

## Growth and reproduction of *Pontoscolex corethrurus* (Muller) with different experimental diets

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**Abstract:** Microcosm laboratory experiments were employed to evaluate the effects of different food substrates (collected in and around rubber plantation sites) on the growth and reproduction of the tropical endogeic earthworm *Pontoscolex corethrurus*. Decomposed rubber leaf litter (*Hevea brasiliensis*), rubber saw dust, bamboo leaf litter (*Bambusa balcooa*) and cow dung were tested in four mixtures that included mineral soil as the main substrate. One pair of young, non-clitellated earthworms per pot ( $n = 3$ ) was used (in a completely randomized experimental design) to test the effects of the following treatments (dry weight proportions in brackets): (1) soil-cow manure (30:2; S<sub>0</sub>); (2) soil-cow manure-rubber leaf litter (30:1:1; S<sub>1</sub>); (3) soil-cow manure-rubber saw-dust (30:1:1; S<sub>2</sub>); and (4) soil-cow manure-bamboo leaf litter (30:1:1; S<sub>3</sub>). Weight and juvenile production were recorded every 15 days over a period of 105 days. Maximum weight for *P. corethrurus* was observed in S<sub>3</sub>, whereas minimum weight was observed in the S<sub>0</sub> treatment. The highest and lowest growth rates were recorded in S<sub>2</sub> (21.14 mg worm<sup>-1</sup> day<sup>-1</sup>) and S<sub>0</sub> (13.36 mg worm<sup>-1</sup> day<sup>-1</sup>), respectively, and the difference in growth rates was significant ( $P < 0.05$ ). The highest and lowest rates of reproduction were obtained in S<sub>2</sub> (1.21 juveniles adult<sup>-1</sup> week<sup>-1</sup>) and S<sub>0</sub> (0.35 juveniles adult<sup>-1</sup> week<sup>-1</sup>), respectively, and this difference was also significant ( $P < 0.05$ ). In order of food preference, growth and reproduction of *P. corethrurus* in different experimental diets followed the order: S<sub>0</sub> < S<sub>1</sub> < S<sub>3</sub> < S<sub>2</sub>. We conclude that in order to maintain a successful field population of this earthworm, it is necessary to add suitable bulking agents such as saw-dust or bamboo leaf litter to the soil.

**Resumen:** Por medio de experimentos de microcosmos de laboratorio se evaluaron los efectos de diferentes sustratos alimenticios (obtenidos en y alrededor de plantaciones de caucho) en el crecimiento y la reproducción de la lombriz de tierra endógena tropical *Pontoscolex corethrurus*. Se probaron hojarasca descompuesta de caucho (*Hevea brasiliensis*), aserrín de caucho, hojarasca de bambú (*Bambusa balcooa*) y estiércol de vaca en cuatro mezclas que incluyeron suelo mineral como sustrato principal. Se utilizó un par lombrices de tierra jóvenes no cliteladas por maceta ( $n = 3$ ) (en un diseño experimental completamente aleatorio) para probar los efectos de los siguientes tratamientos (proporciones en peso seco entre paréntesis): (1) suelo-estiércol de vaca (30:2; S<sub>0</sub>), (2) suelo-estiércol de vaca-hojarasca de caucho (30:1:1; S<sub>1</sub>), (3) suelo-estiércol de vaca-aserrín de caucho (30:1:1; S<sub>2</sub>); y (4) suelo-estiércol de vaca-hojarasca de bambú (30:1:1; S<sub>3</sub>). El peso y la producción de individuos jóvenes fueron registrados cada 15 días durante un período de 105 días. El peso máximo para *P. corethrurus* fue observado en S<sub>3</sub>, mientras que el peso mínimo se observó en el tratamiento S<sub>0</sub>. Las tasas de crecimiento más altas y más bajas se registraron en S<sub>2</sub> (21.14 mg lombriz<sup>-1</sup> día<sup>-1</sup>) y S<sub>0</sub> (13.36 mg lombriz<sup>-1</sup> día<sup>-1</sup>), respectivamente, y la diferencia en las tasas de crecimiento fue significativa ( $P < 0.05$ ). Las tasas reproductivas más alta y más bajase obtuvieron en S<sub>2</sub> (1.21 jóvenes adulto<sup>-1</sup> semana<sup>-1</sup>) y S<sub>0</sub>

