Life-cycle of the African nightcrawler, *Eudrilus eugeniae* (Oligochaeta)

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In order to determine the potential of the earthworm *Eudrilus eugeniae* as waste processor and protein producer, the life history of this species was studied. The development, growth and reproduction of *E. eugeniae* were studied. Cattle dung was used as substrate with a moisture content of 70–80% and a temperature of 25°C. Data were gathered over a period of 300 days. It was found that cocoon production started within 24 h after copulation and can be sustained for at least 300 days. Cocoons are produced at an average rate of 1,65 cocoons per worm per day. The mean incubation period of cocoons is 16,6 days with a hatching success of 84% and 2,7 hatchlings per cocoon that hatched. Sexual maturity is attained by the offspring within 40 to 50 days after hatching. *E. eugeniae* is compared to other vermicomposting species.

Ten einde die potensiaal van die erdwurm *Eudrilus eugeniae* as afvalverwerker en proteïnbron te bepaal, is die lewensgeskiedenis van die spesie bestudeer. Die ontwikkeling, groei en voortplanting van *E. eugeniae* is op 'n substraat van beesmis met 'n voginhoud van 70–80% by 'n temperatuur van 25°C bestudeer. Gegewens is oor 'n periode van 300 dae ingesamel. Daar is bevind dat kokonproduksie binne 24 h na kopulasie begin en vir minstens 300 dae volgehou word. Kokonne word teen 'n gemiddelde koers van 1,65 kokonne per wurm per dag geproduseer. Die gemiddelde uitbroeityd van kokonne is 16,6 dae met 'n uitbroeikoers van 84%. Gemiddeld is 2,7 nakomelinge per kokon wat uitgebroei het, voortgebring. Geslagsrypheid word binne 40 tot 50 dae na uitbroeiing bereik. *E. eugeniae* word ook met ander vermikomposteringspesies vergelyk.

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There is an increasing demand for protein-rich material in the animal feed industry and large amounts of organic waste must also be disposed of without causing pollution.

The potential utilization of certain terrestrial Oligochaeta is receiving increased attention. Research is primarily aimed at protein production (Schultz & Graff 1977; Sabine 1983; Alberts, Reinecke & Venter 1988) and waste processing (Graff 1974; Kaplan, Hartenstein, Neuhauser & Malecki 1980; Haimi & Huhta 1986).

The African nightcrawler, *Eudrilus eugeniae* (Kinberg, 1867), has been identified as a detritus feeder and can be reared in large numbers on organic waste. This opens up the possibility of recovering waste protein if these worms can be used as a protein source in animal feeds. Increased attention is also being given to this species as a possible waste decomposer (Neuhauser, Kaplan & Hartenstein 1979; Graff 1981).

Before the vermicomposting role or the protein potential of *Eudrilus eugeniae* can be established fully various aspects of its biology have to be researched, especially as it is a tropical species with probably limited tolerances for temperate environments. Neuhauser, Kaplan & Hartenstein (1979), Knieriemen (1984), Loehr, Neuhauser & Malecki (1985), Rodriguez, Canetti, Reines & Sierra (1986) and Viljoen & Reinecke (in press) have contributed to our knowledge of various aspects of the life-cycle of this species. The full picture has, however, not yet emerged from these contributing studies. As pointed out by Viljoen & Reinecke (in press) some basic facts about the life-cycle and reproduction of this species are still not documented and very few results with regard to long-term observations and conditions under which these were obtained are available. Apart from contradictions many lacunae in our knowledge are to be found in the literature (see Table 1).

The main aim of this study was to determine the life-cycle of the African nightcrawler, *E. eugeniae* on an organic nutritive source consisting of cattle dung, under favourable conditions with regard to moisture, food availability and temperature. Apart from quantitative observations of the cocoons, their incubation time, viability and hatching success, the growth rate, maturation rate, production rate and number of offspring were determined through extensive experimentation and the total life-cycle is now, for the first time, rendered fully.

Material and Methods

The specimens of *Eudrilus eugeniae* used during the present study came from a stock maintained by Prof. O. Graff in West Germany, which originated from West Africa (Graff 1981). The experimental work was conducted in an environmental control chamber at a temperature of 25°C and a relative humidity of 80% (Viljoen & Reinecke in press).

