Research Communication

Sci. and Cult. 85 (3–4) 105-112 (2019)

Bamboo (*Bambusa polymorpha*) Leaf Litter as a Vermiculture Substrate for Earthworms, *Pontoscolex corethrurus* (Muller) and *Drawida assamensis* Stephenson

Abstract **: Growth and reproduction of two endogeic earthworm species *Pontoscolex corethrurus* and *Drawida assamensis* were studied in different experimental diets [mineral soil(S), soil : cow manure (SC), soil : bamboo leaf litter (SL) and soil : cow manure : bamboo leaf litter (SCL)] in controlled laboratory conditions. Highest rate of growth and cocoon production were recorded in SL diets for both the species although the results were statistically significant (P<0.05) for *P. corethrurus* only. *P. corethrurus* also produced significantly more cocoons (P<0.05) than *D. assamensis*. The rate of reproduction in terms of juvenile produced (Juvenile worm^{-1}week^{-1}) in *D. assamensis* was however substantially higher in SL diet (P<0.05) compared to the other diets. In the worm worked soils of each earthworm species C/N ratio increased in mineral soils (S) and decreased in SCL diet with *P. corethrurus* and SL diet acted upon by *D. assamensis*.**

Keywords: **Vermiculture, endogeic earthworms, *Pontoscolex corethrurus*, *Drawida assamensis*, bamboo leaf litter, growth rate, reproduction rate, C/N ratio.**

Bamboo plantations in North-east India have important role in carbon storage and sequestration[1]. According to Don *et al.*[2] burrowing earthworms (endogeic and anecic) are involved in organic carbon sequestration in soils. Bamboo has widely been adopted as low cost vegetation for agriculture and small scale industry. Recently sixteen earthworm species in the soils of bamboo plantations of West Tripura (India) with dominance of endogeic earthworms, *Pontoscolex corethrurus* and *Drawida assamensis* with relative abundance of 30% and 25% respectively were recorded[3]. Size, biomass, density and frequency of the two species are very close to each other and they often coexist in the bamboo plantations[3]. While *P. corethrurus* is an exotic species in the rubber plantation[4], *D. assamensis*, being a native earthworm, is dominant in the pineapple plantations of Tripura[5]. Coexistence of both the species in greater density and biomass in the bamboo plantations, especially in *Bambusa polymorpha* (locally called '*Bari*' bamboo) indicated suitability of bamboo leaf litter for rearing of these two species. Bamboo leaf litter as vermiculture substrate for tropical earthworm had earlier been reported.[6] Recent studies on earthworm biology[7,8] also recognize cow dung as good nutritional supplement for earthworm species in general. The soil dwelling earthworms are well known as 'ecosystem engineers' for their feeding, burrowing and casting activities to improve the physicochemical properties of soils to support over ground vegetation and underground soil biota. Also because of high production efficiency (approx.37 to 42%) oligochaetes can be used as a source of animal protein for fish and poultry[9]. Recently Debnath *et al.*[10] reported earthworms as a good nutrient source of essential amino acids and essential fatty acids for animals for which they could be utilized as diet supplement. Suitable food additives for growth and reproduction of earthworm species are required for '*in-situ*' earthworm technology. Application of '*in-situ*' earthworm technology using *Pontoscolex corethrurus* in the soils of Sikalmudi tea gardens in South India led to dramatic increase in tea leaf production[11]. On the above ground the present authors aimed to study the comparative growth and reproductive biology of two dominant earthworm species of bamboo plantation using different diet combinations.

Material and Methods : Non clitellate healthy earthworms of *P. corethrurus* and *D. assamensis* species were collected from the soils of bamboo plantations (*Bambusa polymorpha*) from Mahesh khola, West Tripura during May 2017 and acclimated under laboratory conditions for one month. One month old cowdung manure and bamboo leaf litter (*Bambusa polymorpha*) were brought to the laboratory, dried for 15 days and ground and seived to the particle size of <1 mm. The chemical composition of the bamboo plantation soil and different food additives used in the present study are as follows: cow manure N 1.5%, P 0.9% , K 1.2%, C/N 18.0[12],

The journal is in the UGC approved list on broad subject category of Multidisciplinary, Science and Social Sciences.

bamboo leaf litter N 0.98%, P 0.078%, K 0.45%, C 34.6%, C/N 35.3[1], bamboo soil organic carbon (OC) 1.13%, total nitrogen (N) 0.105%, Av. N 0.014%, Av. K 0.011%. Air dried soils of bamboo plantations, ground bamboo leaves and cow dung manure (each ground separately by grinder machine) were mixed as follows to prepare the experimental diets.

1. Control soil (CS)

   Experimental:

2. Soil (S) + cow manure (C), 30:2 w/w (SC)
3. Soils (S) + bamboo leaf litter (L), 30:2 w/w (SL)
4. Soils (S) + bamboo leaf litter (L) + cow manure (C), 30:1:1 w/w (SCL)

In both control and experimental soils earthworms were introduced to study their growth, reproduction and their effects on the food substrates. Four replicas were kept for each diet combination.

