic matter. The functional response of earthworms on the soil surface or in the soil will differ, due to differences in food density, searching rate and handling time. Also the time spent feeding on the soil surface in relation to the time spent feeding on soil organic matter should be known to define the ingestion rate of earthworms. Because the data necessary to define the functional response of earthworms for litter and soil organic matter could not be found in the literature, it was assumed that *L. rubellus* feeds only on litter and changes in food density were simulated by changing *f*.

Multiplying the ingestion rate (Eq. (1)) by the dimensionless assimilation efficiency  $\{\dot{p}_{Am}\}/(\dot{y}_{Xm})$  gives the assimilation rate, which equals the energy input in the reserve:

$$\dot{p}_{\rm A} = \{\dot{p}_{\rm Am}\} f V^{2/3}$$
 (2)

In this equation,  $\{\dot{p}_{Am}\}$  is the maximum surface-area-specific assimilation rate (amount of assimilated energy per unit of time and unit of surface area), which depends on food quality.

#### 2.2.2. Reserve dynamics and growth

Next, the reserve dynamics should be modeled to obtain energy output. The derivation of the reserve dynamics is based on three requirements. Firstly, reserve dynamics should be partitionable. This means that it should be possible to use separate reserves for energy stored in the form of, e.g. lipids or proteins, without changing the total energy input and output of all reserves lumped together. Secondly, the reserve density at steady state should not depend on structural body mass, and thirdly, the use of reserves should not directly relate to food availability. Support for these assumptions is given in Kooijman (2000). The model in this study contained only one energy reserve. The formula derived for the rate of change in energy density (reserve dynamics), based on these requirements, is:

$$\frac{\mathrm{d}}{\mathrm{dt}}[\mathrm{E}] = \frac{\{\dot{p}_{\mathrm{Am}}\}}{\mathrm{V}^{1/3}} \left( f - \frac{[\mathrm{E}]}{[\mathrm{E}_{\mathrm{m}}]} \right) \tag{3}$$

In this equation, [E<sub>m</sub>] stands for the maximum reserve density, which depends on food quality.

Knowing the energy flow to the reserve and the reserve dynamics, it is possible to calculate the output of the reserve or catabolic rate,  $\dot{p}_{C}$  (energy per unit of time):

$$\dot{p}_{\rm C} = [E] \left( \frac{\{\dot{p}_{\rm Am}\}}{[E_{\rm m}]} V^{2/3} - \frac{\rm d}{\rm dt} V \right) \tag{4}$$

As mentioned above, a fraction  $\kappa$  of the catabolic rate flows to somatic maintenance and growth and a fraction  $(1 - \kappa)$  to maintenance of maturity and development or reproduction (Fig. 1). Both somatic and maturity maintenance are essential for the functioning of organisms and they have priority over growth and development or reproduction. Although the exact equations are not given, it is important to know that the DEB-model assumes that the energy flux to maintenance of maturity is a constant fraction of the somatic maintenance costs and that the energy flux to development or reproduction is a constant fraction of the flux to growth. It follows that if the energy flux to development or reproduction is zero, the energy flux to growth is zero, too. Therefore, it is not possible to use the energy flux to development or reproduction to cope with the somatic maintenance costs or the energy flux to growth to cope with maturity maintenance costs. The only option available to an organism that cannot cope with its maintenance costs, is to burn structural mass and thrink.

It is assumed that the energy flow to somatic maintenance is proportional to the volume of the structural body mass. If a constant amount of energy is needed to increase the volume of the structural body mass by one unit, the relation between the catabolic rate and energy flow to maintenance and growth is:

$$\kappa \dot{p}_{\rm C} = [E_{\rm G}] \frac{\rm d}{\rm dt} V + [\dot{p}_{\rm M}] V \tag{5}$$

Parameter  $[E_G]$  represents the energy needed to grow one unit of volume of the structural body mass and  $[\dot{p}_M]$  stands for the maintenance costs per unit of time and per unit of volume of the structural body mass.

Using the compound parameters  $\dot{v} = {\dot{p}_{Am}}/{[E_M]}$ ,  $V_m = (\kappa {\dot{p}_{Am}})/{[\dot{p}_M]}^3$  and  $g = [E_G]/\kappa [E_M]$ , and combining Eqs. (4) and (5) results in the following equation for the rate of change of the volume of the structural body mass:

$$\frac{d}{dt}V = \dot{\upsilon} \frac{V^{2/3}[E]/[E_{\rm M}] - V/V_{\rm m}^{1/3}}{[E]/[E_{\rm M}] + g}$$
(6)

#### 2.3. Toxicokinetic model

Kooijman (2000) proposed to model effects of toxicants on organisms by multiplying one or more target parameters in the DEB-model by a factor (1+s) or (1-s) in which s represents a stress value that depends on the exposure level. The DEB-model contains three possible target parameters:  $[E_G]$ ,  $[\dot{p}_M]$  and  $\{\dot{p}_{Am}\}$ . The first two increase in value due to stress and the latter decreases in value due to stress. Focusing on heavy metals, the dimensionless stress value, s, is assumed to be proportional to the difference between the internal metal concentration in an organism, [M] (mass/volume of the structural body mass), and the internal no effect concentration for a specific target parameter,  $[M]_0$  (mass/volume of the structural body mass):

$$s = \frac{[M] - [M]_0}{c_*}$$
(7)

in which c- (mass/volume of the structural body mass) is the tolerance concentration that belongs to a specific target parameter. It is named tolerance concentration, because its value increases if the toxicity of a compound decreases. Multiplication of or dividing the metal concentrations [M], [M]<sub>0</sub> and c- in Eq. (7) by a constant factor will not change the stress value. Therefore, if no data are available on internal concentrations, scaled internal concentrations can be used (Kooijman, 2000), which are related to the internal concentration by multiplying them by a constant bioaccumulation factor.

