

Effect of the diet on growth and reproduction of *Eisenia andrei* (Oligochaeta, Lumbricidae)

J. Dominguez, M. J. I. Briones and S. Mato

Departamento de Ecoloxía e Bioloxía Animal, Universidade de Vigo. Apto. 874, E-36200, Vigo, Spain

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Summary. The effect of some vegetable bulking agents (straw, pine needles, pine bark, oak leaves and fern fronds) in mixtures with pig slurry (1:1 dry weight) on the growth and reproduction of *Eisenia andrei*, Bouché 1972 was studied in cultures with either 1 or 8 individuals. Unlike individual cultures, with no food limitation and no competition, the cultures with 8 individuals represent a more real situation, with food competition and mating processes. The maximum growth and reproduction rates were achieved, both in the individual and in the group cultures, in the mixtures with straw and pine needles. The earthworms showed low growth rates and very low reproductive rates in the oak leaves and fern mixtures, both in cultures with one individual or 8 individuals.

The high growth rates obtained here are in agreement with others in the literature indicating the high potential of pig wastes for vermicomposting. Our results also confirm that there is a direct relationship between *E. andrei* biomass and clitellum development so that the minimum weight for maturation is approximately 0.4 g. In addition, the remarkably high reproduction rate and cocoon production in the cultures with one individual suggests that mating is not an obligatory requirement for cocoon production.

Key words: *Eisenia andrei*, pig manure, diet, bulking agents, growth rate, reproduction

Introduction

In recent years, the disposal of organic wastes from domestic, agricultural and industrial sources has caused increasing environmental and economic problems. Vermicomposting, the composting of organic wastes through earthworm activity has proved to be successful in processing sewage sludge and solids from wastewater (Neuhauser et al. 1988), materials from breweries (Butt 1993), paper waste (Butt 1993; Elvira et al. 1995a), urban residues, animal wastes (Edwards et al. 1985; Allevi et al. 1987; Edwards 1988; Elvira et al. 1995b; Dominguez & Edwards 1996), as well as horticultural residues from processed potatoes, dead plants and the mushroom industry (Edwards 1983, 1988).

Some of these residues, such as pig manure and sewage sludge, are probably the most productive wastes for growing earthworms. However, most of the time these wastes are in the form of slurries, making it necessary to separate the solids mechanically or by sedimentation. Even after these treatments, the obtained residues have too high a moisture content for the direct use of vermicomposting.

One solution to this problem is the use of agricultural and forest by-products as bulking agents to reduce both the moisture content and the ammonia concentration. They can also improve the C:N ratio by supplying C, and at the same time prevent N losses by ammonia volatilization.

It is well known that the food source influences not only the size of earthworm populations but also their growth and reproduction rates. There is abundant literature on the response of the earthworms to different types of vegetable substrates in the field (Guild 1955; Barley 1959; King & Heath 1967; Swain 1979; Shipitalo et al. 1988), but there is a lack of information about the effect of diet on earthworms during the vermicomposting process.

In this paper, we investigate the effect that some vegetable bulking agents (straw, pine needles, pine bark, oak leaves and fern fronds) mixed with pig slurry (1:1 dry weight) have on the growth and reproduction of *Eisenia andrei* when reared alone or in groups of 8 individuals. Individual cultures represent an ideal growth situation, with no food limitation and no competition whereas the group cultures represent a more real situation, with food competition and mating processes.

Materials and Methods

Individual cultures

Juvenile specimens of *Eisenia andrei*, weighing less than 10 mg live weight, were obtained from a stock culture maintained in the laboratory at a temperature of 25 °C in cow manure. Earthworms were individually placed in 250 cm³ plastic dishes and supplied 100 g of a mixture of pig slurry with straw, fern fronds, oak leaves, pine needles and pine bark (1:1 dry weight, moisture content = 85%). Oak leaf litter and pine needles were collected immediately after fall; senescent fern fronds, and dry wheat straw were also collected in the field. The vegetable materials were chopped and sieved (<2 mm) and twenty replicates for each diet treatment were established. The worms were not supplied with more substrate for the duration of the experiment which ended when weight losses of the earthworms were detected (14 weeks for the mixture with straw and 8 weeks for the mixture with fern).

