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Reproduction of the African earthworm, *Eudrilus eugeniae* (Oligochaeta) – cocoons

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Summary. Although various authors have contributed to our knowledge of the biology of *Eudrilus eugeniae* some basic facts about the life cycle and reproduction are still not fully documented. Quantitative observations were made of the cocoons at 25 °C in different substrates. The incubation period for 166 cocoons was 16.89 days, based on cocoons produced by worms between the ages of 70 and 100 days. These cocoons produced a mean 2.12 hatchlings per cocoon after incubation in cattle manure, moist filter paper and distilled water. A smaller batch of cocoons incubated in cattle manure produced a mean of 2.7 hatchlings per cocoon. The hatching success of the cocoons was 84% in cattle manure, 50% in distilled water, and 48% on moist filter paper. The reproductive capabilities of *E. eugeniae* and *Eisenia fetida* were compared.

Key words: *Eudrilus eugeniae* – Earthworm – Cocoons – Incubation – Reproduction

The African nightcrawler, *E. eugeniae* (Kinberg 1867) is used extensively in commercial vermiculture, especially in the USA (Shields 1976). Increased attention is also being given to this species as a possible waste decomposer and as a protein source (Neuhauser et al. 1979; Graff 1981; Loehr et al. 1985). *E. eugeniae* is one of the most common earthworm species in West African soils (Edwards and Lofty 1972; Mba 1978) and also occurs widely in other parts of the world. In order to clearly establish the role of this species as a waste decomposer, more fundamental research into its general biology is needed. Among the authors who have studied this earthworm are Neuhauser et al. (1979), Graff (1981), Loehr et al. (1985), and Rodriquez et al. (1986). Viljoen and Reinecke (1988) have furnished data on the number, size and growth of hatchlings of *E. eugeniae*; they found 2.2 hatchlings per cocoon after studying 115 viable cocoons. Some basic facts about the life cycle and reproduction of *E. eugeniae* are, however, still not well documented, e.g. the hatching success and incubation period of cocoons has not been determined under controlled conditions.

The aims of the present study were: (1) To make quantitative observation of the cocoons of *E. eugeniae*; (2) to determine the incubation time of the cocoons in different substrates at a controlled temperature; (3) to obtain reliable figures on the viability and hatching success of cocoons under different conditions.

The aim was to examine sufficient cocoons to establish a scientifically sound basis for figures and estimates frequently quoted in the scientific literature. These estimates have seldom been based on extensive experimentation, and have given little regard to variability and the role of intrinsic and extrinsic (environmental) factors. A reliable comparison of the reproductive capability of *E. eugeniae* with that of a compost-dwelling species, e.g. *Eisenia fetida*, can only be made after all aspects of the reproductive biology have been investigated.

Materials and methods

A culture of *E. eugeniae* was maintained in the laboratory in a controlled-environment chamber at a temperature of 25 °C. The worms were kept in cattle manure with a pH of 7 and a moisture content of 70%–80%. They were regularly fed fresh cattle manure. The cocoons used were produced by 10 parent worms (mean biomass 1800 mg) with ages ranging between 70 and 100 days. The mass of each cocoon was determined in water on a Sartorius analytical balance. The cocoons were weighed individually and length and diameter were measured with a slide calliper. Cocooon volume was

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determined in a volumetric cylinder. Before weighing the cocoons were washed lightly in distilled water and most of the debris and organic particles adhering to the sticky hull were removed. The cocoons were randomly divided into three batches and incubated in multicell (repli) dishes. Three media were used for incubation, distilled water (replaced with clean water every 7 days), moist filter paper (kept moist by adding distilled water periodically), and moist, fine-particled cattle manure. These media with the cocoons were kept in a controlled-environment chamber at 25 °C. The cocoons were observed every 24 h. The hatchlings were removed daily and preserved in 70% ethanol.

The hatching success of the cocoons in the different media and the incubation period were determined. The number of offspring per cocoon was counted.

Results and discussion

Characteristics of cocoons

The cocoons of *E. eugeniae* have an irregular oval shape, one side usually being flatter than the other. Each cocoon has long, sharply pointed, fibrous tips at the two ends. The hull is very sticky immediately after formation, so that organic particles and fibres adhere to it. This phenomenon makes it difficult to distinguish the cocoons from the substrate in which they have been deposited, as noted by Graff (1981).