No model could be found in the literature that is able to describe the uptake of Cu by L. rubellus. Neuhauser et al. (1995) found that the elimination of Cu by the earthworm Aporrectodea tuberculata was typical for a two-compartment model, although the uptake of Cu could not be described using a one or two-compartment model. Due to the scarcity of data and to minimize the number of parameters, it was decided to use a simple one-compartment model. Further, it was assumed that the bioaccumulation factor of Cu is constant and the turnover rate of Cu by L. rubellus is high. Because in one-compartment models the time needed to reach an equilibrium concentration depends on the elimination rate (not the uptake rate), it follows that the internal Cu concentration will then always be in equilibrium and equal to the external exposure concentration times the bioaccumulation factor. As a result, the scaled internal concentration will be equal to the external concentration. Using this toxicokinetic model, Klok and De Roos (1996) fitted the DEB-model to data reported in Ma (1983) on the growth of L. rubellus exposed to a number of Cu concentrations and found that using the volume-specific maintenance costs as the target parameter best described the data. Therefore, this parameter was also used as the target parameter in this study.

#### 2.4. Dimensions

In this study, time was expressed in days and energy in Joules. Because no data were available on the volume of the structural body mass of *L. rubellus*, this was approximated by its dry weight expressed in g. Length was approximated by g dry weight, raised to the power 1/3. Because the stress value s is dimensionless, the Cu concentrations in Eq. (7) can be expressed in mg/kg dry weight.

#### 2.5. Parameter estimation

Ma (1983) studied the influence of Cu on the growth of L. rubellus in a sandy loam soil for 28 weeks at T=15 °C with sufficient plant litter as food. Attempts to fit the DEB-model to this dataset failed because of the large number of parameters to be determined. However, because sufficient litter was provided to the earthworms, it can be assumed that f=1. When food density is constant and it is assumed that the initial energy density equals  $f[E_m]$ , the energy density will also be constant and it is possible to rewrite the equation for the growth rate in the DEB-model (Eq. (6)) in a form that contains less parameters. Using  $V^{1/3}$  (g dwt<sup>1/3</sup>, proportional to the length) as the state variable, the resulting equation is:

$$\frac{d}{dt}V^{1/3} = \left(\frac{V_m^{1/3}f}{1+s} - V^{1/3}\right)\dot{r}_B(1+s)$$
(8)

with  $V_m^{1/3} = \kappa \{\dot{p}_{Am}\}/[\dot{p}_M]$ , which is the maximum length at high food densities (f = 1), and  $\dot{r}_B$  (day<sup>-1</sup>), which is the Von Bertalanffy growth rate. At a constant food density,  $\dot{r}_B$  is related to the parameters in the DEB-model as follows:

$$\dot{r}_{\rm B} = \left(\frac{3}{\dot{k}_{\rm M}} + \frac{3fV_{\rm m}^{1/3}}{\dot{\upsilon}}\right)^{-1} \tag{9}$$

in which compound parameter  $k_{\rm M} = [\dot{p}_{\rm M}]/[E_{\rm G}]$  (day<sup>-1</sup>). Substituting the compound parameters by the original parameters gives the following equation for  $\dot{r}_{\rm B}$  as a function of the model parameters:

$$\dot{r}_{\rm B} = \frac{[\dot{p}_{\rm M}]}{3[E_{\rm G}] + 3f\kappa[E_{\rm m}]} \tag{10}$$

Eqs. (7) and (8) were fitted to the growth data of L. rubellus at a number of Cu concentrations in a sandy loam soil reported by Ma (1983), using the maximum likelihood method in combination with the Newton-Raphson iteration procedure. Using scaled internal concentrations, initial parameter values were  $c_0 = 4.45 \text{ mg/kg}$ ,  $c_* = 1193 \text{ mg/kg}$ ,  $V_m^{1/3} = 0.66 \text{ g dwt}^{1/3}$ ,  $V_0^{1/3} = 0.17 \,\text{g}\,\text{dwt}^{1/3}$  and  $\dot{r}_B = 0.018$ . This resulted in the following estimations:  $c_0 = 6.93 \text{ mg/kg}$ ,  $c_* = 475 \text{ mg/kg}$ ,  $V_m^{1/3} =$ 0.8031 g dwt<sup>1/3</sup> and  $\dot{r}_{\rm B}$  = 0.0083. To perform model simulations at varying food densities, it is necessary to know the parameters { $\dot{p}_{Am}$ }, [ $E_M$ ], [ $E_G$ ], [ $\dot{p}_M$ ] and  $\kappa$ . Rewriting  $V_m^{1/3} = \kappa {\dot{p}_{Am}}/{[\dot{p}_M]}$ and Eq. (9) and substitution of the estimated  $V_m^{1/3}$  and  $\dot{r}_B$  gives two independent equations for  $[\dot{p}_{\rm M}]$ :  $[\dot{p}_{\rm M}] = \kappa \{\dot{p}_{\rm Am}\}/0.8031$ and  $[\dot{p}_{M}] = 0.0083(3[E_{G}] + 3\kappa[E_{m}])$ . The parameters  $\{\dot{J}_{Xm}\}$  and  $\{\dot{p}_{Am}\}\$  were estimated using the data on litter consumption reported in Ma (1984). Five adult L. rubellus, with an average individual wet weight of 1.550 g and kept in sandy loam soil at  $T = 15 \degree C$  with sufficient food, consumed 10.54 g of litter during 6 weeks. Assuming that the wet weight: dry weight ratio of L. rubellus with empty gut is 5.4 (Hobbelen, unpublished data) and that poplar litter was used with an energy content of 16148J/gdwt litter (Hobbelen, unpublished data), results in an estimation of 1860 J/(g dwt<sup>2/3</sup> day) for  $(j_{Xm})$ . Assuming that the assimilation efficiency equals 0.5 results in an estimation of 930 J/(g dwt<sup>2/3</sup> day) for  $\{\dot{p}_{Am}\}$ . Bolton and Phillipson (1976a) reported that the energy content of L. rubellus amounted to 17581 J/g dwt. Assuming that this energy content is the sum of the energy stored in reserves and contained in the structural body mass gives  $[E_G] + [E_m] = 17518 J/g dwt$ . Substituting the latter equation and the estimated value of parameter  $\{\dot{p}_{Am}\}$ in the two equations for  $[\dot{p}_{\rm M}]$  and combining these, gives  $[E_m] = (1158\kappa - 436)/0.0249(\kappa - 1)$ . Because  $[E_m] > 0$ , it follows