Group cultures

In a second experiment conducted under similar conditions, 8 juveniles of *Eisenia andrei*, weighing less than 10 mg live weight each, were placed in 250 cm³ plastic dishes and fed 100 g of the mixtures described above. Three replicates were set up for each treatment and the duration of the experiment was 10 weeks.

In both experiments, the weight of the worms (with full alimentary tracts), clitellum development and cocoon production (determined by hand-sorting) were measured weekly. All cocoons were incubated between two dampened filter papers in Petri dishes at 25 °C to enable the measurement of incubation time and viability rate. To compare the growth rates of *E. andrei* in the different mixtures, it was necessary to find a common denominator for comparison. To achieve this, the growth rate (mg weight gained day⁻¹ worm⁻¹) was calculated and the maximum weight achieved was divided by the number of days required to reach the maximum weight.

Chemical analyses of the mixtures

A subsample (approximately 10 g) of each mixture was dried at 105 °C and ashed at 550 °C to determine moisture and organic matter content, respectively. The organic matter content was determined as the difference between ash and dry-weight values. The pH and conductivity were determined by using water diluted samples (1:10) and NH₄⁺-N by using an automatic steam distillator (Buchi 650, Zürich).

Statistical analyses

The growth data were fitted using logistic regressions. One-way analysis of variance (ANOVA) and separation of means based on the least significant difference (LSD, $p \leq 0.05$) allowed determination of significant differences between growth rates in the different mixtures.

Results

Table 1 shows some chemical characteristics of the pig slurry and the mixtures with the different bulking agents. Pig slurry shows high ammonia content and conductivity values which could be detrimental to earthworm survival whereas the mixtures resulted in a marked decrease in these two chemical properties making them potentially more suitable for growing *E. andrei*.

Table 1. Some analytical characteristics of the pig slurry and the mixtures with the different bulking agents

| | Pig slurry | Straw | Pine needles | Pine bark | Fern fronds | Oak leaves |
|---|------------|-------|--------------|-----------|-------------|------------|
| Moisture content (%) | 86.5 | 80.5 | 66.7 | 68.4 | 80 | 76.2 |
| Organic matter (%) | 83.9 | 86.0 | 86.0 | 76.3 | 79.8 | 78.9 |
| pH | 7.7 | 8.6 | 7.1 | 7.5 | 8.3 | 7.2 |
| Conductivity (mS cm ⁻¹) | 2.1 | 0.9 | 0.4 | 0.3 | 1.88 | 0.8 |
| NH ₄ ⁺ -N (mg g ⁻¹) | 5.1 | 0.1 | 0.7 | 0.8 | 0.85 | 0.6 |

Individual cultures

Fig. 1 and Table 2 show the comparison between growth rates of *E. andrei* in the different diet mixtures when cultured individually. It can be seen that earthworms maintained high growth rates and achieved the maximum weights in the mixture with straw (1.09 ± 0.06 g). The mix-

Table 2. Growth of *E. andrei* in individual cultures (n = 20) in the different diet mixtures

| DIET MIXTURES ¹ | Maximum weight achieved (g) ± S.E. ² | Growth rate (mg day ⁻¹) |
|----------------------------|---|-------------------------------------|
| PS + Straw | 1.09 ± 0.06 ^{a3} | 10.65 ± 0.68 ^a |
| PS + Pine Needles | 0.83 ± 0.08 ^b | 8.83 ± 0.42 ^b |
| PS + Pine Bark | 0.70 ± 0.02 ^c | 11.22 ± 0.71 ^a |
| PS + Fern | 0.40 ± 0.03 ^d | 9.14 ± 0.38 ^b |
| PS + Oak leaves | 0.71 ± 0.05 ^c | 9.00 ± 0.47 ^b |

