Moisture preferences, growth and reproduction of the African nightcrawler, *Eudrilus eugeniae* (Oligochaeta)

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As part of a comprehensive study of the biology of the economically important earthworm species *Eudrilus eugeniae*, the water relations of this species were studied. Moisture preferences were studied with the aid of cylindrical moisture towers filled with cattle manure. The cattle manure was dried, ground to a particle size of 500> <1000 μm and moistened. A moisture gradient was allowed to develop in the towers, the worms were added and the towers kept in an environmental control chamber at a temperature of 25°C and a relative humidity of 80%. The moisture preferences of both juvenile and adult worms were studied. The clitellate worms showed a wider preference range than the juveniles and the preference range of the juveniles coincided with the moisture levels at which cocoons were deposited. The highest frequency of occurrence for clitellate worms was between moisture levels of 80 and 82%. For cocoon deposition the highest frequency was at moisture levels between 79 and 80,5% and most juvenile worms showed a preference for a moisture range between 77,5 and 79%. The cocoons produced were incubated and the number of hatchlings per cocoon noted. The biggest number of offspring per cocoon hatched from cocoons originating from the moisture range of 79 to 80,5% coinciding with the highest frequency of cocoon deposition. From the results it appears that this earthworm species could be utilized in organic waste material with a relatively high moisture content.

Die vogverwantskappe van die ekonomies belangrike erdwurmspesie, *Eudrilus eugeniae* is as deel van 'n omvattende studie oor die biologie van hierdie spesie bestudeer. Vogvoorkeure is m.b.v. silindriese vogtorings, gevul met beesmis, bestudeer. Die mis is gedroog, gemaal tot 'n deeltjiegrootte van 500> <1000 μm en bevogtig. Die vogtorings is gelaat sodat 'n voggradiënt daarin kon ontwikkel. Dit is in 'n klimaatbeheerkabinet by 25°C en 'n relatiewe humiditeit van 80% geplaas. Die vogvoorkeure van beide klitellate en juveniele wurms is bestudeer. Die klitellate wurms het 'n wyer vogvoorkeur as die juvenieles vertoon en die voorkeure van die juveniele wurms het ooreenstemming met die vogvlakke waarby kokonne gedeponeer is, getoon. Die hoogste frekwensie van voorkoms van klitellate wurms was tussen vogvlakke van 80 en 82%. Vir kokondeponering was die hoogste frekwensie by vogvlakke van 79 tot 80,5% en die meeste juveniele wurms het voorkeur verleen aan vogvlakke tussen 77,5 en 79%. Die kokonne wat geproduseer is, is geïnkubeer en die getal nakomelinge per kokon aangeteken. Die grootste getal nakomeling per kokon het ontwikkel by kokonne wat vanaf vogvlakke tussen 79 en 80,5% verkry is. Hierdie vogvlakke stem ooreen met dié waarby die hoogste frekwensie kokonne gedeponeer is. Die resultate wat verkry is toon dat hierdie erdwurmspesie gebruik sal kan word in organiese afvalmateriaal met 'n relatief hoë voginhoud.

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As indicated by Viljoen & Reinecke (1989b) very little is known of the water relationships of the detritophagous earthworm species that are of economic importance. The influence of moisture on the growth and reproduction of the West African tropical detritophagous earthworm *Eudrilus eugeniae* was reported on by these authors. Reinecke & Venter (1985) and (1987) did extensive studies on the moisture requirements of another detritophagous species, *Eisenia fetida* and although some work by authors such as Kale, Bano & Krishnamoorthy (1982); Guerrero (1983) and Kale & Bano (1987) refer to the moisture requirements of detritophagous species, no in-depth studies to this effect were done by them. Extensive work was done in this regard on some geophagous species by Lavelle (1975) and Barois & Lavelle (1986).

Moisture is one of a number of physical factors influencing the vermicomposting process. It is therefore important to understand the water requirements of vermicomposting species, especially since they more closely resemble aquatic than truly terrestrial animals (Lee 1985).

Viljoen & Reinecke (1989b) studied the influence of moisture on growth and maturation and provided an extensive survey of the literature on this subject. They did not, however, cover the moisture preferences of *E. eugeniae*. The present study was undertaken to determine the moisture preferences of this species. Simultaneously the growth and reproduction of *Eudrilus eugeniae* were studied under controlled conditions in an experimental design which permitted the worms to move freely within a moisture gradient. A similar gradient was designed in which the worms were restricted to certain moisture levels. In all previous studies on this species the worms were restricted to specific moisture levels. Reinecke & Venter (1987) showed that *E. fetida* worms were often selective and deposited cocoons in certain moisture levels while spending most of their time at other levels.

