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Reproductive biology of eight tropical earthworm species of rubber plantations in Tripura, India

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Abstract: Data on reproductive biology of eight tropical species of earthworms, viz. *Pontoscolex corethrurus* (Muller), *Drawida assamensis* Stephenson, *Drawida papillifer papillifer* Gates, *Eutyphoeus comillahnus* Michaelsen, *Metaphire houlleti* (Perrier), *Dichogaster affinis* Michaelsen, *Octochaetona beatrix* Gates, *Lennogaster chittagongensis* (Stephenson) are presented. The peregrine earthworms viz. *P. corethrurus, D. affinis* and *O. beatrix* are continuous breeders with high fecundity, high hatching success and exhibit uni-parental reproduction. Native earthworm species are either semi-continuous breeders with moderate fecundity (*D. p. papillifer* and *D. assamensis*) or discrete breeders (*E. comillahnus*) with least fecundity. There was significantly higher cocoon production in most species during summer and monsoon. With increase in temperature, incubation period increased in *E. comillahnus* and *O. beatrix* and decreased in *D. affinis*, *M. houlleti* and *D. assamensis* within a temperature range of 20 - 30 °C under laboratory conditions. Incubation period of earthworm species under study showed a significant positive linear relationship ($P < 0.01$) with their body length. The more continuous and high rate of cocoon production, as well as, higher hatching rate in *P. corethrurus*, *O. beatrix, D. affinis* and *M. houlleti* with uniparental reproduction indicate their possible usefulness in vermiculture. The geophagous species such as *P. corethrurus* and *O. beatrix* might be useful for wasteland reclamation, while the phytogeophagous worms viz. *D. affinis, M. houlleti* can be utilized in degradation of organic wastes using soil bedding.

Resumen: Se presentan datos sobre la biología reproductiva de ocho especies tropicales de lombrices de tierra: *Pontoscolex corethrurus* (Muller), *Drawida assamensis* Stephenson, *Drawida papillifer papillifer* Gates, *Eutyphoeus comillahnus* Michaelsen, *Metaphire houlleti* (Perrier), *Dichogaster affinis* Michaelsen, *Octochaetona beatrix* Gates y *Lennogaster chittagongensis* (Stephenson). Las lombrices de tierra peregrinas (*P. corethrurus, D. affinis* y *O. beatrix*) se reproducen de manera continua, con una fecundidad y un éxito de incubación altos, y poseen reproducción uniparental. Las especies de lombrices nativas se reproducen de forma semicontinua con una fecundidad moderada (*D. p. papillifer* y *D. assamensis*) o de forma discreta (*E. comillahnus*) con una fecundidad más baja. La producción de capullos fue significativamente mayor en la mayoría de las especies durante el verano y el monzón. Con el incremento de la temperatura, el periodo de incubación se alargó en E. comillahnus y O. beatrix, y decreció en *D. affinis*, *M. houlleti* y *D. assamensis* dentro de un intervalo de temperatura de 20 - 30 °C en condiciones de laboratorio. El periodo de incubación de las especies de lombrices estudiadas mostró una relación lineal positiva y significativa ($P < 0.01$) con la longitud corporal. La tasa de producción de capullos más continua y alta, así como la tasa de incubación más alta en *P. corethrurus*, *O. beatrix*, *D. affinis* y *M. houlleti* con reproducción uniparental indican su utilidad potencial para la vermicultura. Las especies geófagas como P. corethrurus y *O. beatrix* podrían ser útiles para recuperar tierras degradadas, mientras que las lombrices fitófagas como *D. affinis* y *M. houlleti* podrían ser utilizadas para la degradación de desechos orgánicos, usando camas de suelo.

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Resumo: Apresentam-se os dados sobre a biologia reprodutiva de oito espécies tropicais de minhocas, viz. *Pontoscolex corethrurus* (Muller), *Drawida assamensis* Stephenson, *Drawida papillifer papillifer* Gates, *Eutyphoeus comillahnus* Michaelsen, *Metaphire houlleti* (Perrier), *Dichogaster affinis* Michaelsen, *Octochaetona beatrix* Gates, *Lennogaster chittagongensis* (Stephenson). Asminhocas peregrino, a saber a *P. corethrurus*, *D. affinis* e *O. beatrix* são reprodutoras contínuas, com alta fecundidade, comaltosucesso de eclosão e exibem uma reprodução uni-parental. As espécies de minhocas nativas são quer reprodutoras semi-contínuas com fecundidade moderada (*D. p. papillifer* e *D. assamensis*), ou reprodutoras discretas (*E. comillahnus*), com menor fecundidade. Verificou-se haver uma produção de casulos significativamente maior na maioria das espécies durante o verão e na monção. Sob condições de laboratório verificou-se que com o aumento da temperatura num intervalo de 20 - 30 °C,o período de incubação aumentou em *E. comillahnus* e *O. beatrixe* diminuiu em *D. affinis, M. houlleti* e *D. assamensis*. *O período* de incubação das espécies de minhoca em estudo mostraram uma relação linear positivasignificativa ($P < 0,01$) com o comprimento do corpo. A taxa mais contínua e elevada de produção de casulos, bem como, uma maior taxa de eclosão em *P. corethrurus*, *O. beatrix*, *D. affinis* e *M. houlleti* com reprodução uniparental indicaa sua possível utilidade em vermicultura. As espécies geófagas como a *P. corethrurus* e *O. beatrixpoderão* ser úteis para a recuperação de terrenos baldios, enquanto os vermes fitófagoscomo a *D. affinis* e *M. houlleti* podem ser utilizadas na decomposição de resíduos orgânicos usando camas de terra.

Key words: Cocoon, earthworms, fecundity, hatching success, incubation period, reproductive biology.