Table 1 – Parameter values used in the model simulations							
Parameter	Value	Dimension					
{J <sub>Xm</sub> }	1860	Jgdwt <sup>-2/3</sup> day <sup>-1</sup>					
$\{\dot{p}_{Am}\}$	930	$Jgdwt^{-2/3}day^{-1}$					
[E <sub>m</sub> ]	7845	Jgdwt <sup>−1</sup>					
К	0.25	-					
[E <sub>G</sub> ]	9673	Jgdwt <sup>−1</sup>					
[ṗ <sub>M</sub> ]	290	$Jgdwt^{-1}day^{-1}$					
C <sub>0</sub>	6.93	mg Cu kg <sup>-1</sup>					
C+	475	$ m mgCukg^{-1}$					
Q <sub>10</sub>	3.5	-					
T <sub>ref</sub>	10	°C					

See the text for an explanation of the meaning of the different parameters. Parameter f is excluded from this table, because its value depends on the scenario that is used for the functional response. Stress value s can be calculated using Eq. (7) and its value for different scenarios is mentioned in the text.

that  $\kappa < 0.37$ . Assuming that  $\kappa = 0.25$ , gives  $[E_m] = 7845 J/g dwt$ ,  $[E_G] = 9673 J/g dwt$  and  $[\dot{p}_M] = 290 J/(g dwt day)$ . Table 1 gives an overview of the estimated parameter values and their dimensions.

#### 2.6. Model simulations

#### 2.6.1. Program language

The DEB-model was programmed in MATLAB version 6.5 (The MathWorks, Inc.). Numerical integrations were performed using a built-in solver that is able to deal with stiff differential equations.

#### 2.6.2. Endpoint for the toxicity of Cu

The total amount of consumed litter from birth to the age of 3 years (t = 1095 days) was used as the endpoint for the toxicity of Cu. The toxicity of Cu was indicated using EC50 values, which were defined as the Cu concentrations in the sandy loam soil at which the amount of consumed litter amounted to 50% of the amount at Cu levels below the no effect concentration. EC50s were always calculated by numerical integration. First, the differential equations in the DEB-model were integrated to obtain the total amount of litter consumed by earthworms from birth until the age of 3 years, at a Cu level below the no effect concentration. Next, the Cu concentration was increased by 1 mg/kg and the differential equations in the DEB-model were integrated again to obtain the cumulative consumed litter after 3 years. This last step was repeated until the total amount of consumed litter fell between 49.99 and 50.01% of the maximum amount of litter consumed by non-exposed earthworms.

#### 2.6.3. Temperature and functional response

The influence of temperature on litter consumption by *L*. *rubellus* was simulated at 10 and 20 °C and with a temperature fluctuating between 10 and 20 °C. Functional response *f* was kept constant at 0.5. The influence of functional response *f* on litter consumption by *L*. *rubellus* was simulated for f=0.5, f=1 and for *f* fluctuating between 0.5 and 1. During these simulations, the temperature was kept constant at 15 °C.

The simulations above were performed for both non-exposed earthworms (stress value s = 0) and earthworms exposed to a Cu concentration of 150 mg/kg (stress value s = 0.3; target parameter  $[\dot{p}_{\rm M}]$  is multiplied by factor (1 + s) = 1.3), to see the effect of temperature and f at different Cu concentrations.

The influence of temperature and functional response *f* on EC50s for Cu, with the total amount of litter consumed by *L*. *rubellus* as an endpoint, was studied using six scenarios for temperature and six scenarios for the functional response *f*. EC50s were calculated for all possible combinations of the different scenarios for temperature and functional response.

In three scenarios, temperature was kept constant at 10, 15 or 20 °C and in the other three scenarios, temperature fluctuated between 5 and 15, 10 and 20 or 15 and 25 °C over an annual cycle. The fluctuation of temperature was modeled using a sinus function with the lowest temperature at t=0 and the highest temperature at t=365/2:

$$T = T_{av} + 5\sin\left(\frac{2\pi}{365}t - 0.5\pi\right)$$
(11)

in which T<sub>av</sub> is the average annual temperature.

Parameters in the DEB-model that are affected by temperature are  $\{\dot{p}_{Am}\}$  and  $[\dot{p}_{M}]$ . In this study, it was also assumed that parameter  $\{\dot{J}_{Xm}\}$  depends on the temperature and as a result the assimilation efficiency  $\{\dot{p}_{Am}\}/\{\dot{J}_{Xm}\}$  is constant. So, this means that when L. rubellus finds a piece of litter, the time it needs to consume this piece (handling time) is influenced by the temperature. The dependence of these parameters on the temperature was modeled as  $\dot{k}(T) = \dot{k}(T_{ref})Q_{10}^{(T-T_{ref})/10}$  in which  $\dot{k}$  is parameter { $\dot{p}_{Am}$ }, { $\dot{J}_{Xm}$ } or [ $\dot{p}_{M}$ ] and T<sub>ref</sub> is a reference temperature.  $Q_{10}$  is the factor that should be applied to rates for every 10 °C increase. Uvarov and Scheu (2004) reported a mean Q<sub>10</sub> of 3.4 for juvenile and 3.5 for adult L. rubellus using a diurnally fluctuating temperature regime. Because earthworms will be adult during most of the time interval used for the simulations, this study uses a  $Q_{10}$  of 3.5 in combination with a T<sub>ref</sub> of 15 °C.

In three scenarios for the functional response, f was kept constant at 0.25, 0.5 or 0.75 and in the three other scenarios f fluctuated between 0 and 0.5, 0.25 and 0.75 or 0.5 and 1. Assuming again that a year has 365 days, the fluctuation of f was modeled using a cosinus function with the lowest value of f at  $t = 0.25 \times 365$  days and the highest of f at  $t = 0.75 \times 365$  days:

$$f = f_{\rm av} + 0.25 \cos\left(\frac{2\pi}{365}t\right) \tag{12}$$

in which  $f_{av}$  is the average value of the functional response.

#### 3. Results

#### 3.1. Effect of temperature on litter consumption

Fig. 2A shows that, for scenarios with a constant temperature, the amount of litter consumed by *L. rubellus* from birth to the age of 3 years increases if the temperature increases. This can be explained by the dependence of the ingestion rate  $j_X$  on  $(j_{Xm})$  and  $V^{2/3}$  (Eq. (1)). Parameter  $(j_{Xm})$  increases when the level at which the temperature is kept constant increases. Fig. 2B shows that, according to the DEB model, temperature does not influence the maximum volume that can be reached by *L. rubellus*, but that it is approached faster at higher temperatures, resulting in a larger body mass during most of the time interval from birth to the age of 3 years. So, for constant scenarios, the total amount of consumed litter increases when temperature increases, due to a higher surface-area-specific ingestion rate and faster growth.

