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Towards a better understanding of the life cycle of the earthworm *Aporrectodea caliginosa*: new data and energy-based modelling

Sylvain Bart^{a,*}, Céline Pelosi^{a,b}, Alexandre R.R. Péry^a

^a UMR ECOSYS, INRA, AgroParisTech, Université Paris-Saclay, 78026, Versailles, France

^b UMR EMMAH, INRA, Université d'Avignon et des Pays de Vaucluse, 84914, Avignon, France

* Corresponding author: UMR1402 ECOSYS INRA AgroParistech, Bâtiment 6, RD 10, 78026 Versailles cedex, France. Tel: (+33)1.30.83.32.72. ; Fax: (+33)1.30.83.32.59. E-mail address: sylvain.bart@inra.fr. Present email address: sylvain.bart@york.ac.uk

Highlights:

- New data were provided on the life cycle of *Aporrectodea caliginosa*.
- We showed that the maintenance costs were negligible during the growth period.
- The energy of adult individuals was entirely allocated to cocoon production.
- An energy-based model was calibrated for different feeding conditions.

Abstract

Earthworms have a major role in soils and are used as biological indicators for ecological risk assessment. *Aporrectodea caliginosa* s.s. is one of the dominant earthworm species in agroecosystems and a good candidate for ecotoxicological testing. In order to improve knowledge on its life cycle, we performed growth and reproduction experiments. These data were used to calibrate an energy-based model that displayed good descriptions of *A. caliginosa* life cycle parameters under different feeding conditions (*ad libitum*, food limitations, or absence of food). Here we showed that the maintenance costs were negligible, resulting in a linear growth in length when food was provided *ad libitum* (i.e., without any restriction). We also found that the switch from the juvenile to adult stage depended on the individual weight, and that the threshold weight was not influenced by the amount of provided food (horse dung). Moreover, we provided evidence that once adults, if they were not isolated from other individuals, the energy was entirely allocated to cocoon production, which only depended on the available food amount, without any effect of the earthworm density. Finally, we discussed the usefulness of our energy-based model to support the set up and analyses of ecotoxicological tests and experiments.

Keywords: Lumbricidae, Soil ecology, DEB theory, Life history traits

1. Introduction

Earthworms represent the largest part of animal biomass in most temperate soils (Edwards, 2004; Lavelle and Spain, 2001; Paoletti, 1999). They are considered as typical soil ecosystem engineers (Jones et al., 1994) and provide ecological functions in agrosystems such as organic matter degradation or soil structure improvement (Bart et al., 2019a; Bertrand et al., 2015; Blouin et al., 2013; Palm et al., 2014). Earthworms are indicators of soil biological activity and use as model organisms to assess pesticide effects on soil fauna (OECD 1984; Spurgeon et al., 2003).

Eisenia fetida (Savigny) is recommended as a model species in ecological risk assessment, using a set of standardized tests, because it is easy to breed and has a short generation time (OECD 1984), allowing for quick and cost effective tests. However, it is not representative of the species living in mineral soils (Lowe and Butt 2007), and it is less sensitive to pesticides and metabolites than species found in cultivated fields (Pelosi et al., 2013). Several authors thus proposed *Aporrectodea caliginosa* as a reference species (Bart et al., 2018; Klobucar et al., 2011; Pelosi et al., 2013; van Capelle et al., 2016) since it is representative and one of the dominant earthworm species in arable soils of temperate regions (Bouché, 1972; Decaëns et al., 2011) worldwide (Bart et al., 2018). The taxonomy of *A. caliginosa* has been frequently discussed the last past decades and it is still under discussion (Bart et al., 2018; Fernández et al., 2012; Perez-Losada et al., 2009). For clarity, we here refer to *Aporrectodea caliginosa s. s* as described by Sims and Gerard (1999). Individuals do not show any significant pigmentation but the anterior segments are pale pink in coloration and the reproduction is only biparental (see Bart et al., 2018 for more details and picture). Based on the functional earthworm groups described by Bouché (1977), *A. caliginosa* is an endogeic species living in sub-horizontal temporary burrow systems, in the top 10-15 cm of mineral soils.