Cocoons were incubated in multicell (repli) dishes, using fine-particled cattle manure (prepared from cattle droppings without urine and straw). This cattle manure was also used as substrate to maintain hatchlings and growing worms, as this was deemed a suitable medium by Graff (1981). The nutritive medium in which hatchlings were placed, was prepared by sun-drying and grinding with a liquidizer. The fine particles were sieved to a size of 500–100 μm, found to be favourable for other earthworm species by Venter & Reinecke (1988). Distilled water was added to the medium to create a moisture content of between 70% and 80%. This medium was left to stabilize for at least 48 h before the

Table 1 Published data on the life-cycle of *Eudrilus eugeniae*

	Neuhauser *et al.* (1979)	Graff (1982)	Knieriemen (1984)	Loehr *et al.* (1985)	Rodriguez *et al.* (1986)
Substrate	Sludge and horse manure	Cattle manure	Cattle manure	Sludge	Animal & plant material
Temperature °C	25	25	24	25	30
Moisture	78	–	–	75	–
pH of substrate	–	–	–	–	–
Hatching bomass (mg)	–	4–11	–	–	–
Max. ind. biomass (mg)	2700	3500	3100	–	–
Period of adolescence (days)	49	68	–	–	51
Max. no. cocoons/worm/day	0,1	0,4–0,7	0,3	1,6	–
Incubation period	–	12+	–	–	16,6
No. hatchlings/cocoons that hatched	–	2,2	2,7	2,5	2,08
% Hatching success	–	75	–	73	–
Senescence after (days)	84	200	–	98	210

experimental animals were placed into it.

The worms were reared either singly in glass jars with perforated lids, or in groups of five or 10 worms in plastic containers with gauze lids. When the worms reached the age of 15 days, fresh cattle manure was added to every container in quantities closely related to the number of worms in the container. This was repeated every five or 10 days as was necessary. Some of the older substrate was removed periodically to maintain the original volume of substrate in each container as close as possible. This was deemed necessary to maintain a constant density–volume ratio and to minimize the possible role of such a factor.

The biomass of each hatchling was determined before it was placed into the medium and thereafter every five days up to the age of 60 days. Subsequently the worms were weighed every 10 days. The biomass was determined by removing the worm from the substrate, washing off all particles in a jar with distilled water, removing all water from the worm with tissue paper and then weighing it in a weighing boat with distilled water on a Sartorius analytical balance.

With each weighing the worms were examined to determine the extent of maturation as indicated by clitellum development. As soon as clitellums were fully developed, 10 worms of those kept singly were paired off and observed to see whether copulation took place.

As from Day 35 (when clitellums started to develop) the medium was also searched for cocoons every five days. The cocoons were counted, measured and the mass determined, following which they were placed into multicell containers and covered with fine particled manure with a moisture content of 75%. These cocoons were incubated at 25°C and observed daily to determine whether any hatchlings had emerged. The viability and incubation period of the cocoons as well as the number of offspring were determined in this way (Viljoen & Reinecke in press).

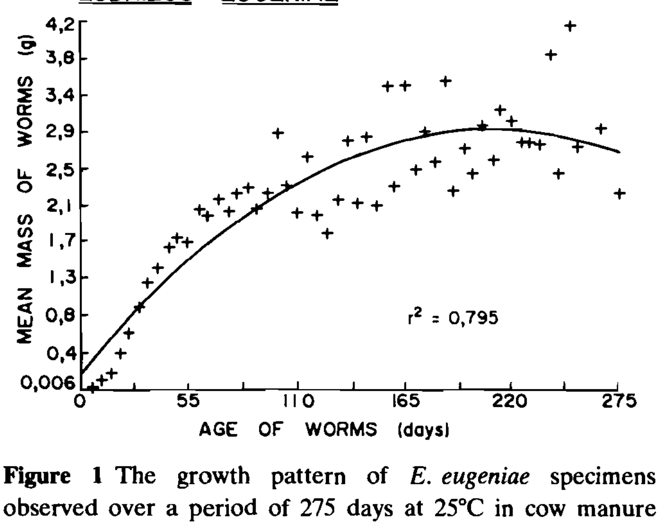

Figure 1 The growth pattern of *E. eugeniae* specimens observed over a period of 275 days at 25°C in cow manure with a moisture content of 75%.

