

The biology and population dynamics of *Eudrilus eugeniae* (Kinberg) (Oligochaeta) in cattle waste solids

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Summary

The growth and reproduction of *Eudrilus eugeniae* (Kinberg) in cattle waste solids was studied by growing groups of 1, 2, 4, 8 or 16 small earthworms in 100 g of waste in small containers in incubators at 15°, 20°, 25° and 30°C. Earthworms were weighed weekly and the numbers of cocoons produced per week assessed. Fecundity, growth, maturation and biomass production were all significantly greater at 25°C than 15°, 20° or 30°. The growth of individual earthworms increased the lower the population density, but the greatest overall earthworm biomass production occurred at the highest population density. The highest ratio of conversion from organic waste to earthworm biomass (dry weight) was 10:1. Cocoon production, the times for cocoons to hatch, the percentage hatch and the number of earthworms hatching per cocoon, at each temperature were recorded. The rates of growth of hatchlings at these temperatures was measured. The greatest number of cocoons per week and the number of hatchlings per cocoon were obtained at 25°C. Cocoons of *E. eugeniae* hatched in only 12 days at 25°C, the earthworms at these temperatures reached sexual maturity in as little as 35 days after hatching and gained weight at maximum rate of 280 mg per week.

Key words: *Eudrilus eugeniae*, earthworms, vermicomposting, cattle waste, temperature, growth rates, reproduction

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Introduction

The disposal of organic wastes from domestic, agricultural and industrial sources is increasingly causing environmental and economic problems. Vermicomposting, the microbial composting of organic wastes through earthworm activity, has proved to be successful in processing sewage sludges and solids from wastewater (Neuhauser et al. 1988; Domínguez et al. 2000), materials from breweries (Butt 1993), paper wastes (Butt 1993; Elvira et al. 1996a), urban food and garden residues, animal wastes (Edwards et al. 1985; Edwards 1988; Elvira et al. 1996b; Domínguez and Edwards 1997), as well as horticultural residues from processed potatoes, dead plants and the brewery and mushroom industries (Edwards 1988). Most of this research has utilized the earthworm species *Eisenia fetida* (Savigny, 1862) and *Eisenia andrei*, Bouché 1972 and the potential of both species as a source of protein, to be used for animal feed, has also been reported (Schulz and Graff 1977; Sabine 1978; Hartenstein 1981; Edwards and Niederer 1988; Edwards 1988). Only one other species, *Lumbricus terrestris* L., has been suggested for this purpose (Fosgate and Babb 1972).

Eudrilus eugeniae is an earthworm species indigenous in Africa but it has been bred extensively in the USA, Canada, Europe and Asia for the fish bait market, where it is commonly called the African nightcrawler. *E. eugeniae* is a large worm that grows extremely rapidly and is reasonably prolific and, under optimum conditions it would be ideal for animal feed protein production; however there has been relatively little work on the biology and ecology of this species (Madge 1969; Neuhauser et al. 1979; Viljoen and Reinecke 1989, 1992; Reinecke et al. 1992; Reinecke and Viljoen 1993).

In this paper we investigated the growth, reproduction and population dynamics of *E. eugeniae* in cattle waste, the influence of temperature and population density on its growth, and summarize its potential for development as a replacement for *E. fetida* or *E. andrei* in organic waste disposal or vermicomposting.

Materials and Methods

The cattle solids used in the experiments contained 18% dry matter after separation from the liquid cattle waste fraction in a commercial manure separator. This is a very suitable moisture content level for growth of species of earthworms that can degrade organic wastes (Edwards 1988).

To assess the effects of temperature on the growth of *E. eugeniae*, four hatchlings, each weighing 0.05 g, were placed in plastic dishes 12.5 cm diam \times 5 cm deep containing 200 g of separated cattle solids (82% moisture content). At regular intervals as the cattle solids were consumed by the earthworms, 100 g were removed and replaced with 100 g fresh solids. Three replicate dishes for each temperature were kept in incubators at 15°, 20°, 25° and 30°C. To compare the growth of *E. eugeniae* with *E. fetida*, at 25°, the optimal temperature for this latter species, four hatchlings of *E. fetida* were also placed in 200 g of separated cattle solids in three similar replicate plastic dishes at each temperature.

