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The growth and reproduction of *Lumbricus rubellus* and *Dendrobaena rubida* in cow manure Mixed cultures with *Eisenia andrei*

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Abstract

The growth and reproduction of the epigeic species *Lumbricus rubellus* and *Dendrobaena rubida* in cow manure and the possible interactions of these species with *Eisenia andrei* are studied. The mean growth rate of *D. rubida* was 3.84 mg day^{-1}, reaching sexual maturity at 54 days and producing an average of 1.45 cocoons per week. After collection, 85% of the cocoons of this species were viable, incubation took an average of 21.7 days and an average of 1.67 worms emerged from each cocoon. The mean growth rate of *L. rubellus* was 8.02 mg day^{-1}, maturing at 74 days and with a mean weekly production of 0.54 cocoons. After an incubation period of 36.5 days, 64% of the cocoons hatched, one worm emerging from each. The mixed cultures tested did not present any advantage over pure cultures. *E. andrei* showed higher growth rates in mixed cultures, while the growth rate of *L. rubellus* and *D. rubida* decreased slightly in mixed cultures as compared to pure cultures.

Keywords: *Dendrobaena rubida*; *Lumbricus rubellus*; *Eisenia andrei*; Vermicomposting; Life cycles; Competitive interactions

1. Introduction

Earthworms have been successfully used in the vermistabilization of urban, industrial and agricultural wastes in order to produce organic fertilizers and obtain protein for animal feed. Although many species could be used for these ends, given their nutritional requirements and reproductive biology, only *Eisenia fetida* and *Eisenia andrei* are widely used. Their growth and reproduction patterns have been widely documented (e.g. Hartenstein, 1978; Edwards, 1988; Venter and Reinecke, 1988; Haimi, 1990; Reinecke and Viljoen, 1991), demonstrating their efficiency in vermicomposting processes. Different aspects of the biology of other species have recently been studied evaluating their suitability for vermicomposting: *Eudrilus eugeniae* (Viljoen and Reinecke, 1989), *Dendrobaena veneta* (Viljoen et al., 1991), *Pontoscolex corethrurus* (Hamoui, 1991) and *Drawidia nepalensis* (Kaushal and Bisht, 1992). *Allolobophora caliginosa*, *Amynthas hawayana*, *Lumbricus terrestris* and *Lumbricus rubellus* have been also mentioned for their potential use in stabilizing organic material (Lee, 1985; Edwards, 1988).

Lumbricus rubellus and *Dendrobaena rubida* are epigeic species common to northwest Spain which exhibit a clear preference for a highly organic substrate (Elvira et al., 1996). Their growth and repro-

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duction in organic substrates have not yet been fully described, although some specific aspects of their biology have been investigated (Evans and Guild, 1948; Gates, 1972; Sims and Gerard, 1985; Bengtsson et al., 1986; Cluzeau and Fayolle, 1989).

In this paper, the growth and reproduction of *D. rubida* and *L. rubellus* are examined to evaluate their suitability for vermicomposting. The advantages and disadvantages of breeding these species in mixed cultures with *E. andrei* are also studied, as the extent to which it is convenient to breed these or other species simultaneously is presently unknown (Loehr et al., 1985).

2. Materials and methods

2.1. Growth and reproduction of *L. rubellus* and *D. rubida*

Cultures were constructed using 5-litre cylindrical containers with a sponge in the bottom which was kept damp. Each container held 800 g (wet weight) of urine-free cow manure with a moisture content of 80%. Eighty newly hatched *D. rubida* and 40 newly hatched *L. rubellus* were placed in each container for the duration of the experiment (3 months) at 20–25°C. Every 15 days, 200 g (wet weight) of substrate was replaced with the same amount of new manure to avoid any nutritional deficiency. The weight of the earthworms (with full guts), clitellum development and cocoon production by handsorting the cultures were measured weekly. One hundred and eighty cocoons of *D. rubida* and 120 cocoons of *L. rubellus* were incubated at 25°C, in groups of ten in Petri dishes between two dampened filter papers. This enabled the incubation time and viability rate of the cocoons to be determined. The number of worms hatched per cocoon was measured starting from 50 cocoons of each species, incubated individually using the above procedure. As a general indication of reproductive capacity, the net reproductive rate or number of worms produced by each adult per week was calculated.