Introduction

Recent research on *in-situ* earthworm based technology for soil amelioration has led to breeding trials involving cocoon production from adults in soil environments (Bhattacharjee & Chaudhuri 2002; Butt 2002; Lavelle *et al.* 1998). Higher fecundity, low incubation period, high hatching success and quick maturation are some of the special features of earthworms useful for this technology. Endogeic and anecic earthworms are the most important biological agents which influence the physicochemical status of the soil to a great extent. However, very few studies have been done on their biology in the tropical conditions as compared to the temperate climate (Edwards & Bohlen 1996). Studies on the life cycles of earthworms are also necessary for effective vermiculture.

Dash & Senapati (1980), Senapati & Dash (1979a and b) studied the morphology of cocoons of four tropical earthworms, *Lampito mauritii*, *Drawida willsi*, *Octochaetona surensis* and *Drawida calebi* and also the effect of soil moisture and temperature on the cocoon hatching success and emergence pattern of juveniles under field and laboratory conditions. Kaushal *et al.* (1995, 1999) observed the effect of different food substrates on cocoon production, incubation time and hatching success in *Drawida nepalensis* and *Metaphire houlleti* respectively. Bhattacharjee & Chaudhuri (2002) studied the breeding strategies of seven tropical earthworm species based on their dynamics of cocoon production, hatching pattern and fecundity under laboratory conditions and suggested possible usefulness of *Perionyx excavatus*, *Dichogaster modiglianii, Drawida nepalensis* and *Lampito mauritii* in vermiculture.

The aim of the present study was to describe the cocoon morphology, cocoon development, hatching success and fecundity in eight earthworm species found in rubber plantations in Tripura, one of the northeastern hill states of India. Ability of single individual earthworms (unmated) to produce cocoons was also studied. Such information could be useful for selection of appropriate species for production of organic manure (vermi-compost).

Materials and methods

The study was carried out on eight tropical earthworm species: *Pontoscolex corethrurus* (Muller), *Drawida assamensis* Stephenson, *Drawida papillifer papillifer* Gates, *Eutyphoeus comillahnus* Michaelsen, *Metaphire houlleti* (Perrier), *Dicho-*

gaster affinis Michaelsen, *Octochaetona beatrix* Gates, *Lennogaster chittagongensis* (Stephenson) found in the state of Tripura. The state has sub-tropical climate with three distinct seasons: winter (November - February), summer (March - June) and monsoon (July - October). Adult earthworms were collected by digging (20 cm x 20 cm x 30 cm) and hand sorting from the 25 year old rubber plantations at Mohanpur Block of Sadar sub-division (22°51´- 24°32´ N latitude and 90°10´- 92°21´E longitude) from August to October 2006. The earthworms were acclimated in a ventilated laboratory until December 2006. The earthworm species were identified with the help of experts at Zoological Survey of India, Kolkata. Among the collected species *M. houlleti, L. chittagongensis, P. corethrurus, D. affinis, D. p. papillifer* and *D. assamensis* were found within 0 - 15 cm depth of soil and hence regarded as top-soil species. *E. comillahnus* and *O. beatrix* were found within 15 - 30 cm depth, hence regarded as sub-soil species.

Culture technique

Six species of earthworms viz. *P. corethrurus*, *D. assamensis*, *D. p. papillifer, E. comillahnus, O. beatrix* and *M. houlleti*, were reared in 4.5 L earthen pots. Each pot was filled with 2000 g of air-dried, ground and sieved (0.05 mm mesh) soil collected from mature rubber plantation (pH 4.8, N 0.01 %, organic C 1.21 %, sandy loam texture) mixed with 200 g of 15-day old cow dung dust (particle size $\leq$ 2 mm) as food at the surface. In case of small sized worms i.e., *D. affinis* and *L. chittagongensis* smaller earthen pots (1 L) were used which contained 500 g of soil and 5 g of cow dung dust. A pair of adult healthy earthworms belonging to the same species with well developed clitellum was introduced into each pot. Five replicates were maintained for each species. The moisture level of the substrate in each pot was maintained at 40 % by spraying fresh water on alternate days. Moisture content of the substrate was measured periodically by the gravimetric method. Mean maximum room temperature (ºC) during the experimental period (year 2007) was as follows: Jan- 20.05 ± 1.26, Feb- 25.93 ± 2.57, Mar- 30.18±1.48, Apr- 30.88 ± 2.30, May- 31.58 ± 3.38, Jun- 30.82 ± 1.83, Jul- 32.18 ± 1.05, Aug- 32.11 ± 0.93, Sept- 31.23 ± 1.66, Oct- 31.69 ± 0.78, Nov- 27.82 ± 1.82, Dec- 23.48 ± 1.63. The cultures were maintained for 1 year (January 2007 to December 2007).

Another experiment of three months duration was set in a BOD incubator at constant 26 ºC temperature to determine the effect of individual body weight on the weight and number of cocoon produced in *P. corethrurus*. Ten replicates (1 adult worm per pot having same type and amount of substrate used before) were maintained. The weight of clitellate worms used in this experiment ranged from 0.4 g to 0.8 g.

Cocoon studies

The contents of each pot were carefully examined on a weekly basis. The adult worms were hand sorted from the culture media and weighed. Cocoons were collected by gentle wet sieving through 0.05 mm mesh sieve. The number of cocoon produced per individual was estimated. The size and weight of cocoons were also measured. Before weighing, the cocoons were washed lightly in distilled water to remove debris adhering to the sticky hull. At each sampling period, the old culture media was replaced with fresh material at weekly interval, so that food was not a limiting factor.