A pair of young, non-clitellate earthworms of each species (*P. corethrurus and D. assamensis*) were introduced in each of the earthen pot (2.5L) containing 1600g of substrate of different diet combinations. Moisture of 30% (near to field level) was maintained by sprinkling of water at regular intervals. The experiment was continued for 150 days (June- October 2017) at room temperature varying from 25-30°C. Casting activities of earthworms were recorded during the experimental period. But casts ejected were not removed from the pots and the culture media were kept unchanged. Earthworms in each culture pot were weighted on fortnightly basis and the incubation media were thoroughly checked with hand lens to find out cocoons if any. The culture media, before introduction of the earthworms and after termination of the experiment were analyzed to study the changes if any in the contents of carbon and nitrogen and also the soil pH. The cocoons produced were separated from the culture media and kept in Petri-dishes under moistened filter paper to study their hatching characteristics. The growth rates (mg weight gained worm^{-1}day^{-1}), rate of reproduction in terms of cocoon produced (number of cocoons worm^{-1} week^{-1}) and in terms of juvenile produced (juveniles worm^{-1} week^{-1}) were calculated using the following formulae:

1. Rate of growth = Maximum worm weight-Initial worm weight/No. of days to attain maximum weight.
2. Rate at which cocoon were produced = Number of cocoons produced/No. of adults at '0'day × Total no. of days required to produce the cocoons × 7.
3. Rate of reproduction = Number of juveniles/No. of adults at '0'day × total no. of days to attain juvenile production × 7.

Significant differences ($p<0.05$) in the pH, organic carbon(OC) and total nitrogen (N) between the pre and post incubation media in control and experimental soil samples and inter-specific comparisons in growth, cocoon production and juvenile production were determined by t-tests. Two-way ANOVA followed by Tukey's multiple paired tests were employed to earmark the source of variations (among the substrates, among the species or interaction between substrate and species) in different biological parameters and also in the physicochemical parameters (pH, C and N content) of the earthworm worked substrates at 5% level of significance.

Results and Discussion : **Effects of Earthworm Species on Soils :** Table 1 shows the results of two-way ANOVA showing the effects of substrate, earthworm species and their interactions on different physicochemical parameters of the soils. Effect of the substrate type (F value- 3.407, P value- 0.043) and more importantly its

TABLE 1: Results of two-way ANOVA showing the effects of substrate and earthworm species and their interactions on different physicochemical parameters of the soils.

Parameter	Source of Variation					
	Among the substrates		Among the earthworm species		Due to interaction between the earthworm species and substrate	
	F-Value	P-Value	F- Value	P-Value	F- Value	P-Value
pH	3.407	**0.043**	0.972	0.338	5.349	**0.009**
Organic carbon	31.227	**0.00000006**	62.655	**0.00000006**	51.805	**0.000000001**
Total Nitrogen	1.072	0.388	0.057	0.814	4.686	**0.015**
C/N ratio	30.641	**0.00000007**	10.540	**0.005**	4.041	**0.025**

Values in ***bold*** *indicate statistically significant results at 5% level of significance.*

interaction with earthworm species (F value- 5.349, P value- 0.009) were the most important sources of variation for the alteration of pH in the cultures. Both diet combination, species concerned and interactive effects of diet and species played a very important role in altering the organic carbon of the earthworm worked soils (F values- 31.227, 62.655, 51.805; P values- 0.00000006, 0.00000006, 0.000000001 respectively). Greatest source of variation in C/N ratio was due to difference in diet combinations followed by the type of the earthworm species which worked on it (F value-30.641, P value-0.0000007; F-value-10.540, P value-0.005). Interactions between diet type and species were also statistically significant (F value-4.041, P value- 0.025).

Table 2 shows changes in pH, organic carbon contents (g %), total nitrogen (g %) and C/N ratio between the initial and final substrates acted upon by *P. corethrurus* and *D. assamensis* and also among the diets along with interspecific comparisons.

pH : In most of the substrates (S, SC, SL), due to activity of either *P. corethrurus* or *D. assamensis* pH was decreased which however was not significant (P>0.05). Significant decrease (P< 0.05) in pH of SCL was brought about only in presence of *P. corethrurus*. Lowering of pH in the worm-worked soils with or without food additive was due to mucus secreted by earthworms that had a "priming-effect" on microbial activity[13] and CO_2 and organic acid produced during microbial metabolism[14,15,16].

Organic carbon (OC) : The activities of the two earthworm species in terms of soil OC change on different experimental diets were different. In presence of native species, *D. assamensis*, OC contents were significantly increased (P< 0.05) in S (3.92 g %) and SCL (3.04 g %) but significantly decreased (P< 0.05) in SL (1.92 g %) compared to the initial soil samples(S 1.95 g%, SCL 2.04 g%, SL 2.69 g %). Exotic *P. corethrurus* worked SL had significant increase (P<.05) in OC content (1.15 g %) compared to that in *D. assamensis* worked SL (0.77 g %). In worm worked S of both earthworm species, OC content was increased but in case of native species *D. assamensis* the level of increase was significantly higher (P< 0.05) than that of *P. corethrurus*. Among the diets, while *D. assamensis* had a much better activity in increasing (P< 0.05) OC contents in all the diet combinations (S 1.97 g%, SC 0.62 g%, SL 0.77 g% and SCL 1 g%) compared to control soils (CS 0.06 g%), *P. corethrurus* increased OC contents significantly (P< 0.05) in soils only when bamboo

Table 2: Difference in pH, organic carbon content (g %), total nitrogen (g %) and C/N ratio between the initial and final soil samples worked by the two species of earthworms-*P. corethrurus* (P.C) and *D. assamensis* (D.A).