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(0.35 jóvenes adulto<sup>-1</sup> semana<sup>-1</sup>), respectivamente, y esta diferencia también fue significativa ( $P < 0.05$ ). En términos de preferencia de alimentos, el crecimiento y la reproducción de *P. corethrurus* con las diferentes dietas experimentales siguieron el orden:  $S_0 < S_1 < S_3 < S_2$ . Concluimos que para mantener una población de campo exitosa de esta lombriz de tierra es necesario añadir al suelo agentes adecuados que proporcionen volumen, tales como el aserrín o la hojarasca de bambú.

**Resumo:** As experiências no laboratório microcosmo foram utilizadas para avaliar os efeitos de diferentes substratos alimentares (coletados em e em torno de locais de plantação de seringueiras) sobre o crescimento e reprodução da minhoca endogénica tropical *Pontoscolex corethrurus*. Folhada de compostagem de seringueira (*Hevea brasiliensis*), serradura de madeira de seringueira, folhada de bambu (*Bambusa balcooa*) e estrume de vaca foram testados em quatro misturas que incluíamos solo mineral como o principal substrato. Usou-se um par de minhocas jovens, desprovidas de clitelo, por vaso ( $n = 3$ ) (tendo-se usado um delineamento experimental inteiramente casualizado), para testar os efeitos dos seguintes tratamentos (proporções em peso seco em parênteses): (1) solo- estrume vaca (30:2;  $S_0$ ); (2) solo- estrume de vaca-folhada de seringueira (30:1:1;  $S_1$ ); (3) solo-estrume de vaca-serradura de madeira de seringueira (30:1:1;  $S_2$ ); e (4) solo-estrume de vaca-folhada de bambu (30:1:1;  $S_3$ ). O peso e a produção de juvenis foram registados a cada 15 dias durante um período de 105 dias. O peso máximo para *P. corethrurus* foi obtido para  $S_3$ , enquanto que o peso mínimo foi observada no tratamento  $S_0$ . As taxas de crescimento mais elevadas e as mais baixas foram registadas em  $S_2$  (21,14 mg de verme<sup>-1</sup> dia<sup>-1</sup>) e  $S_0$  (13,36 mg de verme<sup>-1</sup> dia<sup>-1</sup>), respectivamente, sendo significativa a diferença nas taxas de crescimento ( $P < 0,05$ ). As taxas mais elevadas e as mais baixas de reprodução, foram obtidas em  $S_2$  (1,21 juvenis adulto<sup>-1</sup> semana<sup>-1</sup>) e  $S_0$  (0,35 juvenis adulto<sup>-1</sup> semana<sup>-1</sup>), respectivamente, sendo esta diferença significativa ( $P < 0,05$ ). A ordem de preferência alimentar, crescimento e reprodução de *P. Corethrurus* sob diferentes dietas experimentais foi a seguinte:  $S_0 < S_1 < S_3 < S_2$ . Conclui-se que, a fim de manter uma população de campo bem sucedida desta minhoca, é necessário adicionar ao solo agentes grosseiros apropriados, tais como serradura ou folhada de bambu.

**Key words:** Bamboo leaf litter, growth rate, *Pontoscolex corethrurus*, reproduction rate, rubber saw-dust.

## Introduction

*Pontoscolex corethrurus* (Muller) is the most widely distributed earthworm species in the world, with its ubiquitous occurrence in the soils of rubber plantations of Malaysia, Burma and south and north-east India (Chaudhuri *et al.* 2008; Gates 1972; Julka & Paliwal 2005). Large scale introduction of para rubber in the 19th century into south-east Asia may have accelerated the expansion of its range (Shen & Yeo 2005). Environmental plasticity, a parthenogenetic mode of reproduction, anthropogenic influences, efficient assimilation of low-quality soil, organic matter and an outstanding ability to colonize due to its demographic profile could help to explain the successful invasion of *P. corethrurus* in the tropics (Fragoso *et al.* 1999).