Fig. 3A shows that, for scenarios with a temperature varying between 10 and 20 °C, the amount of litter consumed by *L*. *rubellus* from birth to the age of 3 years, is a little higher compared to a constant temperature of 15 °C. This can be explained by looking at Eq. (1). If body mass is kept constant, fluctuation of  $(\dot{J}_{\rm Xm})$  around its value at 15 °C results in an increased amount of consumed litter after 3 years. This is the result of the way the influence of temperature is modeled. At T = 10 °C,  $Q_{10}^{(T-T_{\rm ref})/10} = 0.53$ , while at T = 20 °C,  $Q_{10}^{(T-T_{\rm ref})/10} = 1.90$ . Therefore, an increase of the temperature from 15 to 20 °C will have more effect on the litter consumption than a decrease from 15 to 10 °C, resulting in a higher amount of consumed litter for a fluctuating temperature regime. Fig. 2B shows that, when temperature fluctuates, the volume of the structural



Fig. 2 – The amount of litter consumed by individual earthworms (A), their structural body mass (B) and the scaled amount of consumed litter (C) during the time interval from birth to the age of 3 years, using a functional response f of 0.5, two Cu concentrations (5 and 150 mg/kg) and three temperature scenarios (10,  $15 \pm 5$  and  $20 \degree C$ ). The amount of consumed litter in C was scaled by dividing it by the maximum amount consumed by non-exposed earthworms at the age of 3 years under similar circumstances. Solid lines: 5 mg/kg Cu, dotted lines: 150 mg/kg Cu, (\*) 20 °C, square: 10 °C, and lines without markers:  $15 \pm 5 \degree C$ .

body mass is in between the volume at constant temperatures of T = 10 °C and T = 20 °C. Fig. 3B shows that the volume of the structural body mass, during the time interval from birth to the age of 3 years, is larger if temperature fluctuates compared to constant temperatures. This is the result of the assumption that cocoons hatch in April. As a result, temperature varies between 15 and 20 °C during the first months (April-September) of the life of L. rubellus and therefore the growth rate during these months is increased compared to a constant temperature of 15 °C. As a result, the volume of the structural body mass is higher during most of the time interval from birth to the age of 3 years. So, it can be concluded that the amount of litter consumed by L. rubellus is higher when temperature fluctuates than for a constant regime with the same average temperature, because the parameter  $\{\dot{J}_{Xm}\}$  is an exponential function of temperature and because it was assumed that cocoons hatch in April.



Fig. 3 – The amount of litter consumed by non-exposed individual earthworms (A) and their structural body mass (B), during the time interval from birth to the age of 3 years, for four combinations of temperature and functional response f. Solid line:  $T = 15 \degree C$  and f = 0.75, dotted line:  $T = 15 \pm 5 \degree C$  and f = 0.75, dashed line:  $T = 15 \degree C$  and  $f = 0.75 \pm 0.25$ , dashed-dotted line:  $T = 15 \pm 5 \degree C$  and  $f = 0.75 \pm 0.25$ .

#### 3.2. Effect of food density on litter consumption

Fig. 4A shows that, for scenarios with a constant food density, the amount of litter consumed by L. rubellus from birth to the age of 3 years increases if food density increases. This can be explained by the dependence of the ingestion rate  $\dot{J}_X$ on f and  $V^{2/3}$  (Eq. (1)). The functional response f increases when the level at which the food density is kept constant, increases. Fig. 4B shows that the maximum volume, that can be reached by L. rubellus, increases with food density. So, the total amount of consumed litter increases when food density increases, due to a higher functional response *f* and a larger volume of the structural body mass. Fig. 3A shows that, for scenarios with a functional response varying between 0.5 and 1.0, the amount of litter consumed by L. rubellus is higher than the amount consumed at a constant f of 0.75, for most points in the time interval from birth to the age of 3 years. During three small time intervals, the amount of consumed litter is approximately equal to or lower than the amount consumed at a constant f of 0.75, so the length of the time interval should be taken into account when the effect of varying food density on the amount of consumed litter is determined. Fig. 4B shows



Fig. 4 – The amount of litter consumed by individual earthworms (A), their structural body mass (B) and the scaled amount of consumed litter (C) during the time interval from birth to the age of 3 years, using a temperature of  $15 \,^{\circ}$ C, two Cu concentrations (5 and 150 mg/kg) and three scenarios for the functional response f (0.5, 0.75 ± 0.25 and 1.0). The amount of consumed litter in C was scaled by dividing it by the maximum amount consumed by non-exposed earthworms at the age of 3 years under similar circumstances. Solid lines:  $5 \,\text{mg/kg Cu}$ , dotted lines:  $150 \,\text{mg/kg Cu}$ , (\*) f = 1.0, square: f = 0.5, and lines without markers:  $f = 0.75 \pm 0.25$ .

that the use of a fluctuating functional response (food density) results in a volume of the structural body mass in between the volume at a constant f of 0.5 and 1.0. When the energy flux from the reserve is too low to cope with the maintenance costs, structural body mass is burned to produce energy. This explains why the structural body mass fluctuates. Comparing the volume of the structural body mass V in Fig. 3B with f fluctuating around 0.75 to the volume at a constant f of 0.75,  $V_{0.75}$ , the time interval during which  $V > V_{0.75}$  is larger than the time interval during which  $V < V_{0.75}$ . Also the absolute difference between the highest values of V and  $V_{0.75}$  is larger than the absolute difference between the lowest values of V and V<sub>0.75</sub> (Fig. 3B). However, at a constant body mass, fluctuation of f around an average value of 0.75 does not increase the amount of consumed litter at a constant body mass, because the ingestion rate is proportional to f (Eq. (1)). Therefore, the fact that the