Directly after their formation the cocoons are soft and greyish-white in colour. They harden rapidly, the hull becoming strong and leathery with the colour changing to orange-brown. The brown colour intensifies with time, becoming dark brown immediately before hatching.

When the cocoon hatches a round aperture is left at one end by the first hatchling that emerges. The diameter of this aperture is usually smaller than that of the emerging worm which has to squeeze through the opening. Hatchlings were often observed retreating into cocoons when disturbed. In contrast to reports by Vail (1974) on *Eisenia fetida* and Reinecke and Visser (1981) on *Aporrectodea caliginosa*, the cocoons never became translucent enough for the embryos or young worms to be seen through the wall, except when viewed under a stereomicroscope with transmitted light of high intensity.

The mean length of the cocoons was 6.02 ± 0.05 mm (range 4.3 – 7.8) and the mean diameter 3.11 ± 0.03 mm (range 2.1 – 4.0), for 210 cocoons collected randomly from the breeding containers at 25 °C. The mean volumetric size of a cocoon was 0.2 cm^3. The mean mass based on the 210 cocoons was 16.99 ± 0.36 mg (range 9.9 – 39.7). No significant correlation was found between cocoon size (or mass) and the number of hatchlings produced.

Hatching success of cocoons

Hatching success varied in the different media used, the highest being in cattle manure, 84% (Table 1), and the lowest on moist filter paper, 48%. When cocoons were placed in distilled water to hatch, many of the worms emerged in an embryonic stage of developement. Vail (1974) found that this phenomenon was common for *Eisenia fetida*. In our study these worms lacked segmentation in the posterior half of the body and red blood pigment appeared to be absent in the circulatory system. Unlike the observations made by Vail (1974), where these embryos died within 24 h, those we observed often survived in distilled water and continued their development. The blood vessels attained a reddish colour after about 48 h and the segment number increased as the worm grew older. The only other published data available concerning the hatching rate of cocoons of *E. eugeniae* was provided by Loehr et al. (1985), who found a hatching success of 73% in aerobically digested sludge.

During preliminary experiments we observed that the hatching success of cocoons produced by worms younger that 60 days was low, the rate of hatching increasing as the worms grew older. Infertile cocoons can be produced by unmated specimens of *E. eugeniae* and since we were not able to distinguish between these and fertile cocoons, this might influence the percentage hatch recorded. Production of cocoons prior to mating has not previously been reported for *E. eugeniae* but is mentioned for some other earthworm species by Evans and Guild (1948). They observed the production of cocoons from unmated sexually mature

Table 1. Number of hatchlings per cocoon and hatching success of cocoons of *Eudrilus eugeniae* in different substrates at 25 °C

Medium	No. of cocoons used	No. of cocoons and % of total no. of cocoons that hatched — No. of hatchlings: 1		2		3		4		5		No. of cocoons hatched	Hatching success (%)	Mean no. of hatchlings: Of total no. of cocoons ± SE	Of cocoons hatched ± SE
		No.	%	No.	%	No.	%	No.	%	No.	%				
Cattle manure	88	22	29.7	20	27.0	17	23.0	10	13.5	5	6.8	74	84.09	2.14 ± 0.19	2.7 ± 0.14
Distilled H_2O	126	29	46.0	15	23.8	12	19.0	3	4.9	4	6.3	63	50.0	1.01 ± 0.11	2.1 ± 0.45
Filter paper	60	13	44.8	14	48.3	2	6.9	–	–	–	–	29	48.3	0.78 ± 0.13	1.6 ± 0.12
Total	274	64	38.6	49	29.5	31	18.7	13	7.8	9	5.4	166	60.6	1.29 ± 0.83	2.12 ± 0.09

specimens in a number of species and found that those from the genera *Allolobophora, Dendrobaena,* and *Octolasium* did hatch, while the cocoons produced by unmated specimens of four different species of the genus *Lumbricus* and by *Eisenia fetida* did not hatch.

