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

3

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14 Highlights:

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

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26 **Abstract**

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

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

52 **1. Introduction**

53 Earthworms represent the largest part of animal biomass in most temperate soils
54 (Edwards, 2004; Lavelle and Spain, 2001; Paoletti, 1999). They are considered as typical soil
55 ecosystem engineers (Jones et al., 1994) and provide ecological functions in agrosystems such
56 as organic matter degradation or soil structure improvement (Bart et al., 2019a; Bertrand et al.,
57 2015; Blouin et al., 2013; Palm et al., 2014). Earthworms are indicators of soil biological
58 activity and use as model organisms to assess pesticide effects on soil fauna (OECD 1984;
59 Spurgeon et al., 2003).

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

76 The use of *A. caliginosa* in risk assessment requires sufficient knowledge and
77 understanding of its life cycle and the drivers of its life history parameters, i.e., growth and
78 reproduction. However, to date, the life cycle of the different earthworm species, and in
79 particular, of *A. caliginosa* is poorly known, as is the influence of environmental factors on life
80 cycle parameters. Soil temperature and moisture are known to be key parameters driving
81 earthworm life cycle (Eriksen-Hamel and Whalen, 2006; Holmstrup et al., 1991; Moreau-
82 Valancogne et al. 2013). Furthermore, food quality and quantity are also recognized as major
83 factors influencing life parameters of *A. caliginosa*. For instance, Boström and Lofs-Holmin
84 (1986) showed that particle size had a strong effect on growth curve of *A. caliginosa* and cocoon
85 production in laboratory experiments. Unfortunately, the relationship between food availability
86 and *A. caliginosa* growth and reproduction is still poorly documented and understood.

87 In ecotoxicological tests exceeding 14 days with *A. caliginosa*, food supply is necessary
88 because earthworms may starve, stop growing or lose weight, especially in experiments with
89 juveniles (Bart et al., 2018). This could increase the risk of false positives. In contrast, too much
90 food could limit the contact of earthworms with the soil since they would not have to explore
91 much of the soil to find food. Understanding and modelling the influence of food quantity and
92 quality on ecotoxicological endpoints such as growth and reproduction is thus essential to use
93 appropriate feeding conditions in experimental designs. Then, modelling the life cycle can
94 allow the development of energy-based models using the Dynamic Energy Budget (DEB)
95 theory (Kooijman 1986, 2000, 2010). Such models are powerful in explaining life-history
96 patterns of animal (Jager et al., 2013). These models are also useful to analyse growth and
97 reproduction toxicity data (Ashauer et al, 2018; Kooijman and Bedaux, 1996; Péry et al.,
98 2002a), with effects monitored at many time points. Finally, such models can be used to
99 extrapolate observed effects at the population level (Beaudouin et al., 2012, David et al., 2019).
100 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).

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110 **2. Materials and methods**

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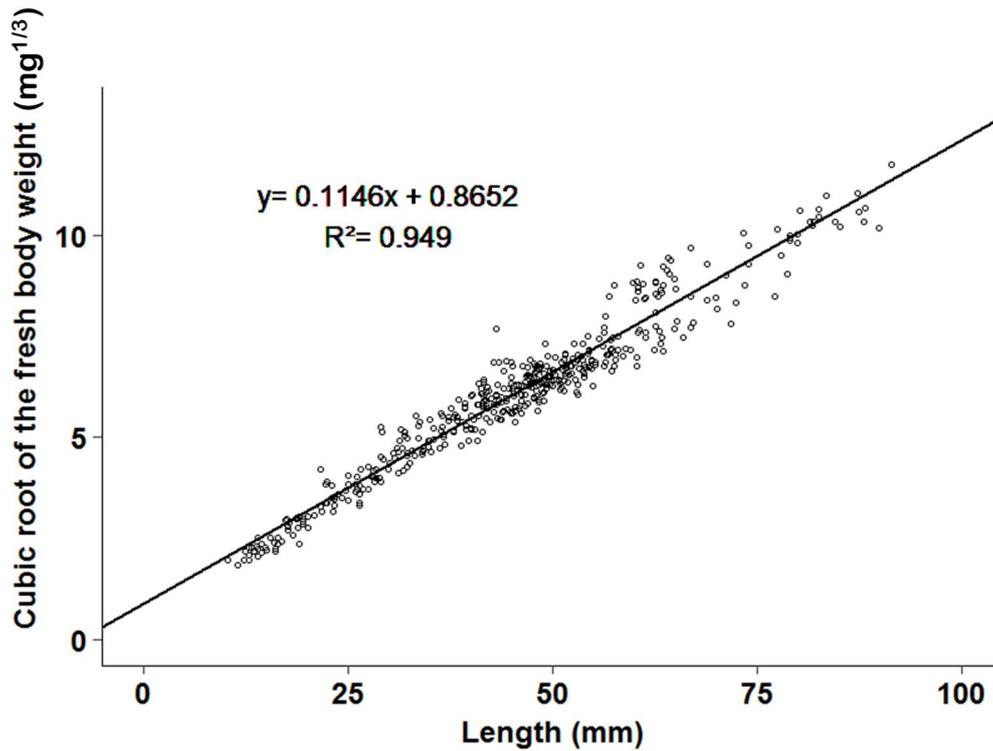
112 *2.1. Animals, soil and food.*

