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Growth and reproduction of *Perionyx excavatus* (Perr.) (Megascolecidae) as factors in organic waste management

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Abstract The life cycle of *Perionyx excavatus* has been studied and the potential of this epigeic earthworm species for breaking down and processing organic wastes is well known. Understanding of its optimal environmental requirements is required in order to optimize and accelerate the vermicomposting process. The rates of growth and reproduction of *P. excavatus*, on a variety of organic wastes, were evaluated in these experiments. The time of maturation and the rates of growth of this species, under various population density pressures and temperatures between 15 °C and 30 °C, were also assessed. Increasing temperatures up to 30 °C accelerated the growth of earthworms and lessened the time to sexual maturity. However, the highest rates of reproduction occurred at 25 °C both in cattle solids and sewage sludge. The mean time to egg hatching decreased and the degree of hatching success increased with increasing temperature. Earthworms grew at similar rates in cattle solids, pig solids and aerobically digested sewage sludge, but the earthworms did not grow well in horse solids and grew only poorly in turkey wastes.

The maximum individual growth rates as a function of earthworm population and the maximum earthworm weights as a function of time with a constant food supply at four different temperatures were assessed.

Key words *Perionyx excavatus* · Earthworms · Organic waste · Vermicomposting

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Introduction

The most effective use of earthworms in organic waste management requires a detailed understanding of the biology of all potentially useful species (Edwards and Bohlen 1996). *Perionyx excavatus* (Perr.) is an earthworm found commonly over a large area of tropical Asia (Stephenson 1930; Gates 1972) although it has been transported to Europe and North America. This is an epigeic species which lives in organic wastes, and high moisture contents and adequate amounts of suitable organic material are required for populations to become fully established and for them to process organic wastes efficiently. The life cycle and the potential of this species for breaking down organic wastes has been documented by various authors under controlled conditions (Kale et al. 1982; Reinecke and Hallatt 1989, Hallatt et al. 1990; Reinecke et al. 1992; Hallatt et al. 1992).

This paper evaluates the detailed growth and reproduction of *P. excavatus* in a range of organic wastes and relates this to existing data on the time to maturation and the rates of growth of *P. excavatus* under various population density pressures and at a range of temperatures. These critical data should enable *P. excavatus* to be used in an optimal manner in organic waste management and in the production of vermicomposts.

Materials and methods

The cultures of the earthworm *P. excavatus* used in these experiments were obtained from a breeding source in Manila (Philippines). Maturation rates, reproductive potential, comparative growth and response to population pressure of *P. excavatus* in five organic animal wastes were studied in relation to temperature and food availability.

Maturation rate

Young *P. excavatus*, <5 mg fresh weight, were placed into a mixture 30:70 (wet weight) of sphagnum peat moss (69% moisture

content) and separated cattle solids (80% moisture content). Four earthworms were placed into each 9 cm × 6 cm crystallizing dish and successive thin layers of the organic waste were added, as soon as the existing top layer had been broken down. Two replicate crystallizing dishes were kept at each of a range of temperatures of 15, 20, 25 and 30 °C and the earthworms were examined weekly for clitellum development.

Reproduction

The reproductive rate of *P. excavatus* was assessed, using two different waste sources: composted cattle solids and aerobically digested sewage sludge, at four different temperatures: 15, 20, 25 and 30 °C. Two earthworms that had been bred in cattle solids were placed in each 6 cm × 9 cm crystallizing dish, together with 12 g of cattle solids. Two such replicate dishes were kept at each temperature. The crystallizing dishes were examined every 3–5 days for cocoons and new batches of cattle solids were replaced after each examination, so that food was not limited. Four earthworms that had been grown in aerobically digested sludge (100 g/dish, 13% solids) were placed in 12 g of sludge into glass dishes 6 cm high by 9 cm in diameter. Four replicate dishes were kept at each of the temperatures: 15, 20, 25 and 30 °C. Each dish was examined biweekly to assess numbers of cocoons produced and fresh aerobically digested sludge was replaced after each examination. All of the cocoons in the cattle solids were weighed collectively and divided by their numbers to obtain an average cocoon weight. They were then incubated individually and examined daily to determine the percentage hatch of the cocoons.