Fig. 5 – The amount of litter consumed by individual earthworms (A), their structural body mass (B) and the scaled amount of consumed litter (C) during the time interval from birth to the age of 3 years for two Cu concentrations (5 and 150 mg/kg) and two combinations of temperature and functional response  $f(T = 10 \pm 5 \degree C$  and  $f = 0.5 \pm 0.25$ ;  $T = 15 \pm 5 \degree C$  and  $f = 0.75 \pm 0.25$ ). The amount of consumed litter in C was scaled by dividing it by the maximum amount consumed by non-exposed earthworms at the age of 3 years under similar circumstances. Solid line: Cu = 5 mg/kg,  $T = 10 \pm 5 \degree C$  and  $f = 0.5 \pm 0.25$ ; dotted line: Cu = 5 mg/kg,  $T = 10 \pm 5 \degree C$  and  $f = 0.75 \pm 0.25$ ; dotted line: Cu = 150 mg/kg,  $T = 10 \pm 5 \degree C$  and  $f = 0.75 \pm 0.25$ ; dashed line: Cu = 150 mg/kg,  $T = 10 \pm 5 \degree C$  and  $f = 0.75 \pm 0.25$ ; dashed line:

amount of litter consumed by *L*. *rubellus* from birth to the age of 3 years is a little increased by using a fluctuating instead of a constant functional response *f* (food density), must be due to the combination of the hatching time of cocoons in April and the modeling of the functional response, with a maximum in autumn and a minimum in spring. This results in an increased volume of the structural body mass of *L*. *rubellus* compared to constant functional response scenarios during more than half of the simulation period.

## 3.3. The effect of fluctuating both temperature and food density on litter consumption

Fig. 5A shows that the amount of litter consumed by L. *rubellus*, from birth to the age of 3 years increases if the annual averages

Table 2 – EC50s (mg/kg) for the effect of Cu on total food consumption from birth until the age of 3 years by *L. rubellus* in a sandy loam soil, for all combinations of six temperature and six functional response (food density) scenarios

Temperature (°C)		Functional response						
	0.25	$0.25\pm0.25$	0.5	$0.5\pm0.25$	0.75	$0.75\pm0.25$		
10	246	284	245	258	247	255		
$10\pm5$	241	278	240	252	242	248		
15	224	286	222	242	223	233		
$15\pm5$	222	282	220	239	221	230		
20	214	258	213	229	213	221		
$20\pm5$	213	252	212	226	212	219		

For each combination of temperature and functional response, the EC50 was calculated relative to the food consumption of non-exposed earthworms using the same combination of temperature and functional response.

around which the temperature and functional response fluctuate increase. Fig. 3A shows that the amount of consumed litter is higher for scenarios with varying temperature and functional response than for constant scenarios. This can be explained by looking at Eq. (1) again. If temperature and f fluctuate, the behaviour of the product  $\{\dot{J}_{Xm}\}f$  together with  $V^{2/3}$ determine the ingestion rate. Assuming that  $\{J_{Xm}\}$  fluctuates around  $\{\dot{J}_{Xm}\}_{av}$  due to the fluctuation of the temperature and f around  $f_{\rm av}$  due to the fluctuation of the food density, the product  $(J_{Xm})f$  is larger than  $(J_{Xm})_{av}f_{av}$  when  $(J_{Xm}) > (J_{Xm})_{av}$ and  $f > f_{av}$  and smaller than  $\{\dot{j}_{Xm}\}_{av}f_{av}$  when  $\{\dot{j}_{Xm}\} > \{\dot{j}_{Xm}\}_{av}$  and  $f > f_{av}$ . When  $\{\dot{J}_{Xm}\} > \{\dot{J}_{Xm}\}_{av}$  and  $f > f_{av}$  or  $\{\dot{J}_{Xm}\} > \{\dot{J}_{Xm}\}_{av}$  and  $f > f_{av}$  it depends on the amplitudes of the fluctuation of  $\{\dot{J}_{Xm}\}$ and *f* if their product is higher or lower than  $\{\dot{J}_{Xm}\}_{av}$ . For the scenarios in Figs. 3 and 5,  $\{\dot{J}_{Xm}\}f$  is higher than  $\{\dot{J}_{Xm}\}_{av}f_{av}$  for 173 days and lower for 192 days per years. However, the absolute difference between the highest value of  $\{\dot{j}_{Xm}\}f$  and  $\{\dot{j}_{Xm}\}_{av}f_{av}$  is larger than the difference between the lowest value of  $\{\dot{J}_{Xm}\}f$ and  $\{J_{Xm}\}_{av}f_{av}$ .

Comparing the volume of the structural body mass V for a scenario with varying temperature and functional response f (Fig. 5B) to the volume of the structural body mass  $V_c$  for a constant scenario (Fig. 3B), shows that the time interval during which  $V > V_c$  is larger than the time interval during which V < V<sub>c</sub>. Also the absolute difference between the highest values of V and  $V_c$  is larger than the absolute difference between the lowest values of V and V<sub>c</sub>. So, although the product  $\{J_{Xm}\}f$  is smaller during most of the year compared to this product for a scenario with a constant temperature and functional response, the amount of litter consumed by L. rubellus from birth to the age of 3 years is increased by using a fluctuating temperature and functional response. Firstly, because the volume of the structural body mass is more often larger than smaller compared to the volume under constant conditions, and secondly, because the difference between maximum value of product  $\{\dot{J}_{Xm}\}f$  for a fluctuating scenario and  $\{J_{Xm}\}_{av}f_{av}$  is higher compared to the difference between the minimum value of  $\{J_{Xm}\}f$  and  $\{J_{Xm}\}_{av}f_{av}$ .

## 3.4. Effects of temperature and food density on the sensitivity to Cu

Figs. 2C, 4C and 5C show the scaled amount of litter consumed by exposed (150 mgCu/kg dry soil) and non-exposed (5 mgCu/kg dry soil) L. rubellus, for scenarios using varying temperature, food density and both varying temperature and food density, respectively. The amount of consumed litter is scaled by dividing it by the total amount of litter consumed by non-exposed earthworms, raised under similar temperature conditions and food density. These figures show that increasing the Cu concentration from 5 to 150 mg/kg dry soil reduces the amount of consumed litter by approximately the same factor for all temperature and food density scenarios. This suggests that the sensitivity of *L. rubellus* to Cu is not affected by temperature and food density.