Big differences (Table 1) were found in the hatching success of cocoons on different substrates. In cattle manure a hatching success of 84% was obtained while only 50% of the cocoons incubated in distilled water hatched and only 48% on moist filter paper. Since these cocoons were from the same parental batch of worms and were chosen randomly the extent of the differences seems to be more than can be attributed to natural variation within the batch. Viljoen and Reinecke (1988) found that ageing of the worms influenced the cocoon production rate markedly. Venter and Reinecke (1987) recorded that the first and last cocoons of *Eisenia fetida* produced by parental worms were less viable than those produced between days 30 and 120. These authors found that the peak in hatching success corresponded with a peak in production. The cocoons in the present study were therefore chosen randomly so as to minimize the possible role of age differences of the parent worms on the hatching success.

A plausible explanation for the observed differences that can be advanced at this stage concerns the role of moisture tension and ionic differences, which were evidently quite different in the different substrates. Specific experiments are required to establish the role of these factors.

Incubation period of cocoons

When the incubation periods for all the cocoons hatched in cattle manure, distilled water and moist filter paper (Table 2) were compiled, a mean value of 16.89±0.32 days was obtained.

The incubation periods of cocoons hatched in cattle manure, distilled water and on moist filter paper are shown on Fig. 1. There were significant differences in incubation times for the three different substrates. The total range of incubation times (Fig. 1) was, however, smaller for the cocoons incubated on moist filter paper than those on the other media. The incubation times of the individual cocoons incubated on the moist filter paper and in the distilled water varied much more than those incubated in the cattle manure, as evident from the standard errors. This also corresponded with a lower hatching percentage in the two water media. In the filter-paper medium a secondary peak was recorded after 21 days (Table 2), which was not paralleled in the other media. The reason for this peak is not clear.

The mean incubation period of 16.89 days observed in this study is much shorter than that of the common compost worm *Eisenia fetida*. Venter and

Table 2. Incubation of cocoons of *Eudrilus eugeniae* at 25 °C in three different substrates

Incubation time (days)	Cattle manure		Distilled water		Filter paper		Total in all media	
	No. of cocoons	% of total	No. of cocoons	% of total	No. of cocoons	% of total	No. of cocoons	% of total
9	2	2.7	1	1.5	–	–	3	1.8
10	–	–	–	–	2	6.9	2	1.2
11	2	2.7	2	3.2	–	–	4	2.4
12	1	1.4	2	3.2	2	6.9	5	3.0
13	5	6.8	3	4.8	1	3.4	9	5.4
14	9	12.2	6	9.5	6	20.7	21	12.6
15	8	10.8	6	9.5	3	10.3	17	10.2
16	14	18.9	7	11.1	3	10.3	24	14.4
17	7	9.5	11	17.5	2	6.9	20	12.0
18	10	13.5	6	9.5	2	6.9	18	10.8
19	5	6.8	5	7.9	1	3.4	11	6.6
20	3	4.1	2	3.2	2	6.9	7	4.2
21	2	2.7	2	3.2	4	13.8	8	4.8
22	2	2.7	–	–	1	3.4	3	1.8
23	1	1.4	3	4.8	–	–	4	2.4
24	1	1.4	1	1.5	–	–	2	1.2
25	1	1.4	1	1.5	–	–	2	1.2
26	–	–	–	–	–	–	–	–
27	1	1.4	1	1.5	–	–	2	1.2
28	–	–	3	4.8	–	–	3	2.4
35	–	–	1	1.5	–	–	1	0.6
Total no. of cocoons	74		63		29		166	

Reinecke (1987) recorded an incubation period of 23 days for the latter species while Reinecke and Kriel (1981) recorded a figure of 26 days. *E. eugeniae* therefore differs in this respect from the well-known compost-inhabiting species.

The only published information on the incubation times of cocoons of *E. eugeniae* is provided in recent studies by Rodriquez et al. (1986) and Knieriemen (1984). Rodriquez et al. (1986) determined an incubation time of 16.6 days for *E. eugeniae* at a temperature of 30 °C. This corresponds very well with our figure of 16.9 days at 25 °C.