Growth in various food sources

Batches of 100 g wet weight of each of five organic wastes were put into 9 cm × 4 cm crystallizing dishes. The wastes studied were: separated cattle solids (80% moisture), separated pig solids (80% moisture), horse solids (82% moisture), turkey waste (shavings-based) (73% moisture) and aerobically digested sludge (110 g, 87% moisture). Two young *P. excavatus*, <20 mg fresh weight, were placed in each dish. Dishes were incubated at 15, 20, 25 and 30 °C, with two replicates dishes for each waste, at each temperature. The earthworms were weighed every 5 days and no new waste was added after each weighing.

Population density and food rationing

Five different population densities of *P. excavatus*, <30 mg, were kept in 6 cm in diameter × 4 cm deep crystallizing dishes, containing 50 g fresh weight of cattle solids (80% moisture). Young earthworms, weighing about 10 mg fresh weight, were also kept in 55 g fresh weight (87% moisture) of aerobically digested sludge, in petri dishes 20 cm high × 10 cm in diameter. There were two replicates for the cattle solids test and four replicates for the aerobically digested sludge tests. Dishes of each type of waste were inoculated with either 1, 2, 4, 8 or 16 earthworms and incubated at either 15, 20, 25 and 30 °C. There were two replicates for each type of waste and different temperature. The earthworms were weighed at 3–5 day intervals and the waste was moistened as necessary to maintain its initial moisture content. The weights of surviving earthworms were recorded on each date and averaged to provide a figure for each population density.

Results and discussion

Maturation rate

Table 1 summarizes the time taken to clitellum formation at the four experimental temperatures. Higher

Table 1 Maturation rate of *Perionyx excavatus* in cattle solids

Temperature °C	% of Clitellate earthworms				
	Week 2	Week 4	Week 5	Week 6	Week 7
15	No clitellate worms after 10 weeks				
20	0	0	12	75	100
25	0	25	50	88	100
30	0	50	63	100	100

temperatures, up to 30 °C, clearly accelerated the rate of development of sexual maturity, but none of the earthworms kept at 15 °C developed a clitellum over the entire study period of 10 weeks. The earthworms that were kept at 15 °C weighed approximately 150 mg fresh weight at the end of the experiment, whereas the earthworms at the other temperatures ranged in weight from 250 mg to 400 mg. This contrasts with results of Reinecke et al. (1992) who reported that higher temperatures slowed down the maturation rate, although they found the most rapid maturation rates at 25 °C, as well as at a fluctuating temperature between 25 °C and 37 °C. The time from hatching to sexual maturity was longer than for *E. eugeniae* (less than 35 days) (Edwards et al., in press) and longer than the 6–8 weeks quoted for *E. fetida* (Hartenstein et al. 1979) or the 5–6 weeks quoted for *E. andrei* (Elvira et al. 1996).

Reproduction

Table 2 summarizes the reproduction rates of *P. excavatus* in cattle solids and aerobically digested sludge. No reproduction occurred in cattle solids at 15 °C, although a few cocoons were produced at 15 °C in aerobically digested sludge. The survival of the earthworms kept at 30 °C in digested sludge was low, indicating that these conditions were stressful. The mean number of cocoons produced per worm per day of 0.82 at 25 °C was 64% greater than the 0.29 cocoons produced per day at 30 °C in the cattle solids and, significantly, 91.71% greater than at 30 °C in the digested sewage sludge. The important difference between rates of cocoon production in the two organic wastes must be related to the quality of the waste, which was one of the factors determining the time taken to reach sexual maturation and for the onset of reproduction (Neuhauser et al. 1979; Edwards 1988). Kale et al. (1982) calculated a mean production of 0.15 cocoons per worm per day at 30 °C over 210 days, Knieriemen (1985) reported a mean production of 0.41 cocoons per worm per day at 27 °C and Reinecke et al. (1992) reported that a mean of 0.33 cocoons were produced per earthworm per day at 25 °C and 0.12 cocoons per earthworm per day at 25–37 °C.

The cocoons produced by *P. excavatus* in cattle solids ranged in weight from 2.5 to 2.6 mg per cocoon, whereas cocoons from earthworms in the aerobically

Table 2 Effect of temperature on cocoon production in *P. excavatus*

Temperature °C	Cattle solids				Digested sewage sludge			
	15	20	25	30	15	20	25	30
Mean cocoon production ¹	0	0.43	0.82	0.29	0.08	0.81	1.69	0.14
Maximum cocoon production ¹	0	1.66	2.78	0.75	0.08	1.37	3.48	0.21

¹ per earthworm per day

digested sludge ranged in weight from 2.0 to 2.2 mg per cocoon. The cocoons produced by *P. excavatus* were small compared to those produced by most other species of earthworm; this made them more difficult to find in the cattle solids than in the sludge.