¹ PS: Pig slurry

² Standard error

³ Treatments followed by the same letters are not significantly different (p ≤ 0.05)

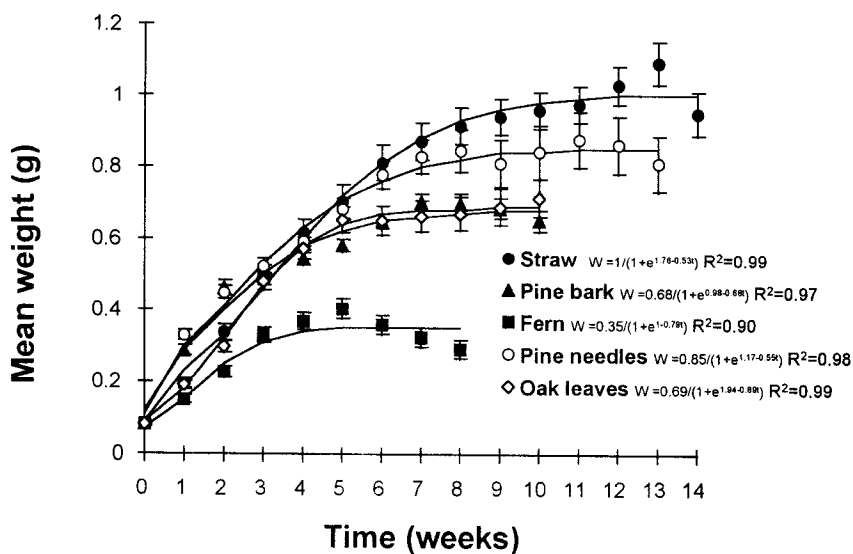


Fig. 1. Growth of *E. andrei* fed on five diet mixtures in the individual cultures (n=20). Solid lines represent fitted logistic equations. The legend includes regression formulas and R² values for each treatment. The general formula of the logistic regression was $W = A/(1 + e^{-b+kt})$, where W is the weight at time t, A is the asymptotic weight, and k and b are constants

ture with pine needles produced the second highest value (0.83 ± 0.08 g), which was significantly different from that obtained in the mixture with pine bark (0.70 ± 0.02 g) although less time was required in the latter. The reason for the observed maximum growth rates in the mixture with pine bark is due to a different duration of the experiment (10 weeks compared with 13 and 14 for the pine needles and straw mixtures respectively). The daily growth rate in the mixtures of pig slurry with straw and pine bark was significantly higher than in the other three mixtures (Table 2).

In the mixture with oak leaves, the maximum weight achieved was not significantly different from the one obtained in the mixture with pine bark although the growth rate was much lower. *E. andrei* showed its lowest maximum weight in the mixture with fern (Table 2).

With regard to clitellum development and reproduction (Fig. 2), in the mixture of pig slurry with straw, 5 % of the population was preclitellate (denoted by the appearance of tubercula pubertatis) after 1 week and the first mature individuals (with the clitellum totally developed) appeared after two weeks and represented 5 % of the total population. In the mixture with pine needles, the first clitellate individuals were observed after 2 weeks and represented a 50 % of the population with the first preclitellate individuals having appeared after 1 week and represented 52 % of the total. In this mixture, clitellum regression was observed after 7 weeks and at the end of the experimental period (13 weeks) 10 % of the individuals had completely lost their clitellum. The mixture of pig slurry with pine bark, followed a similar pattern, although clitellum regression did not occur until week 10, when a 30 % of the population had lost the clitellum.

In the mixture with fern fronds, clitellate individuals did not appear until week 4 and they represented only 25 % of the population. More than 50 % of the individuals remained immature during the 8 weeks of the experiment. In the mixture with oak leaves, the first clitellate individuals were detected after 3 weeks and represented 20 % of the population, 5 % of the population remained immature throughout the whole experiment and clitellum regression occurred after 9 weeks.