Material and Methods

A stock culture of *Eudrilus eugeniae* was maintained in the laboratory in an environmental control chamber at a temperature of 25°C in a cattle manure medium. The worms originated from cocoons obtained from Prof. O. Graff of West Germany.

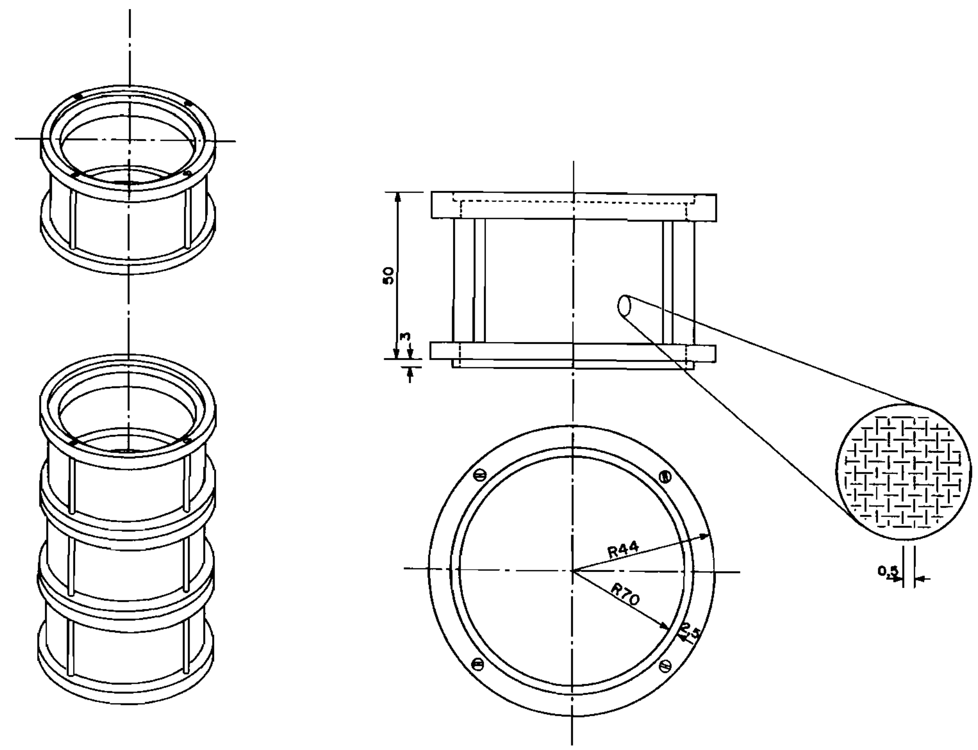

Figure 1 Three dimensional and technical drawings of the segments of the moisture towers constructed for the preference studies.

The moisture preferences were studied with the aid of cylindric moisture towers consisting of six cylinders made of a fine gauze (Figure 1). Each cylinder had a height of 5 cm and a diameter of 7 cm and fitted tightly into each other. These cylinders were open on both sides except for the one used as the lowest segment. The bottom of this cylinder was also covered with gauze to prevent worms from crawling out. Six replicates of these towers were used for the study of clitellate worms and three for juvenile specimens. All the juvenile worms used during the experiment were 5 days old at the start of the experiment.

A second set of cylinders, closed at one end with gauze, was used to construct moisture towers in which six separate sections were thus created. Six replicates of these towers were also used for clitellate specimens and three for juvenile worms.

Sun-dried cow manure without straw or urine was used as medium for these experiments. The manure was ground and sieved to a particle size of 500> <1000 μm. The fine manure was wetted with distilled water to a moisture content of approximately 60%. Moisture gradients were created by filling the towers with this dampened manure and placing them vertically in a water bath. The lower half of the bottom cylinder of each tower was completely submerged in the water. The towers were left to stabilize for seven days before worms were added. Similar moisture columns filled with soil were used by Grant (1955) for preference studies on *Allolobophora caliginosa* and *Pheretima hupeiensis.* Reinecke & Ryke (1970), did the same for *Microchaetus modestus.* Reinecke & Venter (1987) performed moisture preference studies with *E. fetida,* in a cattle manure medium similar to that used during the present study.