Incubation of cocoons

After isolation, cocoons were kept on moist filter paper spread over water soaked cotton (having 85 % moisture) inside a Petri-dish having 15 cm diameter (one cocoon per dish). Number of replicates for each species was twenty. The cocoons were incubated at the same temperature at which they had been produced. During the incubation period, the cocoons were checked on a regular basis to record change in color and emergence of hatchlings. Number of hatchlings per cocoon, their fresh weight, size and site of emergence were also recorded. This also allowed calculation of hatchability and length of incubation period. The incubation period was calculated as time period from cocoon collection until the appearance of first hatchling plus half the time interval between cocoon collection and previous inspection of food media (Butt 1993, 1997) which was 3.5 days in this study.

Maturation and onset of cocoon production in F1 generation worms

The newly hatched juveniles of the earthworm species under study were kept individually (n = 15) in fresh culture media (*Hevea* soil mixed with cow dung dust) kept in suitable earthen pot and reared until they became clitellate and started to produce cocoon. The cocoons they produced were incubated at room temperature. Such study indicated whether unmated worms could produce fertile cocoons.

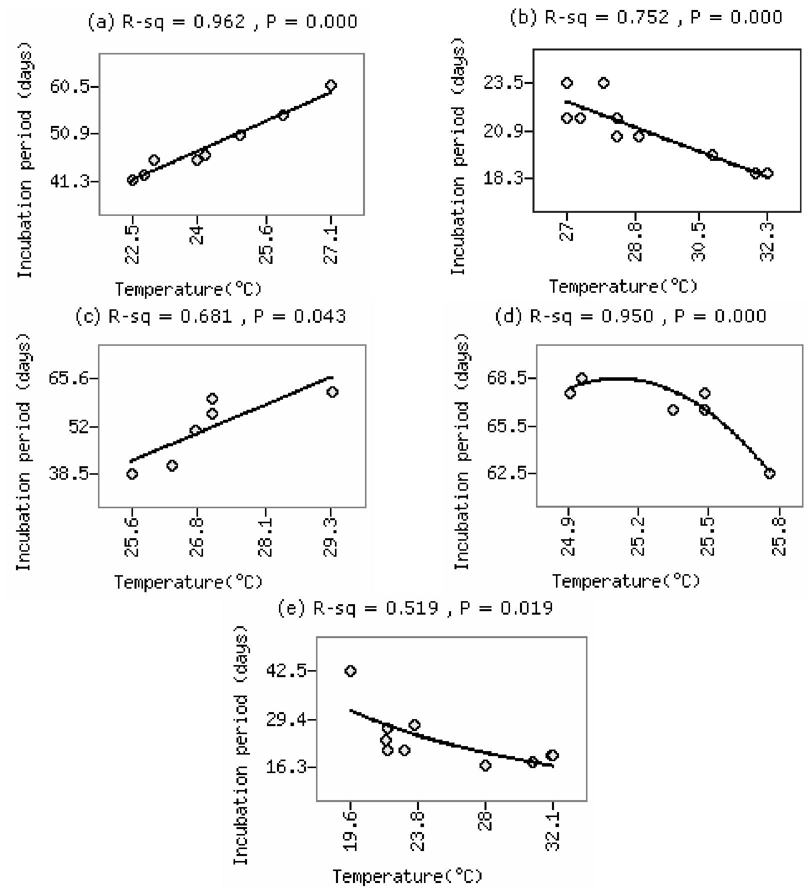

Fig 1. Relationship between incubation period (days) & temperature (ºC) in (a) *Octochaetona beatrix* (b) *Drawida assamensis* (c) *Eutyphoeus comillahnus* (d) *Metaphire houlleti* (e) *Dichogaster affinis*; Note significant positive correlation between room temperature & incubation period in *E. comillahnus* & *O. beatrix* and significant negative correlation between the same variables in *D. assamensis, M. houlleti* & *D. affinis.*

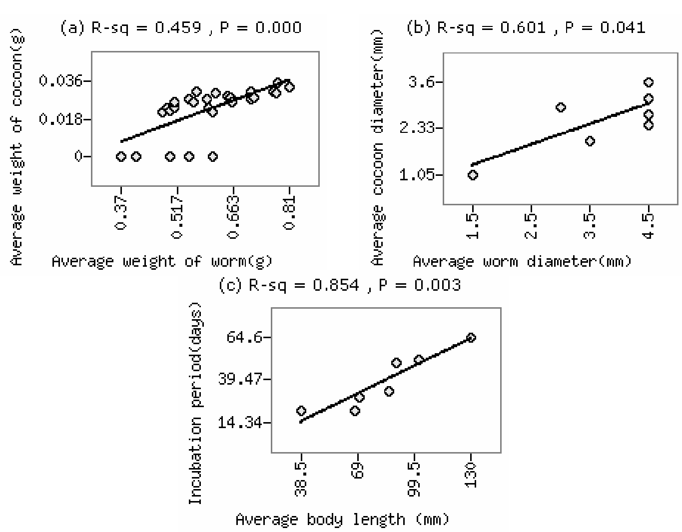

Fig 2. Relationship between (a) worm weight (g) and cocoon weight (g), (b) worm diameter (mm) and cocoon diameter (mm), (c) worm length (mm) and incubation period of cocoon (days); Note the highly significant relationship among the variables.