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).

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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).



143

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

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

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

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

$$162 \quad \frac{d}{dt} l = a \quad (\text{Eqn 1})$$

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

$$169 \quad \text{If } l < C_s, \text{ then } \frac{d}{dt} l = a(1 - b) \quad (\text{Eqn 1a})$$

$$170 \quad \text{If } l > C_s, \text{ then } \frac{d}{dt} l = a \quad (\text{Eqn 1b})$$

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

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

$$177 \quad W_{n+1} - W_n = (p \times Q) + S \quad (\text{Eqn 2})$$

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

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

$$184 \quad S = I_{max} (1 - \exp^{-KW}) \text{ (Eqn 3)}$$

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

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

192

193 2.3. Experimental procedures

194 2.3.1. Growth experiments

195 In all the experiments, the soil was renewed every 28 days and the moisture was adjusted
196 and maintained at 70% of the water holding capacity (WHC) (corresponding to 28% of water
197 content) by monitoring the mass of the vessels throughout the experiments and adjusted with
198 tap water if necessary. The horse dung was also adjusted at 70% of the water holding capacity
199 and mixed with the soil. Earthworms were individually placed in 1 L plastic vessels filled with
200 400 g equivalent dry soil. All the vessels had a removable perforated cover for gas exchanges
201 and were stored in a climate room at 15 ± 1 °C. Individuals were carefully extracted from the
202 vessels by hand every 14 days at the beginning of their growth to be weighed. When they

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

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

210

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

218

219 **Experiment 2.** This experiment was set up to test the influence of the food quality, represented
220 by the size of food particles, on the growth. We expected that with smaller food particles, small
221 juvenile individuals would reach and ingest more food, thus decreasing the value of the
222 parameter b (i.e., the correction factor corresponding to 1 less the fraction of the particles
223 reachable for the individual). To test this assumption, hatchling earthworms ($n = 7$) were fed
224 *ad libitum* (i.e., $3 \text{ g ind}^{-1} 14 \text{ days}^{-1}$) but, contrarily to the *Experiment 1*, the food particles were
225 $< 0.5 \text{ mm}$. Prior to assessing results, we modelled the predicted data in two ways. Firstly, we
226 predicted data of this experiment with Eqn 1 of the growth model and the parameter values

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

232

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

245

246 ***Experiment 4.*** This experiment was designed to estimate the value of the parameter p of the
247 Eqn 2 and its confidence interval. To achieve this goal, we monitored the growth (weight
248 measurement) throughout time of 10 individuals fed with $1 \text{ g ind}^{-1} 14 \text{ days}^{-1}$ (same origin as in
249 the *Experiment 1*). We used Eqn 1 with the parameter values C_s , a , b (estimated in the
250 *Experiment 1*) and S equation (Eqn 3) with parameter values estimated in the *Experiment 3*, and
251 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

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

291

292 *2.4. Model calibration.*

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

298

299 **3. Results**

300

301 *3.1. Growth experiments.*

302

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

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

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

316 Based on the data of the *Experiment 3*, we estimated parameters values for I_{max} and K
317 of the S equation (Eqn 3) at 80.97 and 0.00159 respectively (Fig. 3, Table 1). The S equation
318 (Eqn 3) provided a relevant description of the growth pattern when individuals where fed only
319 with soil (Fig. 2C, full line). The growth model (Eqn 1) with parameter values previously
320 estimated (in *Experiment 1 and 2*) provided a relevant prediction of the growth pattern when
321 individuals were fed *ad libitum*, with a maximum error of 11% between predicted values and
322 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).

