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Influence of Adult *Pontoscolex corethrurus* on Development of Cocoons and Hatchlings

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ABSTRACT

Despite many decades of intensive research on earthworms, some fundamental questions remain unsolved. This study investigates the effect of the tropical *Pontoscolex corethrurus* on its cocoon biomass and hatchling growth. A controlled experiment was set up manipulating the presence of adult *P. corethrurus* based on three treatments, each with 20 replicates: Removal of *P. corethrurus* (RPc), No Removal of *P. corethrurus* (Control) and Controlled Removal, where *P. corethrurus* was removed and reintroduced again (RIPc). In all treatments, *P. corethrurus* deposited cocoons inside specially built chambers. No significant differences were observed in the number of cocoons and hatchlings produced between treatments ($P > 0.1206$ and $P > 0.8707$, respectively). Mean cocoon biomass was significantly ($P > 0.001$) higher in the Control (30.3 ± 3.5 mg) and RIPc (29.1 ± 3.6 mg) treatments relative to RPc (25.1 ± 2.7 mg) treatment. By contrast, mean hatchling biomass in RPc (46.7 ± 27.6 mg) was significantly higher than in RIPc (29.3 ± 9.0 mg) and the Control (31.8 ± 9.5 mg) groups ($P < 0.0270$). Based on these findings, we concluded that the parental presence of *P. corethrurus* significantly contributed to maintain cocoon biomass although it was related with a decrease in hatchling growth.

Keywords: growth, incubation, invertebrates, Oligochaeta, reproduction
Abbreviations: RIPc, earthworms removed and reintroduced; **RPc**, earthworms removed from the container

INTRODUCTION

In many soil types, the earthworms dominate soil fauna and have inhabited terrestrial environments for over 600 million years (Stephenson 1930; Lavelle and Spain 2001; Coleman *et al.* 2004). Mostly, investigations on earthworms have focused on species of the family Lumbricidae. For instance, *Lumbricus terrestris* L. (1758) has been the subject of research for nearly 250 years (Grigoropoulou *et al.* 2007). In recent years, most laboratory experiments in tropical regions have advanced our knowledge on the biological features of tropical species, e.g. *Pontoscolex corethrurus* Müller, 1856 (Lavelle *et al*. 1987), *Polypheretima elongata* Perrier, 1876 (Lavelle *et al*. 1987; Huerta *et al*. 2005), which are generally dominant in natural ecosystems and agroecosystems.

P. corethrurus is a parthenogenic earthworm commonly found in gardens, cropland and fallowland, being widely tolerant of edaphic and climatic changes (Lavelle *et al.* 1987). It is an unpigmented worm, 7-10 cm long and 3-4 mm in diameter. Adults may weigh up to 600-3500 mg fresh mass, and the cocoons' mean weight is about 40 mg fresh weight (Lavelle *et al*. 1987).

However, despite many decades of intensive research on the biological activity of earthworms, especially as "ecosystem engineers" (Jones *et al.* 1994; Lavelle *et al*. 2006; Hastings *et al.* 2007) and their impact on plant growth (Scheu 2003; Brown *et al.* 2004; Ortíz-Ceballos *et al.* 2007), basic questions remain unsolved. One of these is: do earthworms display any behaviour patterns which help increase offspring survival? Cocoons have been documented to be extraordinarily tolerant to low and high temperatures (Stephenson 1930; Lee 1985; Edwards and Bohlen 1996; Holmstrup and Zachariassen 1996). In a study by Ortíz-Ceballos *et al.* (2005), *P. corethrurus* cocoons were observed to dehydrate rapidly when exposed to air. This fact led us to hypothesize that earthworms might protect cocoons. Whether earthworms display any behaviour patterns which help increase offspring survival is not yet clear (Ortíz-Ceballos and Fragoso 2006; Grigoropoulou *et al.* 2007). This study investigates the influence of *P. corethrurus* presence on cocoons and hatchlings development.

MATERIALS AND METHODS

One hundred and ten adult earthworms, *P. corethrurus* were collected by manual hand-sorting from an abandoned mango plantation located 70 km southwest (17° 48′ N, 93° 28′ W) of Villahermosa, Tabasco, Mexico. Each earthworm was placed in individual plastic containers (12 × 12 × 8 cm) until cocoon production was observed. Sixty adult earthworms with a similar biomass (1.21 ± 0.23 g; $P > 0.577$) were selected from this group. Each was individually placed in a styrofoam container (273 ml) along with 300 g, equivalent oven-dry weight and sieved at 2 mm, of well-mixed soil (Fluvisol; 26.9% clay, 31.6% silt, 41.5% sand and 2.7% organic matter) and velvetbean (3% of *Mucuna pruriens*; 2.3% N and 23.3 C/N) and moistened to field capacity (42% H_2O). Containers were incubated at 27 ± 3°C.