The mean time to hatching for *P. excavatus* decreased with increasing temperature, with times of 21.7 days at 20°C, 19.6 days at 25°C, and 16.1 days at 30°C, which corresponds well with the results of Reinecke et al. (1992) who reported mean times to hatching of 17.8 days at 25°C and 15.3 days at 25–37°C and also with those reported by Watanabe and Tsukamoto (1976), who concluded that temperatures above the optimum for growth decreased the incubation period. The percentage of cocoons that hatched also increased with increasing temperature, with 67% hatching at 20°C, 91% hatching at 25°C and 96% hatching at 30°C. Reinecke et al. (1992) reported hatching successes of 72% and 48% at 25°C and 25–37°C respectively. When cocoons hatched, they usually produced only one earthworm per cocoon with a very small percentage of cocoons, <5%, producing two earthworms per cocoon. These results differ from those of Reinecke et al. (1992) who reported that 14% of the cocoons produced two hatchlings and 2% produced three.

Growth in various food sources

To compare the growth rates of *P. excavatus* in a limited range of six different organic wastes, it was necessary to find a good comparative index. To obtain this, the growth rate (mg weight gained day⁻¹ earthworm⁻¹) was calculated and the maximum weight achieved was divided by the number of days required to reach this maximum weight (Table 3). The two kinds of turkey waste that were tested in our experiments appeared to support the least growth of *P. excavatus* and the horse solids were only marginally better than turkey waste in influencing earthworm growth. Earthworms grew at relatively similar rates in cattle solids, pig solids and aerobically digested sludge. Table 4 shows some chemical characteristics of different animal wastes used as food sources. Turkey solids show high ammonia content and conductivity values which could be detrimental to earthworm survival and growth. Horse solids show the lowest nutritional value of all the organic wastes.

Table 3 Growth of *P. excavatus* in organic wastes reared at different temperatures

	Temperature °C	Growth rate (mg wt. worm ⁻¹ ·day ⁻¹)	Maximum weight achieved (mg)
Cattle solids	15	4.0	230
	20	7.3	297
	25	7.2	292
	30	7.6	308
Pig solids	15	5.0	448
	20	6.8	443
	25	4.6	312
	30	5.7	345
Horse solids	15	0.5	35
	20	1.8	70
	25	1.5	70
	30	2.6	105
Turkey waste, shavings base	15	0.1	20
	20	0.6	43
	25	0.6	42
	30	0.6	42
Turkey waste, straw base	15	0.6	33
	20	0.4	28
	25	0.7	42
	30	0.8	48
Aerobic sludge	15	0.8	341
	20	5.7	401
	25	5.9	351
	30	0.9	449

To appreciate how the amount of solids available might affect the rate of weight gain of the earthworms, it was necessary to compare the waste in which the earthworms were reared on a dry weight basis. Table 5 lists the weight gains for *P. excavatus* (wet weight) for each waste (dry weight), to provide an estimate of mg wet weight of earthworms produced per g dry weight of food source. Temperature affected this comparison very little, because time was not a limiting factor in growth. The organic waste in which earthworms grew fastest was aerobically digested sludge, with almost twice the weight increase of earthworms grown in cattle and pig solids. The earthworms did not grow well in horse solids and grew even more poorly in both of the turkey wastes.

None of the earthworms kept at 15°C, in any of the waste materials tested, became clitellate over the entire

Table 4 Some analytical characteristics of the different animal wastes

	Cattle solids	Pig solids	Horse solids	Turkey waste	Sewage sludge
Moisture content (%)	80.40	86.5	78.30	82.30	85.70
Organic matter (%)	85.06	83.9	74.15	78.12	79.54
Total C (%)	41.41	37.9	33.55	35.20	37.92
Total N (%)	2.20	2.60	1.80	2.60	4.50
NH ₄ ⁺ -N (mg g ⁻¹)	3.59	5.10	2.10	6.32	2.19
pH	7.4	7.7	7.0	8.4	6.6
Conductivity (mS cm ⁻¹)	1.4	2.1	0.60	5.12	1.6