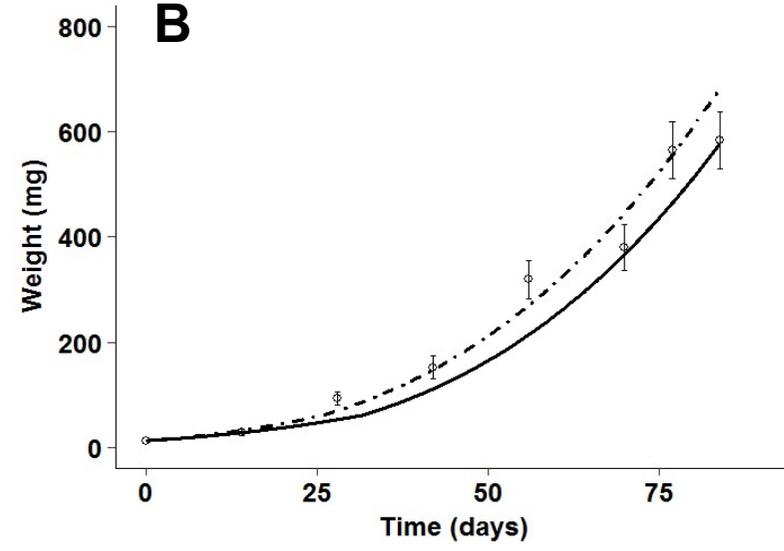
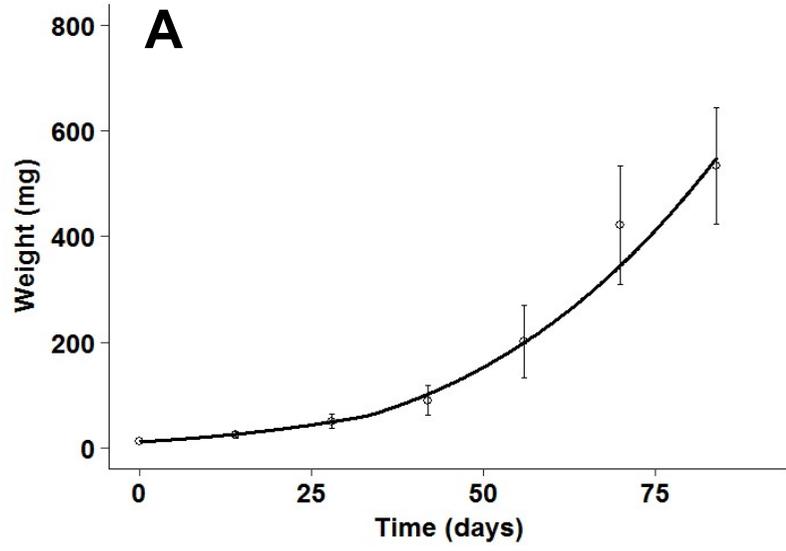
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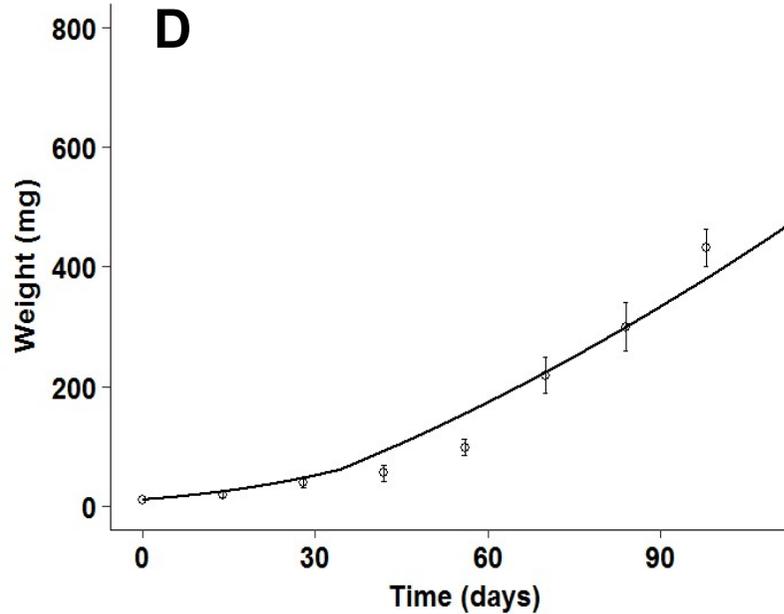
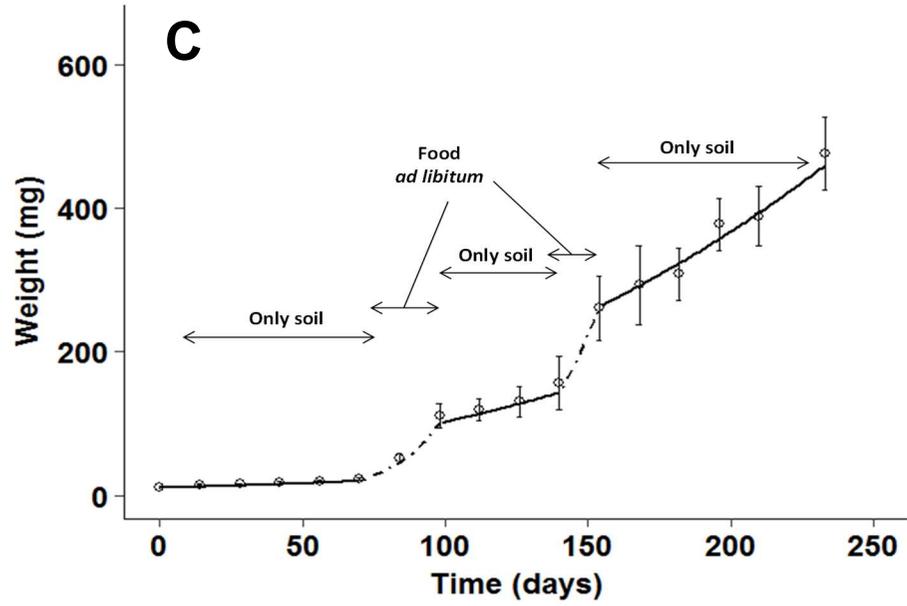
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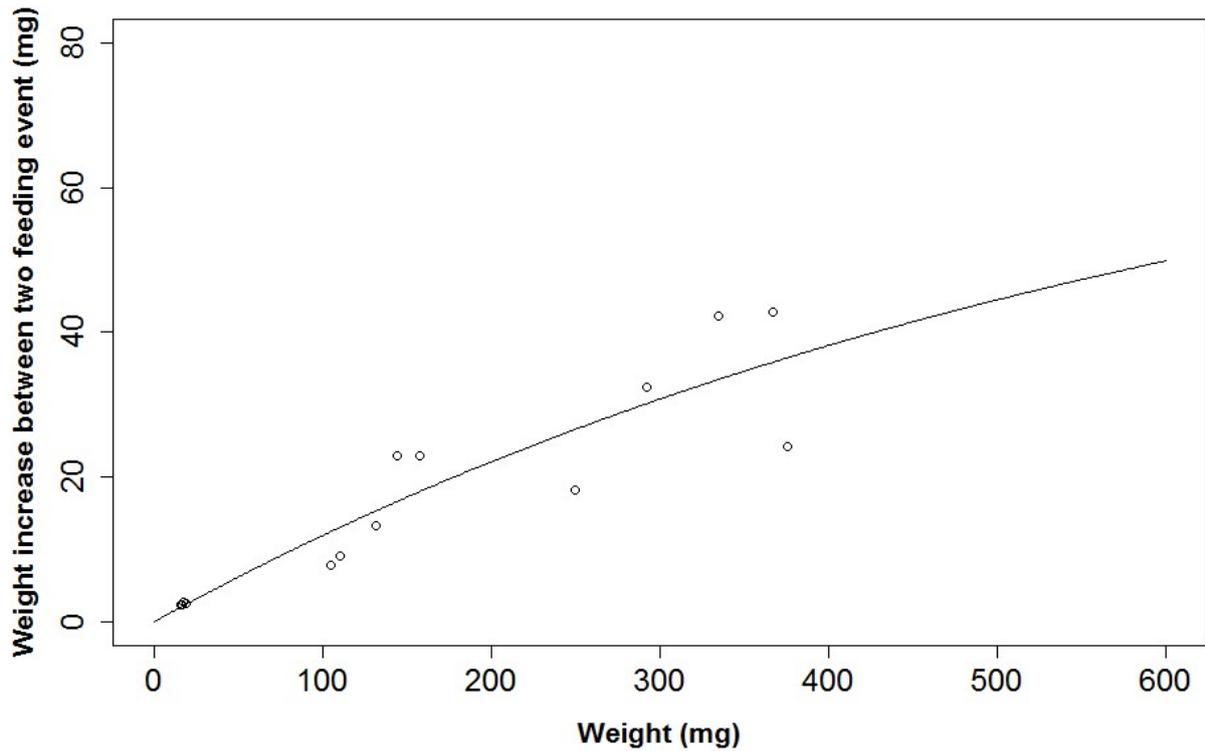
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338 **Figure 2.** **A)** The growth pattern of *A. caliginosa* from the *Experiment 1*, with food *ad libitum*
339 as a function of time since hatching ($n = 10 \pm \text{SD}$). The line represents the description obtained
340 with the growth model (Eqn. 1). **B)** The growth pattern of *A. caliginosa* from the *Experiment*