The use of *A. caliginosa* in risk assessment requires sufficient knowledge and understanding of its life cycle and the drivers of its life history parameters, i.e., growth and reproduction. However, to date, the life cycle of the different earthworm species, and in particular, of *A. caliginosa* is poorly known, as is the influence of environmental factors on life cycle parameters. Soil temperature and moisture are known to be key parameters driving earthworm life cycle (Eriksen-Hamel and Whalen, 2006; Holmstrup et al., 1991; Moreau-Valancogne et al. 2013). Furthermore, food quality and quantity are also recognized as major factors influencing life parameters of *A. caliginosa*. For instance, Boström and Lofs-Holmin (1986) showed that particle size had a strong effect on growth curve of *A. caliginosa* and cocoon production in laboratory experiments. Unfortunately, the relationship between food availability and *A. caliginosa* growth and reproduction is still poorly documented and understood.

In ecotoxicological tests exceeding 14 days with *A. caliginosa*, food supply is necessary because earthworms may starve, stop growing or lose weight, especially in experiments with juveniles (Bart et al., 2018). This could increase the risk of false positives. In contrast, too much food could limit the contact of earthworms with the soil since they would not have to explore much of the soil to find food. Understanding and modelling the influence of food quantity and quality on ecotoxicological endpoints such as growth and reproduction is thus essential to use appropriate feeding conditions in experimental designs. Then, modelling the life cycle can allow the development of energy-based models using the Dynamic Energy Budget (DEB) theory (Kooijman 1986, 2000, 2010). Such models are powerful in explaining life-history patterns of animal (Jager et al., 2013). These models are also useful to analyse growth and reproduction toxicity data (Ashauer et al, 2018; Kooijman and Bedaux, 1996; Péry et al., 2002a), with effects monitored at many time points. Finally, such models can be used to extrapolate observed effects at the population level (Beaudouin et al., 2012, David et al., 2019). The DEB theory separates the use of energy, derived from the food assimilation, to growth,

101 maintenance, and reproduction. The calibration of such models requires data on the life cycle
102 and the influence of food quantity and quality.

103 We here aimed to calibrate an energy-based model, inspired by the DEB theory
104 (Kooijman 1986, 2000, 2010), taking into account the influence of food quantity and quality on
105 *A. caliginosa* life cycle, with dedicated experiments. Data from experiments on growth and
106 reproduction under different food quantities and quality were used to calibrate the energy-based
107 model. We then challenged the model to predict new data obtained in different experimental
108 conditions (different food quantity).

110 2. Materials and methods

112 2.1. Animals, soil and food.

113 Adults of *Aporrectodea caliginosa* s.s. (Sims and Gerard, 1999) were hand sorted from
114 an agriculture field at INRA (Estrées-mons, 49°52' N 3°01'E) and cultured in a climate room at
115 15 ± 1 °C in a natural soil with food added. The soil was a Luvisol (FAO soil classification),
116 sampled from the top 0–20 cm in a permanent meadow in Versailles (48°48' N, 2°5' E). It was
117 air-dried and milled (< 2 mm). Soil physical and chemical properties were as follows: pH 7.5,
118 organic matter 32.6 g kg⁻¹, C/N 12.7, 29% sand, 48% silt, and 23% clay (see Bart et al., 2017
119 for more details). The food was horse dung which was frozen and defrosted twice and then
120 milled (< 1 mm) as presented in Lowe and Butt (2005). Cocoons were obtained through water
121 sieving (1 mm) the culture soil (Bart et al., 2018), incubated at 20 °C in petri dish on wet filter
122 papers (Holmstrup et al., 1991), and checked every two days. New hatchlings were collected
123 and stored in soil at 4 °C for a maximum of 1 week, to slow their development. This procedure
124 allowed synchronizing cohorts of individuals of same level development (i.e., weight).