However, EC50s in Table 2 show that increasing the average annual temperature for both the constant regimes and the fluctuating regimes from 10 to 20 °C results in a decrease of the EC50s between 28 and 34 mg Cu/kg (factor 0.86-0.88) for constant functional response scenarios and between 26 and 34 mg Cu/kg (factor 0.87–091) for varying functional response scenarios. EC50s in Table 2 show that increasing f for constant regimes results in a change of EC50s of at most 2 mg Cu/kg, regardless of the temperature scenario. However, increasing the average value of *f* for fluctuating regimes from 0.25 to 0.75 results in a decrease of EC50s between 29-53 mg Cu/kg (factor 0.81-0.90) for constant temperature scenarios and between 29 and 53 mg Cu/kg (factor 0.82-0.89) for fluctuating temperature scenarios. Table 2 shows that the using of a fluctuating temperature and functional response instead of constant scenarios increased EC50s by 1-58 mg Cu/kg (factor 1.004-1.26).

Table 2 shows that the effect of increasing temperature on EC50s depends on the functional response and that the reverse is also true. The effect on EC50s of increasing the temperature for constant scenarios or the average temperature for fluctuating scenarios varies between 6 and 4 mg Cu/kg, respectively, as a result of changes in the functional response. The effect on EC50s of increasing the functional response (food density) for constant scenarios or the average value of the functional response for fluctuating scenarios varies between 24 and 22 mg Cu/kg, respectively, as a result of changes in the temperature regime.

#### 4. Discussion

#### 4.1. Validity of model assumptions

#### 4.1.1. Basic DEB-model assumptions

The assumptions on which the basic DEB-model is based are listed in Table 3. It will take a considerable research effort

#### Table 3 - The assumptions that lead to the basic DEB model according to Table 3.3 in Kooijman (2000)

#### General

- 1. Structural body mass and reserves are the state variables of the individual; they have a constant composition
- 2. Food is converted into faeces, and assimilates derived from food are added to reserves. These fuel all other metabolic processes, which can be classified into three categories: synthesis of structural body mass, synthesis of gametes, and processes that are not associated with synthesis of biomass. Products that leave the organism may be formed in direct association with these three categories of processes, and with the assimilation process
- 3. At the start of the embryonic stage, an individual has a negligibly small structural body mass (but a substantial amount of reserves)

#### Specific

3a. The reserve density of the hatchling equals that of the mother at egg formation

- 4. Foetuses develop in the same way as embryos in eggs, but at a rate unrestricted by energy reserves. The transition from embryo to juvenile initiates feeding, that from juvenile to adult initiates reproduction, which is coupled to the cessation of maturation. The transitions occur when the cumulated energy invested in maturation exceeds certain threshold values. Unicellulars divide when the cumulated energy invested in maturation exceeds a threshold value
- 5. Somatic and maturity maintenance are proportional to structural body volume, but maturity maintenance does not increase after a given cumulated investment in maturation. Heating costs for endotherms are proportional to surface area
- 6. The feeding rate is proportional to the surface area of the organism and the food handling time and the digestion efficiency are independent of food density
- 7. The reserves must be partitionable, such that the dynamics is not affected; the use of reserves does not depend on food density; the reserve density at steady state does not depend on structural body mass (weak homeostasis)
- 8. A fixed fraction of energy, utilized from the reserves, is spent on somatic maintenance plus growth, the rest on maturity maintenance plus maturation or reproduction (the K-rule)
- 9. Under starvation conditions, individuals always give priority to somatic maintenance and follow one of two possible strategies: they do not change the reserve dynamics (so continue to invest in development or reproduction), or cease energy investment in development and reproduction (thus changing reserves dynamics)

to investigate these assumptions separately. To verify these assumptions, one should be able to determine the energy reserves in an organism and follow energy streams to the reserves and from the reserves to different processes. A number of authors have studied energy budgets in organisms (e.g. for earthworms: Bolton and Phillipson, 1976b; for isopods: Khalil et al., 1995) by quantifying energy input (assimilation) and output in the form of growth, reproduction and respiration. The DEB-model however distinguishes between respiration due to somatic maintenance, maintenance of maturity, overhead costs of growth and reproduction, which will be difficult to do experimentally. It will also be difficult to quantify energy reserves when they are not located at a specific location in an organism.

Another approach to verify the assumptions in the DEBmodel is the goodness of fit of the model predictions to data. Klok and De Roos (1996) showed that the growth and reproduction of *L*. *rubellus* at different Cu concentrations and unlimited food density and kept at 15 °C in sandy loam soil could be described well by the DEB-model. Because the availability of energy for growth and reproduction depends on the energy flux to and out of the reserve, this suggests that the DEB-model is able to describe energy fluxes in *L*. *rubellus*.

#### 4.1.2. Additional assumptions

In this study it was assumed that earthworms are capable of using structural body mass as an energy source, when the energy flux from the reserve is not high enough to cover the maintenance costs. This resulted in a decline of the body mass at low food densities. In reality, a part of the energy spent on the production of an amount of structural body mass will consist of overhead costs and is lost. However, because no data were available to quantify the amount of energy lost on overhead costs of growth, it was assumed that the amount of energy produced by burning a unit of volume of structural body mass was assumed to equal the energy needed for the production of a similar volume of structural body mass. This will have resulted in an overestimation of the volume of the structural body mass and therefore of food consumption.

#### 4.1.3. Temperature dependency of parameters

Modeling the influence of temperature by applying  $Q_{10}$  factors to rate parameters in the DEB-model results in an increasing food consumption at higher temperatures (see Fig. 2A).

Data on the influence of temperature on food consumption are scarce in the literature. Daniel (1991) found that litter consumption by L. *terrestris* increased until an optimum temperature of 22.5 °C, followed by a sharp decline at higher temperatures. So, for the range of temperatures used in this study, this is in agreement with the model output (Fig. 2A). However, Ma (1984) found that the litter consumption by L. *rubellus* in control as well as Cu treated soil was highest at  $15 \,^{\circ}$ C.