Table 5 The maximum wet weight gained by *P. excavatus* per gram dry weight of waste

Temperature °C	Maximum fresh weight (mg·g ⁻¹)					
	Cattle solids	Pig solids	Horse solids	Turkey straw based	Turkey shavings based	Aerobic sludge
15	23.0	42.6	3.9	1.5	2.5	52.4
20	29.8	42.1	7.8	4.3	2.1	61.6
25	29.3	29.8	7.8	4.3	3.3	54.0
30	30.8	32.9	11.7	4.3	3.7	69.0

study period of 10 weeks. The earthworms kept at 20 °C, 25 °C and 30 °C grew sufficiently to become clitellate, but produced very few cocoons, probably because the food potential of the waste was exhausted by the end of the experiment. Earthworm mortality was very low in sludge, cattle and pig wastes. The earthworm mortality rate was so high in horse and turkey wastes that these experiments were terminated early.

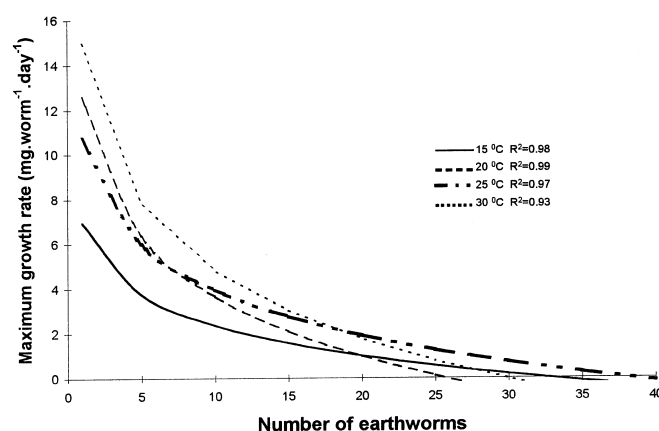
Our results contrast with those of Reinecke et al. (1992) who reported that higher temperatures had a negative influence on the rates of growth of *P. excavatus*. However, their experiments were at temperatures higher or fluctuating (25–37 °C). Reinecke and Hallatt (1989) reported a growth rate of 3.48 mg over the first 30 days at 25 °C in cattle solids, a much slower growth rate than we obtained after 60 days. The rates of increase in growth in their experiments then slowed down slightly, until after 250 days, when a mean biomass per worm of 541.6 ± 27.7 mg fresh weight was attained. After 60 days they reported a mean biomass per worm of 221.1 mg, a value a little less than that of our earthworms under similar conditions. Loehr et al. (1984), using sewage sludge as food source, reported that *P. excavatus* reached its maximum biomass after approximately 100 days at 25 °C. In our study we observed the maximum earthworm biomass at 30 °C after 60 days.

Population density and food rationing

The rates of growth of five different populations of *P. excavatus*, in a surplus of organic wastes at four different temperatures, allowed several different comparisons to be made. It was possible to calculate the maximum growth rates per earthworm, as a function of

earthworm population, and the maximum earthworm weight as a function of time. Figures 1 and 2 summarize the relationships between the maximum earthworm growth rate and the mean maximum weights with population densities at four different temperatures, in cattle solids. There was a positive correlation between the mean maximum earthworm weight and the population density. The mean maximum earthworm growth rate and the population density were negatively correlated.

The maximum growth rates of individual earthworms, in the five different earthworm populations were calculated by taking the maximum weight gain achieved by each population, subtracting the initial earthworm weight, and dividing the weight increase, by the number of days needed to reach the maximum weight, multiplied by the number of earthworms in the population. The maximum growth rate, expressed as