Results

Growth

The growth rate of *Eudrilus eugeniae* was studied over a period of approximately 300 days (Figure 1). The mean biomass of newly hatched worms was 6,2 ± 0,35 mg although some differences were observed between the biomass of the first hatchlings and worms hatching later from the same cocoon (Viljoen & Reinecke 1989). This figure was obtained after weighing 95 hatchlings.

The worms grew steadily for the first 50 days at a mean rate of 34,3 mg per worm per day. The worms lost weight by the 55th day. This coincided broadly with the onset of cocoon production. After this dip in the growth rate the mean biomass increased again, attaining the highest mean biomass of 4133,6 ± 118,3 mg by the 250th day. During the period from Day 55 to the last observation a number of fluctuations occurred in the mean biomass with a mean increase of 2,6 mg per worm per day (Figure 1).

Obvious differences in the growth rate of worms raised singly and those kept in batches of higher densities were observed (Figure 2). Over a growth period of 220 days the mean growth rate of single worms was 14,9 mg per worm per day and that of worms kept at higher densities was 8,8 mg per worm per day. The highest mean biomass attained by the group of single worms was 4294,8 ± 369 mg on Day 185 and that attained by worms kept in groups, 2136,4 ± 113 mg, also on Day 185. One specimen weighed at the age of 295 days attained a mass of 14430,1 mg. This worm was kept singly throughout the study.

Maturation and copulation

The first indications of clitellum development appeared between Days 25 and 30. This is manifested by a slight change in colour in the clitellum area from reddish to yellow. Thereafter the clitellum swells progressively.

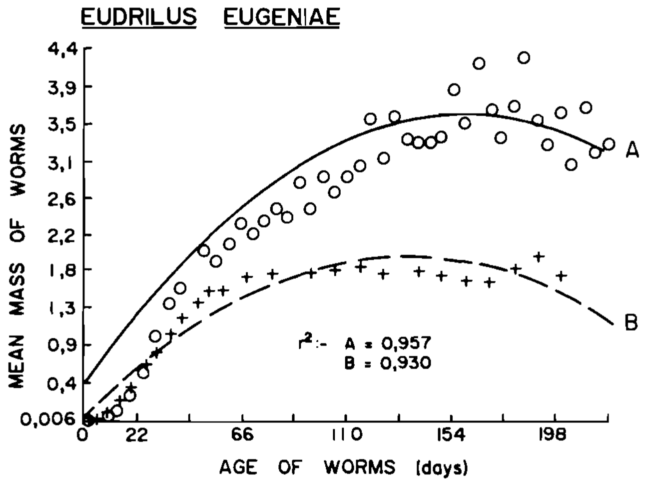

Figure 2 The growth rate of *E. eugeniae* specimens kept singly (O) and in batches of five and 10 worms each (+) over a period of 220 days at 25°C in cow manure with a moisture content of 75%.

In our study the worms were deemed sexually mature when they had fully developed clitellums. This was reached after approximately 45 days. As soon as worms reached this stage irridescent testes were discernable ventrally through the body wall, which normally indicates that copulation has taken place.

We did not find biomass to be a limiting factor for sexual maturity and observed copulating worms weighing 2 g or less. Worms with fully developed clitellums copulated readily within half an hour after they were brought together. No mucous band is formed during the copulation process. The copulating worms positioned themselves with their ventral sides closely together with the two clitellums directly opposite each other.

Cocoon production

Cocoon formation (Figure 3) started within 24 h after copulation had taken place and continued up to the last observation on the 295th day. Although variation occurred in the rate of cocoon production it never stopped completely.

A peak in production rate occurred on the 75th day when the rate was 1,65 cocoons per worm per day. Thereafter the production rate fluctuated quite markedly with a low production rate when the worms were between the ages of 140 and 210 days, the lowest being a production of 0,7 cocoons per worm per day during this period. When the worms reached the age of 230 days 1,7 cocoons per worm per day were produced and this high production rate was again noted by Day 260 whereafter the rate again decreased considerably (Figure 3). Over the whole study period a mean production of 1,3 ± 0,1 cocoons per worm per day was obtained.

Eudrilus eugeniae can produce cocoons even though copulation has not taken place. This occurred where worms were raised in isolation immediately after they had hatched. This is an indication that copulation is not required to trigger cocoon production as seems to be the case with some other terrestrial oligochaetes.