Literature is available on the growth and

reproduction of vermicomposting earthworms (Chaudhuri & Bhattacharjee 2002; Chaudhuri *et al.* 2003; Dominguez *et al.* 2001; Edwards *et al.* 1998; Elvira *et al.* 1996; Haimi 1990; Karmegam & Daniel 2009; Suthar 2009), but information is scant on the growth and reproduction in soil-dwelling earthworms (Butt 1997; Garcia & Fragoso 2003; Lowe & Butt 2005; Monroy *et al.* 2007), which are considered “ecosystem engineers” (Lavelle *et al.* 1998). Inoculating with appropriate species of earthworms to promote soil health is now regarded as an important step towards restoration of degraded soils (Butt *et al.* 1995; Pashanasi *et al.* 1992; Tapia-Coral *et al.* 2006). On the basis of laboratory performance of *P. corethrurus* and *Amyntas corticis* using Mexican soils degraded by the cement industry, Garcia & Fragoso (2002) concluded that both species have potential for soil rehabilitation. Production of

sustainable field populations may be accomplished through mass rearing of the required species under controlled conditions.

*P. corethrurus* is an endogeic earthworm and an efficient decomposer of organic matter, enhancing the nitrogen mineralization process. It is a common species used in managed ecosystems with anthropogenic practices (Tapia-Coral *et al.* 2006). Moreover, this species has an advantage over many native species, which are difficult to collect from the field and culture under laboratory conditions. Lavelle *et al.* (1987) suggested that this worm might also be utilized as a source of protein, since it can convert low-quality soil organic matter to fresh tissue with a 60 - 70 % protein content with great efficiency. In New Guinea, Rose & Wood (1980) reported that *P. corethrurus* has become an indispensable component in the diet of pigs after its introduction in the early 1960s.

It is well known that food sources influence not only size of an earthworm population but also their growth and reproduction rates (Dominguez *et al.* 2000). Since earthworms often constitute an important component of agricultural foodwebs, knowledge of their feeding preferences is to understand the carbon and nutrient cycling in agroecosystems, (Amador *et al.* 2013; Dabral *et al.* 2013). In the present paper we investigated the effect of different types of organic residues found in and around rubber plantation sites (rubber leaf litter, rubber wood saw dust, bamboo leaf litter and cow dung) on the growth and reproduction of *P. corethrurus*. The basis for selection of these residues is as follows:

- Cow dung is a recognized food additive for all earthworms irrespective of their ecological categories (Lowe & Butt 2005; Nath & Chaudhuri 2012).
- Better growth and reproduction of epigeic earthworms (e.g., *Eisenia fetida*, *Eudrilus eugeniae*) and endogeic earthworm (e.g., *P. corethrurus*) in treatments with rubber (*Hevea brasiliensis*) leaves were reported by Chaudhuri *et al.* (2003) and Araujo *et al.* (2004) respectively.
- Massive production of *P. corethrurus* (1.6 - 2.8 kg live earthworms m<sup>-2</sup>) in culture beds containing wood residues (saw dust) was reported by Lavelle *et al.* (1998).
- The rate of growth and reproduction of *Perionyx excavatus* in cow dung - bamboo leaf litter diet was significantly higher than that in cow dung alone (Chaudhuri & Bhattacharjee 2002).