2.2. Description of the energy-based model.

We first assumed that the length-to-width ratio remained constant during growth, and that there was a linear relationship between the body length and the cubic root of the body weight. This assumption, named isomorphism, is reasonable for earthworms. As long as the organism does not change its shape, in practice, any length measure can be used to calibrate the model. The fresh weight is easier and more precise to obtain than length as individuals contract and relax all the time. We thus used the cubic root of the fresh body weight, which is correlated to the length of individuals. To validate this assumption, the length (mm) and weight (mg) of 40 individuals from the breeding culture and previous experiments (prior to this study) were monitored at different ages. For that, individuals were placed on a sheet of graph paper and at least 5 pictures were taken to catch an image of individuals in retracted position. Then, we used the ImageJ software for the length measurement (Schneider et al., 2012). The same individuals were weighted using an analytical balance instrument (± 0.1 mg). The data showed and validated the linear correlation between the cubic root of the fresh body weight and the length ($r^2 = 0.949$, Fig. 1).

Figure 1. Relationship between the cubic root of the fresh body weight and the length of *Aporrectodea caliginosa*.

Secondly, we assumed that the energy costs of maintenance were negligible compared to the energy costs of growth throughout the development of the individuals. Usually, in DEB models, the maintenance requirements are supposed to take precedence over growth and the food uptake is assumed to be proportional to the surface area, whereas maintenance is proportional to volume. Considering a faster increase of the volume than surface area with growth, a growth curve with a maximal asymptotic length is expected, which is captured by the Von bertalanffy growth curve. However, preliminary results with *A. caliginosa* showed a linear growth in length, even for biggest individuals, suggesting negligible maintenance costs (Péry et al., 2002b).

Finally, to determine the switch from juvenile to adult stage, we needed to choose a threshold parameter. Considering that earthworms growth efficiency depends on the environmental condition (including temperature and moisture), we assumed that a weight

threshold for juveniles to become adults (appearance of a fully developed clitellum) was the most reasonable option. Based on these three assumptions and in the case of unlimited food (i.e., *ad libitum* conditions), the assumptions of isomorphic growth and negligible maintenance costs lead to the equation (1) (Péry et al., 2002b):

$$\frac{d}{dt}l = a \quad (\text{Eqn 1})$$

Where l is the length of the organism corresponding to the cubic root of its fresh weight, and a is a constant depending on the ability of the organism to assimilate the food. This parameter can be modulated by food particle size as all food particles are not available for very small individuals, thus reducing their feeding rate. The model considered this process by adding a correction factor to the a parameter for the small individuals (i.e., under a critical size). This lead to the following equations:

$$\text{If } l < C_s, \text{ then } \frac{d}{dt}l = a(1 - b) \quad (\text{Eqn 1a})$$

$$\text{If } l > C_s, \text{ then } \frac{d}{dt}l = a \quad (\text{Eqn 1b})$$

Where C_s is the Critical size, below which the individual cannot access all the food, and b is the correction factor which corresponds to 1 less the fraction of the particles reachable for the individual (if $b = 0$, all the food is available since hatching).

In the case of limited amount of food for earthworms, the daily weight increase was considered proportional to the amount of food that can be taken from the soil plus the added food (i.e., horse dung). This lead to the equation (2):

$$W_{n+1} - W_n = (p \times Q) + S \quad (\text{Eqn 2})$$

Where Q is the quantity of food added to the soil during the feeding event (in mg of horse dung), p is the fraction of this food which can be used to create weight unit, S is the weight unit created

with the food initially available in the soil, and W_n is the individual weight at the feeding event n . The S parameter depends on the soil used in the experiment, the time until the soil is renewed (i.e., 28 days in our experiments) and the weight of the individual. For S , we proposed the following equation:

$$S = I_{max} (1 - \exp^{-KW}) \text{ (Eqn 3)}$$

Where I_{max} is the maximum weight increase between two feeding events, K is a constant and W is the weight of the individual.

Once C_s , a , b , Q , p and the parameters of Eqn 3 (I_{max} and K parameters) were determined, the growth was simulated using the set of equations of the model. For that, the weight increase was first calculated using Eqn 1, assuming *ad libitum* conditions. Then, we checked with Eqn 2 if there was enough food available to obtain the expected growth increase. If not, Eqn 2 was used to calculate the expected weight.

