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COCOON VIABILITY AND EVIDENCE FOR DELAYED HATCHING BY THE EARTHWORM *LUMBRICUS TERRESTRIS* IN A LABORATORY - BASED STUDY

Clitellate Lumbricus terrestris, obtained from 5 commercial suppliers (A-E) and also field collected (F) - grassland in Preston, Lancashire, UK, were kept under controlled environmental conditions (15 °C and 24 h darkness) in a sterilised loam soil and surface-fed with horse manure. Survival, biomass and cocoon production was monitored every 4 weeks over 1 y. Collected cocoons were maintained in water-filled Petri dishes on filter paper. Time to hatch and cocoon viability was recorded over a 2 y period. Cocoon production ranged from 15.1 – 32.2 ind.$^{-1}$ y^{-1}. Cocoon production was initially low followed by a period of high production (12-36 weeks) and then fell (36-52 weeks). Time for cocoon hatching ranged from 132-731 days. Hatching success after 2 years was 58–90% across treatments, with a total viability (including cocoons dissected after the 2 year period) of 88-94%. Evidence of 2 distinct hatching peaks was recorded, separated by a period of approximately 12 months in treatments of most of the commercially obtained earthworms. Cocoon incubation periods are in excess of those previously recorded under similar laboratory conditions (e.g. 90–280 days). Furthermore, viability is also higher than previously recorded (e.g. 67.9–83%). These differences are mainly attributable to the extended length of cocoon observation. Origin, age, unknown pre-treatment (in A-E) and experimental conditions (e.g. a constant temperature regime) may have influenced incubation times. However, it is suggested that asynchronous and delayed hatching within cohorts and ability for cocoons to remain viable for extended periods allows this K-selected species to maximise reproductive potential. This proposed "bet-hedging" strategy is worthy of further laboratory and field-based investigation.

Keywords: *Lumbricus terrestris*, bet-hedging, cocoon development, delayed hatching

I. INTRODUCTION

Lumbricus terrestris (Linnaeus, 1758) is a relatively large (adult mass 3-5 g), long-lived, temperate, anecic earthworm species that usually inhabits a permanent near-vertical burrow system. This K-selected species [20] is found predominantly in undisturbed habitats and under field conditions maturation is achieved in approximately 12 months (in Britain) but may take longer in more extreme climates [4,21]. *L. terrestris* is an obligatory biparental species. Adults usually mate on the soil surface and cocoons are deposited below ground in association with the burrow system. Cocoon production (< 20 coc ind^{-1} y^{-1}) is usually restricted to spring and autumn. Life cycle parameters under field [5] and laboratory [4] conditions are relatively well established and sustainable populations have been successfully cultured under laboratory conditions [13].

The influence of temperature on cocoon development and hatchling emergence is widely recognised. Field observations in Sweden [18] noted a bi-modal synchronised emergence with 2 pronounced peaks in early June and mid-August to September. By contrast, in England, Satchell [19] observed only a single emergence period in spring to early summer. Under controlled laboratory conditions, Meinhardt [15] reported that *L. terrestris* cocoons hatched in 84-91 days a figure supported by Butt et al. [4] who recorded a mean incubation time of 90 days at an optimal incubation temperature of 15°C. Butt et al. [4] recorded cocoon viability of 71% but this was based on maintaining cocoons only for a period of twice (the known) mean incubation period (up to 180 days). There have only been 2 published studies [3, 22] that have recorded *L. terrestris* cocoon incubation and hatching over extended time periods (in excess of 12 months). Both studies recorded cocoon hatching times far in excess of 90 days. Butt [3] observed successful hatching after 100 weeks whilst Svendsen et al. [22] recorded hatching after 114 weeks with a maximum difference between hatching times of cocoons from the same batch of 77 weeks (both studies conducted at 15°C).

The aim of the current laboratory study was to determine the influence of earthworm origin and pre-treatment on cocoon viability and time to hatch for *L. terrestris* over an extended 3 year experimental period. The study formed part of a larger project investigating the use of commercially obtained *L. terrestris* in ecotoxicological studies [14]).

II. MATERIALS AND METHODS

Adult (clitellate) *L. terrestris* were obtained from five commercial suppliers (A-E) and field-collected (F) from grassland in Preston, Lancashire, UK. Twenty *L. terrestris* from each of the suppliers and from the field were cultured (n=4 per vessel), as described by Lowe and Butt [14] for a period of 52 weeks. Every 4 weeks, soil and feed were replaced with fresh material and the culture substrate was wet-sieved for cocoons through a series of graded sieves (6.7, 4.00 and 3.35 mm).