In our study, whenever more than one hatchling emerged from a cocoon, most of them hatched within 3 days, but up to 7 days sometimes elapsed between the emergence of the first and the last hatchling.

Fecundity

In the present study on 166 cocoons of *E. eugeniae* a mean 2.12±0.09 hatchlings emerged per cocoon, with the figures for all three substrates compiled. Many hatchlings emerging from cocoons were not fully differentiated in the posterior half of the body. These cocoons were assumed to have hatched prematurely. When the number of offspring per cocoon is compared with that of *Eisenia fetida*, the latter species seems to have a small advantage with 2.7 hatchlings per cocoon (Venter and Reinecke 1987) although a similar figure was recorded in the present study for cocoons incubated in cattle manure.

The short incubation period, rapid growth rate and size could, however, favour *E. eugeniae* as a commercial breeder and as a potential protein source. The potential of *E. eugeniae* as a vermicomposting species in comparison with *Eisenia fetida* is not only dependent on its reproductive capabilities. The generation time of the two species should also be taken into account when comparing the commercial usefulness. The rate at which sexual maturity is attained will also affect the relative merits of the two species. Studies to this effect are underway. Being a tropical species, *E. eugeniae*'s ability to survive under prevailing environmental conditions outside the tropics would ultimately determine whether it could compete with a species such as *Eisenia fetida*, which is known for its ability to survive harsh environmental conditions and especially low temperatures and fluctuating moisture conditions (Reinecke and Venter 1987).

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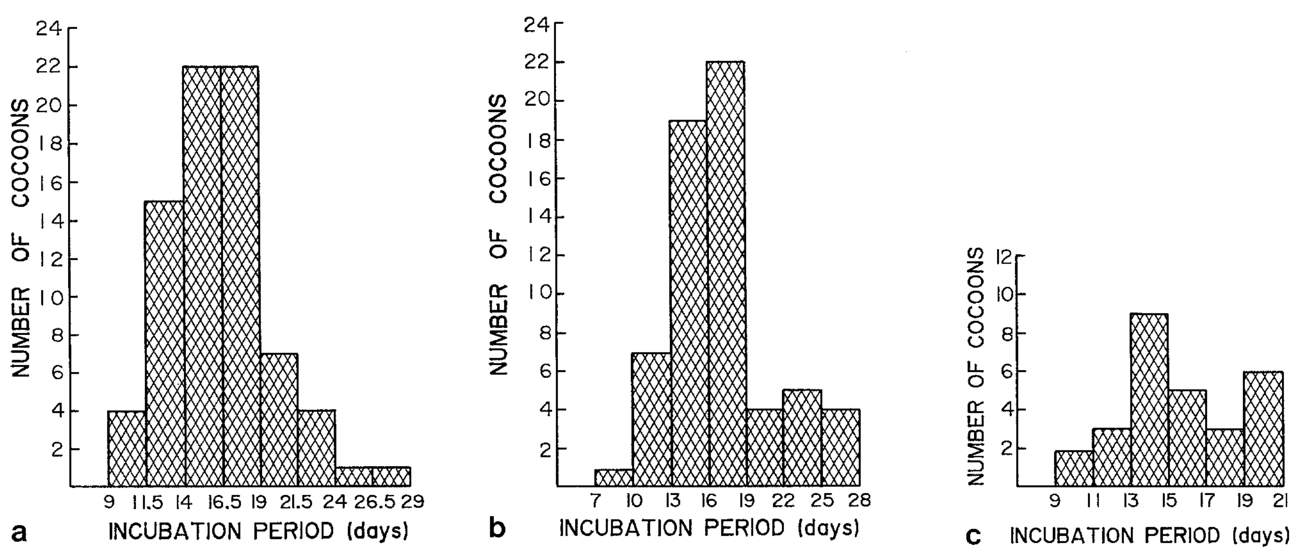

Fig. 1. Incubation times of *Eudrilus eugeniae* in **a** 74 cocoons on cattle manure ($\bar{x} = 16.6 \pm 0.38$); **b** 63 cocoons in distilled water ($\bar{x} = 17.7 \pm 0.66$), and **c** 29 on filter paper at 25 °C ($\bar{x} = 16.1 \pm 0.65$)

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