2.2. Pure and mixed cultures associated with *E. andrei*

Five cultures were set up using the experimental procedure mentioned above: three pure cultures (*E. andrei*, *D. rubida*, and *L. rubellus*) and two mixed cultures (*E. andrei* + *D. rubida* and *E. andrei* + *L. rubellus*). Forty individuals were placed in each container (75% clitellated and 25% immature): 40 of the same species in the pure cultures and 20 of each species in the mixed cultures. The duration of the experiment was 3 months at 20–25°C and cocoon production and individual weight were measured every 15 days. In all of the cultures, the cocoons produced by each species were incubated at 25°C until hatching to determine the viability rates.

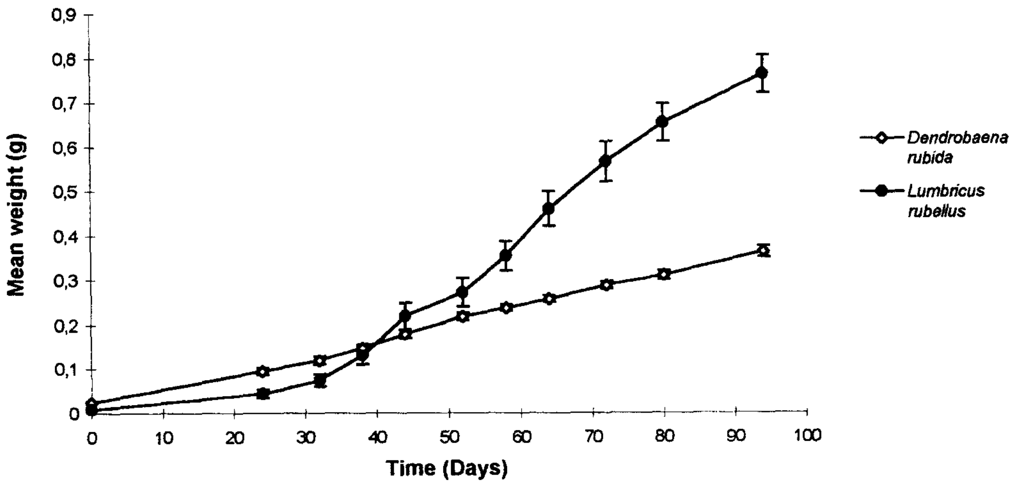


Fig. 1. Growth curves of *D. rubida* and *L. rubellus* in cow manure.

3. Results

3.1. Growth and reproduction of L. rubellus and D. rubida

During the 3 months the experiment lasted, there were few mortalities, these basically occurring during the initial stages of growth, being 12% in *D. rubida* and 18% in *L. rubellus.*

The mean weight gain (fresh weight) for *D. rubida* juveniles was 3.84 mg day^{-1}, reaching a mean weight of 0.357 ± 0.013 g after 94 days. During the first month the growth rate was 3.46 mg day^{-1}, increasing to 4.54 mg day^{-1} in the second month, and decreasing to 3.50 mg day^{-1} in the third month. The mean growth rate for *L. rubellus* was 8.02 mg day^{-1}, reaching a mean weight of 0.762 ± 0.042 g after 94 days. The growth rate for the first 30 days was slow, 2.05 mg day^{-1}, increasing to 10.73 mg day^{-1} during the second month and rising slightly in the third month to 11.36 mg day^{-1} (Fig. 1).