For studies of earthworm population and growth in relation to waste availability and earthworm population density rates, batches of 1, 2, 4, 8 and 16 hatchlings, each weighing 0.05 g, were placed in 100 g of separated cattle solids in plastic dishes of the size given above. A complete range of replicated dishes, with the five earthworm population density rates, was kept at each of four temperatures: 15°, 20°, 25° and 30°C. The dishes were examined either weekly or twice weekly. Earthworms were weighed and the cattle waste was examined carefully for co-

coons which were removed and counted. Cocoons removed from the first experiment were weighed and placed individually in small petri dishes with separated cattle solids, kept at the same temperature at those in which the parent earthworms grew and allowed to hatch. At each temperature, the times taken to hatch, the number of hatchlings produced, and the times taken for hatchlings to reach a weight of 0.05 g were recorded.

All results are given as the average value of a single measurement on each of the three replicates. One-way and two-way analysis of variance (ANOVA) and differences among means based on the Tukey HSD mean separation test ($p \leq 0.05$) were used to determine significant differences between growth rates in the different temperature and stocking rate treatments.

Results

Figure 1 and Table 1 summarize the growth rates of *E. eugeniae* at 15°, 20°, 25° and 30°C, when food availability was not a limiting factor. Earthworm growth at the three higher temperatures was similar initially but much slower at 15°C. After 52 days the mean individual earthworm weights were significantly higher at 25°C (2.44 ± 0.12 g) than at 30°C (2.07 ± 0.11 g), 20°C (2.02 ± 0.09 g) and 15°C (0.58 ± 0.09 g). Most earthworms died in cultures at 30°C by 3 months. After 100 days the mean individual weights were significantly higher at 25°C (3.2 ± 0.26 g) than at 20°C (2.89 ± 0.16 g) and at 15°C (1.70 ± 0.14 g). At the end of the experiment, after 142 days, the earthworm weights were much higher at 25°C (4.41 ± 0.31 g) than at 20°C (3.13 ± 0.21 g) and at 15°C (2.32 ± 0.12 g). Throughout its life cycle, *E. eugeniae* grew much more rapidly than *E. fetida*, in similar environmental conditions, attaining a final weight (4.41 ± 0.31 g) that was much greater than that of *E. fetida* (0.61 ± 0.09 g) (data not shown).

There were no differences among the times to reach sexual maturity at 20°C, 25°C and 30°C, but sexual maturation was much slower at 15°C (Table 1). Cocoon production differed markedly in relation to temperature and the number of cocoons produced by *E. eugeniae* was significantly higher at 25°C than at 20°C with relatively few cocoons produced at 15°C and 30°C. The largest numbers of cocoons were produced at 25°C and at this temperature, the hatching percentage and the number of hatchlings produced per mature earthworm were also significantly higher. These same indices of reproduction success were very low in cultures kept at 30°C (Table 2).

Table 1. Effect of temperature on the life cycle of *E. eugeniae* in cattle solids

| Temperature °C | Incubation period (days) (\pm S.E.) | Time to reach 0.05 g (days) (\pm S.E.) | Maturation time (days) from 0.05 g (\pm S.E.) | Total time to sexual maturity (days) | Time from cocoon laying to sexual maturity (days) |
|-------------------|--|---|---|--|--|
| 15 | 28 ± 3^{a1} | 28 ± 2^a | 95 ± 4^a | 123 ± 6^a | 150 ± 7^a |
| 20 | 21 ± 2^a | 13 ± 2^b | 32 ± 3^b | 45 ± 4^b | 66 ± 5^b |
| 25 | 12 ± 1^b | 11 ± 2^{bc} | 24 ± 2^b | 35 ± 3^b | 47 ± 3^c |
| 30 | 14 ± 2^b | 7 ± 1^c | 31 ± 3^b | 39 ± 4^b | 52 ± 4^c |