In the DEB-model, litter consumption depends on the  $V^{2/3}$  (Eq. (1)). Therefore, in order to predict food consumption, it is essential that the DEB-model is able to describe the influence of temperature on the growth of *L. rubellus*. The model output predicts that, for scenarios with a constant temperature, increasing the temperature level results in an increased growth rate, but similar maximum weight (Fig. 2B). The only data on growth of *L. rubellus* at different temperatures were reported by Ma (1984), who found that growth of adult *L*.

rubellus was higher at 12 °C than at 15 and 18 °C. Van Gestel et al. (1992) found that the growth rate of Eisenia andrei was highest at 15 °C, a little lower at 20 °C, followed by a large decline at 25 °C. So these findings are in contradiction with the model output. However, growth of the earthworms Aporrectodea longa (Baker and Whitby, 2003), A. tuberculata (Wever et al., 2001), Allolobophora chlorotica (Butt, 1997) and Dendrobaena veneta (Fayolle et al., 1997) increased with temperature. At temperatures higher than the optimum temperature for growth, body weight declined fast. The optimum temperatures in the studies mentioned above were higher than 20  $^\circ\text{C}.$  So, the growth of these earthworm species is in agreement with the predictions of the DEB-model if temperature varies within the range used in this study (5-20 °C). Most studies did not follow the growth of earthworms long enough to determine the maximum body weight. Fayolle et al. (1997) showed that D. veneta kept at temperatures between 10 and 25  $^\circ$ C had similar body weights after 189 days, which is in agreement with the model output. So, although the only available data on L. rubellus suggest that the optimum temperature for growth is 12  $^\circ\text{C},$  growth predicted by the model shows a trend similar to literature data on other species.

Perhaps the contradiction between the data reported by Ma (1984) for growth and litter consumption by L. rubellus at different temperatures and the model output can be explained by the short time interval of six weeks over which growth was determined and the use of adult individuals. Most studies on earthworm species that followed growth of juveniles, just hatched from their cocoons, for a longer time period were in agreement with the model predictions. Uvarov and Scheu (2004) showed that the respiration rate of juvenile L. rubellus increased from 10 to 20°C, but that the respiration rate of adult individuals increased with temperature until 15°C was reached and then remained constant. This can explain why growth and litter consumption by L. rubellus, reported by Ma (1984), did not increase when temperature increased from 15 to 20°C, but cannot explain a higher growth rate at temperatures below 15 °C.

#### 4.1.4. Toxicological model

In spite of the lack of data on the uptake kinetics of Cu by *L*. *rubellus* in the literature, Klok and De Roos (1996) were able to reasonably describe the effects of Cu on the growth and reproduction of *L*. *rubellus* using the assumption that its internal Cu concentration is always in equilibrium with the Cu concentration in the environment. Consequently, the results of Klok and De Roos (1996) also support the assumption that effects of Cu depend on the difference between the internal concentration and the internal no effect concentration (Eq. (7)). No data could be found on the relation between internal Cu concentrations and effects on growth and food consumption in earthworms, but Ma (2005) shows that there is a clear negative correlation between fecundity and internal Cu concentrations in *L*. *rubellus*, once a threshold concentration is exceeded.

Janssen and Bergema (1991) found that temperature also affected the uptake and excretion kinetics of metals in ectotherms. Incorporating this in the model could result in variation of the internal metal concentration with temperature and therefore change the effects of Cu as predicted by the model used in this study. Next to this, the bioconcentration factor of Cu decreases with the exposure concentration (Neuhauser et al., 1995). It is difficult to reason how incorporating these factors in the model, will affect model predictions.

## 4.2. Effect of temperature and food density on litter consumption of L. rubellus

Effects of temperature were modeled by applying the factor  $Q_{10}$  to the parameters  $\{\dot{p}_{Am}\}$  and  $[\dot{p}_{M}]$ , and also to  $(\dot{j}_{Xm})$ , because the assimilation efficiency was assumed to be constant. The effect of changing the food density was modeled by multiplying the maximum ingestion rate with functional response f. Therefore, changes in temperature and food density will qualitatively affect the consumption of litter by L. *rubellus* in a similar way for different parameter settings.

The model output shows that the amounts of litter consumed by *L. rubellus* for scenarios with a seasonally fluctuating temperature and/or food density differ little from scenarios with constant temperature and food density equal to these average values (Fig. 2A). Results indicate that the amount of consumed litter from birth to the age of 3 years, depends on the hatching time in combination with the modeling of the fluctuation of the temperature and the functional response (food density). The importance of this dependence was not studied. However, fluctuation of the temperature is expected to increase the amount of consumed litter compared to a constant temperature equal to the average of the fluctuating regime, if the time interval is chosen long enough.

## 4.3. Effect of seasonal variation of temperature and food density on the sensitivity of L. rubellus for Cu

Due to the uncertainty in the estimation of parameter values, predicted EC50s should be interpreted carefully. However, parameters in the DEB-model depend on temperature and food density in such a way, that changes in temperature and food density will qualitatively affect EC50s in a similar way, independent of the parameter settings.

Based on EC50s predicted by the DEB-model, it can be concluded that increasing the temperature for constant and the average annual temperature for fluctuating scenarios increases the sensitivity of the litter consumption by L. *rubellus* for Cu. Increasing the food density only resulted in an increase of the sensitivity of the litter consumption by L. *rubellus* for Cu for scenarios with a fluctuating food density.

These results suggest that effect concentrations of Cu on litter consumption by earthworms determined at a constant temperature can be extrapolated to earthworms in the field experiencing a seasonal variation in temperature. However, they show that the average temperature at which effect concentrations are determined in the laboratory, should be taken into account in risk assessment procedures based on total soil concentrations. The results also suggest that food density is not an important factor in laboratory tests designed to determine effect concentrations of Cu on litter consumption by earthworms, as long as it is constant. However, effect concentrations determined at constant food density will overestimate effects of Cu on earthworms living in the field that experience a seasonally fluctuating food density. In spite of the dependence of EC50s on food density and temperature, mentioned above, the sensitivity to a Cu concentration in the soil of 150 mgCu/kg differed very little between different temperature and functional response scenarios (Figs. 2C, 4C and 5C). Perhaps, this can be explained by the fact that EC50s were higher than 150 mgCu/kg, in the range of 200–300 mgCu/kg, and differences in sensitivity due to temperature and food density increase with exposure to Cu.