The measured reproductive parameters of *E. andrei* in the different diet mixtures are summarized in Table 3. The mean maturation size, defined as the mean individual weight when 50 % of the earthworms had fully developed clitella, and the time required to reach this stage were not significantly different ($p < 0.05$) in the mixtures of pig slurry with straw, pine needles and pine bark. The mixture with oak leaves showed a significantly higher values of these two parameters whereas for the mixture with fern fronds the mean maturation size was significantly lower but the time to reach it was significantly higher; in the mixture with fern fronds only 30 % of the population reached the maturation stage, and the mean maturation size was calculated from these individuals (Table 3).

Regarding the percentage of reproduction, i.e. percentage of worms that laid cocoons, a high variability was observed in the different mixtures. In the mixtures with straw and oak leaves

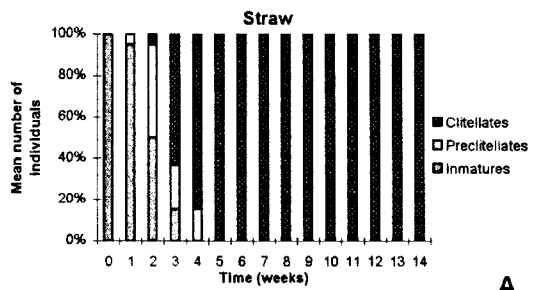
Table 3. Sexual development of *E. andrei* in the individual cultures ($n=20$) in the different diet mixtures

| DIET MIXTURES ¹ | Mean maturation size (g) \pm SD ² | Time (days) | % Reprod. | Cocoons earthworm ⁻¹ | % Viability | N° ind. cocoon ⁻¹ |
|----------------------------|--|-------------|------------------------------|---------------------------------|-------------|------------------------------|
| PS + Straw | 0.50 ± 0.12^{b3} | 21 | 10 2 ^c | 13.5 | 37 | 2.1 |
| PS + Pine Needles | 0.44 ± 0.07^b | 14 | 55 2 ^c | 19.5 | 72 | 3.2 |
| PS + Pine Bark | 0.46 ± 0.10^b | 14 | 35 2 ^c | 5.43 | 44 | 2.6 |
| PS + Fern | 0.40 ± 0.12^c | 35 | 0 | 0 | 0 | 0 |
| PS + Oak leaves | 0.57 ± 0.13^a | 28 | 10 | 9 | 0 | 0 |

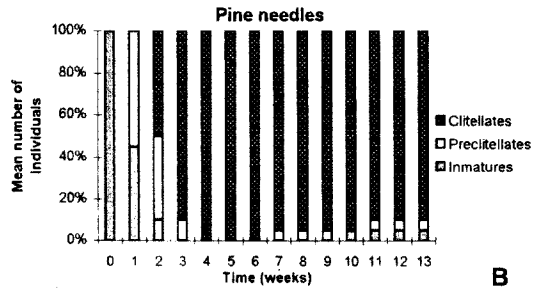
¹ PS: Pig slurry

² Standard deviation

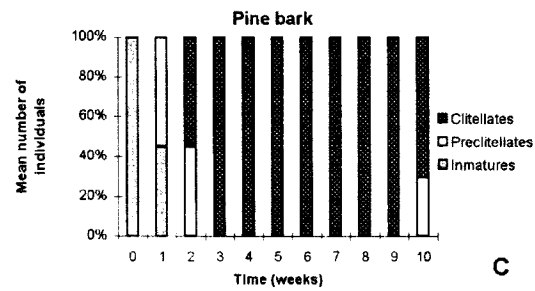
³ Same letters correspond to not significant differences ($p < 0.05$)