341 *2*, with food *ad libitum* as a function of time since hatching ($n = 7$, mean \pm SD). The full line
342 represents the predictive model with food *ad libitum* (Eqn. 1) with parameter values obtained
343 in *Experiment 1*. The dash line represents the model with food *ad libitum* (Eqn 1) with C_s and
344 a parameter values from the *Experiment 1* and the b_2 parameter adjusted to the data. **C)** The
345 growth pattern of *A. caliginosa*, from the *Experiment 3*, since hatching with only soil as food
346 or with food *ad libitum* ($n = 5$, mean \pm SD). The full line is the description of the data using the
347 S equation (Eqn 3) and the dash line is the prediction of the model using Eqn 1 (with parameter
348 values estimated in *Experiment 1 and 2*, i.e., a , b_2 and C_s). **D)** The growth pattern of *A.*
349 *caliginosa* from the *Experiment 4*, feed with $1 \text{ g ind}^{-1} 14 \text{ days}^{-1}$ of food as a function of time
350 since hatching ($n = 10$, mean \pm SD). The line represents the data described by the model using
351 Eqn 1 until individuals were under limited food condition switching to the model with limited
352 food with Eqn 2. The switch between the Eqn 1 and 2 in the model happened at day 35, at a
353 weight of 62 mg.