The time required for 50% of the earthworms to fully develop the clitellum was 51 days for *D. rubida* and 84 days for *L. rubellus* (Fig. 2), the clitellated individuals weighing (mean maturity size) 0.270 ± 0.007 g and 0.770 ± 0.033 g respectively. The mean maturing time for *D. rubida* was 54.08 ± 1.77 days and 74.24 ± 0.33 days for *L. rubellus.*

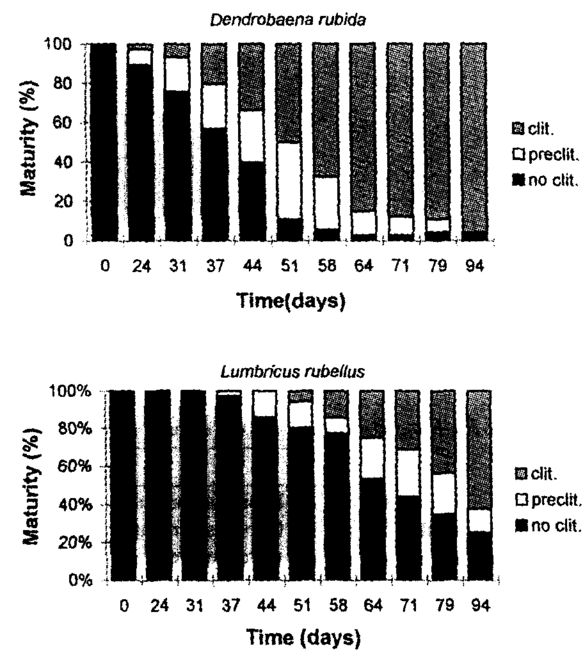


Fig. 2. Percentage of non-clitellated, preclitellated and clitellated individuals of *D. rubida* and *L. rubellus* in cow manure.

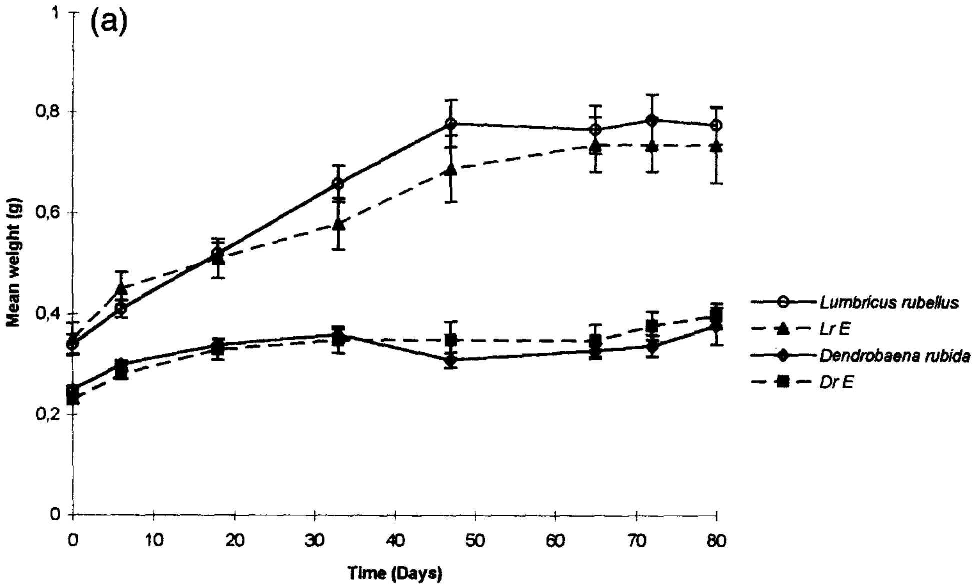
With regards to reproduction rate, *D. rubida* began cocoon production 38 days after the experiment commenced, laying a total of 654 cocoons within the remaining 56 days. The mean cocoon production rate during this time was 1.45 ± 0.372 cocoons per mature worm per week, peaking between days 58 and 64 (3.54 cocoons per mature worm per week). *L. rubellus* began producing cocoons at 64 days and laid 36 in the following 30 days; mean cocoon production rate was 0.54 ± 0.195 cocoons per mature worm per week.

The hatching percentage for *D. rubida* cocoons was 85%, while incubation varied between 15 and 40 days with a mean incubation time of 21.7 ± 0.392 days. For this species the number of hatchlings from a single cocoon varied between one and three, with a mean of 1.67 ± 0.096. In the case of *L. rubellus* the hatching percentage was 64%, while incubation took from 14 to 63 days with a mean incubation time of 36.5 ± 2.125 days. Each cocoon produced only one individual.

