TRACE document

This is a TRACE document ("TRAnsparent and Comprehensive model Evaludation") which provides supporting evidence that our model presented in:

> **Johnston, A.S.A., Hodson, M.E., Throbek, P., Alvarez, T., Sibly, R.M., 2014. An energy budget agent-based model of earthworm populations and its application to study the effect of pesticides. Ecological Modelling. http://dx.doi.org/10.1016/j.ecolmodel.2013.09.012.**

was thoughtfully designed, correctly implemented, thoroughly tested, well understood, and appropriately used for its intended purpose.

The rationale of this document follows:

> Schmolke A, Thorbek P, DeAngelis DL, Grimm V. 2010. Ecological modelling supporting environmental decision making: a strategy for the future. *Trends in Ecology and Evolution* 25: 479-486.

and uses the updated standard terminology and document structure in:

> Grimm V, Augusiak J, Focks A, Frank B, Gabsi F, Johnston ASA, Kułakowska K, Liu C, Martin BT, Meli M, Radchuk V, Schmolke A, Thorbek P, Railsback SF. 2014. Towards better modelling and decision support: documenting model development, testing, and analysis using TRACE. *Ecological Modelling*

and

> Augusiak J, Van den Brink PJ, Grimm V. 2014. Merging validation and evaluation of ecological models to 'evaludation': a review of terminology and a practical approach. *Ecological Modelling*.

If this document include **hyperlinks**, navigation back and forth along previously chosen links works via "ALT" + "←" or "ALT" + "→".

Note: The original publication, Johnston et al. (2014), provides, in the Supplementary Material, a previous version of the TRACE document, which followed the original TRACE format and terminology suggested by Schmolke et al. (2010).

Content

1 Problem formulation

This TRACE element provides supporting information on: The decision-making context in which the model will be used; the types of model clients or stakeholders addressed; a precise specification of the question(s) that should be answered with the model, including a specification of necessary model outputs; and a statement of the domain of applicability of the model, including the extent of acceptable extrapolations.

Summary:

The acquisition and expenditure of energy to life cycle processes depends on a combination of environment- and organism-specific conditions. In addition, exposure of individuals to chemical stress can alter a populations dynamics via physiological pathways. To investigate the sublethal effects of pesticides we develop and evaluate an energy budget agent-based model of the earthworm *Eisenia fetida*. *E. fetida* is used as a model species here due to it's recommended use in lower tier toxicity tests, and therefore ample quantity of literature data available for model development at the individual level.

The model is designed to estimate the effects of varying food supplies, soil temperature, soil moisture and pesticide applications on earthworm population dynamics. The purpose of the model is to extrapolate from lower tier toxicity experiments for the OECD recommended earthworm species *Eisenia fetida*, in order to interpret the sublethal effects of toxicants at the field level. This forms a foundation for the application of the model in predicting pesticide (organic and inorganic) effects on earthworm populations under field conditions. We synthesise knowledge on the effects of pesticides on individual physiology and develop and evaluate methods for identifying the potential underlying physiological parameters toxicity disrupts. The broader model aim is to act as a potential refinement option in current risk assessments of soil invertebrates (SANCO, 2002; 2010). Thus, stakeholders include relevant

agrochemical regulators and plant protection product (PPP) developers and risk assessors. Specific questions addressed by the model are: 1) Which physiological parameters are affected by toxic stress of different pesticides? 2) How does imposed stress on specific physiological parameters within the model translate into changes in growth, reproduction and survival rates? 3) How do variable background conditions (e.g. temperature, food availability) interact with toxic stress at the individual level? Environmental variables considered in the model are food density, soil temperature and soil moisture, which together determine the ecological niche of *E. fetida* (Reinecke & Viljoen, 1990; Tripathi & Bhardwaj, 2004; Edwards & Bater, 1992). As *E. fetida* is of importance in vermicomposting of animal wastes and is the recommended OECD test species (OECD, 1984), literature data is available on individual life cycle processes e.g. growth and reproduction and the variation of these rates under optimal to limiting environmental conditions. Model outputs are compared to literature data to assess the models applicability as a potential foundation for generating useful tools for predictive toxicology. Although extrapolations to the field level are possible when toxic stress is absent, literature data is not available for validating field applications of pesticides for this species. Thus, the model is currently applicable at the laboratory scale only, and outputs on growth and reproduction are evaluated over short periods of chemical exposure (< 56 days) and recorded at regular time-steps. In addition, data on two pesticides, copper oxychloride and chlorpyrifos, are used to evalute the models ability to predict sublethal effects at the individual level. Extrapolation to different pesticides is readily achievable given data on the dose-response relationships between chemical concentration and growth and/or reproduction outputs. Future applications of the model will be within an ecotoxicological framework, simulating field applications of pesticides on the population dynamics of ecologically relevant earthworm species.

2 Model description

This TRACE element provides supporting information on: The model. Provide a detailed written model description. For individual/agent-based and other simulation models, the ODD protocol is recommended as standard format. For complex submodels it should include concise explanations of the underlying rationale. Model users should learn what the model is, how it works, and what guided its design.

Summary:

Here we present the complete model description. It is identical to the one given in Johnston et al. (2014), but nevertheless included here because it is relatively short and allows to keep all supplementary information in one document.

The model description follows the ODD (Overview, Design concepts, Details) protocol for describing individual-based models (Grimm et al., 2006; Grimm et al., 2010). The model was implemented in NetLogo 5.0 (Wilensky, 1999), a free software platform for implementing individual-based models. The NetLogo code has been made available in the Supplementary Material of Johnston et al. (2014).

2.1 Purpose

The purpose of the model is to simulate *Eisenia fetida* population dynamics under varying environmental conditions representative of those encountered in the field and investigate how energy budgets can be used to investigate how pesticides achieve their physiological effects.

2.2 Entities, state variables and scales

This ABM comprises a number of individual *E. fetida* individuals and a model landscape consisting of two-dimensional 0.01 m^2 patches of soil. Individuals are characterized by life cycle stage (cocoon, juvenile or adult), mass and energy reserves, and landscape patches by food density, soil temperature, soil moisture and pesticide concentration. The model proceeds in discrete daily time-steps. Metabolic calculations are in units of energy per unit time (kJ/day).

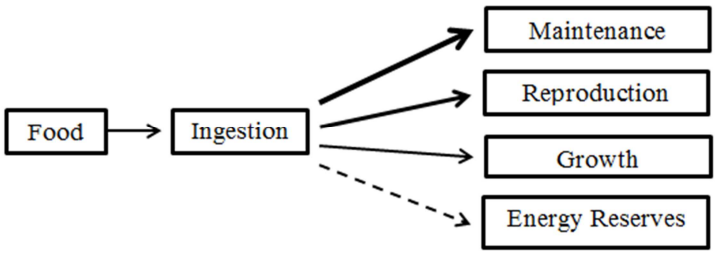

Figure 1. Structure of the energy budget model for adult *E. fetida*, with the thickness of arrows indicating priorities for energy allocation from food. Cocoons and juveniles are also in the model though cocoons do not grow and juveniles do not reproduce. Energy remaining after allocation enters the energy reserves which may be used for other functions when food is limited.

2.3 Process overview and scheduling

Each individual in the ABM has its own energy budget. The energy budget model includes algorithms for how energy uptake and expenditure direct life cycle processes based on fundamental principles of physiological ecology, and generally follows the methodology of Sibly et al. (2013). Individuals assimilate energy from ingested food (*Ingestion and Energy Uptake*) and expend available energy on maintenance (*Maintenance*), growth (*Growth*) and reproduction (*Reproduction*) in the order of priority outlined in Fig. 1. Total available energy is limited by the amount of food an organism can ingest, whilst mass and temperature have scaling effects on individual metabolic rates (Brown et al., 2004). Maintenance is essential for the survival of an individual, and thus has first priority for energy allocation. Juveniles grow until sexually mature, and thereafter adults preferentially allocate energy to reproduction before growth. If energy remains after reproduction and/or growth, energy is stored in the energy reserves as glycogen (Byzova, 1977), which may be used to pay maintenance costs when food is limited (*Energy Reserves and Starvation*).