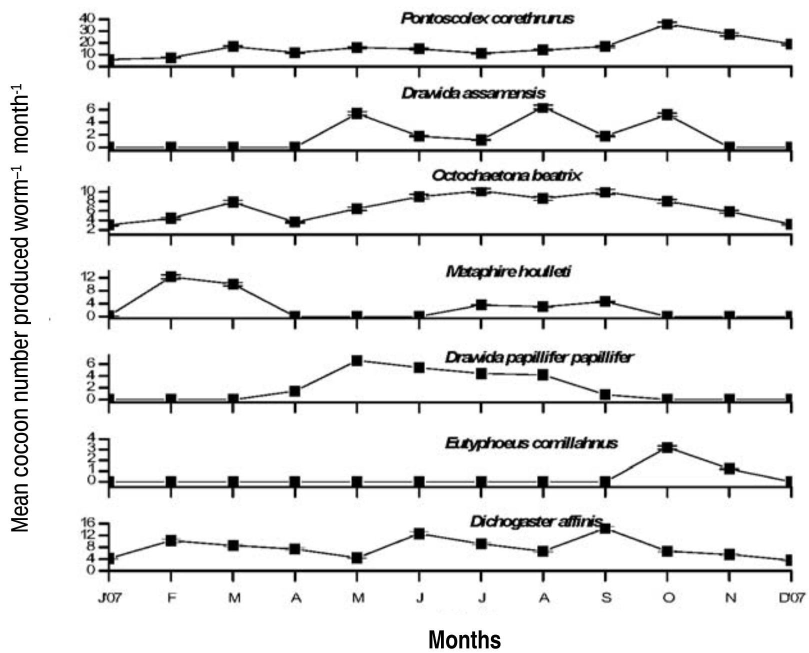

Fig 3. Number of cocoon produced (mean ± SE) per worm per month for seven species of earthworms. Note continuous breeding strategies in *P. corethrurus*, *O. beatrix* & *D affinis;* semicontinuous breeding strategies in *D. assamensis, D. papillifer papillifer* and *M. houlleti* and discrete breeding strategy in *E. comillahnus.*

Statistical analysis

The correlation between the mean body length and incubation period, mean room temperature and incubation period for *D. assamensis, D. p. papillifer, E. comillahnus, D. affinis, O. beatrix, M. houlleti, P. corethrurus* and also the mean body weight (fresh weight) vs number of cocoon produced and mean weight (fresh weight) of cocoons for *Pontoscolex corethrurus* were tested by linear, quadratic and inverse regression analysis. Pairwise t-test was used to know significant differences, if any, in cocoon production of different earthworm species among three different seasons (winter, summer and monsoon).

Results

Cocoon morphology and incubation period

Cocoons in general were spheroidal (*P. corethrurus, L. chittagongensis, E. comillahnus, D. affinis, O. beatrix, D. assamensis, D. p. papillifer*) with two poles (one broad and other pointed one). *M. houlleti* produced irregular and oval cocoons. Ornamentation in the form of protruding structures (*E. comillahnus, O. beatrix, D. assamensis, D. p. papillifer*) or pointed spinous processes (*M. houlleti*) were present on either side of the cocoons. In *D. affinis* ornamentation is much elaborate with a circlet of bristles at the blunt end and an elongated pointed end on the other side. Although cocoons of *D. assamensis* and *D. p. papillifer* were similar in shape, they differed in size, weight and colour (Table 1). Among the eight earthworm species studied, the cocoons of *L. chittagongensis* were the smallest (diameter 1.07 mm, fresh weight 1.87 mg) while those of *O. beatrix* were the largest (diameter 3.6 mm, fresh weight 34.8 mg). There were significant positive ($P < 0.05$) linear correlation between mean body weight of worm and their mean cocoon weight in *P. corethrurus* (Fig. 2a) and also mean worm diameter (just behind the clitellum) and cocoon diameter in all the species studied (Fig. 2b). Freshly laid cocoons of most earthworm species were opaque but those of *P. corethrurus* were semitransparent and milky white. In the cocoons blood capillaries first appeared on the 15th day of incubation in *D. affinis*, 20th day in both *P. corethrurus* and *E. comillahnus* and about 30th day in *M. houlleti.* With progress in the days of incubation, vascularization increased and the cocoons turned reddish brown. Emergence of hatchlings of most species of earthworms was found to occur through an aperture made at the broader

Table 1. Biological features of cocoons of the seven earthworm species studied in Rubber Plantations, Tripura (mean ± SE).