All worms used during the experiments were weighed individually before being placed into the moisture towers. This weighing as well as that during the experiment was done by rinsing the worms in distilled water, drying them on filter paper and then determining the mass in water on a Sartorius analytical balance. In the open moisture towers nine worms were placed onto the top of the upper cylinder, covered with dark plastic material and allowed to move into the medium. After 10 days the distribution of the worms in each tower was determined by separating the six sections and sorting the organic medium of each on a tray. The worms were weighed and in the case of the clitellate worms, any cocoons present were removed, weighed and incubated. The worms were replaced on the top segment of the tower after the same medium was placed back into each segment of the tower. This was repeated every 10 days. In the segmented moisture towers, three individually weighed worms, were placed in each of the six segments of each tower. Every 10 days the worms from each segment were also sorted and weighed as described above. All the moisture towers were kept in an environmental control chamber at 25°C and 80% relative humidity for the duration of the experiments. The procedure described was repeated every 10 days for a period of 60 days. This duration of 60 days was determined by the rate at which the worms utilized the medium. The experiment had to be terminated when food became a restricting factor at the end of this period. This was decided upon when the utilizable substrate was completely replaced by worm castings.

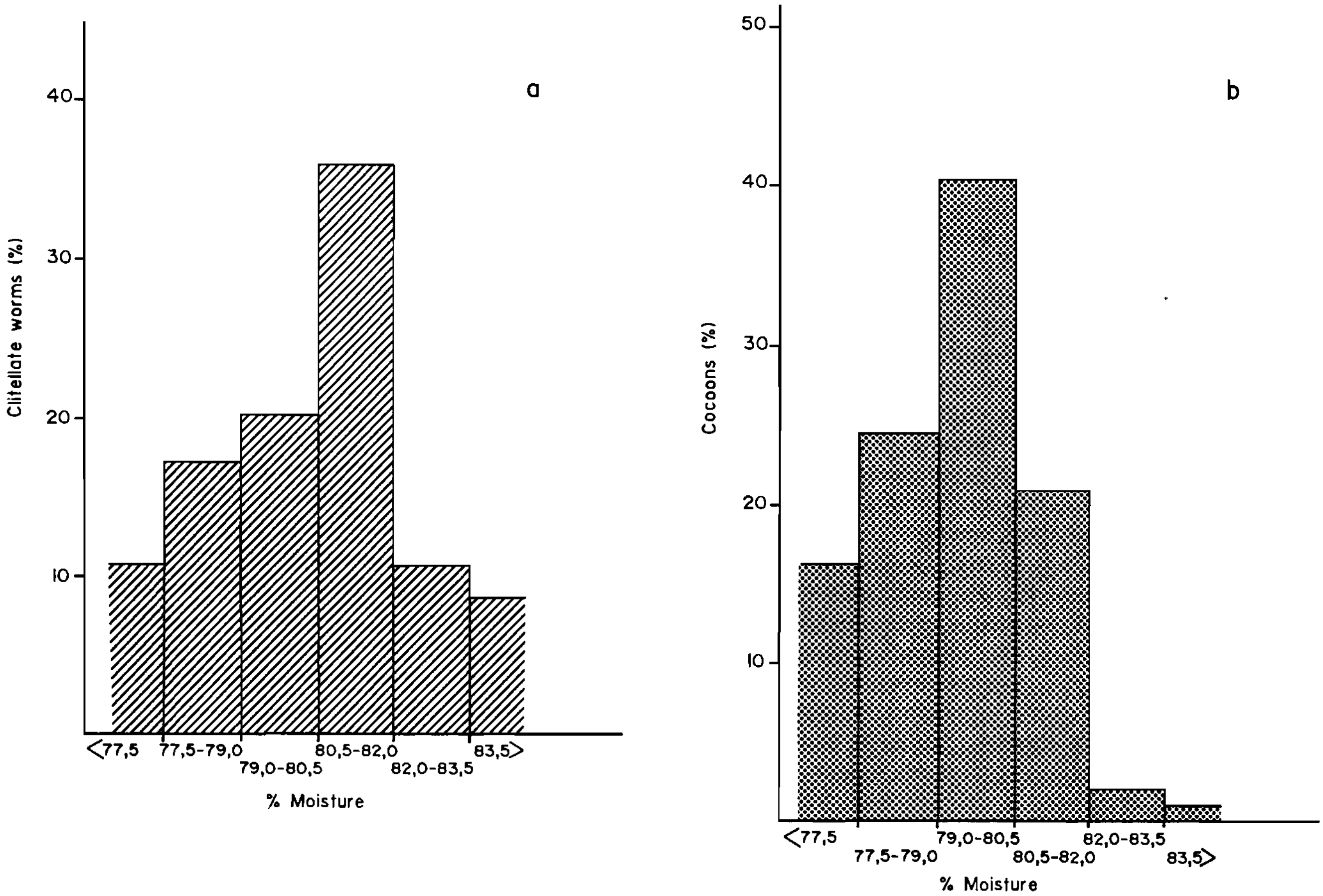

Figure 2 (a) Distribution of 54 clitellate specimens of *E. eugeniae* in an open moisture gradient. (b) Distribution of 434 cocoons of *E. eugeniae* in an open moisture gradient.

The cocoons collected during the study were all incubated in Petri dishes with distilled water at 25°C. These dishes were monitored daily and the hatchlings removed and counted as they hatched.

The results given are a compilation of all the replicates.

Results and Discussion

Moisture preference