#### 4.4. Suggestions for further research

To be able to better verify the assumptions of the DEB-model and quantify the effect of temperature and food density on the sensitivity of *L. rubellus* for Cu, research is needed on the following topics:

- the relation between food consumption and the structural body mass of earthworms;
- the functional response for earthworms feeding on both soil organic matter and leaf litter;
- the effect of variation in food density on growth and reproduction (reserve dynamics);
- the effect of variation in temperature on food consumption by earthworms;
- the uptake and excretion kinetics of Cu in earthworms;
- the relation between the internal concentration of heavy metals and effects on growth and food consumption (taking possible detoxification by storage into account).

#### 5. Conclusions

The qualitative effects of temperature on growth, as predicted by the DEB-model, were in agreement with most data on earthworm species that could be found in the literature. Few data were available on the effect of temperature on the litter consumption by earthworms, but data on the species Lumbricus terrestris were in agreement with the qualitative effects that were predicted by the DEB-model. Assuming that the DEBmodel can describe the food consumption by most earthworm species as a function of their internal Cu concentration and as a function of temperature and food density, model predictions show that the effect of Cu on the litter consumption by earthworms is larger at higher temperatures for both constant and seasonally fluctuating temperature regimes, but that it does not matter if temperature fluctuates or not. If food density fluctuates, increasing the average food density results in an increase of the effect of Cu the litter consumption by earthworms. The modeling approach based on dynamic energy budgets seems to be a useful tool to integrate literature data and to predict effects of environmental variables and toxicants that are difficult to measure experimentally, but relevant in the field.

#### REFERENCES

Baker, G.H., Whitby, W.A., 2003. Soil pH preferences and the influence of soil type and temperature on the survival and

growth of Aporrectodea longa (Lumbricidae). Pedobiologia 47, 745–753.

- Bolton, P.J., Phillipson, J., 1976a. Energy equivalents of earthworms, their egesta and a mineral soil. Pedobiologia 16, 443–450.
- Bolton, P.J., Phillipson, J., 1976b. Burrowing, feeding, egestion and energy budgets of Allolobophora rosea (Savigny) (Lumbricidae). Oecologia 23, 225–245.
- Brussaard, L., 1998. Soil fauna, guilds, functional groups and ecosystem processes. Appl. Soil Ecol. 9, 123–135.

Butt, 1997. Reproduction and growth of the earthworm Allolobophora chlorotica (Savigny 1826) in controlled environments. Pedobiologia 41, 369–374.

Daniel, O., 1991. Leaf-litter consumption and assimilation by juveniles of Lumbricus terrestris L. (Oligochaeta Lumbricidae) under different environmental conditions. Biol. Fertil. Soils 12, 202–208.

- Donker, M.H., Abdel-Lateif, H.M., Khalil, M.A., Bayoumi, B.M., Van Straalen, N.M., 1998. Temperature, physiological time and zinc toxicity in the isopod Porcellio scaber. Environ. Toxicol. Chem. 17, 1558–1563.
- Fayolle, L., Michaud, H., Cluzeau, D., Stawiecki, J., 1997. Influence of temperature and food source on the life cycle of the earthworm *Dendrobaena veneta* (Oligochaeta). Soil Biol. Biochem. 29, 747–750.
- Janssen, P.M., Bergema, W.F., 1991. The effect of temperature on cadmium kinetics and oxygen consumption in soil arthropods. Environ. Toxicol. Chem. 10, 1493– 1501.
- Khalil, M.A., Donker, M.H., Van Straalen, N.M., 1995. Long-term and short-term changes in the energy budget of *Porcellio scaber* Latreille (Crustacea) exposed to cadmium in polluted food. Eur. J. Soil Biol. 31, 163–172.
- Klok, C., De Roos, A.M., 1996. Population level consequences of toxicological influences on individual growth and reproduction in *Lumbricus rubellus* (Lumbricidae Oligochaeta). Ecotoxicol. Environ. Safety 33, 118–127.
- Kooijman, S.A.L.M., 2000. Dynamic Energy and Mass Budgets in Biological Systems. Cambridge University Press, Cambridge, p. 424.
- Ma, W.-C., 1983. Biomonitoring of soil pollution: ecotoxicological studies of the effect of soil-borne heavy metals on lumbricid earthworms. RIN, Annual Report 1982, Arnhem, The Netherlands.
- Ma, W.-C., 1984. Sublethal toxic effects of Cu on growth, reproduction and litter breakdown activity in the earthworm *L. rubellus*, with observations on the influence of temperature and soil pH. Environ. Pollut. (Series A) 33, 207–219.
- Ma, W.-C., 1988. Toxicity of copper to lumbricid earthworms in sandy agricultural soils amended with Cu-enriched organic waste materials. Ecol. Bullet. 39, 53–56.
- Ma, W.-C., 2005. Critical body residues (CBRs) for ecotoxicological soil quality assessment: copper in earthworms. Soil Biol. Biochem. 37, 561–568.
- Neuhauser, E.F., Cukic, Z.V., Malecki, M.R., Loehr, R.C., Durkin, P.R., 1995. Bioconcentration and biokinetics of heavy metals in the earthworm. Environ. Pollut. 89, 293–301.
- Spurgeon, D.J., Hopkin, S.P., 1999. Tolerance of zinc in populations of the earthworm Lumbricus rubellus from uncontaminated and metal-contaminated ecosystems. Archive Environ. Contamin. Toxicol. 37, 332–337.

Spurgeon, D.J., Svendsen, C., Rimmer, V.R., Hopkin, S.P., Weeks, J.M., 2000. Relative sensitivity of the life-cycle and biomarker responses in four earthworm species exposed to zinc. Environ. Toxicol. Chem. 19, 1800–1808.

Uvarov, A.V., Scheu, S., 2004. Effects of temperature regime on the respiratory activity of developmental stages of *Lumbricus rubellus* (Lumbricidae). Pedobiologia 48, 365– 371.

- Van Gestel, C.A.M., Dirven-van Breemen, E.M., Baerselman, R., 1992. Influence of environmental conditions on the growth and reproduction of the earthworm *Eisenia andrei* in an artificial soil substrate. Pedobiologia 36, 109–120.
- Wever, L.A., Lysyk, T.J., Clapperton, M.J., 2001. The influence of soil moisture and temperature on the survival, aestivation, growth and development of juvenile *Aporrectodea tuberculata* (Eisen) (Lumbricidae). Pedobiologia 45, 121–133.