354



355

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

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360

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

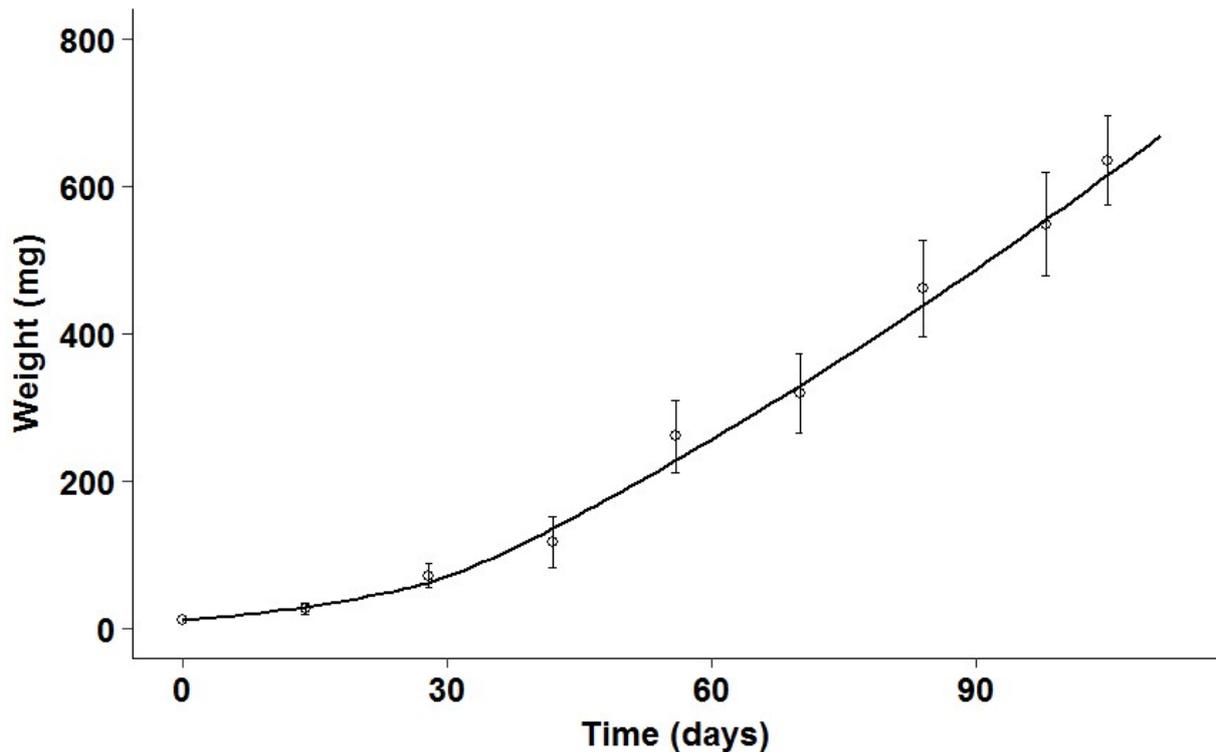
Optimize with data set of	Symbol	Unit	Description	Value	CI 95%
Experiment 1	C_s	$\text{mg}^{1/3}$	Critical size allowing reachability to the all food	3.927	3.262 - 4.531
Experiment 1	a	$\text{mg}^{1/3} \text{ day}^{-1}$	Constant	0.084	0.074 - 0.093
Experiment 1	b	-	Correcting factor for growth constant 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	-

363

364

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

369
370



371
372 **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
373 time since the hatching ($n = 5$, mean \pm SD). The line represents the prediction of the model with
374 Eqn 1 and 2 with parameter values estimated in the *Experiment 1, 2, 3* and 4 (i.e., a , b_2 , C_s , p ,
375 k , I_{max}).

376

377 3.2. Switch to the adult stage.