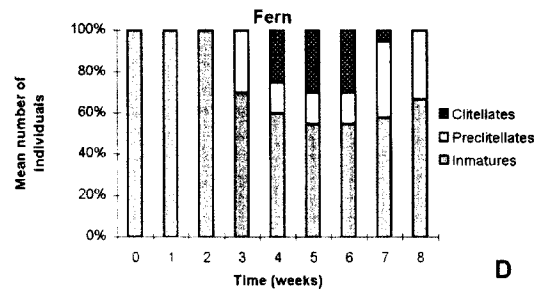
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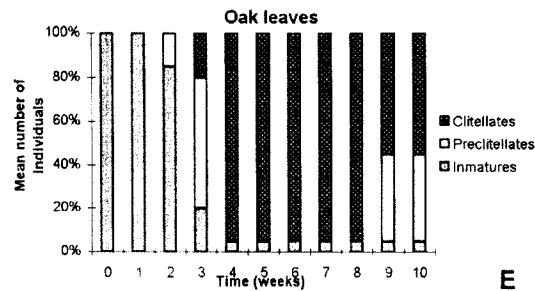
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C



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E

Fig. 2. Percentage of immature, pre-clitellate and clitellate individuals of *E. andrei* fed on pig slurry with the indicated bulking agent in the individual cultures. Note that duration of experiments vary

10 % produced cocoons, in the mixture with pine bark 35 %, in the mixture with pine needles 55 % and none in the mixture with fern.

Cocoon production and viability was significantly higher in the mixtures with straw and pine needles than in the mixtures with pine bark and oak leaves and there was no cocoon production in the mixture with fern. Although, the mixture with pine bark produced a much lower number of cocoons their viability and the mean number of hatchlings per cocoon were high and close to the values obtained for the straw mixture. By contrast, none of the cocoons produced in the mixture with oak leaves hatched (Table 3).

Group cultures

The growth curves of *E. andrei* in the different diet mixtures when cultured in groups of 8 individuals are given in Fig. 3. The worms reached their maximum weights in the mixture with straw (0.62 g) and pine needles (0.60 g), although in the latter less time was needed to reach this maximum value. The smallest size was attained in the mixtures with oak leaves (0.38 g) (Table 4).

After the initial biomass increment a stabilization and, later, weight loss were observed in all mixtures tested. Cocoon biomass does not fully explain the weight loss observed and it seems

Table 4. Growth of *E. andrei* in group cultures (n = 3) in the different diet mixtures

| DIET MIXTURES ¹ | Maximum weight achieved (g) ± S.D. ² | Growth rate (mg day ⁻¹) |
|----------------------------|---|-------------------------------------|
| PS + Straw | 0.62 ± 0.13 ^{a3} | 15.71 ± 0.89 ^a |
| PS + Pine Needles | 0.60 ± 0.09 ^a | 17.86 ± 0.78 ^a |
| PS + Pine Bark | 0.46 ± 0.06 ^b | 13.93 ± 0.65 ^b |
| PS + Fern | 0.50 ± 0.09 ^b | 11 ± 0.66 ^c |
| PS + Oak leaves | 0.38 ± 0.08 ^c | 10.71 ± 0.59 ^c |

¹ PS: Pig slurry

² Standard deviation

³ Treatments followed by the same letters are not significantly different (p (0.05)