## Materials and methods

The experiment was performed with non-clitellated young individuals of *P. corethrurus* collected from the soils of rubber plantations at Anandanagar, Agartala, West Tripura (22°51' - 24°32' N and 90°10' - 92°21' E). Earthworms were acclimated under laboratory conditions for two weeks. Growth and reproduction of *P. corethrurus* was tested in four different substrates that were elaborated by mixing sandy loam soil (sand, 70.4 %; silt, 13.2 %; clay, 16.4 %; organic C, 1.17 %; available nitrogen, 0.01 %) of rubber plantation with four types of organic substances as earthworm feed: cow manure, rubber leaf litter (*Hevea brasiliensis*), rubber saw-dust and bamboo leaf litter (*Bambusa balcooa*). Freshly abscised leaves of rubber tree and bamboo were collected during the peak period of litter fall (February – March 2008) from the plantation floor. Rubber wood saw-dust and cow manure were obtained from a local saw mill and a cow shed, respectively, in the neighborhood of Anandanagar rubber plantation area. The chemical composition of the different food additives used in the present study are as follows:

- Cow dung: N, 1.5 %; P, 0.9 %; K, 1.2 %; C/N 18.0 (Karmegam & Daniel 2009)
- Bamboo leaf litter: N, 0.98 %; P, 0.078 %; K, 0.45 %; C, 34.6 %; C/N, 35.3 (Nath & Das 2011)
- Rubber leaf litter: N, 2.31 %; P, 0.12 %; K, 0.83 %; C, 52.68 %; C/N, 22.75 (Chaudhuri *et al.* 2003).

As *P. corethrurus* is adapted to feed on soil with almost totally decomposed residues, and litter and timber are low-quality residues with medium to slow decomposition rates (Garcia & Frago 2003), leaf litters (bamboo and rubber) and saw-dust were allowed to decompose aerobically under field condition for six months before use, whereas cow dung, because of its higher decomposition rate, was allowed to decompose for two months. The materials used as earthworm feed in the experiment were dried at 60 °C, ground and sieved (< 2 mm). All of the experimental diets included mineral soil as the main substrate. The chosen materials were mixed with soils in two different proportions to provide four treatments (dry weight proportions in brackets): (1) soil-cow manure (30:2; S<sub>0</sub>); (2) soil-cow manure-rubber leaf litter (30:1:1; S<sub>1</sub>); (3) soil-cow manure-rubber wood saw-dust (30:1:1; S<sub>2</sub>); and (4) soil-cow manure-bamboo leaf litter (30:1:1; S<sub>3</sub>). Chemical analyses of these diet mixtures were performed following standard

methodologies (Upadhyay & Sharma 2001). In spite of its abundance under rubber plantations, preliminary experiments showed that *P. corethrurus* was unable to grow and reproduce well when raised in either soil-rubber saw-dust or soil-rubber leaf litter mixtures. Thus cow manure was introduced to all the treatments to improve the quality of culture media, as suggested by Lowe & Butt (2005).

In each treatment ( $n = 3$ ) one pair of young, non-clitellated, *P. corethrurus* of similar weight (400 mg live weight each) and age group was introduced randomly per plastic pot ( $15 \times 15 \times 8$  cm) containing 1600 g substrate. The culture pots were kept in a BOD incubator (REMI, India) at a temperature of 27 °C. A 25 % moisture content was maintained in the substrate by re-wetting as necessary. The worms were not supplied with additional food during the experiment. The experiment was terminated on the 105th day, after a decrease in weight was noticed in all the treatments on day 90. Earthworm biomass (live weight including gut contents) of individual worms and number of juvenile worms were recorded every 15 days. The rate of growth (mg weight gained worm<sup>-1</sup> day<sup>-1</sup>) and the rate of reproduction (juveniles worm<sup>-1</sup> week<sup>-1</sup>) were calculated by using the following formulae:

$$\text{Rate of growth} = \frac{\text{Maximum worm weight} - \text{Initial worm weight}}{\text{No. of days to attain maximum weight}}$$

$$\text{Rate of reproduction} = \frac{\text{Number of juveniles}}{\text{No. of adults at '0' day} \times \text{Total no. of days to attain juvenile population}} \times 7$$

Since not all earthworm cocoons are viable, and there may be more than one juvenile produced per cocoon of *P. corethrurus* (Bhattacharjee & Chaudhuri 2002), the mean of live worms obtained per cocoon is considered a reliable measure of the rate of reproduction of this species.