Juveniles and adults move randomly in the landscape (*Movement*), assimilating a fixed proportion of energy from ingested food that fuels life cycle processes and survival. Feeding by individuals depletes landscape patches and the food density changes accordingly. Cocoons cannot feed or move but pay maintenance costs from energy reserves until they are fully developed at the end of the temperature-dependent incubation period, when they hatch as juveniles (Sousa et al., 2010). Juveniles transform to adults once they reach a body mass threshold for sexual maturity (Ma, 1984; Springett and Gray, 1992). Food was provided in the same amounts as in the experiment being simulated, and food densities in landscape patches depleted as individuals ingested food. When food was not available, energy reserves were used to cover maintenance costs. Once the energy reserves are depleted to a critical level individuals catabolise energy from tissue to meet maintenance demands (*Survival*). Pesticides were applied in the ABM at the concentrations and times specified in the experiment being simulated. Individuals experiencing these concentrations were affected as indicated by

potential 'toxicity submodels'. Fig. 2 gives an overview of processes occurring at the adult stage in each time-step under different feeding conditions.

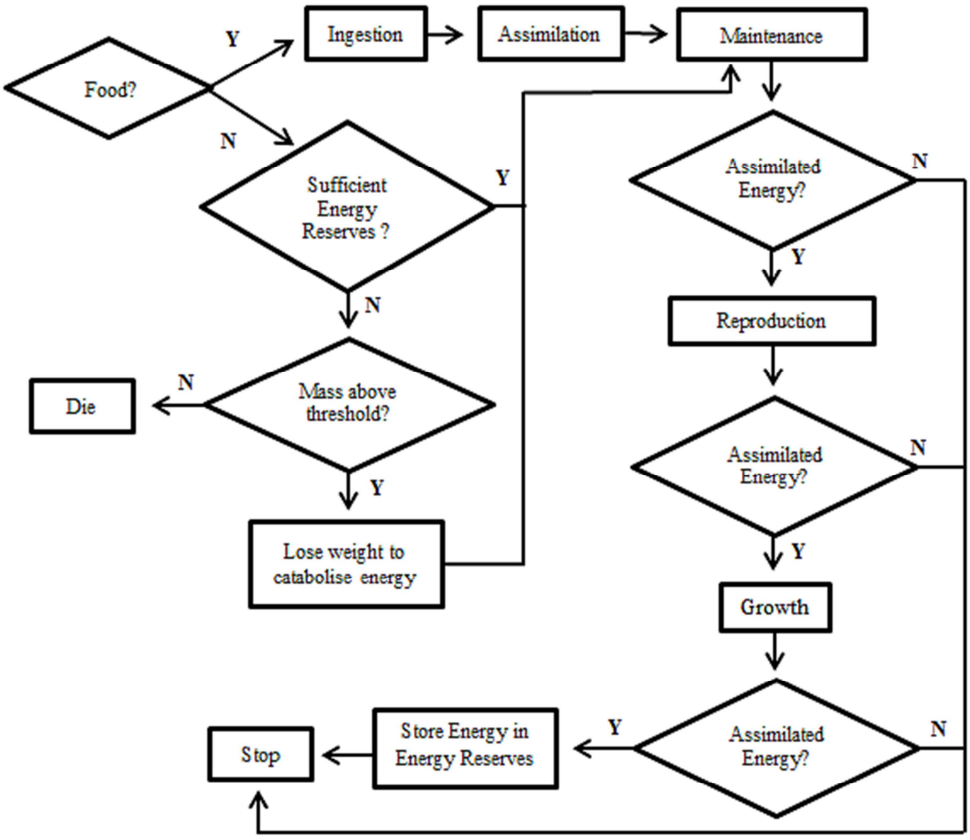

Figure 2. Partial energy flow diagram of *E. fetida* adults within the ABM, showing the processes (rectangles) each individual goes through per time step, with diamonds indicating decision points. Energy reserves are used for maintenance and reproduction in starving individuals.

2.4 Design concepts

Basic Principles. Key processes in the model determine how energy consumption and expenditure direct life cycle processes in response to environmental and pesticide exposure. Individual energy budgets follow fundamental principles of physiological ecology (Sibly and Calow, 1986) and scale with body mass and temperature according to known allometric laws (Sibly et al., 2013). Pesticides achieve their effects by imposing stress on specific physiological parameters following a dose-response relationship obtained from toxicity data.

Emergence. Variation in food availability between patches arises from the random movement and feeding of individuals in the landscape. Population dynamics emerge from differential energy allocation amongst individuals which is affected by food availability, soil temperature, soil moisture and pesticide concentration (Reinecke and Viljoen, 1990; Tripathi and Bhardwaj, 2004; Edwards and Bater, 1992).

Interaction. Individuals need mates (any other adult as earthworms are hermaphrodite (Dominguez et al., 2003)) present in the same patch to reproduce. Adults and juveniles

interact indirectly by competing for food within patches, and both affect patches by depleting food.

Stochasticity. Movement and background mortality are random amongst juveniles and adults, with specified probability density functions.

Observation. Population density, stage class structure (cocoon, juvenile, adult) and individual body masses and reproduction were recorded.

2.5 Initialization

Simulations were initialised with individuals randomly distributed in the landscape. Landscape size and earthworm numbers, life cycle stages and body masses followed the experiments being replicated, outlined in detail in section *2.2*.

Table 1. Default parameter values for the energy budget model with reference to literature data sources

Symbol	Definition	Value	Unit	Reference	Notes
A_e	Assimilation efficiency	0.50	---	Hobbelen and van Gestel (2007)	p. 376 (see 3 Data Evaluation)
B_o	Taxon-specific normalization constant	967	kJ/day	Meehan (2006)	Calculated from Table 2, p. 881 and Eq. 4 (see 3 Data Evaluation)
E	Activation energy	0.25	eV	Meehan (2006)	p. 880
E_c	Energy content of tissue	7	kJ/g	Peters (1983)	p. 235
E_s	Energy cost of synthesis	3.6	kJ/g	Sibly and Calow (1986)	Calculated from p. 54-55
E_x	Energy content of food	21.2	kJ/g	Wang et al. (2011)	p. 173
IG_{max}	Maximum ingestion rate	0.70	g/day/g	Neuhauser et al. (1980)	Derived/re-calculated from Fig. 6, p. 96 (see 3 Data Evaluation)
h	Half saturation coefficient	3.5	g/0.01 m^2	Neuhauser et al. (1980)	
M_b	Mass at birth	0.011	g	Gunadi et al. (2002)	Derived from Table 1, p. 18 and Fig. 1, p. 19
M_c	Mass of cocoon	0.015	g	Hartenstein et al. (1979)	Derived mean from Fig. 5, p. 333
M_p	Mass at sexual maturity	0.25	g	Gunadi et al. (2002)	Derived from Table 1, p. 18 and
M_m	Maximum asymptotic mass	0.50	g	Gunadi et al. (2002)	Fig. 1, p. 19
r_B	Growth constant	0.177	day^{-1}	Gunadi et al. (2002)	Fig. 1, p. 19 fitted to Eq. 5a (see 3 Data Evaluation)
r_m	Maximum rate of energy allocation to reproduction	0.182	kJ/g day	Tripathi and Bhardwaj (2004)	Derived from p. 281 (see 3 Data Evaluation)
T_0	Incubation period	23	days	Reinecke et al. (1992)	Table 3, p. 1298
T_{ref}	Reference temperature	298.15	kelvins	Tripathi and Bhardwaj (2004)	p. 280

2.6 Input data

The model does not utilize any input data for representing external driving factors.

2.7 Submodels

Species-specific parameters were derived from the literature for *E. fetida* as shown in Table 1. Where data were not available for *E. fetida* closely related species were used. For example, the assimilation efficiency estimated by Hobbelen and van Gestel (2007) was for *Lumbricus rubellus*, which we suggest is similar for *E. fetida* given its epigeic feeding strategy and additional support provided in *3 Data Evaluation*. A number of assumptions about the metabolism of individuals were necessary for model development and these are described in the following sections. Further details of parameter calculations are available in *3 Data Evaluation*. The following sections describe the energy budget model, outlined in the above sections and in Fig. 1, in terms of metabolic organisation at the individual level.

Maintenance

The basal metabolic rate (*B*) is the level of metabolism below which an organism cannot survive (Fry, 1971; Calow and Sibly, 1990), and is used here as a measure of maintenance costs. Costs of movement, small in earthworms, are here included in maintenance. *B* is known to scale with body mass (*M*) as a power law and temperature (*T*), measured in grams and kelvins respectively, according to the equation:

$$B = B_0 M^{3/4} e^{-E/kT} \qquad \text{Eq. 1}$$

where B_0 is a taxon-specific normalization constant, $M^{3/4}$ is the scaling with body mass, $e^{-E/\kappa T}$ is the exponential Arrhenius function, E is the activation energy, κ is the Boltzmann's constant (8.62 x 10^{-5} eV K^{-1}) (Table 1) (Peters, 1983; Gillooly et al., 2001; Brown and Sibly, 2012). In what follows it is sometimes convenient to consider effects of temperature relative to a reference temperature, T_{ref}. The effect of temperature is then given by $e^{\frac{-E}{\kappa}\left(\frac{1}{T}-\frac{1}{T_{ref}}\right)}$.