2.3. Experimental procedures

2.3.1. Growth experiments

In all the experiments, the soil was renewed every 28 days and the moisture was adjusted and maintained at 70% of the water holding capacity (WHC) (corresponding to 28% of water content) by monitoring the mass of the vessels throughout the experiments and adjusted with tap water if necessary. The horse dung was also adjusted at 70% of the water holding capacity and mixed with the soil. Earthworms were individually placed in 1 L plastic vessels filled with 400 g equivalent dry soil. All the vessels had a removable perforated cover for gas exchanges and were stored in a climate room at 15 ± 1 °C. Individuals were carefully extracted from the vessels by hand every 14 days at the beginning of their growth to be weighed. When they

reached approximately 500 mg, they were weighted every 7 days to monitor precisely their weight when a fully developed clitellum was observable (corresponding to the switch to adult stage).

We used weight measurements collected throughout time from experiments 1 - 4 to calibrate the growth energy-based model. The experiment 5 was used to challenge the ability of our model to predict new data in different experimental conditions (i.e., food quantity), by comparing real data to the model predictions.

Experiment 1. This experiment was designed to collect data and calibrate the growth model under *ad libitum* food condition. For that, hatchling earthworms ($n = 10$) were fed *ad libitum*, corresponding to $3 \text{ g ind}^{-1} 14 \text{ days}^{-1}$ of horse dung. This food amount was previously determined and was close to the amount of $5 \text{ g ind}^{-1} 14 \text{ days}^{-1}$ proposed by Lowe and Butt (2005) for the same species. The weight measurements throughout time from this experiment were used to estimate the value and confidence intervals of the parameters C_s , a , and b of the growth model (Eqn 1).

Experiment 2. This experiment was set up to test the influence of the food quality, represented by the size of food particles, on the growth. We expected that with smaller food particles, small juvenile individuals would reach and ingest more food, thus decreasing the value of the parameter b (i.e., the correction factor corresponding to 1 less the fraction of the particles reachable for the individual). To test this assumption, hatchling earthworms ($n = 7$) were fed *ad libitum* (i.e., $3 \text{ g ind}^{-1} 14 \text{ days}^{-1}$) but, contrarily to the *Experiment 1*, the food particles were $< 0.5 \text{ mm}$. Prior to assessing results, we modelled the predicted data in two ways. Firstly, we predicted data of this experiment with Eqn 1 of the growth model and the parameter values

estimated in the *Experiment 1* (C_s , a , and b). Secondly, we performed a new calibration of the parameter b , called b_2 here, with the data of this experiment (keeping C_s and a value). We finally compared the two fitted growth curve to the data set with the extra sum-of-squares F-test to determine if the re-estimation of the parameter b led to a significantly better fit of the data, meaning that the food quality has an impact on the growth.

Experiment 3. This experiment was designed to collect data used to estimate the value of the parameters I_{max} and K of the S the equation (Eqn 3). For that, we experimentally monitored the individual weight ($n = 5$) of juvenile earthworms in the soil without any food addition. Thus, only the food naturally available in this soil was available to growing earthworms. Because growth was very slow in these conditions, we alternated periods of growth in soil without added food and growth in soil with *ad libitum* food (same origin as in the *Experiment 2*). We used these *ad libitum* periods to assess the ability of our growth model (Eqn1) to predict the growth pattern of new data. In this way, we predicted the growth with Eqn 1 (i.e., with C_s , a and b_2 estimated in the *Experiments 1* and 2, as the food was the same as in the *Experiment 2*) and then compared to the produced data. We calculated a percent of error between data and model predictions using the weight value predicted by the model and the average weight measurement of the replicates, at each time.

Experiment 4. This experiment was designed to estimate the value of the parameter p of the Eqn 2 and its confidence interval. To achieve this goal, we monitored the growth (weight measurement) throughout time of 10 individuals fed with 1 g ind⁻¹ 14 days⁻¹ (same origin as in the *Experiment 1*). We used Eqn 1 with the parameter values C_s , a , b (estimated in the *Experiment 1*) and S equation (Eqn 3) with parameter values estimated in the *Experiment 3*, and we estimated the parameter p from the data.