**Fig. 1** The relation between maximum growth rate and population density at four temperatures in cattle solids

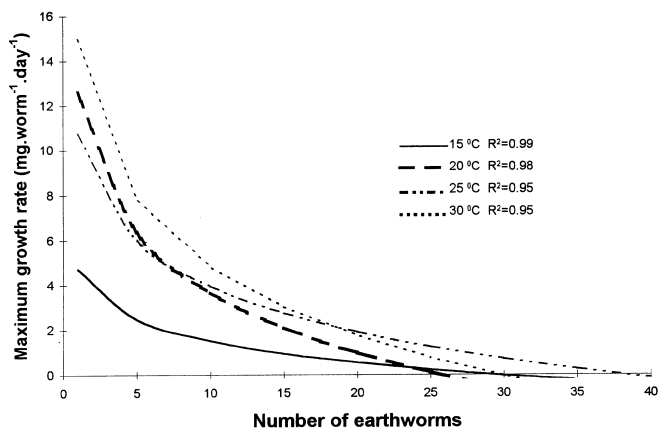


Fig. 2 The relation between mean maximum weight and population density at four temperatures in cattle solids

mg weight gained/worm/day, was fitted to a logarithmic equation: maximum growth rate = $b + m \ln(\text{number of earthworms})$. The curves calculated for growth in cattle solids (Fig. 3) and sludge (Fig. 4) were very similar.

The temperature which corresponded with the lowest maximum earthworm growth rates in both wastes was 15°C, with the highest growth rates, in both wastes, being at 30°C. The earthworm growth rates at 20°C and 25°C were similar for both organic wastes.

One possible interpretation of these data might be based on the maximum weight of *P. excavatus* that could be obtained in response to waste availability in these experiments. A maximum weight achieved by each of the earthworm populations at the four temperatures tested was calculated. This weight was subtracted from the initial average starting weight of each earthworm population. Using the five earthworm population densities tested for each temperature, and the maximum weight achieved by each population, a quadratic equation, expressed as $\text{weight} = a_0 + a_1(\text{population}) - a_2(\text{population})^2$ was obtained for each temperature. The data on earthworm growth in cattle solids (Fig. 5) and aerobically digested sludge (Fig. 6) enabled us to calculate the number of earthworms at each temperature that would give the maximum earthworm bio-

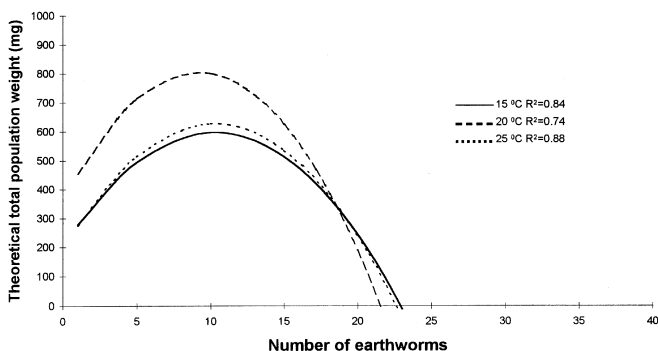


Fig. 3 The maximum growth rates of *P. excavatus* at different temperatures in cattle solids as a function of earthworm number

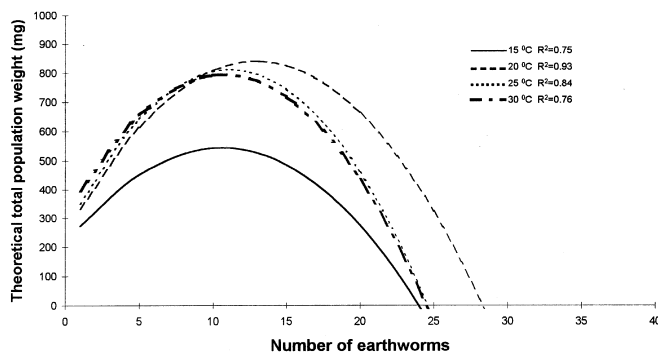


Fig. 4 The maximum growth rates of *P. excavatus* at different temperatures in sewage sludge as a function of earthworm number

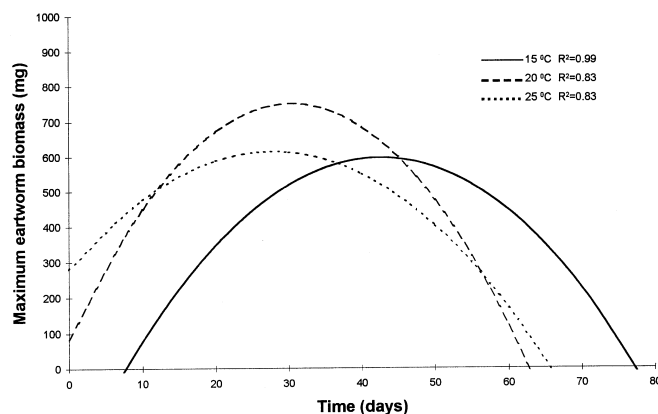


Fig. 5 The theoretical total weight reached by *P. excavatus* at different temperatures in cattle solids as a function of population