1 Treatments followed by the same letters are not significantly different ($p \leq 0.05$)

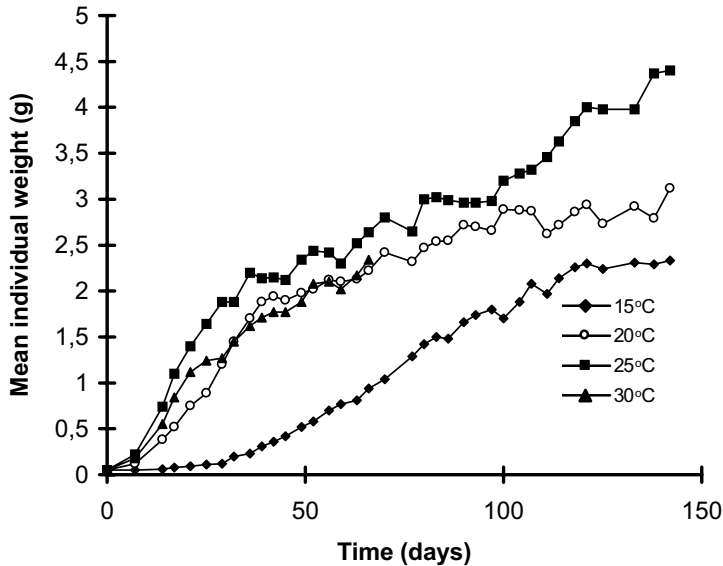


Fig. 1. Growth of *Eudrilus eugeniae* fed on cattle waste solids at four different temperatures (15°C, 20°C, 25°C, and 30°C)

The overall effects of the different temperatures on the life cycle of *E. eugeniae* and the time to sexual maturity were quite marked (Table 1). The incubation time for cocoons was significantly shorter at 25°C and 30°C than at 20°C and 15°, and the most rapid hatchling growth occurred at 30°C with a time to sexual maturity ranging from 35 ± 3 to 123 ± 6 days, overall growth being fastest at 25°C. Clearly, the rate of development was extremely temperature-dependent and although earthworms grew slightly faster at 25°C than at 30° the differences were not statistically significant ($p \leq 0.05$).

The overall earthworm biomass production was significantly greater at 25°C than at any of the other temperatures tested (Table 3, Fig. 2). Earthworm survival in all the experiments was high, with earthworm deaths occurring only in three replicates at 30°C, after 70-80 days, in one replicate at 25°C after 97 days, and in two replicates at 20°C after 125 days, in the rates of growth experiment. Since there was no earthworm mortality in the earthworm population density experiment, it is probable that the deaths in the first experiment were caused by the addition of animal wastes containing small amounts of some toxic element, e.g. a high ammonium content.

Whereas the mean individual weight of earthworms became significantly greater at the lowest population densities, the total biomass production was greatest at the highest earthworm population density and only at 15°C was the growth and time for development of earthworms extended greatly. At 15°C, the lower population densities did not affect the growth rate, although growth was significantly lower at the highest earthworm population densities. At 30°C, the tendency was similar, with a clear inverse relationship between earthworm population density and their growth rates. However, at 20°C the growth rate was significantly greater at the medium earthworm po-

Table 2. Effect of temperature on the reproduction of *E. eugeniae* in cattle solids

| Temperature °C | No of cocoons worm ⁻¹ week ⁻¹ | Mean weight (± S.E. ¹) | % Hatch | N ^o hatchlings per viable cocoon | No hatchlings week ⁻¹ mature adult ⁻¹ |
|-------------------|--|---------------------------------------|------------|---|---|
| 15 | 0.21 ± 0.08 ^{al} | 0.019 ± 0.002 ^a | 75 | 2.13 ± 0.4 ^a | 0.33 ± 0.07 ^a |
| 20 | 1.61 ± 0.27 ^b | 0.024 ± 0.002 ^a | 69 | 2.00 ± 0.26 ^a | 2.22 ± 0.31 ^b |
| 25 | 3.58 ± 0.64 ^c | 0.018 ± 0.002 ^a | 81 | 2.23 ± 0.18 ^a | 6.46 ± 1.52 ^c |
| 30 | 0.75 ± 0.18 ^d | 0.016 ± 0.002 ^a | 19 | 0.14 ± 0.08 ^b | 0.14 ± 0.11 ^a |