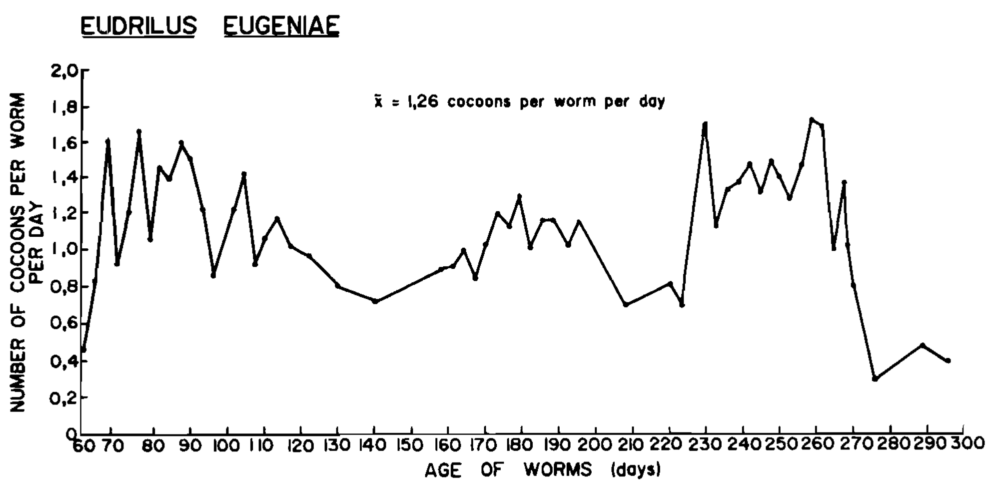

Figure 3 Cocoon production of *E. eugeniae* specimens over a period of 295 days at 25°C in cow manure with a moisture content of 75%.

The rate of cocoon production in these worms was not as high as in worms that had the opportunity to practise amphimixis. One cocoon was produced by each worm every two to three days.

The cocoons of *E. eugeniae* have an irregular oval shape with pointed, fibrous tips at both ends. Directly after formation the soft, whitish cocoon hardens rapidly and attains an orange-brown colour. The brown colour intensifies with time, becoming dark brown directly before hatching.

The cocoons used during this study were measured and weighed to determine any significant correlations between cocoon size (or mass) and the number of hatchlings developing from the cocoon or biomass of the hatchlings produced. No such correlations were found. The mean length of the cocoons was 6,02 ± 0,05 mm, the mean diameter 3,11 ± 0,03 mm, the mean volumetric size, 0,2 cm^3 and the mean mass 16,99 ± 0,36 mg. These figures are based on the measurements of 210 cocoons.

Hatching success, incubation period and number of offspring

A hatching success of 84% was obtained for 88 cocoons incubated at a constant temperature of 25°C in fine particled cattle manure. These cocoons were all produced by worms which had the opportunity to practise amphimixis. No cocoons produced by unmated worms, reared in isolation, produced hatchlings. It seems therefore that even though cocoons are produced by these unmated specimens, *E. eugeniae* cannot reproduce parthenogenetically or by way of self-fertilization. Worms raised in pairs or batches can produce infertile cocoons in the same manner as unmated worms. It was not possible to distinguish between these and fertile cocoons. This should be taken into account when interpreting the percentage hatch obtained in this study.

The incubation period of this batch of cocoons varied

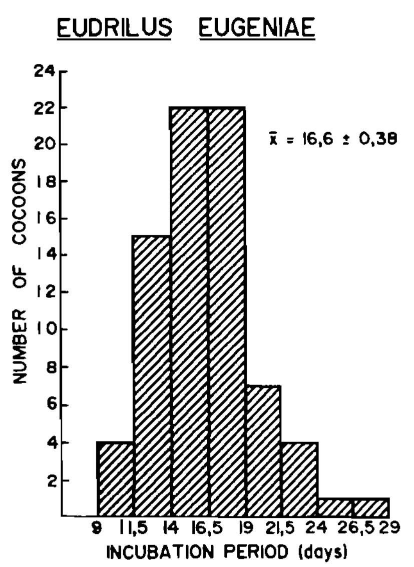

Figure 4 The incubation time of 88 cocoons of *E. eugeniae* at 25°C in cattle manure with a moisture content of 75%.