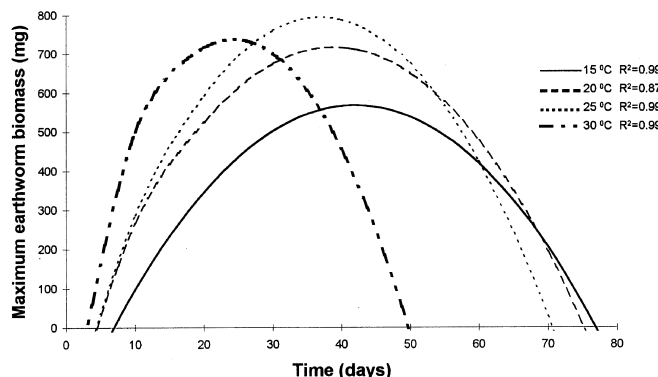


Fig. 6 The theoretical total weight reached by *P. excavatus* at different temperatures in sewage sludge as a function of population

mass in the quantity of waste available. The number of earthworms produced at each temperature was calculated by taking the first derivative of the quadratic equations. This is the point where the slope of the lines are equal to zero and hence the inflection point of the quadratic equation. The optimum numbers of *P. exca-*

vatus to obtain the maximum biomass at each of the four experimental temperatures are summarized in Table 6.

To obtain an estimate of the maximum biomass obtained by *P. excavatus*, as a function of time, it was necessary to use the growth data, for the five different population densities of earthworms at the four different temperatures, in the two organic wastes, cattle manure and aerobically digested sludge. The weights for each of the earthworm populations at days 10, 20, 30, 40 and 50 were subtracted from the original starting weight of the earthworms. For each of the time periods and temperatures examined, a relationship was calculated between the population densities and the weights of the earthworm populations. This relationship appeared as a quadratic equation fitted to the data in the form: $\text{weight} = a_0 + a_1 (\text{population}) - a_2 (\text{population})^2$.

The optimum populations of earthworms, for the amounts of cattle solids and sludge used, were 10 and 11 respectively (Table 6). The quadratic equation for each time period and temperature produced similar final weight calculations, if the earthworm population were within two earthworms of the optimum population number. For example, using the quadratic equations for 15°C and 20 days, with sludge as the food source, gave a mean weight, with a population of 11, of 365 mg. If there were nine earthworms in the population, the weight achieved was 352 mg, while 13 earthworms produced a maximum weight of 353 mg. To progress from the optimal number of earthworms, the maximum weight obtained with a population of 7 earthworms was 317 mg, and the maximum weight obtained with a population of 15 earthworms was 322 mg. Therefore, as long as the earthworm population was within two individuals of the optimal population for the waste

Table 6 Optimum numbers of individuals of *P. excavatus* needed to achieve the maximum biomass in 10 g dry weight of cattle solids and 7.5 g dry weight of aerobically digested sewage sludge

Temperature °C	Number of individuals of <i>P. excavatus</i>	
	Cattle solids	Sewage sludge
15	10	10
20	9	13
25	10	11
30	11	11

available, the maximum weights obtained were close to the optimum.

The equation calculated for each of the four temperatures and the five growth periods, i.e., a total of 20 equations for each food source, was then solved in terms of the earthworm biomass that could be achieved at each time and temperature. This was done by substituting a population of ten earthworms for the cattle waste and eleven earthworms for the aerobically digested sludge. For each temperature, a weighting could be obtained for the five time periods examined. A relationship was developed between the maximum earthworm biomass and time. This relationship was a quadratic equation fitted to the data in the form: $\text{maximum earthworm biomass} = a_0 + a_1 (\text{time}) - a_2 (\text{time})^2$.