Substrate		pH			O.Carbon (Value±SE in g %)			T.Nitrogen (Value±SE in g %)			C/N-ratio		
		Initial	Final	Diff.	Initial	Final	Diff.	Initial	Final	Diff.	Initial	Final	Diff.
CS		4.73± 0.01(a)	4.78± 0.03(a)	0.06± 0.04^{a}	0.73± 0.07(a)	0.73± 0.12(a)	0.06± 0.03^{a}	0.15± 0.004(a)	0.13± 0.01(a)	0.02± 0.01^{a}	4.84± 0.69(a)	5.99± 1.77(a)	1.19± 1.05^{a}
S	P.C	4.74± 0.01(a)	4.68± 0.01(a)	0.06± 0.02^{a}(A)	1.08± 0.03(a)	1.45± 0.08(a)	0.37± 0.10^{a}(A)	0.24± 0.03(a)	0.10± 0.001(a)	0.13± 0.03^{b}(A)	4.64± 0.58(a)	14.5± 0.88(b)	9.85± 1.45^{b}(A)
	D.A	4.85± 0.09(a)	4.92± 0.04(a)	0.19± 0.003^{a}(B)	1.95± 0.05(a)	3.92± 0.04(b)	1.97± 0.05^{b}(B)	0.17± 0.004(a)	0.14± 0.009(a)	0.03± 0.012^{a}(A)	11.26± 0.49(a)	29.68± 2.12(b)	18.41± 2.39^{b}(B)
SC	P.C	5.47± 0.00(a)	5.39± 0.02(a)	0.08± 0.02^{a}(A)	1.54± 0.02(a)	1.35± 0.04(a)	0.19± 0.05^{a}(A)	0.23± 0.00(a)	0.17± 0.00(b)	0.05± 0.002^{ab}(A)	6.69±) 0.08(a	7.93± 0.26(a)	1.24± 0.30^{a}(A)
	D.A	5.60± 0.08(a)	5.54± 0.05(a)	0.19± 0.04^{a}(B)	2.30± 0.04(a)	2.42± 0.48(a)	0.62± 0.12^{c}(A)	0.27± 0.02(a)	0.23± 0.001(a)	0.04± 0.030^{a}(A)	8.58± 0.82(a)	10.91± 2.30(a)	3.75± 0.90^{a}(A)
SL	P.C	5.40± 0.00(a)	5.33± 0.06(a)	0.11± 0.02^{a}(A)	1.92± 0.04(a)	3.07± 0.02(b)	1.15± 0.02^{b}(A)	0.17± 0.01(a)	0.19± 0.008(a)	0.04± 0.0008^{a}(A)	11.36± 1.35(a)	16.5± 0.69(a)	5.14± 1.98^{ab}(A)
	D.A	5.37± 0.02(a)	5.37± 0.01(a)	0.03± 0.005^{a}(A)	2.69± 0.01(a)	1.92± 0.03(b)	0.77± 0.02^{cd}(B)	0.21± 0.01(a)	0.30± 0.04(a)	0.08± 0.03^{a}(A)	12.78± 1.02(a)	6.69± 0.93(b)	6.09± 0.55^{a}(B)
SCL	P.C	5.60± 0.02(a)	5.39± 0.02(b)	0.21± 0.04^{a}(A)	2.38± 0.11(a)	1.56± 0.18(b)	0.82± 0.09^{b}(A)	0.21± 0.02(a)	0.19± 0.008(a)	0.025± 0.018^{a}(A)	12.26± 2.03(a)	8.45± 1.23(b)	3.81± 0.82^{a}(A)
	D.A	5.73± 0.06(a)	5.72± 0.18(a)	0.14± 0.05^{a}(B)	2.04± 0.00(a)	3.04± 0.09(b)	1± 0.09^{d}(A)	0.23± 0.05(a)	0.25± 0.007(a)	0.07± 0.02^{a}(A)	10.18± 2.18(a)	12± 0.18(a)	4.03± 0.73^{a}(A)

CS-control soil S- soil without additives SC-soil with cow-manure SL-soil with bamboo leaf litter SCL-soil with cow-manure and bamboo leaf litter. Dissimilar alphabets (a, b) in brackets indicate statistically significant differences between the initial and final soil samples (paired sample t test). Dissimilar alphabets (a, b, c, d) in superscripts indicate statistically significant differences among the various diet combinations (Tukey's multiple paired tests). Dissimilar capital bold alphabets (A, B) indicate statistically significant differences between the two species (2-sample t test).

leaf litter was added (SL 1.15 g% and SCL 0.82 g%). Thus differential activities of the two earthworm species in different diets is related to their food preferences. Significant increase ($P< 0.05$) in OC content in the SL and SCL by *P. corethrurus* and in all the diets by *D. assamensis* was due to the preferential feeding of soil fractions enriched in organic compounds and also because of addition of intestinal mucus[17]. Significant increase in OC content of the soils following its passage through the gut of earthworm, *P. corethrurus* in SL and *D. assamensis* in S and SCL, enhanced stabilization of organic matter by accelerating its incorporation to organomineral complexes. In contrast, many workers[14,18,19] reported significant decrease in OC content in the worm worked substrates of some earthworm species because of their higher carbon assimilating efficiencies.