The distribution of 54 clitellate specimens of *E. eugeniae* in a moisture gradient in which they could move freely is presented in the histogram in Figure 2a. The percentage moisture in this open gradient ranged from a mean of 40% in the upper regions to 85% in the lowest part of the moisture tower. From Figure 2a can be seen that the worms occurred mainly in the upper moisture regions, within a fairly narrow moisture range. No worms occurred at moisture levels below 68,9%. As can be seen from this histogram (Figure 2a) the distribution was fairly normal within the preferred ranges, with most of the worms occurring in the range of 80 to 82% moisture.

The distribution pattern of cocoons deposited in the moisture gradient (Figure 2b) coincided with the range of moisture preference of the clitellate worms by which they were produced. However, of the 434 cocoons produced only 20,8% were found in the 80 to 82% moisture range whereas 35,5% were deposited in the 79 to 80% range. Although the clitellate worms occurred in the higher moisture levels they tended to deposit more cocoons in the lower moisture regions.

The 51 juvenile specimens showed a narrower distribution within the moisture gradient than that of the clitellate specimens. No juvenile worms occurred at moisture levels above 79%. Of the juvenile worms 68,7% occurred between moisture levels of 77,5 and 79% and 31,3% of the juveniles below 77,5% moisture. The deposition of cocoons in an environment where prevailing moisture conditions would facilitate a higher survival rate of the more sensitive hatchlings, might be the reason for worms depositing more cocoons at the lower moisture levels.

Compared to the findings in a similar experiment with another composting earthworm species, *Eisenia fetida* (Reinecke & Venter 1987) it can be seen that clitellate specimens of *E. eugeniae* have a narrower moisture preference range at higher moisture levels than *E. fetida*. Very few specimens of *E. fetida* occurred at a moisture level higher than 75%. In the case of *E. fetida* cocoons were deposited at moisture levels exactly coinciding with the preference levels determined for juvenile specimens.

Changes in biomass of worms and cocoon production

The changes in biomass of the clitellate worms in the open moisture towers are presented in Figure 3a. The variation exhibited in Figure 3a can be partly attributed to the fact that the worms moved freely in the towers. As