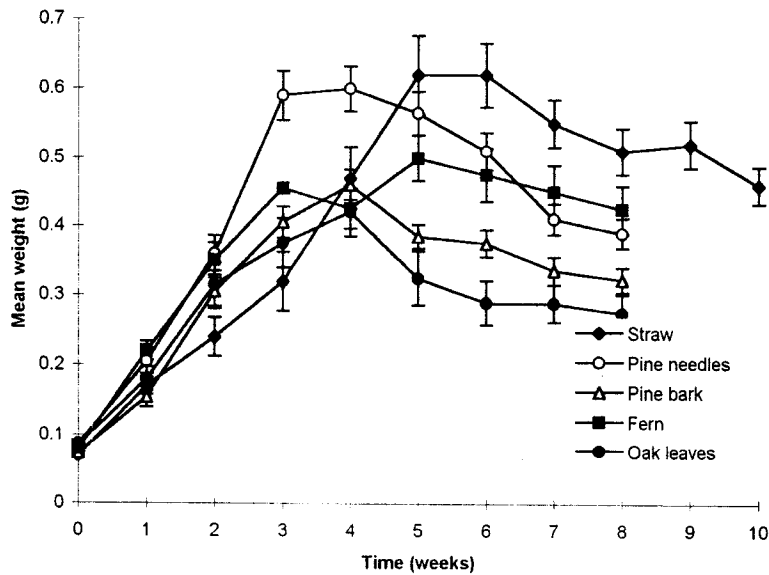
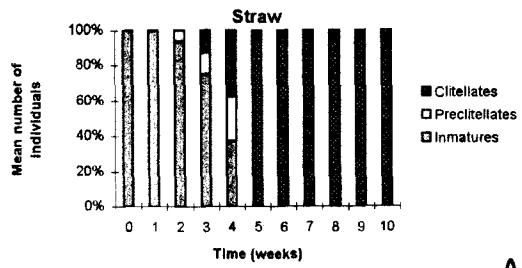
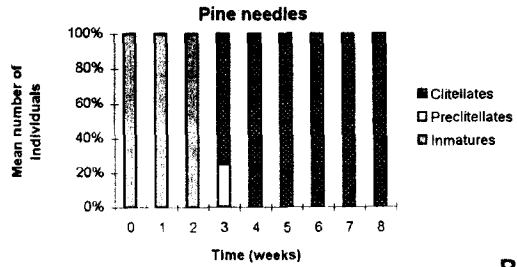


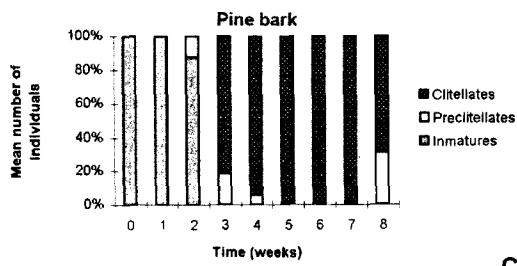
Fig. 3. Growth of *E. andrei* fed on five diet mixtures in the group cultures (n = 3)



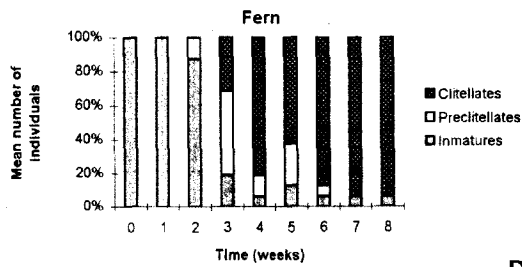
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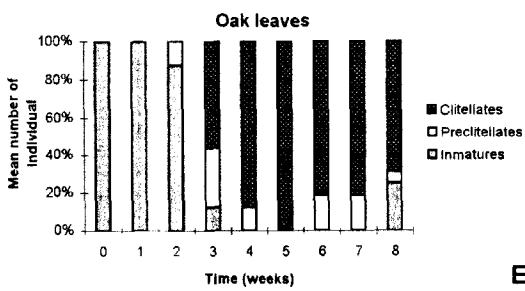
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Fig. 4. Percentage of immature, pre-clitellate and clitellate individuals of *E. andrei* fed on pig slurry with the indicated bulking agent in the group cultures ($n = 3$). Note that duration of experiments vary

more likely that it is a consequence of food limitation during the last weeks rather than to reproductive costs. Besides this diminution, the effect of the diet on growth was basically the same as that observed in the individual cultures, with the exception of the mixture with fern, which showed a higher earthworm growth than did oak leaves mixture (Table 4).