Student's *t*-test was applied to determine differences the number of days allowing for a significant increase in body weight (compared to the initial day). Maximum weights, growth rates and fecundity rates were compared among treatments by one way ANOVA.

## Results

Table 1 shows the major physico-chemical characteristics of the soil mixed with the selected

organic residues. C/N ratio was highest in S<sub>1</sub> and lowest in S<sub>2</sub>, whereas N content was highest in S<sub>2</sub> and lowest in S<sub>0</sub>. In first 15 days, worms became clitellated in all treatments except in S<sub>0</sub>, where they became clitellated 15 days later.

### Growth

In the first 15 days, biomass of *P. corethrurus* increased significantly ( $P < 0.05$ ) in all treatments. Among the four treatments, the biomass value for *P. corethrurus* was highest (1520 mg worm<sup>-1</sup>) in S<sub>3</sub> after 75 days (Table 2). Moreover, S<sub>3</sub> had significantly higher ( $P < 0.05$ ) biomass values than those of S<sub>0</sub>, S<sub>1</sub> and S<sub>2</sub> (Table 2). Although S<sub>0</sub> had the lowest peak biomass, it maintained a positive growth rate along with S<sub>3</sub> until the 75th day, with growth declining thereafter in this treatment (Table 2). Earthworms in treatments S<sub>1</sub> and S<sub>2</sub> gained less weight (1128 mg and 1268 mg, respectively) than S<sub>3</sub>, although their biomass peak appeared 15 days earlier than in the latter.

The highest growth rate was achieved in S<sub>2</sub> (21.14 mg worm<sup>-1</sup> day<sup>-1</sup>; Table 2), although this value was not significantly different ( $P > 0.05$ ) from that for S<sub>1</sub> and S<sub>3</sub>. The lowest growth rate was recorded in S<sub>0</sub> (13.36 mg worm<sup>-1</sup> day<sup>-1</sup>), which was significantly lower ( $P < 0.05$ ) than for all other treatments. The mean growth rate of *P. corethrurus* (considering all treatments) during the pre-reproductive phase (prior to the cocoon deposition period) was  $24.30 \pm 2.42$  mg worm<sup>-1</sup> day<sup>-1</sup>, which was significantly higher ( $P < 0.01$ ) than that during the reproductive phase ( $1.03 \pm 0.68$  mg worm<sup>-1</sup> day<sup>-1</sup>). The general growth rate on different diets followed the order: S<sub>0</sub> < S<sub>1</sub> < S<sub>3</sub> < S<sub>2</sub>.

### Reproduction

In S<sub>1</sub> and S<sub>2</sub> signs of reproduction were noticed on the 30th day with the appearance of cocoons, whereas in the S<sub>0</sub> and S<sub>3</sub> treatments, cocoons were first observed on the 45th day. Juveniles appeared in S<sub>1</sub> and S<sub>2</sub> on the 45th day, and on the 60th day in S<sub>3</sub> (Table 3). A decline in biomass on the 90th day was followed by a juvenile peak on 105th day for all the treatments (Tables 2 & 3). Among different diet mixtures, the highest and the lowest rate of reproduction (juveniles adult<sup>-1</sup> week<sup>-1</sup>) were recorded in S<sub>2</sub> ( $1.21 \pm 0.08$ ) and S<sub>0</sub> ( $0.35 \pm 0.13$ ), respectively (Table 3). The rate of reproduction in S<sub>2</sub> did not differ significantly ( $P > 0.05$ ) from that in S<sub>3</sub>, but was significantly higher ( $P < 0.05$ ) than that in S<sub>0</sub> and S<sub>1</sub>. The fecundity rate in different diets followed the order: S<sub>0</sub> < S<sub>1</sub> < S<sub>3</sub> < S<sub>2</sub>.