By theoretically calculating the net reproductive rate, it was estimated that, in cow manure and under laboratory conditions, each mature worm of *D. rubida* produces 2.06 hatchlings per week (1.45 cocoons per mature worm per week, 85% of cocoons being viable), whereas each mature worm of *L. rubellus* only produces 0.35 hatchlings per week.

3.2. Pure and mixed cultures associated with E. andrei

Fig. 3 compares growth curves for each species in pure and mixed cultures. The growth of *L. rubellus* decreased in the presence of *E. andrei*. However, when *D. rubida* was cultured in association with *E. andrei*, its growth rate increased slightly compared with the pure culture. Higher growth rates were registered in general for *E. andrei* in mixed cultures than in the pure culture (Table 1). The growth rate for *E. andrei* in the pure culture was 17.1 mg day^{-1} during the first 18 days, later decreasing and then stabilizing, with a mean weight loss of −2.02 mg day^{-1}. The growth curve in the presence of *D.*



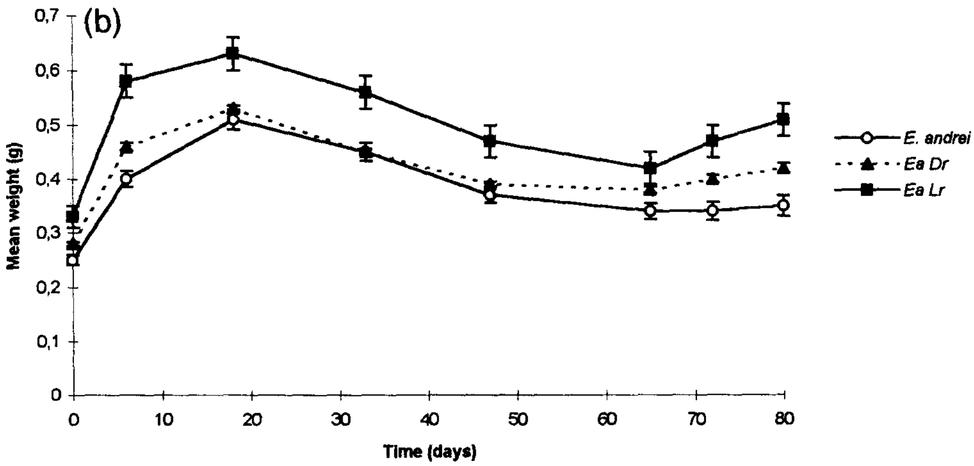


Fig. 3. Growth curves of *D. rubida*, *L. rubellus* and *E. andrei* in pure and mixed cultures. (a) Lr E, *L. rubellus* cultured with *E. andrei*; Dr E, *D. rubida* cultured with *E. andrei*). (b) Ea Dr, *E. andrei* cultured with *D. rubida*; Ea Lr, *E. andrei* cultured with *L. rubellus*.

Table 1
Growth and reproduction rates of *Eisenia andrei*, *Lumbricus rubellus* and *Dendrobaena rubida* in pure and mixed cultures

	Pure cultures		Mixed cultures	
	Growth rate (mg day^{-1})	Reproduction rate (cocoons per mature worm per week	Growth rate (mg day^{-1})	Reproduction rate (cocoons per mature worm per week
L. rubellus	6.1	0.43 ± 0.13	5.3	0.50 ± 0.18
D. rubida	2.4	1.26 ± 0.22	2.9	1.38 ± 0.22
E. andrei + *L. rubellus*	1.25	1.98 ± 0.37	2.23	1.81 ± 0.44
E. andrei + *D. rubida*	1.25	1.98 ± 0.37	1.75	1.89 ± 0.34