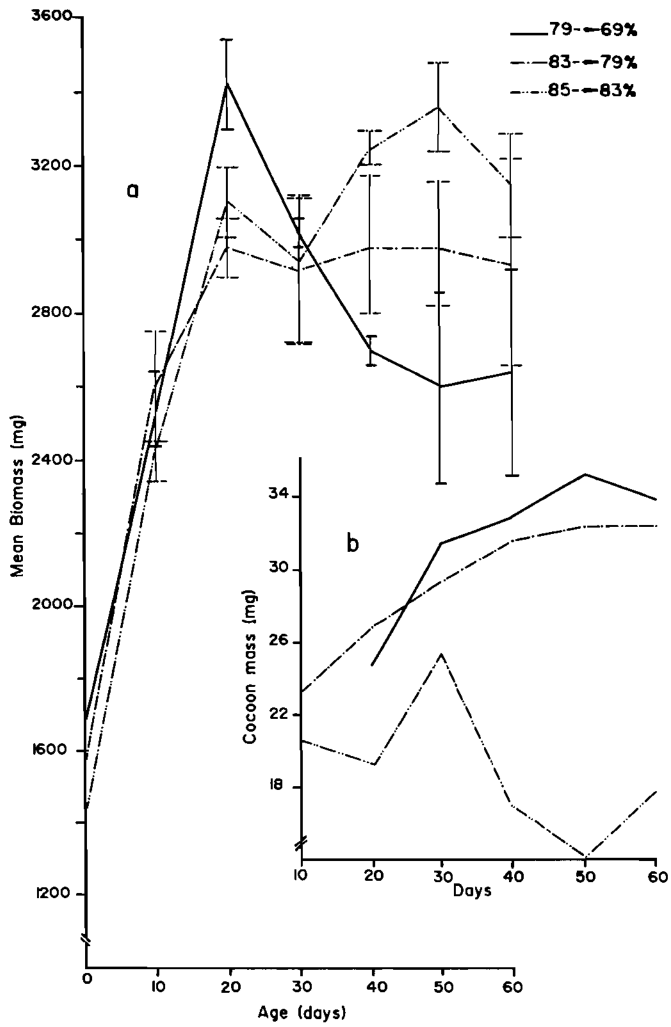

Figure 3 (a) Changes in biomass of clitellate specimens of *E. eugeniae* at three moisture levels in an open moisture gradient. (b) Mean biomass per cocoon produced by clitellate specimens of *E. eugeniae* at three moisture levels in an open moisture gradient.

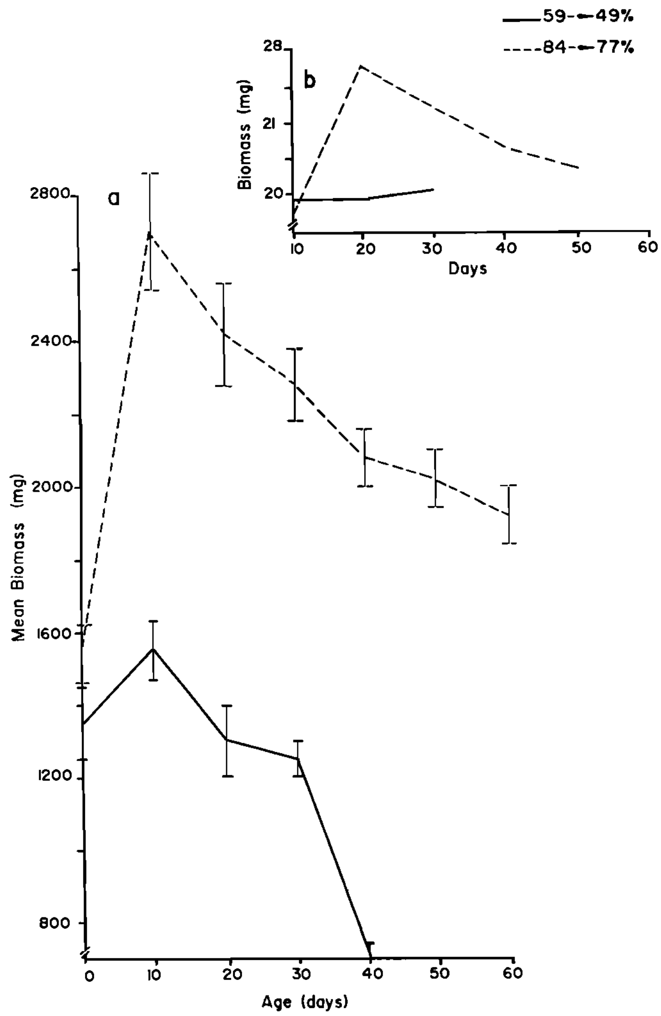

Figure 4 (a) Changes in biomass of clitellate specimens of *E. eugeniae* at two moisture levels in a separated moisture gradient. (b) Mean biomass per cocoon produced by clitellate specimens of *E. eugeniae* at two moisture levels in a separated moisture gradient.

soon as the towers were disturbed during analysis the worms were able to move to other levels. Moisture values in the different sections of the tower also changed slightly with time. The highest mean growth rate over this period occurred at the highest moisture levels. The mean mass of cocoons (weighed in groups) deposited within these three moisture levels is illustrated in Figure 3b. The highest cocoon biomass was obtained at the lower moisture levels. As can be seen from Figure 2b fewer cocoons were also deposited in the levels with a high moisture content. It would therefore seem that although the clitellate worms prefer the higher moisture levels, they were more active reproductively in the lower moisture levels. As these worms could move around freely no deduction concerning a relationship between the worm biomass and the biomass of the cocoons they produced can be made.