Ingestion and energy uptake

Variation in food density affects the rate of ingestion of food up to an asymptote according to a type II functional response (Holling, 1959; Ricklefs and Miller, 2000), so that:

$$\text{Ingestion rate} \propto \frac{X}{(h+X)}$$

where X is food density (g/0.01 m^2) and *h* is a constant that shows how quickly the response curve reaches its maximum as food density increases. Ingestion rate is also proportional to the surface area ($M^{2/3}$) of an individual as the search rate depends on the food gathering apparatus (Kooijman and Metz, 1984; Pilarska, 1977) and to temperature, giving:

$$\text{Ingestion rate} = IG_{max}\, e^{\frac{-E}{\kappa}\left(\frac{1}{T}-\frac{1}{T_{ref}}\right)} \frac{X}{(K+X)} M^{2/3} \qquad \text{Eq. 2}$$

where IG_{max} is the maximum ingestion rate recorded of a 1 g *E. fetida* under optimal feeding conditions (g/day/g) (Table 1). Ingestion rate is measured in g/day and this is converted into kJ/day depending on the energy content of the food. After ingestion, food is processed by the digestive system and a proportion, assimilation efficiency, becomes available for allocation to the various functions shown in Fig. 1. The value of the assimilation efficiency (A_e) (Table 1) depends on diet but not body mass (Hendriks, 1999).

Growth

After expenditure to maintenance and, at the adult stage, to reproduction, individuals allocate remaining energy to somatic growth. The maximum growth rate of an individual under optimal conditions is assumed to follow the von Bertalanffy (1957) growth equation:

$$M = M_m\left(1 - \left(1 - \left(\frac{M_b}{M_m}\right)^{1/3}\right) e^{-r_B t/3}\right)^3 \qquad \text{Eq. 3a}$$

where M_b and M_m denote mass at birth and maximum mass respectively and r_B is the Bertalanffy growth constant, obtained by fitting Eq. 3a to data recording the increase in individual biomass over time under optimal conditions. The maximum growth rate per time-step is obtained from:

$$\Delta M = r_B e^{\frac{-E}{\kappa}\left(\frac{1}{T} - \frac{1}{T_{ref}}\right)}\left(M_m^{1/3} M^{2/3} - M\right) \qquad \text{Eq. 3b}$$

(Sibly et al, 2013). The energy costs of growth are determined from the new mass calculated from Eq. 3b and the energy costs of production (E_c + E_s) (Table 1). Eq. 3b shows how the maximum rate at which resources can be allocated to growth changes as an individual increases in mass. If insufficient energy is available to support maximal growth, growth rate is reduced accordingly.

Reproduction

Reproduction is assumed to take priority over growth in adults, because in the absence of a sexual partner, earthworms grow larger (Neuhauser et al., 1980). Energy allocated to reproduction by adults goes directly to the production of an egg until oviposition inside a cocoon (note this is a slight simplification since *E. fetida* can insert more than one egg into a cocoon). The maximum rate of energy allocation to reproduction per day increases linearly with adult mass (Mulder et al., 2007):

$$\Delta R = r_m e^{\frac{-E}{\kappa}\left(\frac{1}{T} - \frac{1}{T_{ref}}\right)} M \qquad \text{Eq. 4}$$

where r_m is the maximum rate of energy allocation to reproduction per unit of adult mass (kJ/g/day). The energy cost of producing a hatchling is M_c (E_c + E_s) (Table 1) and the hatchling's energy reserve content is initially M_c E_c, which is utilized for maintenance during the incubation period.

Energy reserves and starvation

If any assimilated energy remains after expenditure on relevant life processes (Fig. 1) it is stored in an individual's energy reserves. Energy is stored as glycogen (Byzova, 1977), costing E_s = 3.6 kJ to store 1g with an energy content of E_c = 7 kJ (Sibly and Calow, 1986; Peters, 1983). When energy is not available from ingested material, maintenance costs are taken from energy reserves, allowing individuals to survive for some time under starvation (Sousa et al, 2010; Gunadi et al., 2002). Furthermore, as evidence supports the assumption that reproduction continues even when food is limiting (Reinecke and Viljoen, 1990), the energy reserves are assumed to be utilized for reproduction above a threshold of 50% of an individual's maximum energy reserves, taken as $\frac{M}{2}E_c$ (e.g. Peters, 1983). If food limitation

continues and the energy reserves decline below 50% of an individual's maximum energy reserves, individuals are considered to be in a state of starvation. Under these conditions tissue is catabolised to cover maintenance costs, resulting in net weight loss (Gunadi and Edwards, 2003); individuals die if their mass falls to that at birth (M_b) following Reinecke and Viljoen (1990).

Movement
On the basis that Kobetičová et al. (2010) found movement in *E. fetida* individuals to be random, we modelled individual movements as random in direction from a uniform distribution between -90 ^{o} and 90 ^{o} and distance travelled as 0.5 patches per time-step.

Survival
The survival of individuals living in field populations is determined by the availability of energy resources to maintain life cycle processes alongside temperature and soil moisture specific mortality rates. Individuals die of starvation if their energy resources are depleted, and additional mortality rates were imposed using the regression equation derived from Presley et al. (1996):

$$\text{Mortality Rate (\%)} = 12.7 - 0.0010\,SM - 0.0861\,T + 0.000009\,SM^2 + 0.000147\,T^2 \qquad \text{Eq. 5}$$

where SM is soil moisture (%) and T is soil temperature (K). Individual adults and juveniles die according to Bernoulli processes with daily mortality rates given by Eq. 5.

3 Data evaluation

This TRACE elements provides supporting information on: The quality of numerical and qualitative data used to parameterize the model, both directly and inversely via calibration, and of the observed patterns that were used to design the overall model structure. This critical evaluation will allow model users to assess the scope and the uncertainty of the data and knowledge on which the model is based.

Summary:

Energy budget parameters for *E. fetida* have been directly derived from relevant literature data. As much of the data does not directly relate to energy equivalents, calculations were necessary to transform the literature data in to compatible units. The parameters represent energy acquisition and expenditure under optimal and constant environmental conditions. In suboptimal conditions, environmental variables (e.g. food availability) limit energy ingestion and subsequent allocation to life cycle processes. Methods used to parameterize the dose-response relationship between pesticide concentration and physiological parameters is also outlined below.

Where parameter values have not been directly taken from the literature data source in Table 1, calculations were necessary to obtain a best estimate.

Assimilation efficiency, A_e
Assimilation efficiency determines how much energy from the ingested food (determined by IG_{max} and E_X; Table 1) becomes available for expenditure to metabolic processes (Fig. 1). As *E. fetida* feeds on resources high in organic matter, the assimilation efficiency is assumed to be relatively high. Here we follow Hobellen and van Gestel (2008) who used a value of A_e = 50% for the epigeic species *L. rubellus.* This is less than the value of 70% recorded for *L.*

rubellus eating alder leaves by Dickschen and Topp (1987), but we consider it realistic as the energy content of cow manure, a common food of *E. fetida* in the conditions simulated here, is much higher than that of plant material.

Normalization constant for maintenance, B_0
Regression analysis of earthworm data by Meehan (2006 p. 881) yielded

$$\ln(B) = 5.70 + 0.71\ln(M) - 0.25/\kappa T \qquad \text{Eq. 8}$$

in the notation of Eq. 1, where B is measured in J/hour and M in mg. Evaluated at M = 1 g and T = 298.15 K (25 °C) gives B = 0.0577 kJ/day, which can be used to yield a B_0 value of 967 kJ/day.

Ingestion Rate, IG_{max}
The maximum ingestion rate (IG_{max}) was calculated from growth data of individual *E. fetida* recorded by Neuhauser et al. (1980) (Fig. 6, p. 96) under varying densities of cow manure. Calculations are shown in Table 2. Earthworms continued to growth for 8 weeks at each food ration, and so it is assumed that ingestion occurred until day 56. Thus, the mean amount of food ingested (g) per day at each food ration is calculated as original food ration (g)/56 days (column 2, Table 2). The mean biomass (g) of individuals (column 3) was taken from Fig. 6 of Neuhauser et al. (1980). Individual ingestion rates were re-calculated for 1g individuals (column 4, Table 2), as all metabolic rates scale with mass, which gives a value for IG_{max} of 0.70 g/(day/g) (Table 1).

Table 2. Calculations for the estimation of maximum ingestion rate (IG_{max}) in g/day for *Eisenia fetida*. Derived from Neuhauser et al., 1980.