¹ Treatments followed by the same letters are not significantly different ($p \leq 0.05$)

Table 3. Effects of temperature on growth and biomass production of *Eudrilus eugeniae* in cattle waste solids

| Temperature °C | N ^o of hatchlings worm ⁻¹ | Mean weight gain (g) | Hatchling biomass production (g) | Mean weight gain of mature worm | Total biomass production per mature worm (g) |
|-------------------|---|-------------------------|--|------------------------------------|--|
| 15 | 0.33 | 0.11 | 0.036 | 0.0017 | 0.0377 |
| 20 | 2.22 | 0.15 | 0.333 | 0.012 | 0.345 |
| 25 | 6.46 | 0.21 | 1.357 | 0.023 | 1.380 |
| 30 | 0.14 | 0.28 | 0.039 | 0.014 | 0.053 |

pulation densities (2 and 4 earthworms per 100 g of waste) and at 25°C the growth rate was significantly higher at a population density of two earthworms per 100 g of waste and then decreased inversely with the higher population densities (Fig. 2). The mean maximum individual weight of the earthworm was significantly greater at the lowest earthworm population densities, independent of the temperature. The times for conversion of separated cattle solids into biomass of earthworm tissues varied with temperature (Fig. 3) and the conversion ratios of cattle solids into earthworm tissues also differed with population density and temperature. Clearly, the highest earthworm population density rates and temperatures resulted in the greatest biomass conversion efficiencies (Table 4), but the adverse effects of earthworm mortality at 30°C would indicate an optimal choice of 25°C for maximum biomass production.

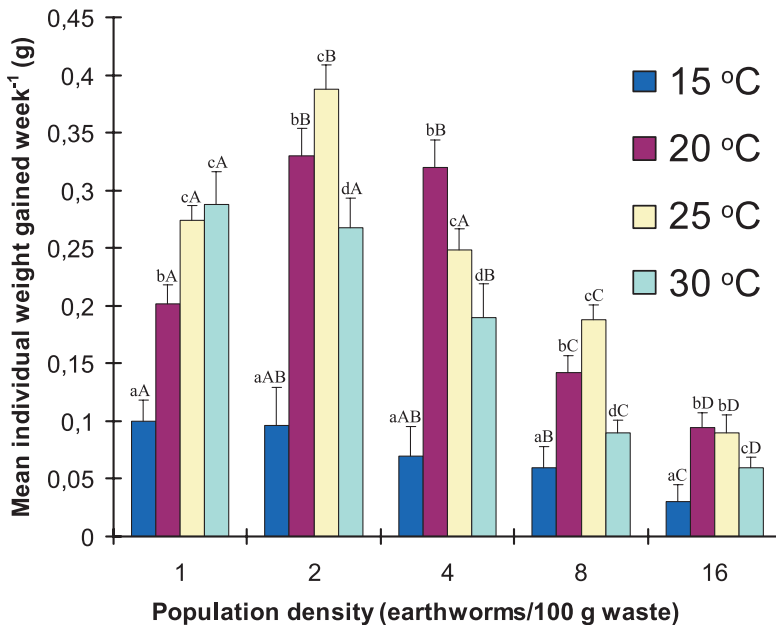


Fig. 2. Influence of temperature (15°C, 20°C, 25°C, and 30°C) and population density on the growth rate of *Eudrilus eugeniae*. The two-way ANOVA model indicated that main effects are significant ($p < 0.001$) and the interaction was also significant ($p < 0.001$). Mean values with different lower case letters indicate significant differences between temperature treatments. Mean values with different capital letters indicate significant differences between population densities