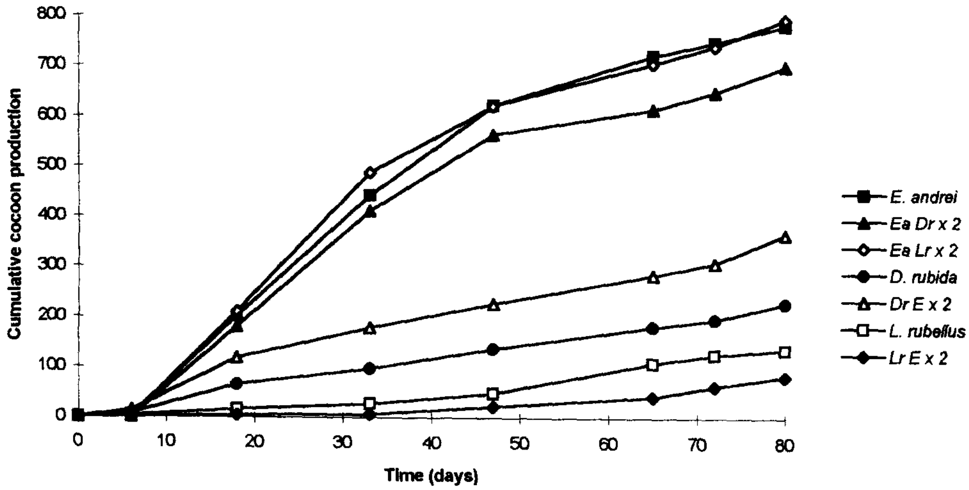


Fig. 4. Cumulative cocoon production of *D. rubida*, *L. rubellus* and *E. andrei* in pure and mixed cultures. Dr E, *D. rubida* cultured with *E. andrei*; Lr E, *L. rubellus* cultured with *E. andrei*; Ea Dr, *E. andrei* cultured with *D. rubida*; Ea Lr, *E. andrei* cultured with *L. rubellus*.

rubida is similar, with an initial increase of 17.9 mg day^{-1} and weight loss at a rate of -1.01 mg day^{-1}. In the mixed culture with *L. rubellus*, the initial growth rate was higher (23 mg day^{-1}) while weight loss was lower (-0.36 mg day^{-1}).

Fig. 4 shows cocoon production in pure and mixed cultures. The value of each point in the mixed cultures is multiplied by two as the initial number of worms of each species in the mixed cultures is half the number in the pure cultures). In the pure cultures, cocoon production for *E. andrei* was higher than for the other two species. The presence of a second species in the mixed cultures did not greatly affect the rate of cocoon production (Table 1).

The percentage of cocoons hatched for *D. rubida* and *L. rubellus* was not affected by the presence of *E. andrei*, being 84.5% in the pure culture and 85.4% in the mixed culture for *D. rubida*, and 64.5% and 63.3% respectively for *L. rubellus*. The viability of *E. andrei* cocoons was higher in pure culture (92%) than in the presence of *D. rubida* (89%) or *L. rubellus* (82%).

4. Discussion

The results obtained show that both *D. rubida* and *L. rubellus* are species that are capable of growing and reproducing successfully in substrates with a high organic content; however, this does not guarantee that these species are of use for vermiculture. As well as showing preference for organic media, they should exhibit rapid growth rates, high reproductive rates and a high tolerance of environmental factors.

Compared to other species, *D. rubida* has a slow growth rate although it reaches sexual maturity relatively quickly (54 days). Cluzeau and Fayolle (1989) found that the sexual maturation of this species occurs by 44 ± 10 days. The average age of sexual maturity in other epigeic species has been reported as follows: 65 days for *D. veneta* (Viljoen et al., 1991); 60 days for *E. fetida* (Venter and Reinecke, 1988); 45 days for *Eudrilus eugeniae* (Viljoen and Reinecke, 1989); 42 days for *Perionyx excavatus* (Hallatt et al., 1990); 34 days for *D. nepalensis* (Kaushal and Bisht, 1992).