In a previous pilot experiment using the same quantity and quality of soil (300 g of soil mixed with 3% of *Mucuna pruriens*), we registered cocoons production at every 10, 15, 23 and 35 days, we found that after 22 days cocoon production did not increase. Also we observed that if cocoon was removed or the earthworm changed to a different container, earthworms deposited more cocoons (Ortiz-Ceballos *et al.* 2005). So, we determined the 22 days period adequate for our experiments.

After 22 days, during which earthworms produced cocoons, experimental manipulation of the earthworms was undertaken. Each of the following treatments (randomized) included 20 replicates:

1. Removal of *P. corethrurus* (RPc): the earthworm was removed from the container.
2. No *P. corethrurus* removal (Control): no manipulation.

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Short Communication

Fig. 1 Chamber constructed by *Pontoscolex corethrurus* containing cocoon and associated casting.

3. Controlled removal of *P. corethrurus* (RIPc): the earthworm was removed and immediately reintroduced, in an attempt to assess any potential effect of the removal method.

In the manipulation treatments (RPc and RIPc), the block of soil was carefully removed from the container, air was then blown into burrows until the earthworm exited at which point it was removed immediately from the container. After the day of manipulation, soil moisture was monitored at 5-day intervals to keep it constant. The experiment ended 38 days after the initial manipulation.

When the experiment was completed (sufficient time was allowed for the hatchlings to emerge from the cocoon), the earthworm and hatchlings were collected manually, washed and dried and then counted and weighed; while that cocoons only were counted and weighed immediately.

Earthworm, cocoon and hatchling weight data were evaluated by an analysis of variance (one-way ANOVA). The number of cocoons and hatchlings were fitted to the Poisson distribution model. Differences between means were assessed using Tukey's HSD test ($P < 0.05$). All analyses were run with Statistica 6.0 (StatSoft Inc.).

RESULTS

All earthworms were active and healthy on the day of manipulation (22 days after the initiation of the test) and after 60 days (test completion). Each earthworms gained weight (5 ± 2.4 mg) after 22 days in RPc, but lost weight at the end of the experiment (60 days) in the Control and RIPc (mean of weight loose 362 ± 194 and 357 ± 225 mg, respectively) treatments. All Control earthworms and in manipulated treatments (85% RPc and 70% RIPc) produced cocoons at the end of the experiment (60 days). In all cases, cocoons (n = 240; 3.9 ± 1.6 mm long and 3.5 ± 0.3 mm wide) were found inside circular chambers, along with associated casting chamber (**Fig. 1**).

No significant differences were found in the number of cocoons and hatchlings between treatments ($F = 2.21$, $P > 0.1206$ and $F = 0.14$, $P > 0.8707$, respectively). Control, RIPc and RPc treatments produced a similar number of cocoons (5.3 ± 3.1; 5.1 ± 2.1 and 3.5 ± 1.3, respectively) and hatchlings (3.0 ± 1.6; 2.9 ± 3.1 and 2.6 ± 1.3) per earthworm.

Mean cocoon biomass was higher in the Control (30.3 ± 3.5 mg) and RIPc (29.1 ± 3.6 mg) treatments relative to the RPc (25.1 ± 2.7 mg) treatment (**Fig. 2**). By contrast, mean hatchling biomass was higher in the RPc (46.7 ± 27.6 mg) treatment compared to the Control (31.8 ± 9.5 mg) and RIPc (29.3 ± 9.0 mg) treatments. This increase in cocoon and hatchling biomass depended on earthworm manipulation ($F = 10.45$, $P < 0.0001$ and $F = 3.98$, $P < 0.0270$, respectively).

At the end of the experiment, the Control and RIPc treatments produced an average of 8.3 ± 4.3 of progeny (cocoons + hatchlings, with a maximum of 15 and 12, respectively). The RPc treatment produced 6.0 ± 2.2 (with a maximum of 6 cocoons and 5 hatchlings).