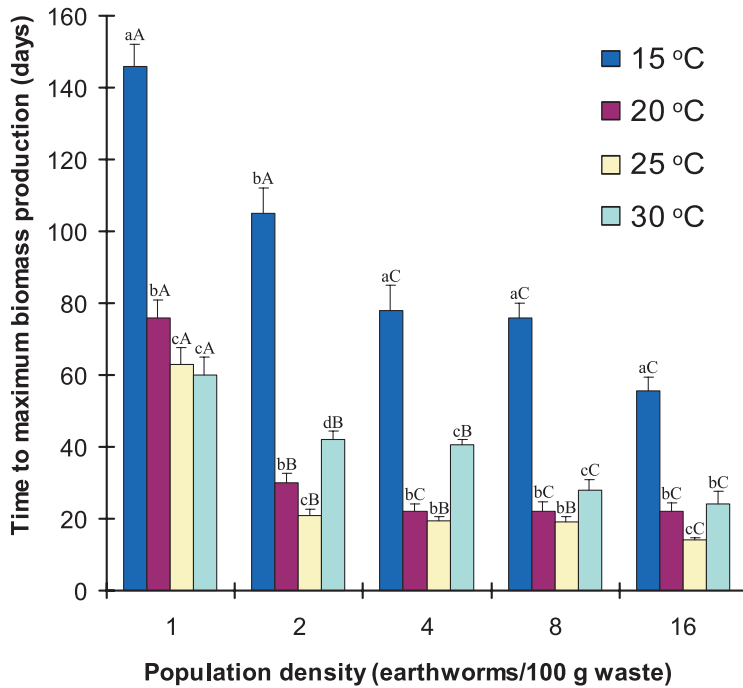


Fig. 3. Influence of temperature (15°C, 20°C, 25°C, and 30°C) and population density on the time to produce maximum biomass of *Eudrilus eugeniae*. The two-way ANOVA model indicated that main effects are significant ($p < 0.001$) and the interaction was also significant ($p < 0.001$). Mean values with different lower case letters indicate significant differences between temperature treatments. Mean values with different capital letters indicate significant differences between population densities

Discussion

Growth

Individuals of *E. eugeniae* grew well in separated cattle solids, with the growth closely following a sigmoid curve of the types described by Bertalanffy (1957). Individual earthworms continued to increase in weight with virtually no mortality for 22 weeks; this differed markedly from data reported by Neuhauser et al. (1979) who reported that maximum weight was attained after 8 weeks, and that thereafter a loss of weight and considerable mortality occurred, with only about 40% surviving for as long as 16 weeks. Reinecke et al. 1992 reported continuous growth and maximum weight up to 21 weeks at 25°C.

Eudrilus eugeniae increased in total biomass much more rapidly than *E. fetida*, a species which grows relatively well in most organic wastes. Moreover, *E. eugeniae* reached sexual maturity in as little as five weeks compared with *E. fetida* which took 6-8 weeks to produce its first cocoon (Edwards 1988). This is a more rapid develop-

Table 4. Conversion ratios (\pm SE of the mean) of separated cattle solids to earthworm tissue (based on 18% D. M.) and significance of two-way ANOVA model effects

| Temperature °C | CONVERSION RATIO | | | |
|----------------|--------------------------------|--------------------------------|-------------------------------|-------------------------------|
| | 160 | 80 | 40 | 10 |
| 15 | 7.9 \pm 0.17 ^{aA†‡} | 8.4 \pm 0.18 ^{aA} | 7.7 \pm 0.24 ^{aAB} | 6.7 \pm 0.12 ^{aB} |
| 20 | 8.9 \pm 0.25 ^{abA} | 9.4 \pm 0.23 ^{abAB} | 8.5 \pm 0.12 ^{bAB} | 7.7 \pm 0.21 ^{abB} |
| 25 | 9.2 \pm 0.2 ^{bcA} | 9.96 \pm 0.35 ^{ba} | 9.1 \pm 0.18 ^{bcA} | 7.2 \pm 0.17 ^{abB} |
| 30 | 10.1 \pm 0.31 ^{cA} | 10.0 \pm 0.12 ^{ba} | 9.6 \pm 0.17 ^{cA} | 7.7 \pm 0.24 ^{bB} |
| | | | | 5.0 \pm 0.23 ^{aC} |
| | | | | 5.3 \pm 0.24 ^{aC} |
| | | | | 5.6 \pm 0.26 ^{aC} |
| | | | | 5.4 \pm 0.37 ^{aC} |