The net reproductive rate for *D. rubida* has been estimated at 2.06 hatchlings per mature worm per week. The cocoon production rates in *D. rubida* are usually found to be higher than those in this study: 2.31 cocoons per week (Bengtsson et al., 1986), 3.22 cocoons per week (Cluzeau and Fayolle, 1989); and regarding the number of worms emerged per cocoon, Gates (1972) found that only one worm emerged from 75% of the cocoons of *D. rubida*, with 2–4 hatchlings in the remaining cocoons. The net reproductive rate of this species is lower than the 4.8

worms per week of *E. fetida* (Venter and Reinecke, 1988), the 26.2 worms per week of *E. eugeniae* (Viljoen and Reinecke, 1989) and the 7.7 worms per week of *P. excavatus* (Hallatt et al., 1990) but higher than the 0.4 worms per week of *D. veneta* (Viljoen et al., 1991) and the 1.7 worms per week of *D. nepalensis* (Kaushal and Bisht, 1992). According to Cluzeau and Fayolle (1989), one of the factors that contribute to the high fertility of *D. rubida* is that its reproduction may be biparental, amphimitic or uniparental, either by parthenogenesis (Omodeo, 1952) or by self-fertilization (André and Davant, 1972).

L. rubellus exhibited a slow growth rate and long maturation time (74 days). Cluzeau and Fayolle (1989) found that this species required a minimum of 91 ± 22 days to reach sexual maturity. In addition, the net reproductive rate estimated in this study was 0.35 hatchlings per worm per week, due to the low cocoon production rate (0.54 cocoons per week) and only one worm emerging from each cocoon. Other researchers have pointed out cocoon production rates for this species which vary from 0.49 (Cluzeau and Fayolle, 1989) to 1.75 cocoons per week (Evans and Guild, 1948). *L. rubellus* is not an opportunistic species, with obligatory biparental reproduction (Sims and Gerard, 1985) which contributes to its low reproductive rates.

The early maturation and high reproductive rate of *D. rubida* thus make it a suitable species for vermicomposting. On the other hand, the slow maturation and low reproductive rate of *L. rubellus* mean that it is not very suitable for use in vermicomposting, although its size and vigour could make it of potential interest as fish bait.

Results showed that the possible competitive interactions clearly affect survival and growth rate to a greater extent than reproduction, probably due to food competition and involving physiological processes (Abbott, 1980).

Pure cultures of *E. andrei* showed a higher growth rate than the mixed ones, probably related to a higher population density. Domínguez and Edwards (1996) observed decreasing growth rates of *E. andrei* with increasing population density, and the same effect was reported for *E. fetida* (Neuhauser et al., 1980; Reinecke and Viljoen, 1990). In mixed cultures, however, food competition was the most influential factor with the lowest growth rate observed when *D. rubida* was present.

When *L. rubellus* was cultured in mixture with *E. andrei*, a lower growth rate was recorded compared to pure cultures; this can be explained as a consequence of a negative effect of *E. andrei* on *L. rubellus*. Similarly, Hamilton et al. (1988) recorded a lower growth rate of *L. terrestris* when mixed with *E. fetida*.

With regard to *D. rubida*, a less marked negative effect was observed when living with *E. andrei* and it might therefore be inferred that it is a more successful competitor than *L. rubellus*.

Other authors (Neuhauser et al., 1980; Rouelle et al., 1987) have found that certain excretory compounds can accumulate and provoke negative effects on the growth of other species. For example, toxic bactericidal compounds in the celomatic fluid of both *E. fetida* and *E. andrei* have been reported. Rouelle et al. (1987) found a thiamine-destroying factor in the faeces of *E. andrei* and *Dendrobaena* sp., which could negatively affect growth when ingested by other earthworms. In this paper, food competition seems to be the factor which best explains the observed interactions.

In view of all this, if we take into consideration the effect of density on the growth rates, it is possible to conclude that no advantage was found in mixed cultures over pure cultures. Loehr et al. (1985) measured the weight increase and reduction in the proportion of volatile solids of the substrate in pure and mixed cultures for *E. fetida, E. eugeniae* and *P. excavatus* and found that no advantage was gained in polycultures.

It is possible that mixed cultures could be more appropiate when the selected species belong to different ecological categories and thus competition for the same food resources could be avoided. The use of this kind of culture could be a more suitable technique in agrosystems management, soil restoration and soil amelioration.

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