**Table 1.** Selected initial chemical properties of the experimental diets.

| Properties    | Experimental diets |                |                |                |
|---------------|--------------------|----------------|----------------|----------------|
|               | S <sub>0</sub>     | S <sub>1</sub> | S <sub>2</sub> | S <sub>3</sub> |
| pH            | 5.70 ± 0.02        | 5.43 ± 0.01    | 5.45 ± 0.0     | 5.4 ± 0.01     |
| Organic C (%) | 2.56 ± 0.07        | 4.00 ± 0.14    | 3.48 ± 0.03    | 3.26 ± 0.04    |
| Total N (%)   | 0.11 ± 0.01        | 0.16 ± 0.01    | 0.18 ± 0.01    | 0.15 ± 0.01    |
| C/N ratio     | 24.23 ± 1.39       | 24.51 ± 0.49   | 19.37 ± 0.54   | 22.36 ± 1.12   |

Values are mean of three replicates ± standard error; S<sub>0</sub> – soil-cow manure (control), S<sub>1</sub> – soil-cow manure-rubber leaf, S<sub>2</sub> – soil-cow manure-rubber sawdust, S<sub>3</sub> – soil-cow manure-bamboo leaf.

**Table 2.** Changes in biomass and rate of biomass production of *P. corethrurus* fed with different experimental diets.

| Diets          | Biomass (mg fresh weight) |           |            |            |             |             |            |            | Rate of biomass production (mg worm <sup>-1</sup> day <sup>-1</sup> ) |
|----------------|---------------------------|-----------|------------|------------|-------------|-------------|------------|------------|---|
|                | Days 0                    | 15        | 30         | 45         | 60          | 75          | 90         | 105        |   |
| S <sub>0</sub> | 400±0.29                  | 684±24.94 | 811±29.08  | 1099± 9.06 | 1265±20.68  | 1402*±21.99 | 1245±29.46 | 1203±21.80 | 13.36 <sup>a</sup> ±0.54  |
| S <sub>1</sub> | 400±0.58                  | 874±22.65 | 1125±28.75 | 1291±35.32 | 1528*±33.84 | 1465±34.13  | 1308±42.67 | 1150±45.00 | 18.81 <sup>b</sup> ±0.71  |
| S <sub>2</sub> | 400±1.26                  | 840±18.94 | 1244±31.55 | 1471±32.83 | 1668*±28.73 | 1558±38.93  | 1443±28.33 | 1227±33.72 | 21.14 <sup>b</sup> ±0.84  |
| S <sub>3</sub> | 400±0.76                  | 917±25.33 | 1162±38.96 | 1495±41.98 | 1692±22.07  | 1920*±27.95 | 1700±30.55 | 1405±22.51 | 20.27 <sup>b</sup> ±0.68  |

Values are mean of three replicates ± standard error; \*biomass peak; Values followed by the same letter are not significantly different (*P* > 0.05). S<sub>0</sub> - soil-cow manure (control), S<sub>1</sub> - soil-cow manure-rubber leaf, S<sub>2</sub> - soil-cow manure-rubber sawdust, S<sub>3</sub> - soil-cow manure-bamboo leaf.

**Table 3.** Number of adults and juveniles and reproduction rate of *P. corethrurus* fed with different experimental diets.

| Diets          |        |     |     |             |             |              |              |               |                           | Rate of reproduction (No. of juveniles adult <sup>-1</sup> week <sup>-1</sup> ) |
|----------------|--------|-----|-----|-------------|-------------|--------------|--------------|---------------|---------------------------|---|
|                | Days 0 | 15  | 30  | 45          | 60          | 75           | 90           | 105           |                           |   |
|                | A J    | A J | A J | A J         | A J         | A J          | A J          | A J           | A J                       |   |
| S <sub>0</sub> | 2 0    | 2 0 | 2 0 | 2 0         | 2 0         | 2 2.67±1.4   | 2 8±2.23     | 2 10.67*±2.52 | 2 0.35 <sup>a</sup> ±0.10 |   |
| S <sub>1</sub> | 2 0    | 2 0 | 2 0 | 2 0.67±0.67 | 2 3±1.52    | 2 7.67±2.24  | 2 12.67±3.18 | 2 14.33*±2.85 | 2 0.48 <sup>a</sup> ±0.09 |   |
| S <sub>2</sub> | 2 0    | 2 0 | 2 0 | 2 0.33±0.33 | 2 6.67±1.36 | 2 19.33±2.33 | 2 26.33±0.67 | 2 36.33*±2.40 | 2 1.21 <sup>b</sup> ±0.07 |   |
| S <sub>3</sub> | 2 0    | 2 0 | 2 0 | 2 0         | 2 1±0.58    | 2 7.67±1.20  | 2 15±2.65    | 2 28.33*±3.11 | 2 0.94 <sup>b</sup> ±0.08 |   |