Nitrogen : N contents, in different diet substrates processed by both the species, were decreased in most of the cases which however were not statistically significant except in SC processed by *P. corethrurus*. In this substrate N content was significantly lower ($P< 0.05$) compared to initial substrate. Reduction of total nitrogen (N) in the earthworm processed substrate is probably due to NH_3 volatilization and incorporation into earthworm tissue [14,15].

C:N Ratio : *P. corethrurus* and *D. assamensis* significantly decreased ($P< 0.05$) C: N ratio in the SCL and SL respectively. The lowering of C: N ratios in SCL and SL diets in presence of earthworm species were probably achieved by the combustion of carbon substances during respiration[14]. Increase in C: N ratio in most of the experimental diets is in accordance with inability of earthworms to digest most of the plant materials rich in lignin they ingest and also due to assimilation of nitrogen from the diets to form body protein.

Growth and Reproduction in soils with Different Food Additives : Earthworms feed upon a wide variety of organic materials for food and can extract sufficient nourishment from organic matter and micro-organisms in soils to survive. The kind and amount of food available influences not only the size of earthworm population but also the species present and their growth and reproduction[17]. Table 3 shows the key biological parameters observed in *P.corethrurus* and *D.assamensis* in different experimental diets and statistical differences among them. Results of two-way ANOVA on the biological parameters shown in the table indicate that variation in the highest individual weight and weight gain in these species occurs due to the type of the substrate on which the earthworms were cultured (F values- 15.948, 13.682; P values- 0.0000006, 0.000005 respectively) . For parameters like rate of body weight increase, cocoons laid and juveniles produced, both types of substrates (F values- 12.424, 48.447 and 49.301 and P values- 0.000004, 0.00000000001 and 0.00000000002) and the earthworm species (F values- 10.415, 220.666, 25.313 and P values- 0.003, 0.000000000000013, 0.000003 respectively) were the source of variations. Interactive effects of both substrate type and earthworm species were also important source of variation for altering the rate of cocoons and juveniles produced (F values- 20.650, 15.879 and P values- 0.00000007, 0.0000006 respectively).

Growth : Depending upon the nutrient quality of diets, highest body weight was attained at different days in *P. corethrurus* and *D. assamensis*. In the nutrient poor soils(S) highest body weight achieved was significantly lower ($P< 0.05$) than that in soils with different food additives such as SC, SL and SCL in both the species except in-between *D. assamensis* processed S and SC diets where there was no significant difference ($P>0.05$) [Table-3]. Among the other diets (SC, SL, SCL) there was however no significant difference ($P>0.05$) in terms of highest individual weight or weight gain. This indicates that addition of organic matter in the form of either bamboo leaf litter or a combination of cow manure and bamboo

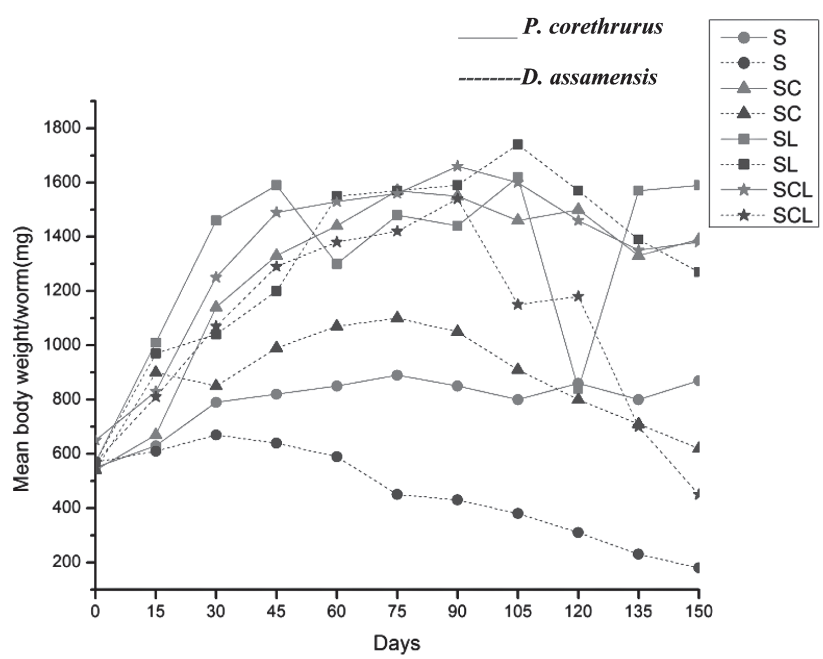

Figure 1: Graphical representation showing variation in mean body weight of *P. corethrurus* and *D. assamensis* at different time intervals with different diet combinations.

TABLE 3: key biological parameters observed in *P. corethrurus* (P.C) and *D. assamensis* (D.A) in different experimental diets and statistical differences among them.