Food Ration (g)	Ingested food (g/day)	Mean individual biomass (g)	Ingestion rate (g/(day/g))
5	0.089	0.21	0.42
10	0.179	0.30	0.60
15	0.268	0.42	0.64
20	0.357	0.51	0.70

Half Saturation Coefficient, K
The maximum ingestion rate (IG_{max}) was calculated from growth data of individual *E. fetida* recorded by Neuhauser et al. (1980) (Table 2). The data follow a Holling Type II response curve in which the value of the half saturation coefficient (h) corresponds to the food ration at which ingestion is half its maximum, here calculated as 3.5 g (Fig. 3).

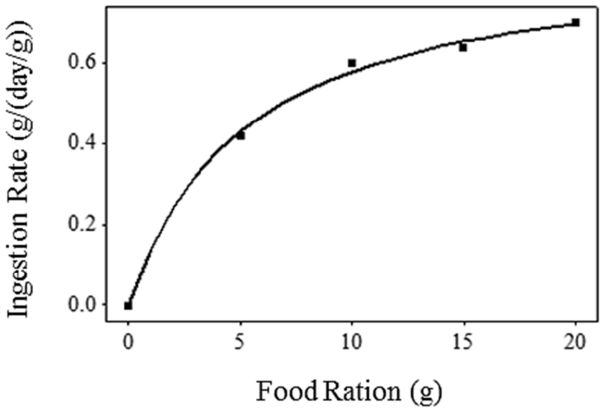

Figure 3. Ingestion rates for *E. fetida* under varying food densities (derived from Neuhauser et al., 1980), with the fitted curve representing the Holling Type II functional response.

Growth Constant, r_B

The growth constant was obtained by least-squares regression fit of the von Bertalanffy growth equation (Eq. 4a) to data from Gunadi et al. (2002) at 20 °C, giving $r_B = 0.15$ (Fig. 4). The Arrhenius function was used to correct this to 0.177 at 25 °C, considered the optimal temperature for development of *E. fetida* (Tripathi and Bhardwaj, 2004).

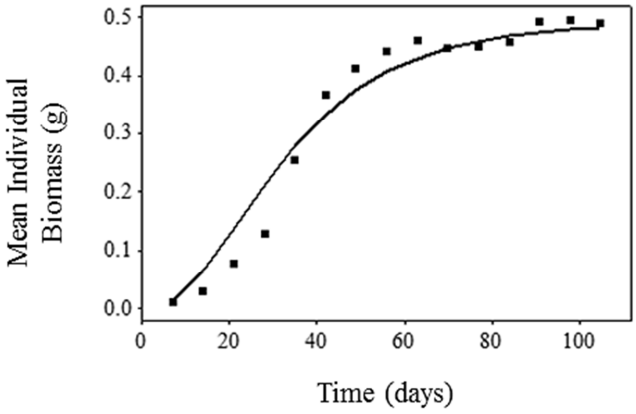

Figure 4. von Bertalanffy growth curve (line) fitted to growth data from Gunadi et al. (2002) (points), yielding a value for rB of 0.15 at 20 °C ($R^2 = 0.97$).

Maximum rate of allocation to reproduction, r_m

Tripathi and Bhardwaj (2004) (p. 281) recorded a maximum cocoon production of 4.4 per individual per month with an average 3 hatchlings emerging from each cocoon. This gives a reproduction rate of 13.2 hatchlings per individual per month and 0.44 hatchlings individual^{-1} day^{-1}. Taking the energy costs of producing one cocoon as $M_c(E_c + E_s) = 0.159$ kJ, this gives a value for energy allocated to reproduction by an average individual as 0.07 kJ day^{-1}. Taking 0.385 g as the average adult mass gives $r_m = 0.182$ kJ/g day^{-1}.

Dose-response curves

The model is designed to simulate laboratory based toxicology experiments from the literature, typical of lower tier risk assessment. Pesticide applications are simulated by applying a chemical concentration to each patch at the specified concentration and time/s. Individuals experience the patch concentration on contact and the effect of this concentration persists unchanged for the duration of the experiment. The physiological effects of a pesticide were identified using toxicity submodels (Table 3), which were evaluated using data from the experiment being simulated. We converted individual biomass and cocoon production values during different treatment concentrations in each case study to percentages of the control value, to identify the reduction in sublethal endpoint due to chemical exposure. The data was then generally well fitted by exponentially declining curves, of the form:

$$R(C) = e^{(kC)} \qquad \text{Eq. 9}$$

where *R(C)* is the effect at a specific concentration (*C*) recorded as % compared to control, *k* is a chemical-specific coefficient calculated by regressing log (%trait compared to control/100) against chemical concentration (*C*) in mg/kg. Eq. 9 represents the dose-response

relationship between chemical concentration and a life cycle trait (growth or reproduction), presented in Fig. 5. However, the toxicity data does not specify which physiological parameter was affected by exposure to result in the observed response in that life cycle trait and as the type of physiological response impacts the population's response, we wanted to identify this.

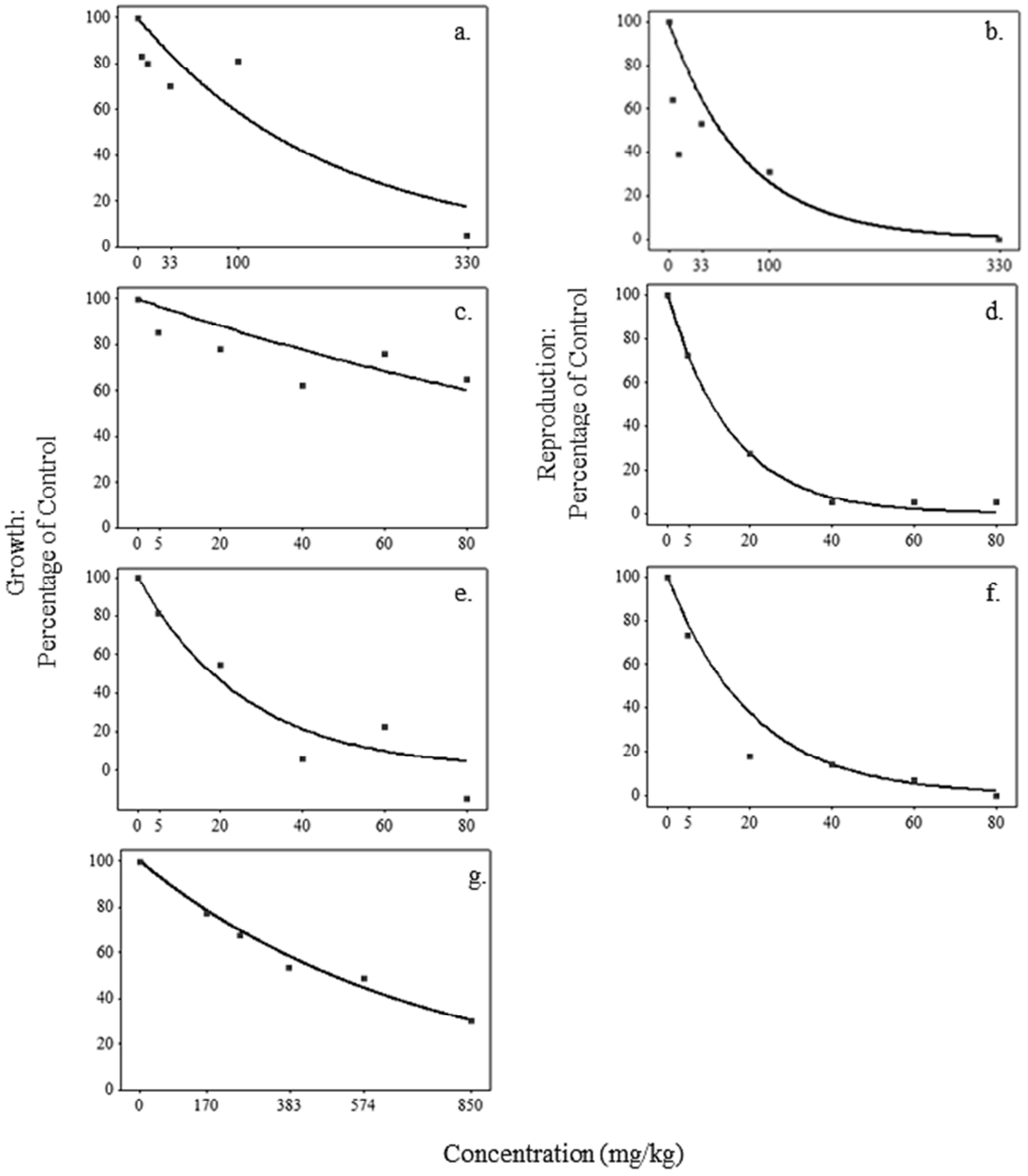

Figure 5. Modelling dose-response curves. Curves fitted to experimental laboratory data for a, c, e & g: growth and b, d & f: reproduction, for a & b: copper oxychloride by Helling et al. (2000); c & d: chlorpyrifos by Zhou et al. (2007), e & f: chlorpyrifos by Zhou et al. (2011) and g: copper oxychloride by Maboeta et al. (2004). R^2 values for regression curves in a, b, c, d, e, f & g are: 0.81, 0.73, 0.65, 0.99, 0.92, 0.96 and 0.99 respectively. Reproduction and growth data are represented as a reduction in life cycle trait compared to the control under different concentrations. Regression coefficients determining these curves are used to investigate the putative metabolic pathway for each pesticide.