252

253 **Experiment 5.** This experiment was designed to test the ability of the model to predict a new
254 data set (not used for the calibration of the parameter values) of a new limited food condition.
255 For that, five individuals were fed with a different amount of food (compared to the first
256 experiments) corresponding to 1.5 g ind⁻¹ 14 days⁻¹. The model predictions based on the
257 parameters estimated in the first four experiments were compared to data obtained in this
258 experiment 5. We calculated a percent of error between the data and the model using the weight
259 value predicted by the model and the average weight measurement of the replicates, at each
260 time.

261

262 2.3.2. *Switch to adult stage*

263 Data obtained in the previous growth experiments (weight measurements) with the three
264 differing food amounts tested (3 g added *ad libitum* in experiments 1 and 2, whilst 1 g in
265 experiment 4 and 1.5 g in experiment 5), were used to determine the weight at which juveniles
266 switched to adult stage (i.e., appearance of a fully developed clitellum). For each individual, we
267 calculated the average of the latest weight recorded as a juvenile and the first weight recorded
268 as an adult. We first tested the impact of food condition on the earthworm weight that
269 determined the switch from juvenile to adult stage, with Kruskal-Wallis tests. Then, these two
270 weights were used to provide the percent of individuals reaching maturity. Finally, a linear
271 regression was fitted on the data to provide the parameter values of the linear equation.

272

273 2.3.3. *Reproduction experiment.*

274

Experiment 6 was designed (i) to understand the energy allocation strategy between growth and reproduction at the adult stage, (ii) to estimate the impact of food amount on cocoon production, and (iii) to test the impact of earthworm density on cocoon production, which could lead to some bias when comparing and interpreting results of ecotoxicological reproduction test. For that, we used individuals of the *Experiments 1* and *4* (fed with 1 and 3 g ind⁻¹ 14 days⁻¹, respectively). When they became adults, the 10 individuals of each experiment were separated into two groups. Four individuals were kept isolated in vessels (same conditions as in the growth experiment) and the growth was monitored by weighting the individuals every 14 days. A second group of 6 individuals were pooled first by 2 for 84 days (3 vessels with 2 individuals, for each food condition), then by 3 for 84 days (2 vessels with 3 individuals, for each food condition), and finally by 6 (all individuals in one vessel, for each food condition) for 84 days. The total food amount was adapted to suit the amount needed per individual per unit time. Growth was monitored by weighting the earthworms individually every 14 days and the cocoon production was monitored when renewing the soil (i.e., every 28 days, leading to 3 cocoon collections for each of the 3 densities tested) by wet sieving it through a 1 mm mesh size (Bart et al., 2018).

2.4. Model calibration.

The model equations were implemented in the R software R Core Team (2015) and the set of differential equations were solved using the deSolve package (Soetaert et al., 2010). All the data for a given experiment were fitted simultaneously. Optimization of the parameter values was performed with the least square method. The 95% Confidence Interval (CI) of the parameters were estimated with the Bootstrap method (Efron, 1979).

3. Results

3.1. Growth experiments.

All the growth curves are presented in fresh weight for more realism in representing the earthworm growth. The figure 2 presents the results of the experiment 1-4 used for the calibration of the model, and the description of the data by the model. The parameters values optimized from these experiments are shown in Table 1.

Based on the results of the *Experiment 1*, the model accurately described the growth data (Fig. 2A) with the estimated parameter values (Table 1). The growth rate in length (i.e., in weight cubic root of the fresh weight) of small earthworms (below C_s) was 39% lower than the rate of bigger individuals (above C_s).

From the *Experiment 2*, we showed that, with the parameter values estimated from the data of the *Experiment 1*, the growth model for *ad libitum* food condition (Eqn 1) underestimated the beginning of the growth pattern (full line, Fig. 2B). The re-estimation of the parameter b , called b_2 , allowed for a better description of the data (dash line, Fig. 2B) as there was a significant improvement of the fit (F-test, p-value < 0.01).