Parameter		S	SC	SL	SCL	Among the substrates		Among the species		Interaction	
						F-Value	P-value	F-Value	P-value	F-Value	P-value
Initial Mean body weight(mg)	P.C	557.50± 54.21^{a}(A)	542.50± 29.54^{a}(A)	577.50± 16.00^{a}(A)	650.00± 22.73^{a}(A)	1.945	0.149	0.536	0.470	0.829	0.490
	D.A	572.50± 13.76^{a}(A)	542.50± 34.97^{a}(A)	572.50± 34.24^{a}(A)	577.50± 10.30^{a}(A)						
Highest individual weight(mg)	P.C	895± 17.07^{a}(A)	1575± 55.75^{b}(A)	1592.50± 176.27^{b}(A)	1662.50± 114.48^{b}(A)	15.948	0.0000006	2.681	0.114	1.612	0.212
	D.A	672.50± 37.94^{a}(B)	1105± 174.18^{ab}(B)	1740± 218.82^{b}(A)	1547.50± 187.63^{b}(A)						
Weight gain (mg)	P.C	337.50± 44.22^{a}(A)	1032.50± 45.16^{b}(A)	1015± 170.41^{b}(A)	1012.50± 113.97^{b}(A)	13.682	0.000005	2.005	0.169	1.596	0.216
	D.A	100± 51.15^{a}(B)	562.50± 197.28^{ab}(A)	1167.50± 228.77^{b}(A)	970± 195.23^{b}(A)						
No. of days required to attain maximum body weight(days)	P.C	75	75	45	90	–	–	–	–	–	–
	D.A	30	75	105	90						
Rate of body weight increase(mg worm^{-1} day^{-1})	P.C	4.49± 0.58^{a}(A)	13.76± 0.60^{b}(A)	22.55± 3.78^{c}(A)	11.24± 1.26^{ab}(A)	12.424	0.000004	10.415	0.003	2.899	0.055
	D.A	3.33± 1.70^{a}(A)	7.49± 2.62^{a}(A)	11.11± 2.17^{a}(B)	10.77± 2.16^{a}(A)						
Rate at which cocoon produced (cocoon worm^{-1}week^{-1})	P.C	0.168± 0.06^{a}(A)	1.562± 0.19^{b}(A)	2.19± 0.14^{c}(A)	2.16± 0.16^{bc}(A)	48.447	0.00000000001	220.666	0.000000000000013	20.650	0.00000007
	D.A	0±0^{a}(B)	0.332± 0.05^{b}(B)	0.513± 0.06^{b} (B)	0.330± 0.11^{b} (B)						
Rate of Juveniles produced worm^{-1} week^{-1}	P.C	0.094± 0.01^{a}(A)	0.330± 0.03^{b}(A)	0.278± 0.01^{b}(A)	0.323± 0.02^{b}(A)	49.301	0.00000000002	25.313	0.000003	15.879	0.0000006
	D.A	0±0^{a}(B)	0.126± 0.01^{b}(B)	0.385± 0.04^{c}(A)	0.172± (B) 0.01^{b}						

S- Soil without additives SC-soil with cow-manure SL-soil with bamboo leaf litter SCL-soil with cow-manure and bamboo leaf litter. Dissimilar alphabets in superscripts (in rows) represent significant difference among the diet combinations (Tukey's multiple paired test). Dissimilar alphabets in brackets (in columns-paired) represent significant difference among the two species (2-sample t-test). P-values in bold represent statistically significant differences at 5% levels of significance (Two-way ANOVA).

leaf litter to soil, significantly increased body weight in *P. corethrurus* and *D. assamensis*. Among the four types of diets, only in mineral soils (S), *P. corethrurus* had more than three times weight gains than that in *D. assamensis*, while in other diets (SC, SL, SLC) difference in weight gain was at par(P>0.05). Moreover, in contrast to native species *D. assamensis*, exotic earthworm, *P. corethrurus* had maintained sustained body weight almost throughout the experimental period in mineral soils while the native species, *D. assamensis* after achieving highest weight gain in the soil (S) on 30th day, its body weight gradually declined (Figure 1). This clearly indicates the higher nutrient assimilation efficiency in exotic earthworm *P. corethrurus* than that in *D. assamensis* in soils which is due to well known gut associated symbiotic microflora in the former earthworm species [20]. Although in SC, SL, SLC there was no significant differences (P>0.05) in weight gains among the two earthworm species, it is clear enough from Table 3 that both *P. corethrurus* and *D. assamensis* had good growth response when bamboo leaf litter was added to soils. Growth rate of *P. corethrurus* in SL diet was significantly higher (P<0.05) than all the other diets (S, SC and SLC) [Table 3]. In spite of achieving highest weight gain in SL, growth rate in *D. assamensis* (11 mg worm^{-1}day^{-1}) was not significantly different (P>0.05) from all other diets. Suitability of the diets promoting growth in

TABLE 4: Growth and reproduction rate in some tropical endogeic and anecic earthworms.