† Means in the same column followed by the same letter (lower case) are not significantly different at $P \leq 0.05$ (Tukey HSD test)

‡ Means in the same row followed by the same letter (capital) are not significantly different at $P \leq 0.05$ (Tukey HSD test)

temperature $p < 0.001$

stocking rate $p < 0.001$

temperature * stocking rate $p < 0.001$

ment than for any species of earthworm that has been reported to date and such growth rates make a very fast rate of population multiplication possible. The maximum weight gain of *E. eugeniae* was 280 mg per week, compared to the highest weight gains reported for *E. fetida* of 60-80 mg per week (Graff 1974; Watanabe and Tsukamoto 1976), 80-90 mg per week for *E. andrei* (Elvira et al. 1996a), 55-60 mg per week for *Lumbricus rubellus* (Elvira et al. 1996b). The rate of weight gains for other species that have been studied has been much less than either of these species. Reinecke et al. (1992) reported a maximum weight gain for *E. eugeniae* of 150 mg per week, at 25°C, significantly less than our results. Thus, based on our laboratory data *E. eugeniae* would be a good candidate species for earthworm protein production for animal feed.

Reproduction

The maximum rate of cocoon production by *E. eugeniae* was 3.6 cocoons per week at 25°C. This is a similar rate to that of *E. fetida*, which has been reported to produce 3.8 cocoons per week, but *E. fetida* reproduced most rapidly at 20°C (Edwards 1988). The maximum rate of cocoon production in our study was much faster than the 0.7 cocoons per week that were reported for *E. eugeniae* by Neuhauser et al. (1979), but less than for *Perionyx excavatus* which has been reported to produce up to 19.5 cocoons per week (Edwards et al. 1988). In another study, Neuhauser et al. (1988) reported a maximum rate of cocoon production by *E. eugeniae* of 3.8 per week at 25°C. Reinecke et al. (1992) reported a rate of cocoon production by *E. eugeniae* of 3.22 cocoons per earthworm per week, at 25°C, and Knieriemen (1985) recorded a mean rate of cocoon production of three cocoons per earthworm per week. Rodriguez et al. (1986) calculated that seven cocoons were produced per earthworm per week at 30°C.

The percentage earthworm hatch of 81% at 25°C was high, and although the number of hatchlings per cocoon (more than two) was comparatively low, the 6.5 hatchlings produced per week at 25°C, compared well with the productive by *E. fetida* of 10.4 cocoons per week at 20°C (Edwards 1988) and *P. excavatus* of 5.2 cocoons per week at 20°C (Edwards et al. 1998). The hatching success of *E. eugeniae* that we found was very similar to the 78% reported by Reinecke et al. (1992) who observed that the number of hatchlings per cocoon were usually more than two. Neuhauser et al. (1988) also reported that *E. eugeniae* produced two live earthworms per cocoon.

Life Cycle

The shortest time from producing cocoons to sexual maturity, for *E. eugeniae* was 47 ± 3 days. The time from hatching to sexual maturity was 35 ± 3 days, which agrees with the results of Reinecke et al. (1992) who reported that *E. eugeniae* started producing cocoons after 46 days. This is a shorter time than the 6-8 weeks previously quoted for *E. fetida* (Hartenstein et al. 1979), and the 51 days reported for *E. eugeniae* (Mba 1984). It is very much less than the 29-42 weeks quoted for the soil-dwelling species *Allolobophora chlorotica*, (Satchell 1967) 10-14 months for *Millsonia anomala* (Lavelle 1971) and 12-24 months for *Bimastos zeteki* (Murchie 1960). Earthworm survival was excellent, with little mortality occurring at temperatures other than 30°C. This agrees with the results of Reinecke et al. (1992), but contrasts with the data of Neuhauser et al. (1979) who suggested that few individuals survived longer than 12 weeks, regardless of population density. All the evidence from our larger scale cultures is that *E. eugeniae* is a long-lived earthworm.