To find the most likely physiological parameter affected in each case study we investigated the various possibilities, here called toxicity submodels. Inspection of Fig. A1 indicates that

chemicals can affect ingestion, assimilation, maintenance, growth or reproduction, the rates of which are governed by physiological parameters IG_{max}, A_e, B_0, r_B or r_m respectively (Table 1). Here, we investigate four potential toxicity submodels, describing how altering specific physiological parameters modifies individual growth and reproduction rates (Table 3). The four submodels tested here were selected on the basis that modifying the specific parameters has effects on growth and reproduction simultaneously, rather than one metabolic rate alone. This was done by supposing that the chemical-specific toxicity coefficient (*k*) obtained by fitting Eq. 9 to the data shown in Fig. 5 determines the relationship of the chemical concentrations with a physiological parameter, rather than with the life cycle trait, calculated as:

$$P_c = \frac{P_0}{100} e^{(kC)} \qquad \text{Eq. 10}$$

where P_c is the parameter value at concentration (C), P_0 is the parameter value under control conditions as indicated in Table 1 and k is the toxicity coefficient determining the dose-response relationship. Effects on the sublethal endpoints growth and reproduction then emerge from model simulations. For example, a decline in the value of the parameter IG_{max} with increasing chemical concentration would reduce individual ingestion, thus reducing the amount of energy available for allocation to metabolic processes. Following the preferential allocation principles for earthworms this would lead to reduced growth but have little impact on reproduction as adults allocate energy preferentially to reproduction before growth. Toxicity submodel T4 requires an increase in the value of the maintenance parameter B_0 to eliminate/detoxify the toxin or repair damage (rather than a decline as in toxicity submodels T1-T3 which follow the dose-response curves in Fig 5). Here we assumed that above a concentration of 100 mg/kg there is a linear relationship between B_0 and C so that:
$B_0 = B_{0\,control}$, if $C \leq 100$;
$B_0 = B_{0\,control} \times 0.01\,C$, if $C > 100$.

Table 3. Tested toxicity submodels used to identify the physiological pathways disrupted by pesticides. In each case the specified physiological parameters were affected according to dose-response curves parameterised as in Fig A3. $IG_{max}$is maximum ingestion rate, r_m is maximum rate of energy allocation to reproduction, r_B is the von Bertalanffy growth constant and B_0 is a taxon-specific normalization constant used for calculating maintenance rates.

Toxicity Submodel	Parameter	Predicted Observations in Adult Life Cycle Traits
T1	IG_{max}	Growth more reduced than reproduction
T2	IG_{max} & r_m	Growth and reproduction similarly reduced
T3	r_m & r_B	Reproduction more reduced than growth
T4	B_0	Growth more reduced than reproduction or accelerated weight loss under resource limitation

4 Conceptual model evaluation

This TRACE elements provides supporting information on: The simplifying assumptions underlying a model's design, both with regard to empirical knowledge and general, basic principles. This critical evaluation allows model users to understand that model design was not ad hoc but based on carefully scrutinized considerations.

Summary:

The conceptual model is represented in Figure 1 and 2. The design concepts underlying model design are presented in section 2, Model description. Further information regarding simplifying assumptions is presented in section 3, Data evaluation.

5 Implementation verification

This TRACE elements provides supporting information on: (1) whether the computer code implementing the model has been thoroughly tested for programming errors, (2) whether the implemented model performs as indicated by the model description, and (3) how the software has been designed and documented to provide necessary usability tools (interfaces, automation of experiments, etc.) and to facilitate future installation, modification, and maintenance.

Summary:

In order to ensure that the computer code implementing the model works according to its specification in the ODD model description, a series of tests has been performed. These tests included syntax checking of the code, visual testing through NetLogo interface, the use of print statements and spot tests with agent and patch monitors to check against calculations in Excel, stress tests with extreme parameters values and environmental variables, chemical exposure and concentrations and independent code reviews.

The model was thoroughly tested to verify that the model behaves as expected. Initial model testing focused on the underlying components of the energy budget model on which individual life histories depend. Alongside checking metabolic rates under different conditions against Excel spread-sheets, we altered components that produce predictable changes in output. For example, if the reproduction submodel is removed individuals grow to a larger size. More general testing used information from the literature on mortality at different temperatures, life-spans, maximum reproduction rates and carrying capacities of field populations, to verify overall model performance. Major tests conducted due to problems occurring during model development are outlined below. Print statements (display of key variable values) and spot checks with both agent and patch monitors (manually checking of agent or patch-specific variables per time-step) were used to check the correct implementation of metabolic algorithms (e.g. mass and temperature relationships) and agent-patch interactions (e.g. depletion of patch food densities equivalent to the ingested food density of agents per time-step). The model code was further independently checked for bugs.

Growth

Under parameterisation (3. Data evaluation) the growth constant parameter was taken as 0.15 without the consideration of temperature effects on this value. Thus, when growth simulations were conducted for verification against literature data recorded at 25 °C growth was under-predicted. The growth constant was converted to 0.177 under 25 °C using the Arrhenius function, which when tested against growth under 20 °C (Gunadi et al., 2002) and 25 °C (Reinecke and Viljoen, 1990) produced a better fit to the data. This further provides a consistent set of parameters, relevant to the reference temperature 25 °C (Table 1).

Reproduction

Initially, cocoons were assumed to contain 3 ova with an energy content of $3(M_c(E_c + E_s))$. Adults thus had to obtain the associated amount of energy to reproduce. Yet cocoon production under variable feeding conditions suggests that once food becomes a limiting factor adults produce cocoons containing only 1 ovum. Further model testing against the cocoon production experiments conducted by Reinecke and Viljoen (1990) under variable food densities showed that depositing cocoons with single ova gave a better fit to the data.

Starvation
Little information exists on the metabolism of earthworms under starvation. Thus, it was necessary to use the available data to determine relevant metabolic algorithms. Initially, the loss of mass under starvation was assumed to follow an inverse relationship to the growth equation (Eq. 3b). Yet, closer inspection and testing against weight loss data from Gunadi et al. (2002) showed this to be over-predicted. As the basal metabolic rate (BMR) represents the minimum energy requirement for survival under limiting food conditions, weight loss was assumed to be proportional to the energy costs of BMR.

Software
The model has been implemented in NetLogo (Wilensky 1999), a free software platform. The program is available in the Supplementary Material of Johnston et al. (2014). After installing NetLogo 5.0, which is available for all major operating systems, users can run our model and use the graphical user interface and an integrated tool to perform simulation experiments ("BehaviorSpace", Wilensky and Shargel, 2002). The developers of NetLogo always provide transition guides to new version of NetLogo, and keep old versions for download. Modifications of the program require knowledge of NetLogo.

6 Model output verification

This TRACE elements provides supporting information on: (1) how well model output matches observations and (2) how much calibration and effects of environmental drivers were involved in obtaining good fits of model output and data.

Summary:

> **In this section it is described how many and which parameters were inversely determined via calibration. As the energy budget parameters in Table 1 were all directly calculated from literature data sources, information on these parameters are confined to section 3, Data evaluation. Here, details on the modelling of the toxicity submodels are presented. To inversely determine the most plausible toxicity submodel (by altering physiological parameters according to the dose-response relationships in section 3, Data evaluation), we set up the model as in the corresponding empirical study and evaluated the model output against several patterns observed in the respective laboratory populations (following "Pattern-Oriented Modelling (POM)" and "Akaike Information Criterion (AIC)".**

Calculations for evaluating the fit of the four potential toxicity submodels (Table 3) to literature data on growth and reproduction are presented in the following section. The model was simulated for each experiment using the different toxicity submodels. In each submodel, the specified parameter values were altered according to the dose-response curves obtained by the data in Fig 5. The value of that parameter under control conditions (0 mg/kg) was the value estimated in Table 1. For example, in toxicity submodel T1 the value of IG_{max} at 0 mg/kg is 0.70 g/(day/g) (Table 1), whilst if a chemical is reported to have an effect of e.g.