Parameters	*Pontoscolex corethrurus*	*Drawida p. papillifer*	*Drawida assamensis*	*Metaphire houlleti*	*Eutyphoeus comillahnus*	*Dichogaster affinis*	*Octochaetona beatrix*
Adult size (mm)	72- 100 x 4-5	45- 90 x 3-4	60- 80 x 4- 5	100- 160 x 3-6	70- 135 x 2- 4	35- 42 x 1- 2	60- 120 x 4- 5
Cocoons (n=20)							
i) Shape	i) Spheroidal	i) Onion shaped	i) Onion shaped	i) Irregular oval	i) Spheroidal	i) Spheroidal	i) Spheroidal
ii) Length (mm)	ii) 4.18 ± 0.15	ii) 2.9 ± 0.04	ii) 4.02 ± 0.26	ii) 3.0 ±0.07	ii) 3.5 ± 0.22	ii) 2.05 ± 0.05	ii) 4.35 ± 0.13
iii) Breadth (mm)	iii) 3.13 ± 0.23	iii) 1.98 ± 0.06	iii) 2.68 ± 0.21	iii) 2.4 ± 0.12	iii) 2.9 ± 0.23	iii) 1.05 ± 0.05	iii) 3.6 ± 0.14
iv) Color	iv) Milky white	iv) Light brown	iv) Dark brown	iv) Light yellow	iv) Dark grey	iv) Light yellow	iv) Olive green
v) Weight (mg)	v) 30.10 ± 0.97	v) 17.30 ± 1.11	v) 18.10 ± 0.58	v) 13.41 ± 0.62	v) 21.46 ± 3.21	v) 2.07 ± 0.13	v) 34.81 ± 1.54
vi) Ornamentation	vi) Absent	vi) Protrusions at both ends	vi) Protrusions at both ends	vi) Pointed spine like structure at one end	vi) Short pointed structure at both apices	vi) Elongated pointed end on one side and circlet of bristles on the other	vi) Short protrusions at both ends
Frequency of cocoon production	Continuous	Semi continuous	Semi continuous	Semi continuous	Discrete	Continuous	Continuous
Cocoon production worm^{-1} year^{-1}	98.0	11.5	10.8	16.8	2.2	46.5	40
Incubation period (days)	32.6 ± 0.70	20.8 ± 0.55	29.2 ± 0.59	64.6 ± 0.96	51.21 ± 3.25	21.28 ± 1.27	49.2 ± 1.90
Hatching success (%)	91.23 (n = 981)	18.26 (n = 115)	33.33 (n = 108)	91.66 (n = 168)	63.64 (n = 22)	81.51 (n = 465)	80.95 (n = 400)
Hatchlings cocoon^{-1}	1	1	1.30 ± 0.07	1.23 ± 0.03	1	1	1
Hatchling size (n=20)							
i) Length (mm)	i) 18.5 ± 1.06	i) 13.1 ± 0.52	i) 11.03 ± 0.79	i) 16.2 ± 0.72	i) 25.1 ± 0.83	i) 15.8 ± 0.32	i) 26.6 ± 0.84
ii) Breadth (mm)	ii) 1.25 ± 0.11	ii) 1.0 ± 0.00	ii) 1.02 ± 0.02	ii) 1.0 ± 0.00	ii) 1.1 ± 0.1	ii) 0.6 ± 0.06	ii) 1.0 ± 0.00
iii) Weight (mg)	iii) 30.0 ± 0.86	iii) 12.27 ± 0.63	iii) ND	iii) 11.0 ± 0.74	iii) 46.0 ± 3.41	iii) 5.10 ± 1.08	iii) 46.5 ± 1.17
Room temperature during incubation (°C)	28.53 ± 1.30	24.84 ± 0.95	20.60 ± 0.48	25.41 ± 0.11	26.75 ± 0.52	26.19 ± 1.53	24.56 ± 0.48
Room temperature during hatching (°C)	27.35 ± 1.73	28.75 ± 0.85	28.75 ± 0.85	30.25 ± 1.05	22.14 ± 0.30	26.33 ± 1.41	21.83 ± 0.26

ND – not determined

end of the cocoon. The hatchlings retreat back into their cocoons when disturbed at the time of emergence. Newly hatched juveniles showed least or no pigmentation.

Development time of cocoon was short in top soil earthworm species i.e., *D. affinis* (21 days), *D. p. papillifer* (21 days), *D. assamensis* (29 days) and *P. corethrurus* (33 days). Development time was long in subsoil species like *O. beatrix* (49 days), *E. comillahnus* (51 days) and *M. houlleti* (65 days) (Table 1). Surprisingly, some cocoons (17 in number) of *M. houlleti* produced during February 2007 had prolonged incubation period of about 168 days and hatched during August 2007.

A significant ($P < 0.05$) positive correlation between mean room temperature and incubation period in *E. comillahnus* and *O. beatrix* and significant ($P < 0.01$) negative correlation between the same variables in *D. assamensis*, *M. houlleti* and *D. affinis* (Fig. 1a - 1e) were observed. Incubation period of different species of earthworms showed a significant ($P < 0.01$) positive correlation with their body length (Fig. 2c). Thus species with small (*D. affinis*), medium (*O. beatrix, E. comillahnus*) and larger (*M. houlleti*) body length showed incubation period of 21 days, 49 days, 51 days and 65 - 168 days respectively.

Hatching success was strikingly higher in *P. corethrurus* (91 %), *M. houlleti* (91 %), *D. affinis* (81 %), *O. beatrix* (81 %), *E. comillahnus* (63 %) compared with *D. p. papillifer* (18 %) and *D. assamensis* (33 %) (Table 1). In most of the earthworm species one hatchling emerged from a single cocoon. Rare emergence of more than one hatchling per cocoon was recorded in *D. assamensis* and *M. houlleti*.

Seasonality of cocoon production

P. corethrurus, D. affinis and *O. beatrix* showed year round cocoon production (continuous breeder) under laboratory cultures (Fig. 3). Cocoon production in *D. p. papillifer* and *D. assamensis* continued from April to October (semi-continuous breeder) and was ceased during the winter months (November to February) when both the species entered a state of inactivity (quiescence). Bimodal cocoon production was recorded in *M. houlleti* that produced cocoons once during February and March and again from July to September (Fig. 3). *E. comillahnus* showed unimodal (discrete) cocoon production during October-November under laboratory conditions. Seasonal variations in cocoon production by different earthworm species is shown in Table 2. *P. corethrurus* was the highest cocoon producer among different earthworm species in each of the three seasons. While *D. p. papillifer* produced maximum cocoons during summer, other species (*P. corethrurus, D. affinis, O. beatrix, D. assamensis*) showed maximum production during monsoon (Table 2). There was significant increase in cocoon production during summer compared to winter in *D. affinis* ($P < 0.05$, t = 3.08), *D. p. papillifer* ($P < 0.01$, t = 10.4) and *D. assamensis* ($P < 0.01$, t = 12.35). Cocoon production was significantly higher ($P < 0.05$) during the monsoon compared to the winter in *D. p. papillifer* ($P = 0.006$, t = 5.37), *D. assamensis* ($P = 0.001$, t = 8.08), *O. beatrix* ($P = 0.004$, t = 6.08) and *P. corethrurus* ($P = 0.02$, t = 3.66). In most of the species viz. *D. affinis*, *P. corethrurus*, *M. houlleti*, *D. assamensis, D. p. papillifer,* however, the difference in cocoon production during summer and monsoon was not significant ($P > 0.05$). Interestingly, there was no significant difference ($P > 0.05$) in cocoon production among the three seasons in *M. houlleti*.