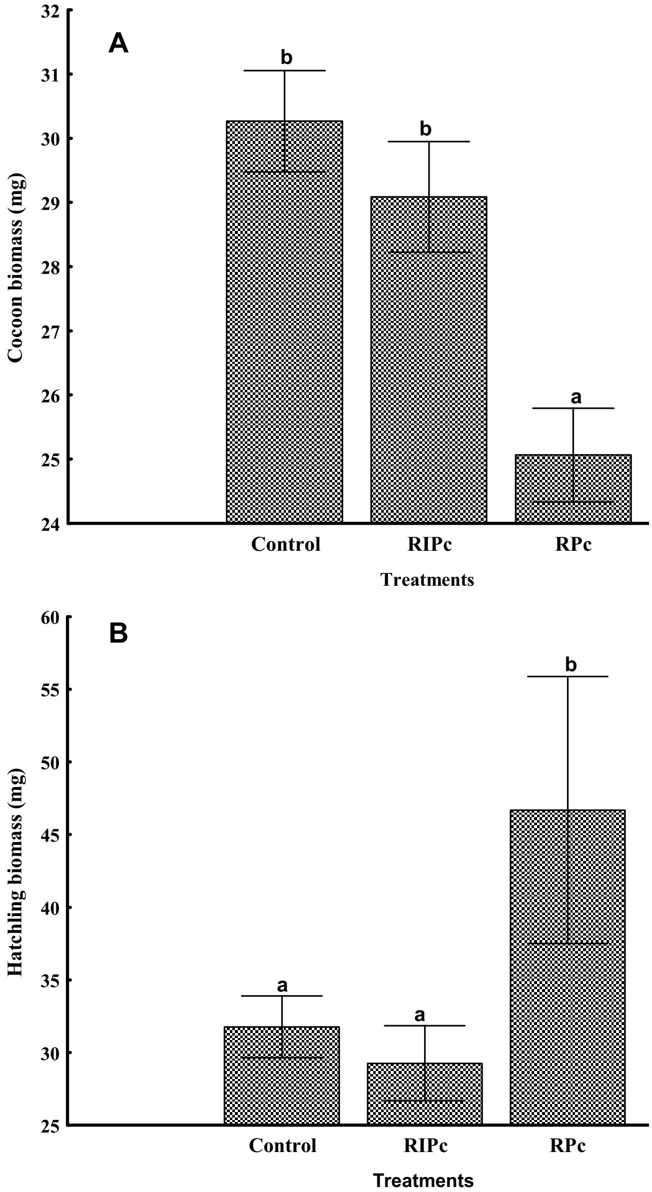

Fig. 2 Effect of *Pontoscolex corethrurus* parental presence on (A) mean cocoon biomass and (B) hatchling growth. Control: no manipulation; RIPc: earthworm removed and reintroduced; RPc: earthworm removed. Different letters indicate significant difference ($P < 0.05$; Tukey's HSD).

DISCUSSION

The present study demonstrates that the presence of *Pontoscolex corethrurus* has a significant effect on cocoon biomass and hatchling growth. Individual cocoon production in the Control group and RPc and RIPc treatments was similar to that reported by several authors (Lavelle *et al.* 1987; Bhattacharjee and Chaudhuri 2002; García and Fragoso 2003). However, cocoon production may increase (0.5 ind^{-1} day^{-1}) when biotic (earthworm age) and abiotic (moisture, temperature, food) conditions and the interaction between them (density dependence) are optimum (Ortiz-Ceballos, unpublished data). Other demographic studies on *P. corethrurus* suggest a trade-off between biomass and cocoon number in response to food quality (García and Fragoso 2003) and soil moisture (Fragoso and Lozano 1992). The results here did not reveal any evidence of the above, as the highest cocoon number and biomass were observed in the Control treatment. Likewise, in recent work by Grigoropoulou *et al.* (2007), the parental presence of *L. terrestris* had no significant effect on cocoon viability; however, the cocoon biomass was not determined.

In this study, *P. corethrurus* deposited all cocoons inside chambers, as observed by Ortiz-Ceballos and Fragoso (2006). By contrast, *L. terrestris* coats cocoons with excreta (Ramisch and Graff 1985; Holmstrup and Zachariassen 1996; Grigoropoulou *et al.* 2007). Both strategies may serve as a protection to maintain humidity, and have been developed based on the ecological category to which these two earthworm species belong, *P. corethrurus* being endogeic (species that inhabit mineral soil horizons, feeding on soil more or less enriched with organic matter – Lee 1985) and *L. terrestris* anecic (those which lives in burrows, in mineral soil layers, but come to the surface to feed on dead leaves, which they drag into their burrows – Lee 1985). The chambers found in this study had not been previously observed, perhaps due to the method used for cocoon collection (Huerta *et al.* 2005; Grigoropoulou *et al.* 2007), as these chambers resemble those built when earthworms are quiescent or dormant (Evans and Guild 1947; Edwards and Bohlen 1996; Jiménez *et al.* 1999, 2000).

Ortíz-Ceballos and Fragoso (2006) suggested that, after depositing cocoons inside chambers, *P. corethrurus* entered into the cocoons chambers perhaps to ingest microorganisms (protection against pathogens) or to deposit mucus and fine soil particles onto the chamber's inner walls (physical protection). The latter may explain why cocoons did not lose weight in the presence of *P. corethrurus* and to the detriment of parental biomass (Control and RIPc). Furthermore, these findings suggest that weight loss (desiccation) would be even higher if cocoons were not deposited inside chambers, as observed by Ortiz-Ceballos *et al.* (2005).

Mean hatchling biomass was higher where *P. corethrurus* was removed (RPc), suggesting a negative effect of parental presence on hatchling growth, similar to reports by Grigoropoulou *et al.* (2007). This leads to the assumption that, under stressed conditions, parent-offspring competition may take place. Ortiz-Ceballos and Fragoso (2006) suggest that *P. corethrurus* fed on excreta (enriched in microorganisms), in addition to newborn hatchlings. This may lead to a competition of resources between parents and offspring.

CONCLUSION

Based on the results reported in this study, it is concluded that *P. corethrurus* presence during incubation significantly contributes to maintain cocoon biomass. However, parental presence negatively affected hatchling growth.

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