50% in Fig 5 the value will be altered to 0.35 g(day/g). Model simulation outputs of mean individual biomass (g) and number of cocoons produced for each toxicity submodel are compared to the experimental data of the respective study to evaluate the goodness of fit. We follow the generally methodology of Anderson (2008) for model selection. Initially, sum of square values for the respective submodel fits to growth and reproduction data were calculated (Table 4).

Table 4. Sum of squares calculated for model outputs of each potential toxicity submodel compared to the literature data in each case study for *G*: growth and *R*: reproduction.

Toxicity Submodel	**Helling et al. (2000)**		**Maboeta et al. (2004)**	**Zhou et al. (2007)**		**Zhou et al. (2011)**	
	G	***R***	***G***	***G***	***R***	***G***	***R***
T1	0.30	300.4	1.067	0.174	2624	0.015	600.9
T2	0.88	139.9	1.067	0.158	36.55	0.025	374.6
T3	0.13	38.92	1.067	0.014	3.83	0.002	2.27
T4	0.90	228.5	0.018	0.193	623.4	0.043	376.4

In order to account for the difference in scales measured (number of cocoons, individual biomass), sum of square values required normalising, so that each variable is equally weighted by their respective variance. Table 5 presents normalised sum of square values along with variance ratios between growth and reproduction data.

Table 5. Sample size, standard deviations, variances and ratios of variances between growth and reproduction values.

	Helling et al. (2000)		**Maboeta et al. (2004)**	**Zhou et al. (2007)**		**Zhou et al. (2011)**	
	G	***R***	***G***	***G***	***R***	***G***	***R***
Sample Size	54	5	30	24	6	6	6
Standard Deviation	0.185	5.90	0.068	0.041	7.31	0.05	7.77
Variance	0.034	34.80	0.0046	0.002	53.5	0.003	60.37
Ratio	1	1018	0.0046	1	32227	1	24582

Sum of square values for growth and reproduction data were combined and normalized in each case study by: $\frac{\sum^{\hat{\varepsilon}_i^2}/_n}{Ratio}$, where ε_i is the normalized difference between the literature data and model output (sum of squares) and n is the sample size (Table 5), for growth and reproduction, presented as $\hat{\sigma}^2$ values for each toxicity submodel in Table 6.

Table 6. Normalized $\hat{\sigma}^2$ values for combined growth and reproduction in each case study compared to the respective toxicity submodels.

Toxicity Submodel	**Helling et al. (2000)**	**Maboeta et al. (2004)**	**Zhou et al. (2007)**	**Zhou et al. (2011)**
T1	0.065	7.73	0.0085	0.0033
T2	0.044	7.73	0.0053	0.0034
T3	0.010	7.73	0.0005	0.0002
T4	0.062	0.13	0.0071	0.0046

AICc values, expressed as: $AICc = n\log(\hat{\sigma}^2) + 2n'\left(\frac{n}{n-n'-1}\right)$, where n' is the number of parameters, here represented by the number of toxicity coefficients used in the simulations, are calculated in Table 7 for the $\hat{\sigma}^2$ values in Table 5. Numbers of parameters were two in toxicity submodels T2 & T3 and one in the case of T1 & T4. The difference between toxicity

submodels (Δ_i) was calculated as: $\Delta_i = AICc_i - AIC_{min}$ where AIC_{min} is the minimum value of *AICc*, to identify the best performing model (Anderson, 2008) (Table 7). Best performing models are represented by values of zero, and increasing values show increasing variance between model outputs and the literature data.

Table 7. AICc and Δi values for each toxicity submodel and case study, the best performing model represented by a Δ*i* value of zero. * indicates the best performing toxicity submodel for a given case study.

Toxicity	Helling et al. (2000)		Maboeta et al. (2004)		Zhou et al. (2007)		Zhou et al. (2011)	
Submodel	*AICc*	Δ*i*	*AICc*	Δ*i*	*AICc*	Δ*i*	*AICc*	Δ*i*
T1	-159.2	108.3	63.5	122.6	-140.9	82.7	-66.2	30.7
T2	-180.1	87.4	65.8	124.9	-152.8	70.8	-62.9	34
T3	-267.5	0*	65.8	124.9	-223.6	0*	-96.9	0*
T4	-162.0	105.5	-59.1	0*	-146.3	77.3	-62.2	34.7

When adequate food was provided effects of both copper oxychloride and chlorpyrifos on growth and reproduction were best described by supposing physiological parameters r_m and r_B were affected, using toxicity submodel T3. When food was limited, increasing weight loss at higher concentrations was best described by toxicity submodel T4. Table 8 gives evidence ratios (*ER*s) for each toxicity submodel and case study. ER values quantify the level of evidence for supporting an alternative model by comparing the outputs with the best performing model. Here, Table 8 shows that the odds against all toxicity models but the best performing being better are very high, with evidence ratios > 10^6:1. Higher values provide less support for a model.

Table 8. Evidence ratio (*ER*) values for each toxicity submodel. Values translate how well the models fit the experimental data, with higher *ER* values representing less support.

Toxicity Submodel	Food Availability Sample Size	Helling et al. (2000)	Maboeta et al. (2004)	Zhou et al. (2007)	Zhou et al. (2011)
		Optimal	Limited	Near optimal	Near optimal
		59	30	30	12
T1	IG_{max}	3.3×10^{23}	4.1×10^{26}	9.1×10^{17}	4.6×10^{6}
T2	IG_{max} & r_m	9.5×10^{18}	1.3×10^{27}	2.4×10^{15}	2.4×10^{7}
T3	r_m & r_B	1	1.3×10^{27}	1	1
T4	B_0	8.1×10^{22}	1	6.1×10^{16}	3.4×10^{7}

7 Model analysis

This TRACE elements provides supporting information on: (1) how sensitive model output is to changes in model parameters (sensitivity analysis), and (2) how well the emergence of model output has been understood.

Summary:

The sensitivity of the model to the values of its parameters is presented in Table 9. The model was run with the parameter values of Table 1 (N=100) and again with parameter values increased one at a time by 10% (N=100) and changes in model outputs (adult biomass, juvenile biomass and cocoon production per adult) are shown in Table 9. Also shown in Table 9 are the sensitivity of the model to the baseline values of the environmental variables varied individually; these were soil temperature: 25 °C; soil moisture: 60%; and food density: 20g per patch.

All simulations were run for one year under the field study conditions outlined in Johnston et al. (2014). The sensitivity analysis shows the model to be generally robust to changes in parameter values (sensitivities < 1) (Table 9). All output variables were most sensitive to parameters affecting temperature relationships, with activation energy (sensitivities 1.09 – 1.18), the reference temperature (-0.95 – 1.42) and soil temperature (-0.52 – 0.48) having most impact. An increase in activation energy reduces maintenance costs, making more energy available for higher growth and reproduction rates, whilst an increase in the reference temperature alters the Arrhenius function. All environmental variable values yielded sensitivities in the range -1 to +1. These results show the importance of temperature for earthworm population dynamics.

Table 9. Sensitivity analysis showing ratio of % changes in mean output variables to 10% changes in parameter values, with standard errors. Thus sensitivities between -1 and +1 represent changes in outputs between -10% and +10% of baseline values respectively.