Cocoons from each replicate were placed in labelled Petri dishes on filter paper (Whatman no 1), provided with excess water to prevent dehydration and maintained at 15°C in 24 h darkness [1]. Dishes were checked regularly for hatchlings, allowing calculations to be made on viability and length of incubation. The latter was calculated as time to hatch after collection plus 14 days (half of the time between sampling periods) [2]. Water was replenished as required, and due to occasional microbial growth, filter paper and Petri dishes replaced. On emergence, hatchlings and spent cocoons were removed from the dishes. After 2 years incubation, any remaining un-hatched cocoons were dissected and viability was recorded.

An Anderson-Darling normality test was used to assess normality of hatching times within the 6 treatments (A-F). Further inferential statistical analyses were not undertaken as experimental design did not allow individual cocoons to be assigned to the adult that produced them, as earthworms were kept in groups of four.

III. RESULTS

Mean incubation durations (Table 1) were in excess of those previously recorded for this species under similar laboratory conditions with cocoons hatching over an extended range (maximum range 599 days in treatment E). All treatments contained cocoons that hatched after 700 days of incubation and viable cocoons (containing a live hatchling on dissection) that remained unhatched at the end of the 2 year period. Cocoon hatching after 2 years was variable and ranged from 58% (E) to 90% (F). However, total viability was more consistent and ranged from 88% (E) to 94% (A).

Table 1 / *Tabela 1*

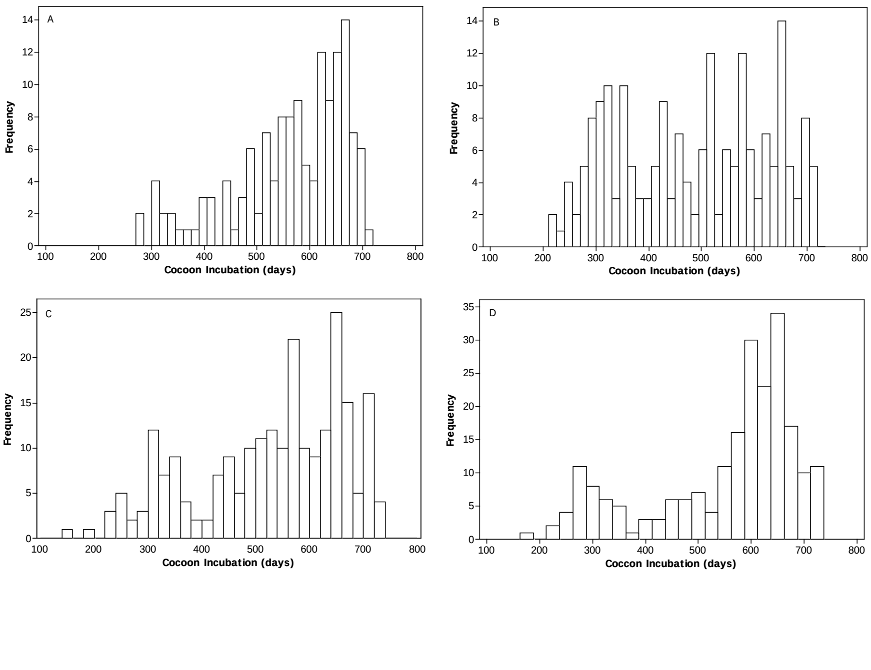
Cocoon production, incubation, hatching, and viability data for adult *L. terrestris* obtained from five commercial (A-E) and 1 field-collected (F) source

Produkcja kokonów, inkubacja, wylęganie oraz żywotność dorosłych L. terrestris uzyskanych z pięciu komercyjnych (AE) i polowego źródła (F)

Treat-ment *Pocho-dzenie*	Total Nº of cocoons *Całkowita liczba kokonów*	Mean time to hatch (days)* *Średni czas do wylęgu (dni) **	Hatching range (days) (min to max) *Zakres wylęgu (dni) (min - max)*	% hatched after 2 years *% wyklutych po 2 latach*	Cocoon viability (%) *żywotność kokonów (%)*	Normality test (p value) *Test śmiertelności (wartość p)*
A	302	566 (103)	442	86	94	<0.005
B	388	478 (146)	504	83	91	<0.005
C	643	535 (131)	577	72	91	<0.005
D	442	552 (138)	544	68	90	<0.005
E	453	538 (160)	599	58	88	<0.005
F	367	446 (88)	479	90	91	0.017

* Standard deviation in parentheses / *Odchylenie standardowe w nawiasach*

Incubation times for cocoons hatching within a 2 year period did not follow a normal distribution (p<0.05 in all treatments). Incubation time distributions for the 6 treatments are shown in Figure 1. Distributions in treatments B-E suggest evidence for two hatching peaks separated by a period of 10-12 months.