Earthworm species	Substrate used	Growth rate	Rate of reproduction		Reference
		(mg/worm/day	(cocoon/worm/ week)	(Juvenile/ adult/week)	
Drawida nepalensis	Cow manure : oak litter	3.52	4.9	–	Joshi & Dabral(2008)[24]
	Oak litter	10.6	5.6	–	Kaushal *et al*.,(1995)[21]
	Pine litter	10.8	7.0	–	Kaushal *et al*.,(1995)[21]
Metaphire houlleti	Cow manure : Oak litter	4.23	0.28	–	Joshi & Dabral(2008)[24]
	Soil:cow dung : rubber leaf litter	12.42	0.01	–	Nath & Chaudhuri(2012)[8]
	Oak litter	3.73	0.16	–	Kaushal *et al*.,(1999)[22]
Metaphire posthuma	Field soil	5.4	0.24	–	Bisht *et al*.(2007)[26]
	Cow manure	8.4	0.16	–	Bisht *et al*.(2007)[26]
	Poultry droppings	7.1	0.15	–	Bisht *et al*.(2007)[26]
Pontoscolex corethrurus	Soil: cowdung : rubber leaf litter	8.37	0.26	–	Nath & Chaudhuri(2012)[8]
	Cow manure : rubber leaf litter	18.81	–	0.48	Nath & Chaudhuri,(2014)[27]
Drawida assamensis	Soil : cow dung : rubber leaf litter	3.55	-	-	Nath & Chaudhuri(2012)[8]
Drawida p. papillifer	Soil : cow dung : rubber leaf litter	4.44	-	-	Nath & Chaudhuri(2012)[8]
Polypheretima elongata	Pasture soil	-	0.52	0.69	Bhattacharjee & Chaudhuri(2002)[25]
Dichogaster modiglianii	Pasture soil	-	1.41	1.41	Bhattacharjee & Chaudhuri(2002)[25]

the two species are in the following order for *P. corethrurus*: SL>SC>SLC>S and *D. assamensis*: SL>SLC>SC>S. Rate of growth and reproduction in some tropical endogeic and anecic earthworm species is shown in Table 4. High growth rate with sustained biomass production of the two earthworm species in SL (Figure1) was due to high C: N ratio (35) and nitrogen content (0.98%) in bamboo litter[1]. Growth rate in *P. corethrurus* (22.55mg/worm/day) and *D. assamensis* (11.11 mg/worm/day) in bamboo leaf litter is much higher than the growth rate of *P. corethrurus* (8.37 mg/worm/day) and *D. assamensis* (3.55 mg/worm/day) in the rubber leaf litter diet[8], *Drawida nepalensis* in oak litter (10.60 mg/worm/day)[21] and *M. houlleti* in oak litter (3.73 mg/worm/day).[22] Growth rate in *D. assamensis* is however close to that in *D. nepalensis* reared in oak litter (10.6 mg/worm/day) and pine litter (10.80 mg/worm/day)[21].

Reproduction : Earthworm species following conjugation produce cocoons inside which development takes place and after certain period of incubation juveniles hatch out. The mean cocoon production in *P. corethrurus* and *D. assamensis* in different diet combinations at different time interval are shown in Figure 2. After attaining certain biomass on the 15th day of experiment, laying of cocoons by the two species started on the 30th day. This indicated that following gaining of certain biomass, earthworm species starts to produce cocoon. In general, peak of biomass production and cocoon production coincided in both *P. corethrurus* and *D. assamensis* upto 75th day after which peak days of growth and cocoon generation in the two earthworm species differed (Figure 1 and 2). *P. corethrurus* always maintained a much higher peak of cocoon generation compared to *D. assamensis*. Much higher fecundity in *P. corethrurus* compared to that in *D. assamensis*[23] is responsible for higher peak of cocoon generation in the former. While *P. corethrurus* had three distinct peaks of cocoon generation on the 45th, 75th and 105th day, peak day of cocoon generation in *D. assamensis* in different substrates was at much later days i.e. on 75th, 90th and 120th day (Figure 2). Although in our present study *D. assamensis* did not lay any cocoon in the bamboo soils (S) [Table 3] under laboratory conditions, Chaudhuri and Bhattacharjee (2011)[23] recorded cocoon generation in *D. assamensis* under laboratory conditions in the *Hevea* soils. In the soils with different food additives (SC, SL, SLC), the rate of cocoon generation (Table 3) was at par ($P>0.05$) in *D. assamensis*. In each diet the rate of cocoon generation in *P. corethrurus* was significantly higher ($p<0.05$) than that in *D. assamensis.*

Rate of reproduction in terms of cocoon generation in *P. corethrurus* (2.19 cocoon worm^{-1} week^{-1}) in bamboo leaf litter is much lower than that in *Drawida nepalensis* in pine litter (7 cocoons worm^{-1}week^{-1})[21], oak litter (5.6 cocoons worm^{-1}week^{-1}) [21], but much higher than *Metaphire*