The influence of different moisture levels on the biomass of clitellate worms in the moisture towers with separate segments is shown in Figure 4a. Cocoon deposition in the different levels is shown in Figure 4b.

The mean moisture content of the different sections at the start of the experimental period was, from top to bottom of the towers, respectively 48,2; 54,4; 58,1; 59,9; 76,6 and 83,2%. After 10 days no worms survived in the upper three sections or in the lowest. After 40 days the moisture level of segment four had dropped to 48,9% and the biomass of the worms decreased drastically from day 30 to 40. By day 50 all the worms in this level had died. In the fifth segment the moisture level rose from 76,6 to 81,3% during this period and the biomass of these worms also decreased as shown in Figure 4a.

Although the worms preferred the higher moisture levels and had a higher growth rate in these levels as long as freedom of movement was possible, when the worms were restricted to them, these levels were not as favourable. The reason for this could be oxygen availability, which is also suggested by a study on *E. eugeniae* Viljoen & Reinecke (1989a), where the worms were subjected to various, constant moisture levels in closed containers. During their study the highest growth rate and cocoon production occurred at a moisture level of 80% while worms grew much slower at higher moisture levels and did not produce any cocoons.

As can be seen in Figure 4b, cocoons were only produced in segments four and five (where worms survived for more than 10 days). No cocoons were, however, produced in segment four after 30 days when the moisture content dropped below 50%, even though the worms survived for another 10 days. The mean mass of cocoons produced at the lower moisture level was much lower than the mean mass of those produced in segment five. By day 20 when the moisture level was still close to 80% the heaviest cocoons were produced, but as the moisture level in this segment rose, the cocoon mass was progressively less after each 10-day period. In the case of the segmented moisture towers where worms could not move around freely, our findings are in agreement with that of Reinecke & Venter (1987) concerning the relationship between worm size and cocoon size. They found that larger worms produced larger cocoons, in the case of *E. fetida.*

The cocoon production per worm per day in the open and segmented moisture towers are compared in Figure 5. Where the worms were restricted, even within favourable moisture levels, their cocoon production was lower than where freedom of movement was possible.

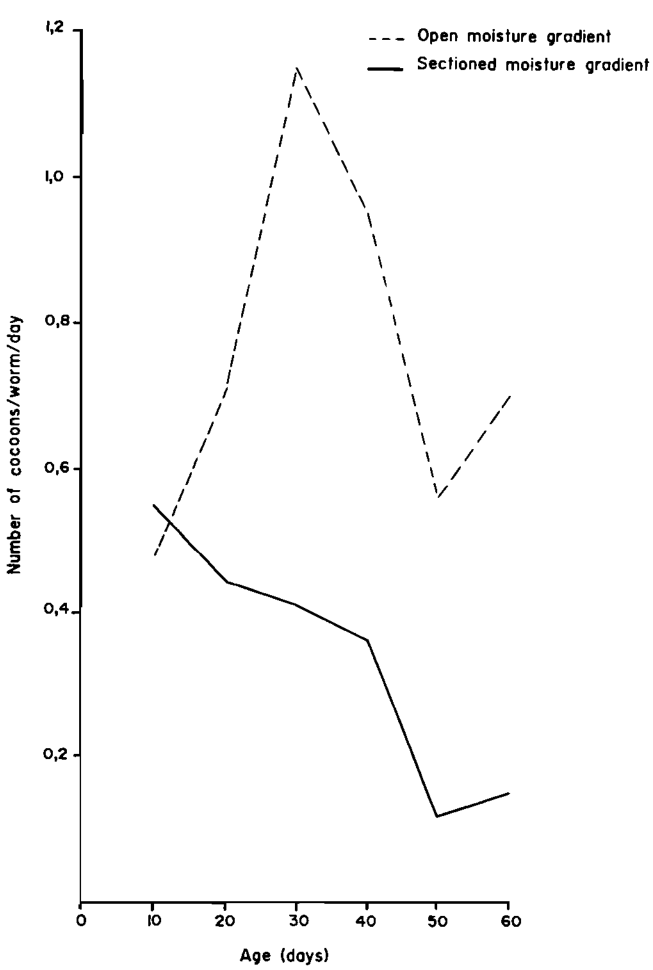

Figure 5 Comparison between the number of cocoons of *E. eugeniae* produced per worm per day in open and sectioned moisture gradients.