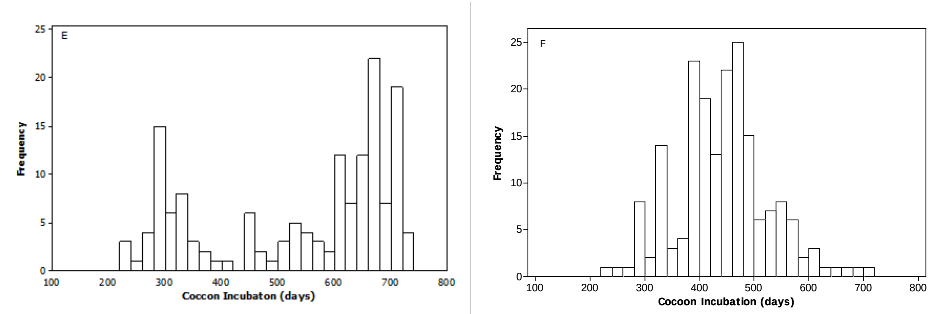


Fig. 1. Frequency of *L. terrestris* cocoon hatching over time for 6 treatments A-F (A-E employed earthworms purchased from different commercial suppliers, F used field-collected earthworms)
Fig. 1. *Frekwencja wykluwania kokonów L. terrestris. Kokony pochodzą od dżdżownic z różnych źródeł A-F (jak w tabeli ; A-E źródła komercyjne; (F) pobranie w polu)*

IV. DISCUSSION

Mean cocoon incubation times (446 – 566 days) in all experimental *L. terrrestris* treatments were significantly greater than the 90 days recorded by Meinhardt [15] and Butt et al. [4]. In treatments A,B,E and F the first cocoons did not hatch until after 200 days of incubation. This stark difference in findings is difficult to interpret as cocoons in the current study and Butt et al. [4] were maintained under very similar laboratory conditions. However, cocoons in the latter were produced by recently matured individuals, whilst in the former, adults were field-collected and of unknown age and origin (with the exception of F). Svendsen et al. [22] suggested that cocoon incubation time increases with the age of the parents and this may partly explain the extended period of time required for the first cocoons to hatch.

In the current study, a proportion of cocoons in all treatments remained viable throughout the 2 year study period. In treatments A, B and F cocoon viability (83-90%) (after 2 years) was in excess of that previously recorded for this species. Furthermore, on cocoon dissection, cohort offspring viability increased to 88-94%, indicating that cocoons of this species can remain viable for more than 24 months (under specified laboratory conditions). This observation is supported by Svendsen et al. [22] who recorded hatching of *L. terrrestris* cocoons after 114 weeks under similar laboratory conditions. They also observed that within a batch of cocoons produced by the same pair of worms over 1 month, the incubation time varied considerably with maximum difference between hatching times of cocoons in the same batch as much as 60 weeks (compared with 86 weeks in the current study).

Climatic conditions are known to influence earthworm activity and development. In adverse soil moisture (and temperature) conditions earthworms have developed a range of survival strategies. Certain species are able to enter into obligatory diapause (e.g. *Aporrectodea longa*) with other species able to adopt a less permanent quiescent state (e.g. *Aporrectodea caliginosa)* [7]. *L. terrestris* does not aestivate but has been reported to reduce casting and surface activity and reduce cocoon production in response to drought [19] and reside at depth it its burrow under extremes of cold [16]. Parmalee and Crossley [17] have suggested that cocoons could act as the main survival stage during drought for certain non-aestivating species (*e.g. Lumbricus rubellus*) as all juveniles and adults may die. Embryonic development is also known to be delayed at low temperatures [1] and/or under dry conditions [9]. Furthermore, Edwards and Bohlen [6] have suggested that cocoon development might be influenced by the availability of food for the parent worm. However, with the exception of the latter, these factors may not explain delayed hatching in this study as all cocoons were maintained at a temperature considered optimal for development and were provided with excess water to prevent dehydration. In addition, Jensen and Holmstrup [11] demonstrated that incubation times of cocoons (*A. caliginosa, Allolobophora chlorotica* and *Dendrobaena octaedra*) placed on moist filter papers were not systematically slower than in soil.