Based on the data of the *Experiment 3*, we estimated parameters values for I_{max} and K of the S equation (Eqn 3) at 80.97 and 0.00159 respectively (Fig. 3, Table 1). The S equation (Eqn 3) provided a relevant description of the growth pattern when individuals were fed only with soil (Fig. 2C, full line). The growth model (Eqn 1) with parameter values previously estimated (in *Experiment 1 and 2*) provided a relevant prediction of the growth pattern when individuals were fed *ad libitum*, with a maximum error of 11% between predicted values and data (Fig. 2C, dash line).

323 The Eqn 1 predicted the beginning of the growth pattern of data of the *Experiment 4* and
324 then Eqn 2 described the rest of the growth curve (Fig. 2D) with the parameter p estimated at
325 0.048 (table1).

Figure 2. **A)** The growth pattern of *A. caliginosa* from the *Experiment 1*, with food *ad libitum* as a function of time since hatching ($n = 10 \pm \text{SD}$). The line represents the description obtained with the growth model (Eqn. 1). **B)** The growth pattern of *A. caliginosa* from the *Experiment 2*, with food *ad libitum* as a function of time since hatching ($n = 7$, mean $\pm \text{SD}$). The full line represents the predictive model with food *ad libitum* (Eqn. 1) with parameter values obtained in *Experiment 1*. The dash line represents the model with food *ad libitum* (Eqn 1) with C_s and a parameter values from the *Experiment 1* and the b_2 parameter adjusted to the data. **C)** The growth pattern of *A. caliginosa*, from the *Experiment 3*, since hatching with only soil as food or with food *ad libitum* ($n = 5$, mean $\pm \text{SD}$). The full line is the description of the data using the S equation (Eqn 3) and the dash line is the prediction of the model using Eqn 1 (with parameter values estimated in *Experiment 1 and 2*, i.e., a , b_2 and C_s). **D)** The growth pattern of *A. caliginosa* from the *Experiment 4*, feed with $1 \text{ g ind}^{-1} 14 \text{ days}^{-1}$ of food as a function of time since hatching ($n = 10$, mean $\pm \text{SD}$). The line represents the data described by the model using Eqn 1 until individuals were under limited food condition switching to the model with limited food with Eqn 2. The switch between the Eqn 1 and 2 in the model happened at day 35, at a weight of 62 mg.

Figure 3. Individual weight increase in mg between two feeding events (i.e., every 14 days) with the food available in the soil in term of the individual weight. The line represents the description obtained with the S equation (Eqn 3).

Table 1. Parameter values of the equation 1, 2 and 3, optimized with the different experiments and their confidence intervals (CI 95%)

Optimize with data set of	Symbol	Unit	Description	Value	CI 95%
Experiment 1	C_s	$\text{mg}^{1/3}$	Critical size allowing reachability to the all food	3.927	3.262 - 4.531
Experiment 1	a	$\text{mg}^{1/3} \text{ day}^{-1}$	Constant	0.084	0.074 - 0.093
Experiment 1	b	-	Correcting factor for growth constant for individual under the Critical size (C_s)	0.391	0.297 - 0.496
Experiment 2	b_2	-	Correcting factor for growth constant for individual under the Critical size (C_s)	0.256	0.202 - 0.309
Experiment 4	p	-	Proportion of food transformable into weight unit	0.048	0.044-0.052
Experiment 3	$I_{\max}$	mg	Maximal weight increase between two feeding event (14 days)	80.97	-
Experiment 3	K	mg^{-1}	Constant	0.00159	-

The use of our growth model, and parameter values optimized in *Experiments 1, 2, 3* and *4* (i.e., a , b_2 , C_s , p , K , I_{max}), to predict the growth pattern of the *Experiment 5* with individuals fed with $1.5 \text{ g ind}^{-1} 14 \text{ days}^{-1}$, is present in Fig. 4. The difference between the predicted values of our model and the data (Fig. 4) never exceeded 18% of error.

Figure 4. Growth pattern of *A. caliginosa* fed with $1.5 \text{ g ind}^{-1} 14 \text{ days}^{-1}$ of food as a function of time since the hatching ($n = 5$, mean $\pm$ SD). The line represents the prediction of the model with Eqn 1 and 2 with parameter values estimated in the *Experiment 1, 2, 3* and *4* (i.e., a , b_2 , C_s , p , k , I_{max}).