In relation to sexual development and reproduction (Fig. 4), in the mixture of pig slurry and straw the first clitellate individuals appeared after 3 weeks (12.5% of the total population) and the first preclitellate individuals after 2 weeks (6.5%). In the mixtures with pine needles and pine bark, the clitellate worms were first detected after 3 weeks (75% and 81.25%, respectively) and by 4–5 weeks all individuals were mature. In the pine bark mixture the first preclitellate individuals were detected after 2 weeks (5%) and a clitellum regression after 8 weeks, with 31.25% of the individuals reverting to a preclitellate stage. In the mixture with oak leaves, the first clitellate worms appeared after 3 weeks (56.25%) and the first preclitellate individuals after 2 weeks (12.5%); clitellum regression was also observed and by 8 weeks only 69% of the population was clitellate. The population did not fully mature during the 8 weeks of the experiment in the mixture with fern. The first preclitellate individuals appeared after 2 weeks and represented 12.5% of the total.

Table 5 shows a series of parameters related with the sexual development and reproduction of *E. andrei* in the different diet mixtures when this species was bred in groups of 8 individuals. The mean maturation size, the time to reach it and cocoon production were significantly higher ($p < 0.05$) in the mixtures of pig slurry with straw and pine needles than in the other three mixtures. Cocoon viability and the mean number of hatchlings per cocoon was also significantly higher in these two mixtures, followed by the mixtures with pine bark. The mixture with oak showed the lowest values of viability and number of hatchlings but cocoon production was slightly higher than that for the fern mixture (Table 5).

Table 5. Sexual development of *E. andrei* in the group cultures ($n=3$) in the different diet mixtures

| DIET MIXTURES ¹ | Mean maturation size (g) \pm SD ² | Time (days) | Cocoons earthworm ⁻¹ | % Viability | N ^o ind. cocoon ⁻¹ |
|----------------------------|--|-------------|---------------------------------|-------------|--|
| PS + Straw | 0.62 \pm 0.16 ^{a 3} | 35 | 18.1 | 66 | 3.76 |
| PS + Pine Needles | 0.59 \pm 0.08 ^a | 21 | 16.7 | 59 | 3.47 |
| PS + Pine Bark | 0.40 \pm 0.06 ^b | 21 | 5.4 | 29 | 2.75 |
| PS + Fern | 0.42 \pm 0.08 ^b | 28 | 1.6 | 12 | 2 |
| PS + Oak leaves | 0.37 \pm 0.10 ^b | 21 | 2.5 | 4.9 | 1.5 |

¹ PS: Pig slurry

² Standard deviation

³ Same letters correspond to not significant differences ($p < 0.05$)

In view of the results discussed above and those from the analysis of variance it is possible to identify two groups of mixtures based on the mean maturation size and the main reproductive parameters. The first group comprises the mixtures of pig slurry with straw and pine needles which showed the highest maturation sizes, cocoon production rates, cocoon viability and number of hatchlings per cocoon. The second group includes the mixtures with fern, oak leaves and pine bark where the measured parameters were significantly lower. Furthermore, in the mixture with fern not all individuals became fully mature, more time was necessary to reach maturation and less cocoons were laid.

Discussion

The bulking agent had an important effect on the growth and reproduction of *E. andrei*. The maximum growth and reproduction rates were obtained in the mixtures of pig slurry with straw and pine needles both in individual as group cultures, and one possible explanation

for this is the organic matter content and the differing digestibility of each bulking substrate.

The observed differences in the growth of *E. andrei* between the individual and group cultures, when fed on the same diet, should be related to a population density effect. According to this, a similar growth pattern for the same mixture under the two treatments would be anticipated. This is true for the straw and pine needles mixtures where the individual weights and the growth rates were higher. Nevertheless, in the mixture with oak leaves, while the growth of *E. andrei* in the individual cultures can be considered good and in fact, it is comparable to that obtained for the mixtures with pine materials, in the group cultures, the growth rate was the smallest, being much lower than the optimum value. This could be explained as a result of the absence of food competition in the individual cultures.