The relationship for the maximum earthworm biomass, as a function of time, is given for cattle solids (Figure 7) and aerobically digested sludge (Figure 8). These curves illustrate how *P. excavatus* could grow at different temperatures over time, in a limited amount of waste. Such data enable the optimal temperatures

Fig. 7 The maximum biomass of a population of 10 *P. excavatus* as a function of time at four different temperatures in cattle solids

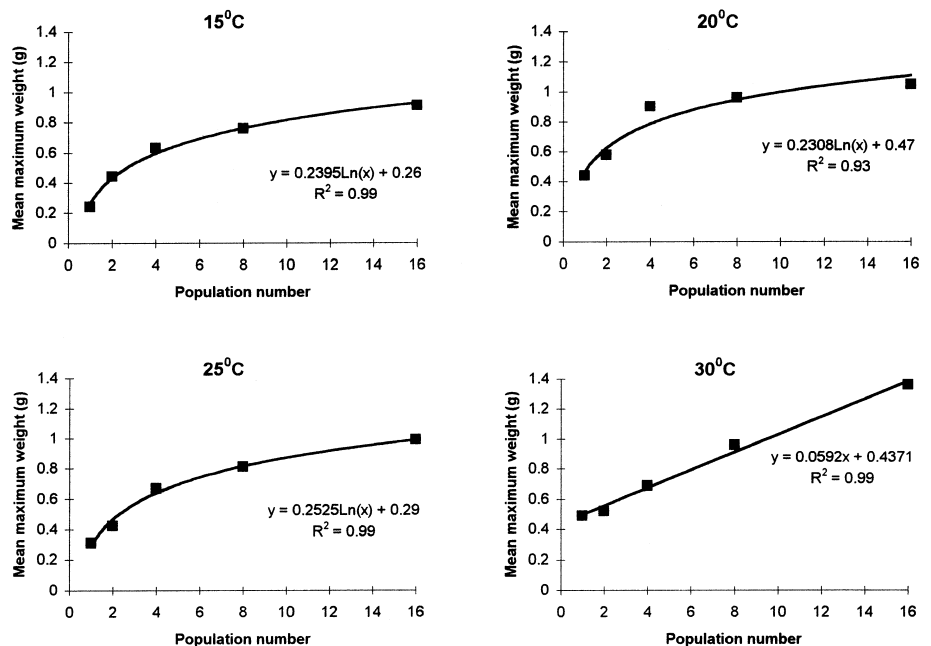
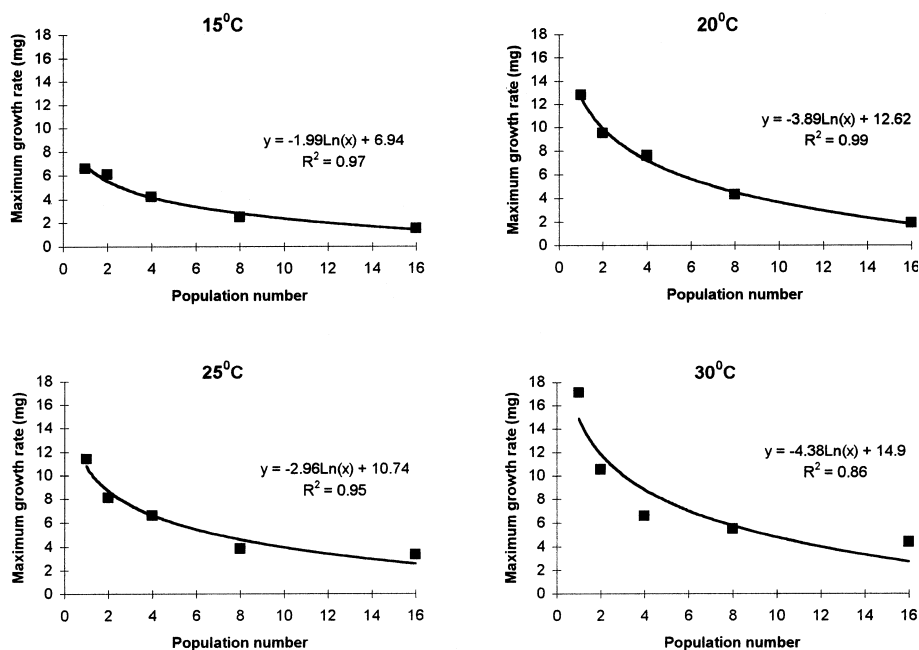


Fig. 8 The maximum biomass of a population of 11 *P. excavatus* as a function of time at four different temperatures in sewage sludge



and earthworm stocking rates for maximum biomass production in the wastes to be calculated.

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