	Output Variables		
Parameter	**Adult Biomass**	**Juvenile Biomass**	**Cocoons per Adult**
Assimilation efficiency (A_e)	0.03 ±0.08	0.02 ± 0.14	0.04 ±0.09
Taxon-specific normalization constant (B_o)	0.12 ±0.10	0.09 ±0.08	0.18 ±0.14
Activation energy (E)	1.09 ±0.31	1.18 ±0.19	1.17 ±1.11
Energy content of tissue (E_c)	0.12 ±0.11	-0.41 ±0.19	0.04 ±0.12
Energy cost of synthesis (E_s)	0.13 ±0.11	-0.01 ±0.03	-0.06 ±0.12
Energy content of food (E_X)	-0.06 ±0.04	0.08 ±0.07	0.11 ±0.08
Maximum ingestion rate (IG_{max})	-0.26 ±0.09	0.01 ±0.09	-0.10 ±0.11
Half saturation coefficient (h)	0.01 ±0.02	0.01 ±0.06	0.03 ±0.05
Mass at birth (M_b)	-0.02 ±0.06	-0.01 ±0.16	-0.10 ±0.04
Mass at sexual maturity (M_p)	0.07 ±0.09	0.26 ±0.11	0.01 ±0.09
Maximum asymptotic weight (M_m)	0.03 ±0.07	0.35 ±0.12	-0.05 ±0.10
Mass of cocoon (M_c)	0.19 ±0.13	-0.08 ±0.01	-0.10 ±0.18
Growth constant (r_B)	-0.02 ±0.10	0.08 ±0.12	0.08 ±0.10
Maximum rate of energy allocation to reproduction (r_m)	0.02 ±0.07	0.01 ±0.09	0.03 ±0.01
Incubation period (T_0)	0.02 ±0.03	0.01 ±0.10	-0.02 ±0.08
Reference temperature (T_{ref})	1.42 ±0.13	-0.95 ±0.13	1.03 ±1.04
Environmental Variable			
Soil Temperature (T)	0.48 ±0.15	-0.52 ±0.09	0.25 ±0.22
Soil Moisture (SM)	0.01 ±0.10	0.01 ±0.08	0.02 ±0.01
Food Density (X)	-0.17 ±0.08	0.13 ±0.09	-0.09 ±0.03

8 Model output corroboration

This TRACE elements provides supporting information on: How model predictions compare to independent data and patterns that were not used, and preferably not even known, while the model was developed, parameterized, and verified. By documenting model output corroboration, model users learn about evidence which, in addition to model output verification, indicates that the model is structurally realistic so that its predictions can be trusted to some degree.

Summary:

A number of patterns on the individual life cycle processes and population dynamics of *E. fetida* have been identified as reproducing well the available literature data. The studies used to evaluate model output use variable laboratory conditions (e.g. temperature, food density). The energy budget model is parameterized with data relating to optimal environmental conditions, and so good model fits to variable conditions show our model to realistically represent

***E. fetida* physiology. Good model fits to sublethal effects of the pesticides copper oxychloride and chlorpyrifos further show the methods for identifying how chemicals achieve their effects. At the population level, good fits to population density, biomass and stage structure show the potential of the model to extrapolate to more natural conditions. Simulation details of all results are available in Johnston et al. (2014).**

Individual life cycle processes

Comparisons of model outputs with literature data on growth and reproduction are presented in Figs. 6 & 7. Simulation of Gunadi et al.'s (2002) experiment shows a good match to data in both the increasing phase (optimal food) and the descending phase when individuals lost body mass because the food supply was depleted (Fig. 6a).). Model predictions of mass loss during starvation are less accurate in Figs 6b and c, but the discrepancies are in opposite directions, so it would not be possible to fit both datasets well.

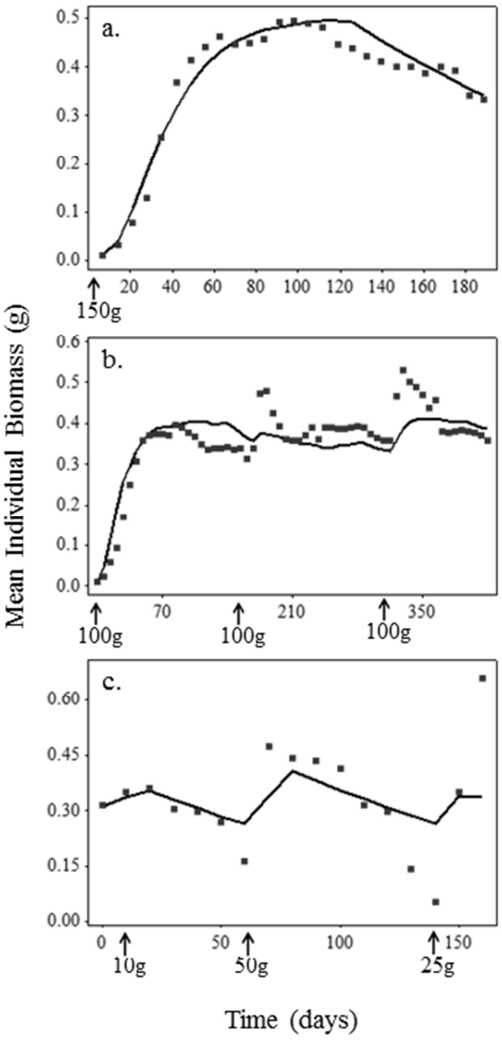

Figure 6. Comparison between model outputs (lines) and recorded growth data (points) from a) Gunadi et al. (2002) b) Gunadi & Edwards (2003) and c) Reinecke & Viljoen (1990). Arrows indicate the time and amounts of food supplied.

Reinecke & Viljoen (1990) recorded the reproduction rate of *E. fetida* under optimal (20g cow manure every 10 days) and limiting conditions (Fig. 7). Model outputs for reproduction under both optimal and limiting food conditions fit the experimental data well.

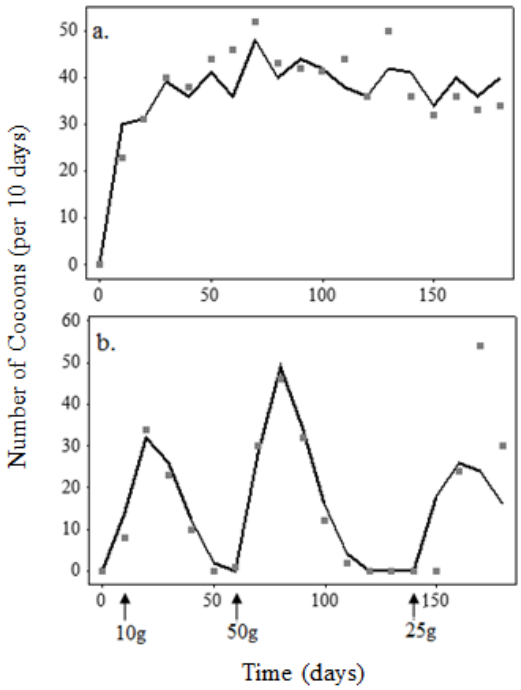

Figure 7. Comparison between reproduction data (number of cocoons produced by 10 individuals in 10-day intervals) from the a) control group with optimal feeding conditions (20g cattle manure every 20 days) and b) experimental group with limiting food conditions in Reinecke & Viljoen (1990) (points) and model simulations (lines). Arrows indicate the time and mass of food supplied.

Figure 8 and 9 shows growth and reproduction data for *E. fetida* from experimental studies under various exposures of copper oxychloride and chlorpyrifos together with the outputs from the best performing toxicity submodel simulations run under the same conditions. Simulation of the Helling et al. (2000) experiment shows good model fits to growth data (Fig. 8a, b) and reproduction data (Fig. 9a) under control and maximum concentrations, although at intermediate concentrations experimental responses do not increase monotonically with concentration. However these results are generally well predicted by submodel T3 in which the parameters controlling allocation of energy to growth and reproduction are directly affected. Effects of copper oxychloride on growth in Maboeta et al. (2004) (Fig. 8c) were not explained by imposing stress on physiological parameters directing the allocation of energy (IG_{max}, r_B, r_m). As the authors in this case study gave a high density of 20 adult *E. fetida* a limited supply of food at the beginning of their experiment there were minimal changes in biomass in the control treatment, indicating that energy ingestion was restricted. The data shows an increase in weight loss with chemical concentration, explained by our energy budget model as the catabolisation of tissue for increasing maintenance requirements. This mechanism is described by submodel T4, resulting in the model outputs presented in Fig. 8d which capture the span of the response. Growth data presented by Zhou et al. (2007) (Fig. 8e) shows great variation in individual biomass between treatment concentrations of chlorpyrifos, with the standard errors for each treatment overlapping. Yet, based on the mean biomasses recorded the model provides a reasonable fit to the growth data (Fig. 8f) and a good fit to the reproduction data (Fig. 9b).