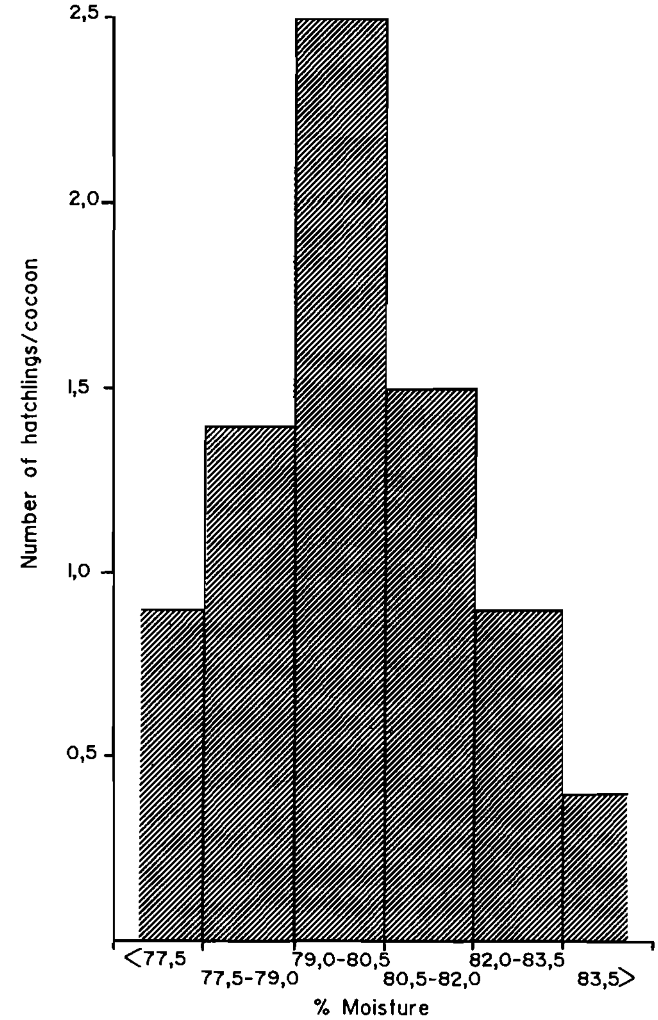

Figure 6 Number of hatchlings per cocoon of *E. eugeniae* produced in different moisture levels of an open moisture gradient.

Worms that were able to move around freely in the moisture towers had more opportunities to copulate successfully. This could have had a stimulating effect resulting in the higher reproduction rate that was observed. The conspicuous difference between these two groups could also be attributed to the effect of worm density. It has been found that *Eisenia fetida,* when cultured in worm beds, tend to become less reproductive as worm density increases (Reinecke & Viljoen 1990).

Hatchlings

As a criterion for comparing fecundity between the different moisture levels, the number of hatchlings per cocoon was observed. In Figure 6 a histogram is presented of the number of hatchlings per cocoon in the open moisture towers. A fairly normal distribution can be seen within the overall moisture range from which these cocoons were collected. In comparing this histogram with that of Figure 2b it is interesting to note that the highest number of hatchlings per cocoon was produced from cocoons originating from the same moisture level in which the largest number of cocoons were deposited, implying that more favourable moisture conditions could perhaps influence the number of offspring.

Conclusion

The general conclusion is that *E. eugeniae* prefers higher moisture levels than *Eisenia fetida*. Clitellate specimens of *E. eugeniae* have a narrower moisture preference range than those of *E. fetida*. The ability to move freely in a bigger volume of substrate had a significant influence on the growth and reproduction of *E. eugeniae*. It seems evident that worm density affects both growth and reproduction, as was found for *E. fetida* (Reinecke & Viljoen 1990) independant of the role of food availability. Spatial distribution, therefore, must be taken into account when aiming to achieve maximum growth and reproduction at favourable moisture levels. *E. eugeniae* has potential for commercial breeding. Its potential use in the biotechnology of waste control or as a possible protein source will depend on whether high numbers of worms in preferably outdoor culture beds can be maintained. If other factors such as temperature and food availability are optimal, this species could be utilized in organic waste material with a relatively high moisture content (such as cattle slurry) which is less favourable for the well known composting species *Eisenia fetida*. The narrower moisture preference range could, however, limit its use in environments where the moisture content can fluctuate.

Acknowledgements

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