Values are mean of three replicates ± standard error; A - Adult, J - Juvenile; \*juvenile peak; Values followed by the same letter are not significantly different (*P* > 0.05). S<sub>0</sub> - soil-cow manure (control), S<sub>1</sub> - soil-cow manure-rubber leaf, S<sub>2</sub> - soil-cow manure-rubber sawdust, S<sub>3</sub> - soil-cow manure-bamboo leaf.

### Discussion

Sexual maturity of *P. corethrurus* was attained on the 15th day of experimentation with the development of clitellum in all the diet mixtures except in the mixture with only cowdung (S<sub>0</sub>) where the clitellum appeared on the 30th day. This

suggests the nutritional superiority of all other treatments (S<sub>1</sub> - S<sub>3</sub>) over the cow dung feed substrate (S<sub>0</sub>). According to Bohlen (2002), a nutritionally superior feed substrate shortens the time to sexual maturity in animals. Positive growth rates for 60 - 75 days and a steady increase in reproduction rates up to the 105th day were main-

tained in these treatments. Appearance of the biomass peak earlier than the reproduction peak in the treatments, irrespective of the type of diet, probably indicates that earthworms begin their reproduction after attainment of a certain level of biomass. A significantly higher growth rate during the pre-reproductive phase compared to the reproductive phase indicates expenditure of energy at the cost of reproduction. A rapid pre-reproductive phase of growth, followed by a phase of steadily decreasing growth once sexual maturity was attained, was also reported for earthworms by Edwards & Bohlen (1996) and Monroy *et al.* (2007).

The highest growth rate of *P. corethrurus* was observed in S<sub>2</sub>, which had the highest N content and the lowest C:N ratio. By contrast, the lowest growth rate for this species was observed in S<sub>0</sub>, which had the lowest N content and a higher C:N ratio. Several studies have shown that the levels of polyphenols (Edwards & Bohlen 1996) and the lignin : N ratio (Tian *et al.* 1993) are negatively correlated with abundance of earthworm populations. Some phenolic compounds, especially the toxic phenyl hydrazine, are present in rubber plant material (Stern 1967). As the food substrates for *P. corethrurus* were prepared by mixing decomposed food materials viz. rubber leaf litter, rubber saw-dust, bamboo leaf litter and cow dung along with mineral soil as the main substrate, the treatments on the initial day had a low C:N ratio (close to 20) and possibly low polyphenol and lignin contents, which may have increased the acceptability of food substrates to this geophagous species. In spite of the low organic C and N contents of the food substrates, sustained growth and reproduction of *P. corethrurus* were observed in all the treatments, likely due to three factors: (i) the food was supplied in excess, (ii) rubber leaf litter, rubber saw-dust and bamboo leaf litter are long-term N release residues, and (iii) *P. corethrurus* has the capacity to use soil organic carbon efficiently (even at low concentrations) due to its microflora-associated mutualistic digestive system (Lavelle *et al.* 1987). *P. corethrurus* in the treatment with rubber leaf litter (S<sub>1</sub>) had a high rate of biomass production but the lowest rate of reproduction. Similarly Chaudhuri & Bhattacharjee (2002) reported a very low rate of reproduction in spite of a high biomass production for the composting worm, *Perionyx excavatus*, cultured in kitchen waste mixtures. This suggests that a medium that supports good biomass production is not necessarily a good medium for reproduction (Dominguez *et al.* 1997; Haimi 1990).