3.2. Switch to the adult stage.

Statistical analyses revealed that the food amount did not influence the weight at which the individuals switched from juvenile to adult stage (Fig. 5A, p -value = 0.115). The linear

curve model representing the percentage of adults in function of the weight of the individuals provided a satisfactory description of the data (Fig. 5B). This linear curve model can be associated with the growth model to predict the switch from juvenile to adult of an individual according to its weight. With the model, individuals would switch from juvenile to adult stage at a weight between 569 mg and 668 mg, and under *ad libitum* food condition it would happened 78 to 85 days after the hatching.

Figure 5. (A) First recorded weight as adult (in red) and last recorded weight as juvenile (in blue) of the individuals in growth experiments, and **(B)** the percent (%) of adult individual in terms of individual weight, the line being the model to describe the data which are the points.

3.3. Reproduction experiment.

Energy allocation strategy. The earthworms maintained individually in vessels at the adult stage did not produce cocoons and kept on growing at both food treatments (red lines, Fig. 6). The individuals pooled in vessels produced cocoons and stopped their growth at both food treatments (blue lines, Fig. 6).

Figure 6. Growth pattern of adult individuals isolated ($n = 4$, mean $\pm$ SD) or pooled for reproduction ($n = 6$, mean $\pm$ SD). Pooled individuals were pooled by 2 for 84 days, then

by 3 for 84 days and finally by 6 the last 84 days. Individuals were fed with two different food amounts (1 and 3 g ind⁻¹ 14 days⁻¹).

Cocoon production. The mean cocoon production was on average 1.6 and 2.4 cocoons ind⁻¹ week⁻¹ with 1 and 3 g food ind⁻¹ 14 days⁻¹ respectively. When the density was 6 individuals per vessel, the cocoons production decreased by 50% but only for the individuals fed with 1 g ind⁻¹ 14 days⁻¹ (Fig. 7).

Figure 7. Cumulative cocoon production of 6 individuals, with two various food conditions (1 and 3 g ind⁻¹ 14 days⁻¹) pooled by 2 for 84 days, then by 3 for 84 days and finally by 6 the last 84 days.

4. Discussion

Interest in the earthworm *A. caliginosa* has increased in ecology risk assessment (Bart et al., 2018). We here provided new data (growth and reproduction) and a modelling approach allowing to describe the life cycle of *A. caliginosa* under different food regimes. The production of new data was specifically designed to allow assessment of the impact of food and earthworm density on the reproduction and the energy allocation between reproduction and growth at the adult stage.

4.1 Growth of *A. caliginosa*, model calibration

At the juvenile stage, the DEB theory (Kooijman, 2000) considers that all energy is allocated to the maintenance cost and growth which is captured by the Von bertalanffy growth curve. Different authors used these assumptions for different earthworm species: *Dendrobaena octaedra* (Jager and klok, 2010), *Eisenia fetida* (Jager et al., 2006), *Lumbricus rubellus* (Hobbelen and van Gestel, 2007; Klok, 2008) and *Aporrectodea caliginosa* (Johnston et al., 2014). However, it has not been tested whether it is based on the biology of the considered species. In our study, we tested a different modelling approach based on negligible maintenance costs. This approach was successfully demonstrated as correct for several aquatic species (*Chironomus* species, Péry et al., 2005), and our results showed that this approach works also for *A. caliginosa*. Undeniably, our model provided a good description of the growth data measured in the different experiments, confirming that the maintenance costs are negligible. Our model was able to account for food limitations (even absence of additional food). In particular, it was able to predict the growth pattern from a new data set with a different food amount (i.e., limited quantity). When coupled with the equation determining the probability for

a juvenile individual to switch to adult stage according to its weight, the model allowed, for any condition of feeding and soil, to predict the time for a juvenile individual to reach maturity.