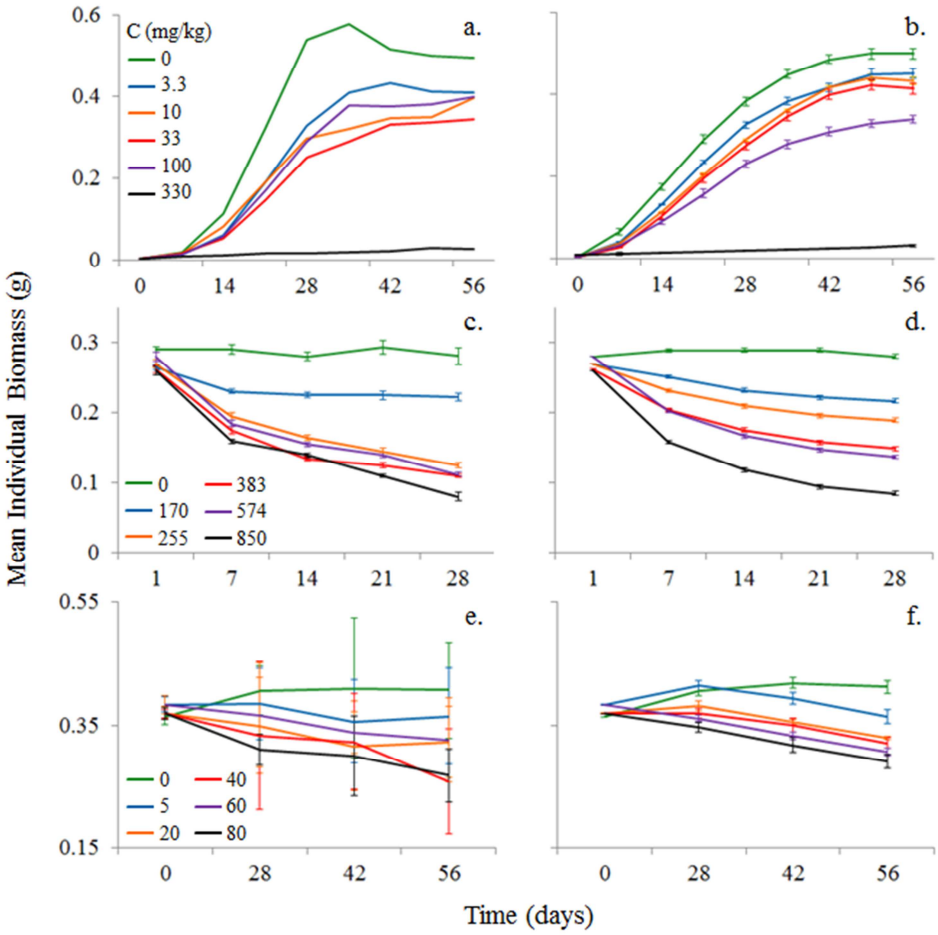

Figure 8. Comparison of experimental toxicity data (left-hand panels) and model simulations of toxicity experiments (right hand panels). (a, b) the effects of copper oxychloride (Helling et al. 2000) modelled using submodel T3; c, d) copper oxychloride (Maboeta et al. 2004) using T4; and (e, f) chlorpyrifos (Zhou et al. 2007) using T3.

Zhou et al. (2011) provided the same experimental conditions as Zhou et al. (2007) and recorded mean individual biomass and cocoon production after 56 days exposure as shown in Fig. 9 c & d. Submodel T3 again provides a good fit to the data.

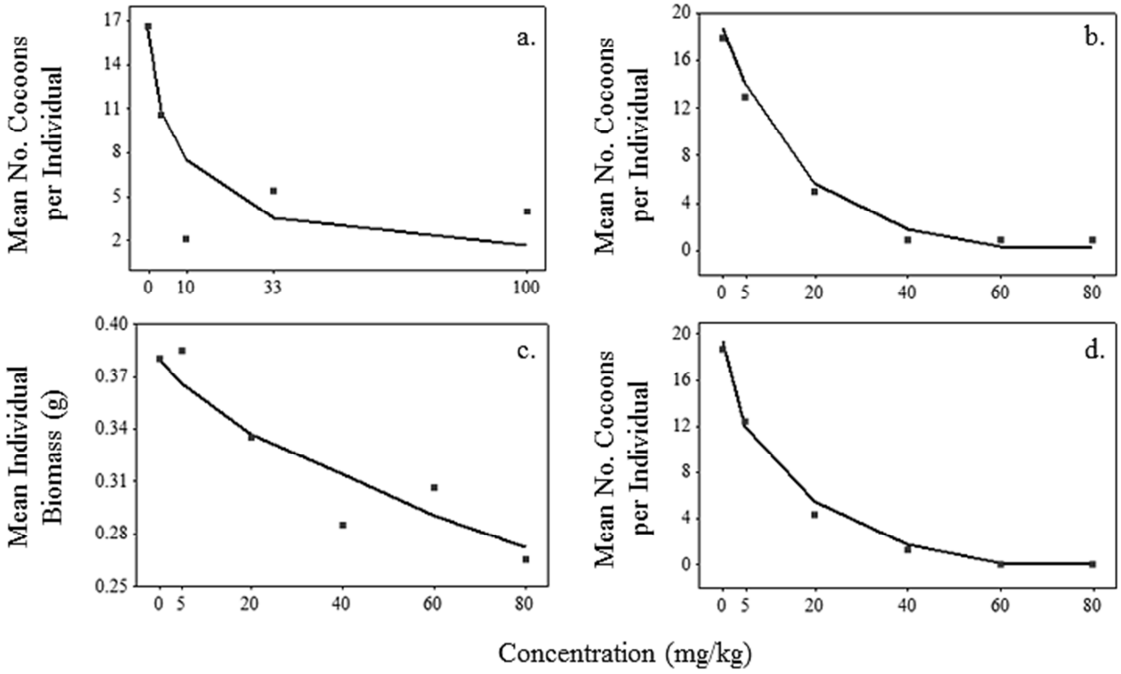

Figure 9. Comparison of model simulations (lines) with toxicity submodel T3 on reproduction (a, b & d) and growth (c) data (points) recorded after 56 days exposure to copper oxychloride (a) and chlorpyrifos (b, c & d) from a) Helling et al. (2000), b) Zhou et al. (2007) and c & d) Zhou et al. (2011).

Population dynamics

Patterns of seasonal changes in population density and biomass (Fig 10) are generally well predicted by the model, although adult density and biomass and cocoon density (Figs 10c, d & g) are slightly underestimated in spring. The higher cocoon densities observed in spring may be due to higher temperatures occurring within the manure heap under high population densities, not considered in the model.

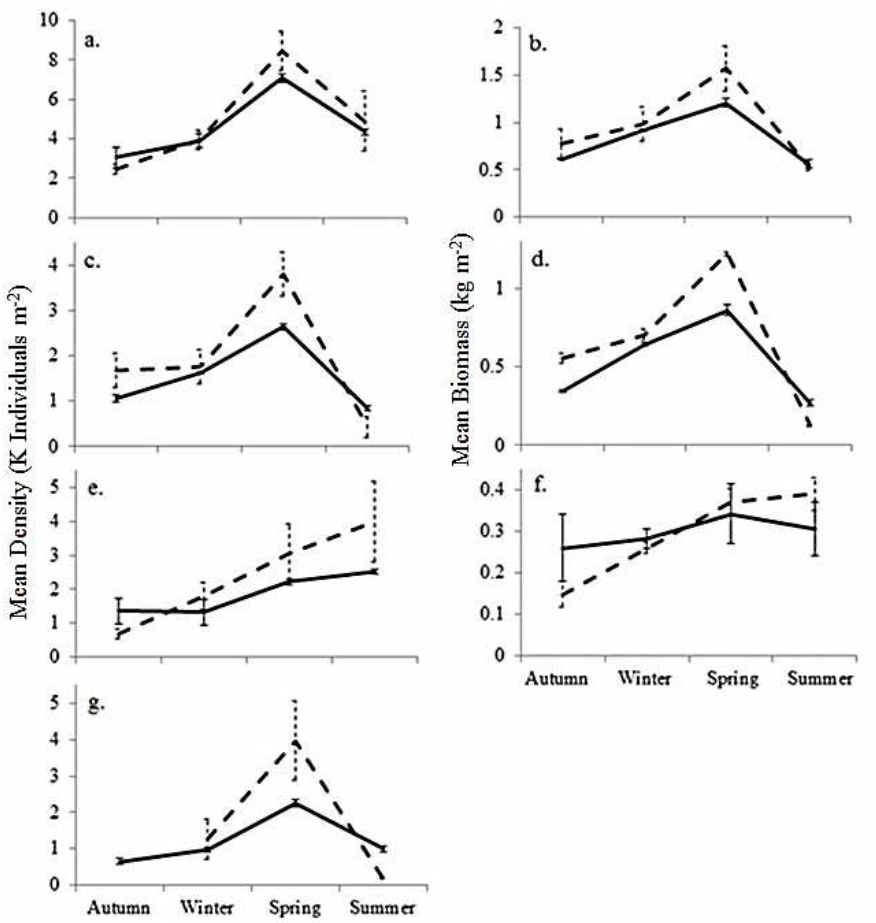

Figure 10. Comparison between (left-hand panels) field population density data and (right-hand panels) population biomass data from Monroy et al. (2006) (dashed line) and model simulations (solid lines) with standard errors. a,b: total population; c,d: adults; e,f: juveniles; g: cocoons. Juveniles here comprise the hatchlings, juveniles and preclittelates that were counted separately in the field.

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