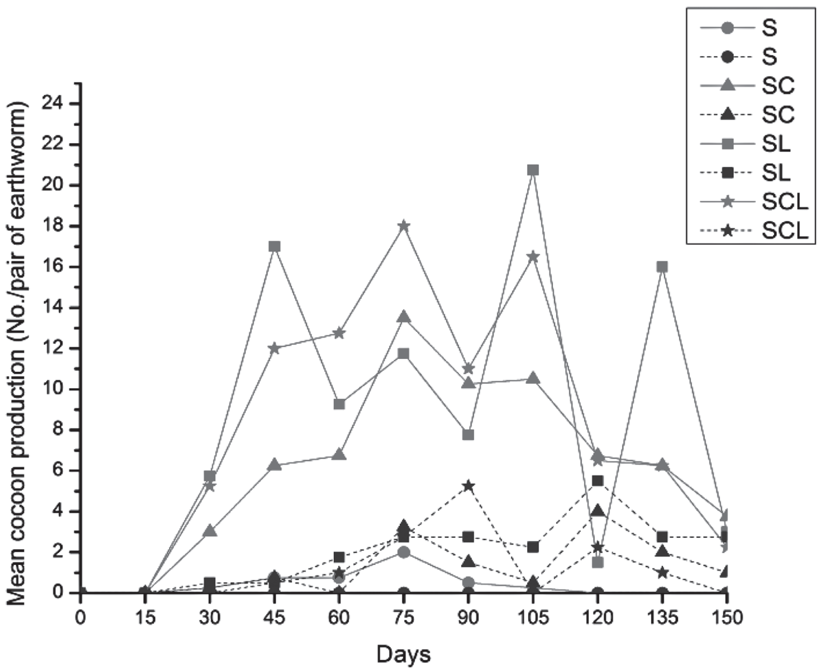

Figure 2: Graphical representation showing variation in mean cocoon production in *P. corethrurus* (P.C) and *D. assamensis* (D.A) at different time intervals with different diet combinations.

houlleti in cow manure : oak litter (0.28 cocoons worm^{-1}week^{-1})[24], oak litter (0.16 cocoon worm^{-1}week^{-1})[22], *P. corethrurus* in rubber leaf litter (0.26 cocoons worm^{-1}week^{-1})[8] .

Rate at which juveniles are produced (juvenile worm^{-1} week^{-1}) in earthworms depends upon hatching success. Although the rate of juvenile produced in *P. corethrurus* in SC, SL and SCL diet was at par (P>0.05), the same in the mineral soil (S) was significantly lower (P<0.05) than that in those diets because of low nutrient content in the soil (S). Due to absence of cocoon production in S by *D. assamensis*, no juvenile of it was recorded in the soil substrate. Rate of juvenile produced in SL (0.38 juveniles worm^{-1} week^{-1}) by *D. assamensis* was significantly higher (P<0.05) than those in SC and SCL. In S, SC and SCL, *P. corethrurus* had significantly higher (P<0.05) rate of juvenile production than in those in *D. assamensis*. Rate at which juveniles of *P. corethrurus* were produced in bamboo plantation soils without any additive [S] (0.094 juveniles worm^{-1} week^{-1}) was much lesser than the rate at which juveniles were produced in pasture soils by *Polypheretima elongata* (0.69 juveniles worm^{-1}week^{-1}) and *Dichogaster modiglianii* (1.41 juveniles worm^{-1}week^{-1})[25].

Among the two studied earthworm species, sustainable as well as, significantly higher growth and reproduction in *P.corethrurus* than *D.assamensis* are probably linked with adaptive strategies like efficient assimilation capacity for wide range of diets, continuous breeding with high fecundity, short incubation and high hatching success [23] in the former compared to the latter. So, vermiculture of *P.corethrurus* as a source of protein (vermitin) in poultry, fishery and piggery is recommended. Significant increase (P< 0.05) in soil carbon and C: N ratio in presence of both *P.corethrurus* and *D.assamensis* indicate their important role in soil carbon conservation. Thus both the species may successfully be applied to soils for land reclamation also.

Acknowledgment : The authors express their sincere thanks to Dr Ratan Saha, Professor, College of Fisheries, Lambucherra, Tripura for analyzing the nitrogen content of the soil samples and Dr J.M. Julka, Solan for identification of earthworms. ❐

P. S. CHAUDHURI[1]*
SOURABH CHAKRABORTY[1]

[1]Earthworm Research Laboratory,
Department of Zoology, Tripura University,
Suryamaninagar, West Tripura-799022, India.

*Corresponding Author: priya_1956@rediffmail.com

Received : 31 May, 2018

Revised : 16 January, 2019

1. A. J. Nath and A. K. Das, Carbon storage and sequestration in bamboo-based small holder home gardens of Barak Valley, Assam. Current Science 100, 229-233(2011).
2. A. Don, B. Steing, I. Schoning, K. Pritisch, M. Joschko, G. Gleixner,522 and E.D. Schulze, Organic carbon sequestration in earthworm burrows. Soil Biology & Biochemistry 40, 1803–1812(2008).
3. S. Chakraborty and P.S. Chaudhuri, Earthworm communities in the bamboo plantations of West Tripura (India). Proc Zool Soc, 70(2), 105-118 (2017) Springer.
4. S. Nath and P.S.Chaudhuri, Human-induced biological invasions in rubber (*Hevea brasiliensis*) plantations of Tripura (India) - *Pontoscolex corethrurus* as a case study. Asian journal of Experimental Biological science 1(2), 360-369(2010).
5. A. Dey and P.S. Chaudhuri, Earthworm community structure of pineapple (*Ananas comosus*) plantations under monoculture and mixed culture in West Tripura, India. Tropical Ecology 55(1), 1–17 (2014).
6. P.S. Chaudhuri and G. Bhattacharjee, Capacity of various experimental diets to support biomass and reproduction of *Perionyx excavatus*. Bioresource Technology 82, 147-150 (2002).
7. C.N. Lowe and K.R. Butt, Culture techniques for soil dwelling earthworms: A review. Pedobiologia 49(5), 401-413(2005).