When food competition increases, the resources per individual decrease, exceeding the importance of the degree of palatability on the growth rate and making this effect only evident in the case of bulking substrates with a higher content in condensed tannins, polyhydric phenols and soluble polyphenols (e.g. oak leaves) (Brown et al. 1963; Edwards & Lofty 1977). King & Heath (1967) reported that the amount of water-soluble polyphenols in litter was inversely proportional to the rate at which it was consumed, and that the litter became much more palatable after a few weeks of weathering. Leaves with high concentrations of condensed tannins are less palatable (Satchell & Lowe 1967; Pugh 1974; Edwards & Heath 1975) as they reduce both the availability of soluble proteins and polysaccharides and the activity of the digestive enzymes (Swain 1979).

Ferns are primitive vascular plants, unable to selectively absorb nutrients and therefore its toxicity in the present study could be due to accumulation of numerous chemical substances in its tissues. Our results showed that the conductivity of this mixture was much higher than in the remaining four. Further studies dealing with the decomposition dynamics of this plant material in natural ecosystems are needed in order to understand how its chemical composition affects organic matter turnover.

Thus, it can be concluded that food requirements play an important role in the growth and reproduction of *E. andrei*, and the observed differences in reproduction proved to be indirectly derived from differences in the growth rate. The weight losses observed during the reproductive period seem more related to food limitation than to reproductive costs and thus, in the case of the mixtures with oak leaves, fern fronds and pine bark, in spite of the smaller (or minimum) reproductive cost there were considerable weight losses. In contrast, in the mixtures with straw and pine needles, the important energetic expenses for reproduction did not lead to the clitellum regression during the experimental period.

Elvira et al. (1996), growing 8 individuals of *E. andrei* on a mixture of fresh cow manure and straw, found greater growth rates ($16.75 \text{ mg day}^{-1}$) and maximum weights (0.59 g) than in cow manure ($12.25 \text{ mg day}^{-1}$ and 0.43 g respectively). This again confirms the nutritional importance of the bulking agent.

Comparing our results with other in literature, it can be easily seen that our high growth rates are similar to those obtained for other pig slurry mixtures, pointing out the high potential of pig wastes for vermicomposting (Edwards 1988).

In relation to clitellum formation, Neuhauser et al. (1980) suggested that food availability and population density determined the time to reach sexual maturity for earthworms, and this was also confirmed in this work. The time needed for clitellum development varied in direct relationship with the nutrient abundance. This difficult the comparisons with previous works using different organic substrates and obtaining longer periods of time for reaching sexual maturity (e.g. Venter & Reinecke 1988; Edwards 1988; Haimi 1990; Elvira et al. 1996).

Our results confirm the general rule, also reported in the literature, establishing a direct relationship between the weight of *E. andrei* and the clitellum development so that the minimum weight for maturation is approximately 0.4 g (Mitchell 1983; Reinecke & Venter 1985; Venter & Reinecke 1988). Additionally, it seems more probable that the maturation size is also dependent on nutritional quality of the substrate and, in general increases with increasing food quality. The fact of the oak litter and fern mixtures, when containing one individual or

8 individuals, showed a very low reproductive rate could indicate a negative effect of the mixing agent on reproduction of *E. andrei*.

As far as cocoon production is concerned, it should be noted that the high values obtained in the cultures with one individual is in disagreement with the previously postulated statement that mating is an obligatory requirement for cocoon production in *E. andrei* (Evans & Guild 1948; Venter & Reinecke 1988). However, Sims & Gerard (1985) describe *E. fetida* as facultatively self-fertilizing.

Although we found that the maximum growth and reproduction rates were achieved in the mixtures with straw and pine needles, in order to better understand the mechanisms by which the bulking agents influence the growth and reproduction of *E. andrei* further studies will be carried out in the future.

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