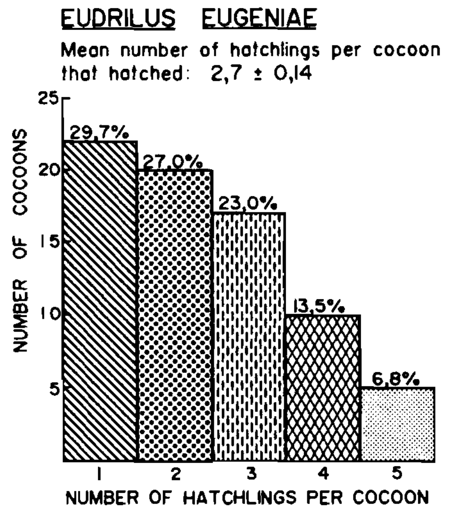

Figure 5 Number of hatchlings per cocoon produced by *E. eugeniae* at 25°C in cattle manure as well as percentage occurrence, based on 88 cocoons.

from 9 to 27 days with a mean of 16,6 ± 0,38 days (Figure 4).

The number of hatchlings produced by all the cocoons incubated was 2,14 ± 0,19 hatchlings per cocoon. The number of hatchlings produced by cocoons that hatched was 2,7 ± 0,14 hatchlings per cocoon (Figure 5). The largest number of hatchlings to emerge from a single cocoon was five. Upon emergence from the cocoon, the hatchlings had a pink, yellowish colour with the hind-most segments not fully differentiated.

Discussion

Growth

From the growth curve (Figure 1) of all the worms studied, it can be deduced that the worms underwent a fast increase in biomass during the first days with a gradual diminishing of the growth rate thereafter. This is in accord with the findings of Mba (1983) who observed an acceleration of growth up to about seven weeks for *E. eugeniae* hatchlings reared on cassava peels and Knieriemen (1984) for *E. eugeniae* fed on different animal waste products.

The decrease in worm biomass observed after 55 days, can possibly be the result of the onset of cocoon production, a tendency also noted by Graff (1981) and Mba (1983). An increased fluctuation in worm biomass was observed during the rest of the growth period. This can possibly be explained by the differences in nutritive value of the fresh cattle droppings with which the worms were fed every five or 10 days.

It should also be noted that there was no complete flattening of the growth curve towards a plateau by the time the last observation was made, but observations made of these same experimental animals after 300 days showed a definite commencement of an ageing process, with the worms diminishing in weight despite ample availability of food. From Figure 3 it can also be seen that the rate of cocoon production was very low at this stage, coinciding with a possible senescence of the worms.

From these results it seems that the life span of *E. eugeniae* is not longer than 300 days on cattle manure at 25°C. Neuhauser *et al.* (1979), Mba (1983) and Loehr *et al.* (1985) also observed that this worm species has a relatively short life span. No previous study of senescence is, however, reported on in the literature.

Knieriemen (1984) found that population density can influence the biotic potential of *E. eugeniae*. He also observed that freedom to move about influenced the reproductive capability, independent of population density. In our study (Figure 2) worm density had a definite influence on growth rate.

Compared to other vermicomposting species *E. eugeniae* seems to have a clear initial growth advantage over species such as *E. fetida* (Knieriemen 1984; Loehr *et al.* 1985; Venter & Reinecke 1988), *Perionyx excavatus* (Knieriemen 1984; Loehr *et al.* 1985) and *Dendrobaena veneta* (Loehr *et al.* 1985) and reaches a higher biomass within a shorter period of time (Viljoen & Reinecke in press). Although this worm species might not have such an extended growth period and long life span as is the case with, for example, *E. fetida* (Venter & Reinecke 1988), the fast growth rate early in the life-cycle and high mean biomass reached at this stage could give it a clear advantage over other vermicomposting species as a potential protein producer. Its true potential will, however, depend on its ability to tolerate various environmental conditions.

Maturation

Reared under the conditions mentioned, the worms attained sexual maturity within 45 days after hatching. This is faster than is the case with *E. fetida* which becomes sexually mature after 60 days (Venter & Reinecke 1988), seven to eight weeks (Edwards, Burrows, Fletcher & Jones 1984) and 10 weeks (Neuhauser *et al.* 1979). It is also faster than *D. veneta* which matures after 10 weeks (Loehr *et al.* 1985).