The results indicated that the parameter corresponding to the growth rate should be optimized for any new food type (quality and quantity). This is particularly required for taking into account the part of the food available for small juveniles. This result is in accordance with Bostrom and Lofs-Holmin (1986) who showed that food particle size had a strong influence on the growth of *A. caliginosa*. Moreover, the equation for the soil (Eqn 3) should also be recalibrated for any new soil because of the organic matter content available (i.e., the food) is different for every soil type.

4.2 Reproduction and energy allocation in adult individuals

The results obtained from the reproduction experiment highlighted that when in contact with each other, adult individuals stopped growing, allocating all of their energy to the production of cocoons. In ecology, this adaptive behavior corresponds to the r strategy which is related to a high growth rate, a production of many offsprings and a relatively low probability of surviving to adulthood (MacArthur and Wilson, 1967). This strategy has been already highlighted for other endogeic earthworm species (Satchell, 1980).

Results showed that adult individuals produced on average 2.4 and 1.6 cocoons ind⁻¹ week⁻¹ for modality with respectively 3 g and 1 g food ind⁻¹ 14 days⁻¹. These results are in accordance with other authors (Bart et al., 2019b; Lofs-Holmin, 1982; Lowe and Butt, 2005; Spurgeon et al., 2000) who reported that cocoon production for *A. caliginosa*, in field soils at 15 °C, was between 0.6 to 2.6 cocoons ind⁻¹ week⁻¹. According to our results, we here provided evidence that the cocoon production by *A. caliginosa* depended on the amount of available food. Our results on reproduction also highlighted that at the highest tested density (i.e., 6 individuals

per vessel), the cocoon production decreased for individuals fed with a limited amount of food but not for individuals fed *ad libitum*. This can be explained by the food available in soil, which was shared by the individuals in the vessel. The food quantity per individual thus decreased with increasing density, leading to a decrease in cocoon production. For individuals fed *ad libitum*, because the amount of food provided (horse dung) was sufficient (the food provided by the soil is negligible in this case), no decrease in cocoon production was observed, as the food quantity per individual remained *ad libitum*. Moreover, considering that *A. caliginosa* is found in the first 20 cm of soil and a medium soil density of 1.2, the highest tested density of earthworms would correspond to 3200 ind⁻¹ m⁻². This is much higher than densities found in the field, which range from 50 to 400 ind⁻¹ m⁻² (Amossé et al., 2018; Edwards and Bohlen, 1996). We thus conclude that reasonable earthworm density does not affect cocoon production, in agreement with Klok et al. (2008) for *L. rubellus* species.

4.3 Interest in ecotoxicology

The model proposed in this study can be used to analyse ecotoxicological effects data throughout time. In ecotoxicology, growth and reproduction data are usually analysed through statistically based models, with *ad hoc* curves to estimate EC_x or NOEC values, for a given duration of exposure, including for earthworms (De Silva et al., 2009; Garcia et al., 2008; Rico et al., 2016). These estimated parameters are thus time-dependent, and do not allow for any extrapolation of the effects at longer time steps. Energy-based models have been first proposed by Kooijman and Bedaux (1996) to analyse toxicity data from growth and reproduction tests at several time steps. Such models provided a relevant description of the data for different species including fish, nematodes, chironomids, and earthworms (Goussen et al., 2015; Hobbelen and van Geslel, 2007; Jager and Klok, 2010; Kooijman and Bedaux, 2010; Péry et al., 2003). In

those models, the chemicals are supposed to affect one of the parameters of the energy-based model, as a function of their mode of action. For instance, an energy-based model was used by Péry et al. (2003) to assess if copper impacted the growth efficiency or the food assimilation of chironomids, using models very similar to the one proposed here. Moreover, these models allow the estimation of a No Effect Concentration (NEC) which does not depend on the duration of the test and make easier the extrapolation to other conditions (e.g. other time measurements) or other levels of biological organization (at population level). The model proposed here was developed for these purposes and thus should be of interest in ecology and ecological risk assessment for the analyses of toxicity data. To be used for population dynamics models, new information would be necessary such as the life span of individual earthworms. Indeed, it is worth noticing that individuals were still alive at the end of the reproduction experiment, and are now more than 2 years old.

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