378

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

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

392

393

394 *3.3. Reproduction experiment.*

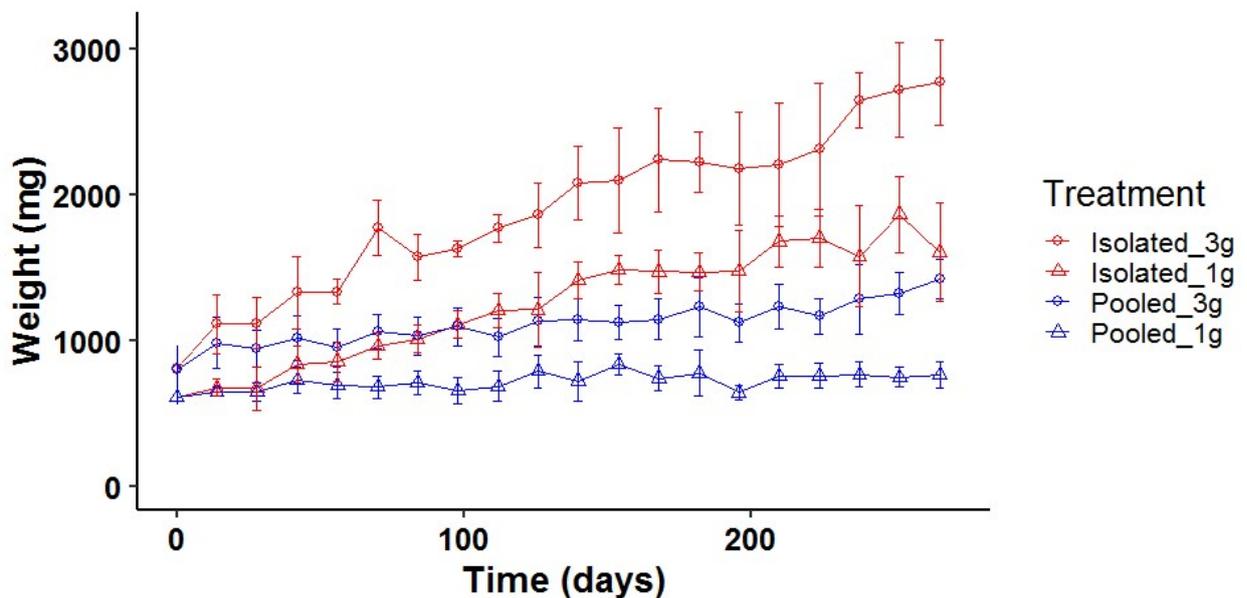
395

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

400

401

402



403

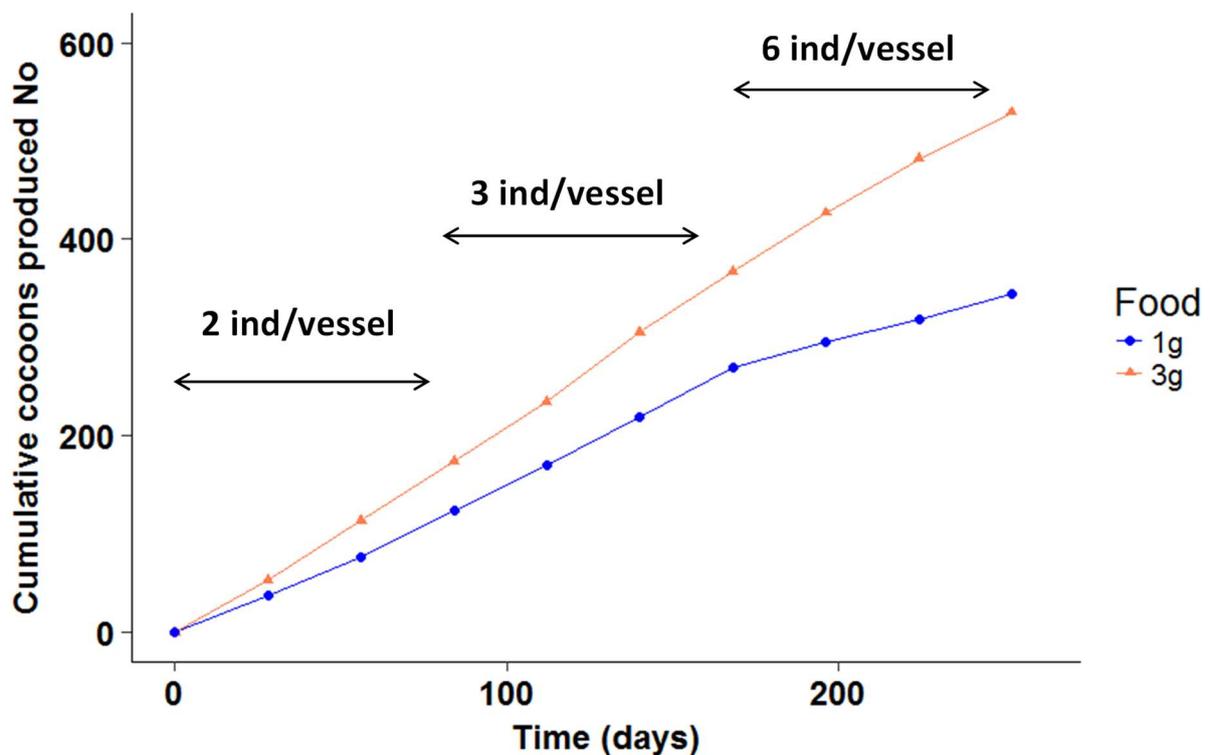
404 **Figure 6.** Growth pattern of adult individuals isolated ($n = 4$, mean \pm SD) or pooled for
405 reproduction ($n = 6$, mean \pm SD). Pooled individuals pooled were pooled by 2 for 84 days, then