Effects of Temperature

Eudrilus eugeniae is a tropical eudrilid earthworm from Africa. Thus, it is not surprising that its fecundity, growth, maturation and biomass production were all greatest at 25°C, which is higher than the optimal temperatures quoted for other species that inhabit temperate habitats. Another tropical species, *P. excavatus* was most productive at 25°C (Edwards et al. 1998). Earthworms coming from temperate regions cannot tolerate such high temperatures, although *E. fetida* is most productive at 20°C if both reproduction and rates of growth are taken in account (Edwards 1988).

Surprisingly, some individuals of *E. eugeniae* died at 30°C, a temperature which *E. fetida* can withstand, although we have evidence from studies that *E. fetida* can adapt to even higher temperatures. Most temperate species of earthworms have optimal developmental temperatures between 12°C and 20°C (Graff 1953). Viljoen and Reinecke (1992) also reported high mortality of *E. eugeniae* at temperatures above 30°C after 20 days and Loehr et al. (1985) also found this species could not withstand temperatures above 30°C and that the best survival was at 20°C. Madge (1969) reported that *E. eugeniae* could survive for one hour at 37°C and one day at 34°C. Rates of growth of *E. eugeniae* were very poor at 15°C compared with those of *E. fetida* which grew almost as fast at 15°C as at 20°C or 25°C, although much slower at temperatures above 25°C. Maximum biomass production by *E. eugeniae* occurred at 25°C and 30°C. Knieriemen (1985) reported that *E. eugeniae* could stay alive at 15°C but it did not grow. Viljoen and Reinecke (1992) reported the highest rates of growth of *E. eugeniae* at 29°C whereas Loehr et al. (1985) determined 25°C as the optimal temperature for the growth of this species.

Population Densities

The greater the earthworm population density in a culture the slower was the growth of individual earthworms at any particular earthworm population density, up to a maximum of 16 earthworms in 100 g of separated solids. At earthworm population densities much above this, such as 32 earthworms in 10 g of solids, earthworms tended to lose weight or even die. Clearly the maximum population density, in terms of earthworm weight per unit weight of waste, lies within these limits. At higher population densities, although the maximum biomass was greater, *E. eugeniae* took much longer to reach this peak weight. Similar results were obtained for *E. fetida* by Neuhauser et al. (1980) and by Reinecke and Viljoen (1990), for *E. andrei* (Dominguez and Edwards 1997) and also in our laboratory for *Perionyx excavatus* (Edwards et al. 1998). Reinecke and Viljoen (1993) reported similar results on the influence of earthworm density on the growth and reproduction of *E. eugeniae*. The limit on populations of all earthworm species, in organic wastes, seems to be related to earthworm biomass per unit of waste rather than to overall numbers. The maximum overall productivity probably depends upon the total biomass and nutritive value of microorganisms growing on the waste (Edwards and Fletcher 1988).

Conversion ratios

The earthworm biomass production was very temperature-dependent, maximum production being attained at the two highest population densities and highest temperatures. However, temperature exerted a much smaller effect on the waste to earthworm

conversion efficiency than population density. The greatest organic waste to earthworm conversion ratio, of 10:1 (10%), was recorded at the most dense earthworm populations and was greater than the 5:1 recorded for *E. fetida* in potato waste (Edwards 1983), and in activated sewage sludge (6:1) (Hartenstein 1983), but not much different from the 10:1 for *E. fetida* in pig solids (Edwards 1988; Dominguez and Edwards 1997).

The conclusions of our investigations are that *Eudrilus eugeniae* is a fast-growing and productive earthworm in animal waste, that is ideally suited as a source of animal feed protein as well as for rapid organic waste conversion. It is more productive in terms of rates of growth than other species and would seem to be a suitable candidate for vermicomposting systems, in regions where the optimal temperature of 25°C is both feasible and economic. Although the large size of *E. eugeniae* makes it much easier to handle and harvest, than commonly-used species such *E. fetida* and *P. excavatus*, it is much more sensitive to disturbance and handling and may occasionally migrate from breeding beds. Since it has been grown commercially for fish bait for a long time in the U.S. shows that is comparatively easy to rear. It is probably one of the two preferred species together with *P. excavatus* for vermiculture and vermicomposting in tropical climates.

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