According to Neuhauser *et al.* (1979) density determines sexual maturation. Mba (1978) indicated that about 3 g body weight is the minimum weight limit required by a worm to attain sexual maturity.

Cocoon production

Eudrilus eugeniae maintained a mean cocoon production rate of 1,3 cocoons per worm per day over the whole study period.

Fluctuations in the production rate of cocoons occurred quite often (Figure 3). This could be ascribed to the fluctuation in the quality of the food provided at various stages of the study. It seems that ageing of the worms did have a very marked influence on the cocoon production rate only at the end of the active life span, from about the age of 270 days, when the cocoon production rate lowered dramatically and ceased after Day 300.

Hatching

The hatching success of 84% obtained for cocoons of *E. eugeniae* during this study was favourable and the incubation period (16,6 days) (Figure 4) was shorter than that of other commercial species (Reinecke & Kriel 1981; Venter & Reinecke 1988).

A mean number of 2,7 hatchlings per cocoon that hatched was obtained during the present study. This confirms the finding of Knieriemen (1984) and is close to that of Loehr *et al.* (1985).

In comparison with other vermicomposting species, *E. eugeniae* had a high fecundity in this study in terms of number of hatchlings. *Eisenia fetida* had 3,5 (Graff 1982); 3,8 (Loehr *et al.* 1985); 2,7 (Venter & Reinecke 1988) hatchlings per cocoon. *Dendrobaena veneta* had only 1,1; *Perionyx excavatus* had only 1,1 and *Pheretima hawayana* had 1,2 hatchlings per cocoon (Loehr *et al.* 1985).

Life-cycle and vermicomposting potential

From the above data it is clear the *Eudrilus eugeniae* has a fairly short life-cycle with a high reproductive rate when reared on cattle manure. This life-cycle is rendered diagrammatically in Figure 6.

The duration of the life-cycle as described here, as well as the survival of the worms will of course be dependent on the various abiotic factors and the availability and quality of food. From our results it seems that cattle manure is a very favourable source of food for *E. eugeniae*. This was supported by results obtained by Neuhauser *et al.* (1979), Loehr *et al.* (1985) and Mba (1978, 1983).

When comparing the potential of *E. eugeniae* as waste processor and protein producer to that of other vermicomposting species, taking into consideration its overall reproductive capacity and growth potential, the results are encouraging. Loehr *et al.* (1985) who

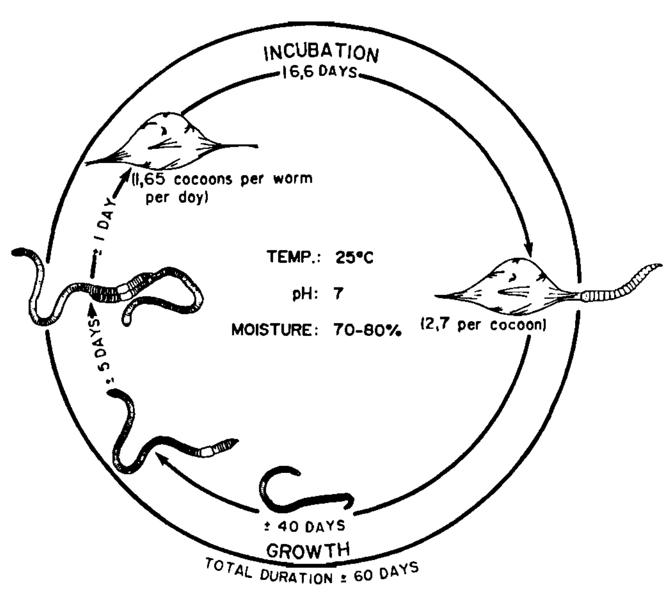

Figure 6 Diagram of the life-cycle of *E. eugeniae* at 25°C in cattle manure with a moisture content of 70–80%.

compared this capacity for five worm species found *E. fetida* to be the most appropriate species to use for the above purposes. This study was, however, done with sludge as medium.

It seems that the utilization of *E. eugeniae* as alternative vermicomposting species will highly depend on its temperature tolerances and not so much on its growth rate and reproductive capacity. In-depth studies of the temperature requirements of this species are needed to establish whether it could be utilized under different climatic conditions to the same extent as *E. fetida*.

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