Although the earthworms showed high cocoon production during summer and monsoon, but the number and period of peak in cocoon production differed among the species. Thus cocoon production showed one peak in *E. comillahnus* (October), two peaks in *M. houlleti* (February and September) and three peaks in *D. affinis* (February, June, September), and *D. assamensis* (May, August and October) (Fig. 3).

Fecundity

The number of cocoon produced per adult per year (fecundity) was found to be the highest in case of *P. corethrurus* that produced 98 cocoons adult^{-1}year^{-1} under laboratory conditions (Table 1). *E. comillahnus* had the lowest fecundity value of 2.2 cocoons adult^{-1}year^{-1}. Of the other species, *D. affinis* had the next highest fecundity followed by *O. beatrix, M. houlleti, D. p. papillifer* and *D. assamensis* (Table 1). Mean body weight of *P. corethrurus* had significant positive ($P < 0.05$) linear correlation with the number of cocoon produced.

Mode of reproduction

Among the F1 juvenile worms of the species under study, maintained singly in culture pots, only those of *P. corethrurus, D. affinis, M. houlleti* and *O. beatrix*, following maturation produced fertile cocoons because their cocoons gave rise to successful emergence of juveniles. This indicates that these earthworm species do not need partner for reproduction. They either exhibit parthenogenesis or self-fertilization. *E. comillahnus, D. assamensis, D. p. papillifer* could not produce cocoon when kept singly.

Discussion

The earthworms produce cocoons by cross-fertilization, self fertilization or through parthenogenesis. This study reveals that *P. corethrurus, D. affinis, M. houlleti* and *O. beatrix* have ability for self fertilization or parthenogenesis as these species produced fertile cocoons without mating. Other species viz. *E. comillahnus, D. assamensis, D. p. papillifer*, in spite of being clitellate, could not produce cocoon when kept singly indicating that in these species, reproduction is biparental. There are reports of parthenogenesis in *P. corethrurus* (Gates 1972) and *M. houlleti* (Kaushal *et al.* 1999; Joshi & Dabral 2008). According to Lavelle *et al.* (1998) almost all exotic species of earthworms in humid tropics are reported to be least facultative parthenogenic, while native species only produce viable cocoons if fertilization occurs.

The shape, size, development time and hatching success of cocoons differed greatly among earthworm species. Both the highest and lowest size and weight of cocoons were found in octochaetid group of earthworm species *O. beatrix* and *D. affinis* respectively. As expected, there were strong positive linear correlations between mean worm body weight vs. mean weight of cocoons and mean worm diameter vs. mean cocoon diameter. Lavelle (1981) and Senapati & Sahu (1993) found a positive relationship between the size of adults and the size of cocoons produced by earthworms. Edwards & Bohlen (1996), however, proposed that cocoon size is not always correlated with adult size. This is especially true in case of some species (e.g. *M. houlleti*) under the genus *Metaphire* where larger worms often produced smaller cocoons. The elaborate pointed ends and bristles of cocoons in epianecic *M. houlleti* and epigeic *D. affinis* are the adaptive features of those species that enable them to adhere to the litter of their surroundings. Similar ornamentation of cocoons in other epigeic species have been reported by Viljoen & Reinecke (1989) in *Eudrilus eugeniae* and Bhattacharjee & Chaudhuri (2002) in *Dichogaster modiglianii* and *Perionyx excavatus*.

The development time of cocoons varied considerably among earthworm species. It was short in top soil species viz., *D. affinis* (21 days), *D. p. papillifer* (21 days), *D. assamensis* (29 days) and *P. corethrurus* (33 days) and long in subsoil species i.e. *O. beatrix* (49 days), *E. comillahnus* (51 days). Cocoon of epianecic species *M. houlleti* had prolonged incubation period of 65 to 168 days. Kaushal *et al.* (1995) reported an incubation period of 30 days for the cocoons of *Drawida nepalensis* which was very close to the value (29 days) of incubation period of *Drawida assamensis* of our present study. Senapati & Sahu (1993) and Bhattacharjee & Chaudhuri (2002) reported mean incubation period of 7 days and 14 days for tropical epigeic species like *Dichogaster bolaui* and *Dichogaster modiglianii* respectively. Kaushal *et al.* (1999) and Bisht *et al.* (2007) observed that cocoon development time of a species varied also with the media for incubation. Thus cocoon development time (in days) of *M. houlleti* was 22 in distilled water, 33 (moist filter paper), 33 (cow manure), 36 (oak litter) and 40 in horse manure (Kaushal *et al.* 1999) and that of *Metaphire posthuma* was 23 (field soil), 27 (wet cotton), and 30 in distilled water (Bisht *et al.* 2007). Remarkable difference in cocoon development time of *M. houlleti* between our present study and that of Kaushal *et al.* (1999) might be related to the occurrence of different parthenogenetic morphotypes under the same species (Gates 1972). Prolonged cocoon development period of 65 to 168 days in *M. houlleti* in the present study was probably a reproductive strategy of this morphotype to overcome unfavorable summer temperature through dormant stage. Lavelle (1978) reported that cocoon incubation period of megascolecid worm *Millsonia anomala*

Table 2. Number of cocoons produced (mean ± SE) per earthworm species during three seasons.