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

408

409 **Cocoon production.** The mean cocoon production was on average 1.6 and 2.4 cocoons ind⁻¹
410 week⁻¹ with 1 and 3 g food ind⁻¹ 14 days⁻¹ respectively. When the density was 6 individuals per
411 vessel, the cocoons production decreased by 50% but only for the individuals fed with 1 g ind⁻¹
412 14 days⁻¹ (Fig. 7).

413



414

415 **Figure 7.** Cumulative cocoon production of 6 individuals, with two various food conditions (1
416 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
417 84 days.

418

419

420

421 4. Discussion

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

428

429 4.1 Growth of *A. caliginosa*, model calibration

430

431 At the juvenile stage, the DEB theory (Kooijman, 2000) considers that all energy is
432 allocated to the maintenance cost and growth which is captured by the Von bertalanffy growth
433 curve. Different authors used these assumptions for different earthworm species: *Dendrobaena*
434 *octaedra* (Jager and klok, 2010), *Eisenia fetida* (Jager et al., 2006), *Lumbricus rubellus*
435 (Hobbelen and van Gestel, 2007; Klok, 2008) and *Aporrectodea caliginosa* (Johnston et al.,
436 2014). However, it has not been tested whether it is based on the biology of the considered
437 species. In our study, we tested a different modelling approach based on negligible maintenance
438 costs. This approach was successfully demonstrated as correct for several aquatic species
439 (*Chironomus* species, Péry et al., 2005), and our results showed that this approach works also
440 for *A. caliginosa*. Undeniably, our model provided a good description of the growth data
441 measured in the different experiments, confirming that the maintenance costs are negligible.
442 Our model was able to account for food limitations (even absence of additional food). In
443 particular, it was able to predict the growth pattern from a new data set with a different food
444 amount (i.e., limited quantity). When coupled with the equation determining the probability for

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

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

454

455 *4.2 Reproduction and energy allocation in adult individuals*

456

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

463 Results showed that adult individuals produced on average 2.4 and 1.6 cocoons ind⁻¹
464 week⁻¹ for modality with respectively 3 g and 1 g food ind⁻¹ 14 days⁻¹. These results are in
465 accordance with other authors (Bart et al., 2019b; Lofs-Holmin, 1982; Lowe and Butt, 2005;
466 Spurgeon et al., 2000) who reported that cocoon production for *A. caliginosa*, in field soils at
467 15 °C, was between 0.6 to 2.6 cocoons ind⁻¹ week⁻¹. According to our results, we here provided
468 evidence that the cocoon production by *A. caliginosa* depended on the amount of available food.
469 Our results on reproduction also highlighted that at the highest tested density (i.e., 6 individuals

470 per vessel), the cocoon production decreased for individuals fed with a limited amount of food
471 but not for individuals fed *ad libitum*. This can be explained by the food available in soil, which
472 was shared by the individuals in the vessel. The food quantity per individual thus decreased
473 with increasing density, leading to a decrease in cocoon production. For individuals fed *ad*
474 *libitum*, because the amount of food provided (horse dung) was sufficient (the food provided
475 by the soil is negligible in this case), no decrease in cocoon production was observed, as the
476 food quantity per individual remained *ad libitum*. Moreover, considering that *A. caliginosa* is
477 found in the first 20 cm of soil and a medium soil density of 1.2, the highest tested density of
478 earthworms would correspond to 3200 ind⁻¹ m⁻². This is much higher than densities found in
479 the field, which range from 50 to 400 ind⁻¹ m⁻² (Amossé et al., 2018; Edwards and Bohlen,
480 1996). We thus conclude that reasonable earthworm density does not affect cocoon production,
481 in agreement with Klok et al. (2008) for *L. rubellus* species.

482

483 *4.3 Interest in ecotoxicology*

484

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

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

507

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514

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