8. S. Nath and P.S. Chaudhuri, Effect of rubber leaf litter diet on growth and reproduction of five tropical species of earthworms under laboratory conditions. J. AppL. Biosci. 38(2), 174-178 (2012).

9. M.C.Dash, S. P. Dash, Fundamentals of Ecology, Third Edition, MC Graw-Hill, New Delhi (2009), Page- 94.

10. M. Debnath, P. S. Chaudhuri, P. P. Chakraborty and S.K. Sil, Nutrient content of native earthworm, *Eutyphoeus gammiei* (Beddard) of Tripura (India). International Journal of Pharmaceutical sciences 8(11), 4832-4838 (2017).

11. B.K. Senapati, P. Lavelle, S. Giri, B. Pashanasi, J. Albrecht, E. Blanchart, M. Mahieux, L. Rousseaux, R. Thomas, P.K. Panigrahi and M. Venkatachalam, In-soil Earthworm Technologies for Tropical Agroecosystems. In: Lavelle P., Brussard L. and Hendrix P.(eds) Earthworm Management in Tropical Agroecosystems. CAB International, Wallingford, UK, pp199-237(1999).

12. N. Karmegam and T. Daniel, Growth, reproductive biology and life cycle of the vermicomposting earthworm, *Perionyx ceylanensis* Mich. (Oligochaeta: Megascolecidae). Bioresource Technology 100, 4790-4796 (2009).

13. D. Trigo, I. Barois, M.H. Garvin, E. Huerta, S. Irisson and P. Lavelle, Mutualism between earthworms and soil microflora. Pedobiologia 43, 866-873(1999).

14. P.S. Chaudhuri, T.K. Pal, G. Bhattacharjee and S.K. Dey, Chemical changes during vermicomposting (*Perionyx excavatus*) of kitchen waste. Tropical Ecology 41, 107-110 (2000).

15. P.S. Chaudhuri, T.K. Paul, G. Bhattacharjee and S.K. Dey, Nutrient changes during vermicomposting by *Perionyx excavatus* of the aquatic weed *Trapa bispinosa*. Philippine Journal of science 130(2), 127-133(2001).

16. R. Hartenstein, Production of earthworms as a potentially economical source of protein. Biotechnology and Bioengineering 23(8), 1797-1811(1981).

17. C. A. Edwards and P. J. Bohlen, Biology and Ecology of Earthworms. Chapman and Hall, London. (1996).

18. J. Domínguez, State of the Art and New Perspectives on Vermicomposting Research, In: Edwards CA(Ed), Earthworm Ecology, CRC Press, ISBN 1-884015-74- 3, Boca Raton, Florida, pp 401-424 (2004).

19. S. Suthar, Influence of different food sources on growth and reproduction performance of composting epigeics: *Eudrilus eugeniae*, *Perionyx excavatus* and *Perionyx sansibaricus*. Applied Ecology and Environmental Research 5(2), 79-92 (2007).

20. P. Lavelle, I. Barois, I. Cruz, C. Fragoso, A. Hernandez, A. Pineda and P. Rangel. Adaptive strategies of *Pontoscolex corethrurus* (Glossoscolecidae, Oligochaeta), a peregrine geophagous earthworm of the humid tropics. Biology & Fertility of Soils 5, 188-194 (1987).

21. B. R. Kaushal, S. Kalia and S. P. S. Bisht, Growth and cocoon production by the earthworm *Drawida nepalensis* (Oligochaeta: Moniligastridae) in oak and pine litter. Pedobiologia 39, 417-422 (1995).

22. B.R. Kaushal, S. Bora and B. Kandpal, Growth and cocoon production by earthworm *Metaphire houlleti* (Oligochaeta) in different food sources. Biology and Fertility of Soils 29, 394-400 (1999).

23. P. S. Chaudhuri and S. Bhattacharjee, Reproductive biology of eight tropical earthworm species of rubber plantations in Tripura, India. Tropical Ecology 52, 49-60 (2011).

24. N. Joshi and M. Dabral, Life cycle of earthworms *Drawida nepalensis*, *Metaphire houlleti* and *Perionyx excavatus* under laboratory controlled conditions. Life science Journal 5(4), 83-86(2008).

25. G. Bhattacharjee and P. S. Chaudhuri, Cocoon production, morphology, hatching pattern and fecundity in seven tropical earthworm species – a laboratory based investigation. Journal of Bio-Science 27, 283-294(2002).

26. R. Bisht, H. Pandey, D. Bharti, S.P.S. Bisht and B.R. Kaushal, Reproductive potential of the earthworm *Metaphire posthuma* (Oligochaeta) in different food substrates. Tropical Ecology 48 (1), 107-114 (2007).

27. S. Nath and P.S. Chaudhuri, Growth and reproduction of *pontoscolex corethrurus* (Muller) with different experimental diets. Tropical Ecology 55(3), 305-312 (2014).