Seasons	Earthworm species						
	D. affinis	*E. comillahnus*	*D.p. papillifer*	*M. houlleti*	*O. beatrix*	*D. assamensis*	*P. corethrurus*
Winter	23.6 ± 1.222	1.2 ± 0.583	0	12.2 ± 1.463	16.4 ± 2.87	0	59.4 ± 2.358
Summer	33 ± 2.702	0	13.4 ± 1.288	10 ± 0.632	26.8 ± 2.818	7.2 ± 0.583	59 ± 5.413
Monsoon	36.8 ± 5.054	3.2 ± 0.97	9.4 ± 1.749	11.2 ± 1.934	36.8 ± 3.2	14.6 ± 1.806	77.8 ± 4.872

was three weeks, but if the cocoons did not hatch before the onset of a dry season, development was delayed and hatching took place at the beginning of the next season. Cocoon development time of *P. corethrurus* in the present study (33 days) is comparable with earlier observations 34, 29 and 40 days by Barois *et al.* (1987), Bhattacharjee & Chaudhuri (2002) and Lavelle *et al.* (1987) respectively.

Temperature affects the incubation period of cocoons. With increase in room temperature, incubation period increased in subsoil endogeic worms, *E. comillahnus* and *O. beatrix* and decreased in epigeic (*D. affinis*), epianecic (*M. houlleti*) and topsoil endogeic species (*D. assamensis*). Reinecke *et al.* (1992) reported a mean incubation period of 17.8 days and 15.3 days for cocoons of tropical earthworm *P. excavatus* incubated at 25 ºC and 25-37 ºC respectively. They further reiterated that the temperature higher than 25 ºC decreased the mean incubation period in the epigeic worms *Perionyx excavatus*, *Eudrilus eugeniae* and *Eisenia fetida*. Holmstrup *et al.* (1991) reported that in temperate endogeic earthworm *Allolobophora chlorotica* embryonic development occurred in 34-38 days at 20 °C compared with 400 days at 5 °C. According to Senapati & Sahu (1993) incubation period, in general, ranges from 3 to 30 weeks in temperate and 1 to 8 weeks in tropical earthworms. In fact, mean temperature during embryonic development does not necessarily coincide with the mean temperature for juvenile emergence. Thus mean temperature during juvenile emergence of *D. p. papillifer* (28 °C), *D. assamensis* (28 °C) and *M. houlleti* (30 °C) were higher than their mean temperature for their embryonic development. On the other hand, mean temperature during juvenile emergence in *E. comillahnus* (22 °C), *O. beatrix* (21 °C) and *P. corethrurus* (27 °C) were lower than their mean incubation temperature. Bhattacharjee & Chaudhuri (2002) reported marginally lower temperature during juvenile emergence of *Perionyx excavatus* (29 °C), *Pontoscolex corethrurus* (29 °C), *Drawida nepalensis* (29 °C) and *Eutyphoeus gammiei* (28 °C) compared with their mean temperature during development. It is suggested that a threshold temperature for cocoon hatching exists for most earthworm species, but that embryonic development can take place even though temperature lies below or above that threshold. The ability of the embryo to develop at a particular temperature range should be regarded as an adaptation to the particular habitat in which the species is living, making it possible for the juveniles to emerge as soon as environmental conditions become favorable.

Compared to the native earthworm species (*D. p. papillifer, D. assamensis, E. comillahnus*) hatching success was much higher in peregrine earthworms viz. *P. corethrurus, M. houlleti, D. affinis* and *O. beatrix*. Our values on hatching success in *M. houlleti* (92 %) and *P. corethrurus* (91 %) are close to figure of 82 % (Kaushal *et al.* 1999) and 97 % (Lavelle *et al.* 1987) respectively. Hatching success of *D. affinis* (81 %) is comparable to the value 78 % in *D. modiglianii* reported by Bhattacharjee & Chaudhuri (2002).

The species under study, with few exceptions, produced one hatchling per worm, although emergence of two juveniles from the cocoons of *D. assamensis* and *M. houlleti* was not rare. Kaushal *et al.* (1995) reported emergence of two juveniles from cocoons of *Drawida nepalensis*. Bhattacharjee & Chaudhuri (2002) recorded emergence of two juveniles per cocoon in *Lampito mauritii*, *Polypheretima elongata*, *Perionyx excavatus* and *Drawida nepalensis*. Edwards (1988) reported mean number of hatchlings per cocoon as 3.3, 2.3 and 1.1 for the cocoons of the epigeic earthworm *Eisenia fetida, Eudrilus eugeniae* and *Perionyx excavatus* respectively. Elvira *et al.* (1996) reported 1.67 hatchlings per cocoon for *Dendrobaena rubida*.

Earthworms are continuous or semi-continuous breeders, producing ova at most times of the year (Olive & Clark 1978). *Pontoscolex corethrurus*, *Dichogaster affinis* and *Octochaetona beatrix* showed continuous breeding strategies with high fecundity, high hatching success and uniparental reproduction, which are the adaptive features of these peregrine species. *P. corethrurus* and *D. affinis* have already established themselves as powerful invasive species in the tropics due to capacity of tolerance to the human disturbance and also their demographic profile (Chaudhuri *et al.* 2008; James & Hendrix 2004; Nath & Chaudhuri 2010). Bhattacharjee & Chaudhuri (2002) reported continuous breeding strategies with high fecundity in peregrine worms viz. *Perionyx excavatus, Pontoscolex corethrurus, Dichogaster modiglianii* and *Polypheretima elongata.* Epianecic species *Metaphire houlleti* and native earthworms *D. p. papillifer, D. assamensis* are semicontinuous breeders and *Eutyphoeus comillahnus* with restricted distribution in north-east India is a discrete breeder. Semi-continuous breeding of widely distributed native species viz. *Lampito mauritii* and *Drawida nepalensis* and discrete breeding of native worm *Eutyphoeus gammiei* were reported by Bhattacharjee & Chaudhuri (2002). The dramatic increase in cocoon production by most earthworm species in summer and monsoon with corresponding peaks during March and September-October were probably due to favourable temperature conditions in Tripura at that time. Bhattacharjee & Chaudhuri (2002) also reported significant increase in cocoon production in *P. excavatus, P. corethrurus, L. mauritii, P. elongata, D. nepalensis* and *D. modiglianii* during summer and monsoon with corresponding peak of cocoon production during April and July. The least number of cocoon production by different earthworm species in the winter months (except *Metaphire houlleti*) were due to fall in temperature. In fact, temperatures beyond optimum levels act as cues for decreased neuro-secretion, thus affecting cocoon production in earthworms (Olive & Clark 1978).