Although Lowe & Butt (2005) observed better growth and reproduction of the endogeic earthworms, *Allolobophora chlorotica* and *Aporrectodea caliginosa*, using cattle dung as a food additive, in the present study, a low rate of growth and reproduction in *P. corethrurus* was observed in the treatment with cow dung compared to the other treatments. This might be because the preferred culture medium may vary between earthworm species (Chaudhuri *et al.* 2003). The growth rate in the present study (13.36 - 21.14 mg worm<sup>-1</sup> day<sup>-1</sup>) was much higher than the growth rates of *Drawida nepalensis* (10.6 - 10.8 mg worm<sup>-1</sup> day<sup>-1</sup>) in oak or pine litter (Kaushal *et al.* 1995) and *P. corethrurus* (3.0 - 13.6 mg worm<sup>-1</sup> day<sup>-1</sup>) in different experimental diets (Garcia & Fragoso 2003). We note that *Drawida nepalensis* and *P. corethrurus* have similar body size and belong to the same ecological category. Interestingly, as in the present study, Garcia & Fragoso (2003) also reported the highest growth rate of *P. corethrurus* when saw-dust was included in the diet. Better performance of *P. corethrurus* on mixtures that included rubber saw-dust and bamboo leaf litter is probably related to the activity of cellulolytic and lignolytic microflora in earthworm-worked soil and its gut-associated processes (Kale 1998; Zhang *et al.* 1993).

Considering that *P. corethrurus* is a continuous breeder with 91 % hatching success and 1 hatchling/cocoon (Chaudhuri & Bhattacharjee 2011), a reproductive rate ranging from 18.25 - 63.1 cocoons adult<sup>-1</sup> year<sup>-1</sup> (converting the value of 0.35 - 1.21 juveniles adult<sup>-1</sup> week<sup>-1</sup>) falls within the reported ranges of 13.0 - 99.0 cocoons adult<sup>-1</sup> year<sup>-1</sup> (Garcia & Fragoso 2002, 2003; Lavelle *et al.* 1987). Interestingly, the reproductive rate of *P. corethrurus* on a diet comprised partly of saw-dust (1.21 juveniles adult<sup>-1</sup> week<sup>-1</sup>) is close to that of the composting worms *E. fetida* (1.3 juveniles adult<sup>-1</sup> week<sup>-1</sup>) and *E. eugeniae* (1.4 juveniles adult<sup>-1</sup> week<sup>-1</sup>), and higher than that of *P. excavatus* (0.2 juveniles adult<sup>-1</sup> week<sup>-1</sup>) on a diet containing rubber leaf litter diet (Chaudhuri *et al.* 2003). The bamboo leaf litter diet, which produced a growth rate of *P. corethrurus* that was not significantly lower than for rubber saw-dust, might be considered as a second food of choice for this species. Interestingly in the bamboo leaf litter diet, *P. excavatus* also showed very high rate of growth and reproduction (Chaudhuri & Bhattacharjee 2002). High growth and reproduction rates for *P. corethrurus* with rubber wood saw-dust and bamboo leaf litter diets indicates that these organic residues are more suitable for mass

culture of this species compared to cow dung. Similar results relating to mass production of *Lampito mauritii* and *P. corethrurus* in culture beds with saw dust as feed substrate were reported by Ismail (1997) and Lavelle *et al.* (1998).

In spite of the fact that *P. corethrurus* is endogeic and geophagous, its demographic profile is typically of the r-type (Lavelle *et al.* 1987), as reflected in its high rate of growth and reproduction in rubber residues, accounting for the fact that it constitutes more than 61 % of the biomass and 72 % of the population densities of earthworm communities in rubber plantations (Chaudhuri *et al.* 2008).

Our results suggest that soil amended with either cow dung-rubber saw-dust or cow dung-bamboo leaf litter diet mixtures is superior to cow dung alone for the culture of geophagous species like *P. corethrurus*.

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