The rate of cocoon production i.e. fecundity varies with species, amount and quality of food supply as well as soil temperature (Evans & Guild 1948). In the present study, the highest and the lowest cocoon production under fluctuating laboratory conditions (20 °C to 30 °C) was by the exotic peregrine and topsoil endogeic worm *Pontoscolex corethrurus* (98 cocoons adult^{-1} year^{-1}) and the native subsoil endogeic worm *Eutyphoeus comillahnus* (2.2 cocoons adult^{-1} year^{-1}). Continuous and high cocoon production in *Pontoscolex corethrurus* and low, discrete cocoon production in *Eutyphoeus comillahnus* respectively may be related to their r- and K-selection breeding strategies respectively (Pianka 1970). High and low fecundity of *Pontoscolex corethrurus* and *Eutyphoeus comillahnus* have been reflected well in their high and low density values (*Pontoscolex corethrurus* 78 m^{-2}, *Eutyphoeus comillahnus* 1.36 m^{-2}) in rubber plantation of Tripura (Chaudhuri *et al.* 2008). According to Lavelle *et al.* (1987) the demographic profile of *Pontoscolex corethrurus* is typically of the r- type, which gives populations a colonization capacity greater than that of a native species. The present value (98 cocoons adult^{-1} year^{-1}) on fecundity of *Pontoscolex corethrurus* is close to 100 cocoons adult^{-1} year^{-1} (Lavelle *et al.* 1987), 118 adult^{-1} year^{-1}, (Bhattacharjee & Chaudhuri 2002) and far above 68 cocoons adult^{-1} year^{-1} reported by Barois *et al.* (1999). Lowest fecundity (1 cocoon adult^{-1} year^{-1}) so far known from tropical species was that of giant earthworm, *Eutyphoeus gammiei* of Tripura (Bhattacharjee & Chaudhuri 2002). Higher fecundity with high hatching success in *P. corethrurus, D. affinis,* and *M. houlleti* seem to be important adaptive features of these peregrine species of earthworms for their successful colonization and worldwide distribution. In spite of being top soil species low rate of cocoon production with low hatching success in *D. p. papillifer* and *D. assamensis* are probably related to their endemicity.

Bhattacharjee & Chaudhuri (2002) reported comparatively higher fecundity in topsoil endogeic species viz. *P. corethrurus* (118 cocoons adult^{-1} year^{-1}). *D. nepalensis* (29 cocoons adult^{-1} year^{-1}) and *L. mauritii* (43 cocoons adult^{-1} year^{-1}). According to Satchell (1967) there is a distinct relationship between the number of cocoon produced and their location in the soil profile. Thus, the species such as *E. comillahnus,* which can move into deeper soil and are protected from adverse conditions produce fewest cocoons, whereas those living near the surface (e.g. *D. affinis* and *P. corethrurus*) and facing unpredictable conditions produce many more cocoons. Lee (1985) correlated the higher risk of mortality in early life of earthworm species with its higher rate of cocoon production. Time of maturation of cocoons and cocoon production vary with species, population density, age structure and external factors specially soil temperature, moisture and energy content of the available food (Lee 1985).

Lavelle *et al.* (1998) proposed a relationship between reproductive strategies and ecological categories in tropical earthworms. They distinguished four groups of earthworms. These are group 1: large native endogeic and anecic species (16-32 g fresh weight) with low fecundity (0.5 - 3.1 cocoons adult^{-1} year^{-1}) and one hatchling per cocoon; group 2 : medium sized species (1.2 - 6 g) with intermediate fecundity (1.3 - 45 cocoons adult^{-1} year^{-1}); group 3: small mainly polyhumic endogeic species (0.17 - 1.25 g fresh weight) with intermediate fecundity (10 - 68 cocoons adult^{-1} year^{-1}) and usually one hatchling per cocoon; and group 4 : generally small, mainly exotic and epigeic species (80 - 550 mg fresh weight) with very high fecundity (50 - 350 cocoons adult^{-1} year^{-1}). From our studies it appears that *O. beatrix* and *E. comillahnus* belong to group 2, *D. p. papillifer, D. assamensis* and *M. houlleti* to group 3 and *P. corethrurus*, *D. affinis* to group 4. No species under present study belonged to group 1. *E. comillahnus, D. p. papillifer* and *D. assamensis* with discrete breeding strategies, low fecundity and moderate to low hatching success are not suitable species for vermiculture under fluctuating environmental conditions. Their reproductive success might be increased if cultured in controlled environmental chamber with suitable diet and maintaining optimum temperature and moisture.

Continuous and high rate of cocoon production coupled with higher hatching rate in *P. corethrurus, D. affinis, O. beatrix* and *M. houlleti* indicate their suitability in vermiculture technology. The geophagous species such as *P. corethrurus* might be useful for waste-land reclamation and to increase plant productivity. This worm might also be used as a source of protein, since it is able to convert low quality organic matter to fresh tissues with 60-70 % protein content with great efficiency (Lavelle *et al.* 1987). In New Guinea, Rose & Wood (1980) showed that *P. corethrurus* has become an indispensable component in the diet of pigs after having been introduced in the early 1960s. *D. affinis* and *M. houlleti* are phytogeophagous and so can be utilized in degradation of organic wastes using soil bedding.

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