Butt [3] recorded extended periods of cocoon incubation and suggested that the results may be ‘an artefact of the incubation technique’ and considered it ‘difficult to explain in terms of evolutionary advantage’. However, it is suggested that asynchronous and delayed hatching within cohorts and ability for cocoons to remain viable for extended periods may allow this K-selected species to maximise reproductive potential. Rundgren [18] suggested that the bi-modal pattern of *L. terrestris* emergence observed under field conditions in Sweden, may reduce risks associated with emergence in adverse environmental conditions and/or scarcity of food. Svendsen et al. [22] also stated that variable incubation times may be a physiological adaptation to fluctuating environmental conditions, but also suggested that it may also enable populations to survive stress associated with agricultural management practices. This “bet-hedging” strategy has also been observed in other invertebrate species. Hakalahti et al. [10] studied the egg hatching dynamics in the ectoparasitic crustacean *Argulus coregoni*. This species of fish louse over-winters as eggs and emerges in the spring to infect fish populations. Observations at Finnish fish farms have indicated that the hatching pattern of eggs was extended by several months, which was confirmed by controlled laboratory experiments. Clutches of eggs hatched over a period of 7 months with potentially viable eggs remaining at the end of the 451 day observation period. The authors speculated that ‘some eggs were genetically programmed for later hatching’ and that this strategy maximises progeny survival through unpredictable patterns of host availability. Extended periods of hatching have also been recorded in the eggs of the Nearctic Stonefly *Megarcys signata* [23] and the northern and western corn root worms (*Diabrotica barberi and Diabrotica virgifera virgifera*) [12].

In this current study, *L. terrestris* cocoons were maintained at a temperature considered optimal for development and hatching. Therefore it is suggested that observed delayed hatching may not be related to environmental cues but rather a “pre-programmed” response (bet-hedging strategy) to maximise reproductive potential driven by low fecundity and variable environmental conditions.

However it also must be accepted that origin, age, unknown pre-treatment and controlled experimental conditions may have influenced cocoon incubation times and it is advocated that the proposed alternative “bet-hedging” strategy in this and similar earthworm species is worthy of further laboratory and field-based investigation.

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ŻYWOTNOŚĆ KOKONÓW I DOWODY NA OPÓŹNIONY WYLĘG U DŻDŻOWNICY LUMBRICUS TERRESTRIS L. W BADANIACH LABORATORYJNYCH

Streszczenie

Dojrzałe dżdżownice Lumbricus terrestris *L. zostały zakupione u pięciu komercyjnych hodowców (A-E) a także zebrane w środowisku naturalnym (F) – użytek zielony w Preston, Lancashire, w Anglii. Trzymano je w kontrolowanych warunkach (15°C i 24 godziny w ciemności w wyjałowionej glebie gliniastej. Były karmione obornikiem końskim.*
Przeżywalność, biomasę i produkcję kokonów monitorowano co 4 tygodnie przez okres jednego roku. Otrzymane kokony utrzymywano w wypełnionych wodą płytkach Petriego na papierze filtracyjnym. Czas wylęgu i żywotność kokonów obserwowano ponad 2 lata. Produkcja wahała się od 15,1 - 32,2 kokony / osobnika / rok.
Początkowo produkcja kokonów była niska, następuje obserwowano (przez 12-36 tygodni) okres wysokiej produkcji a potem spadek (w 36-52 tygodniu). Czas wykluwania z kokonów wahał się od 132-731 dni. Po 2 latach badań stwierdzono 58-90% wykluwalności we wszystkich obserwowanych przypadkach (w tym kokony badane po okresie 2 lat - 88-94%). Odnotowano 2 odrębne piki wylęgowe oddzielone przez okres około 12 miesięcy „leczenia" większości dżdżownic zakupionych u komercyjnych hodowców. Wykazane okresy inkubacji kokonów przekraczają uprzednio zarejestrowane w podobnych warunkach laboratoryjnych (np. 90-280 dni). Ponadto stwierdzona efektywność wykluwania jest wyższa niż wykazana poprzednio (np. 67.9-83%). Różnice te są w głównej mierze wynikiem znacznie dłuższego okresu obserwacji kokonów. Pochodzenie, wiek, nieznane traktowanie (w przypadku AE) oraz warunki doświadczalne (np. stały rozkład temperatur) mogą mieć także wpływ na czas inkubacji. Doświadczenie sugeruje jednak dodatkowo, że asynchroniczne i opóźnione wylęgi ciągu kohort Lumbricus terrestris *i ich zdolność do pozostawania przy życiu przez dłuższy czas, pozwalają tym dżdżownicom o strategii rozrodczej K, na maksymalizowanie potencjału reprodukcyjnego.*
Obserwowana strategia "bet- hedging " wymaga dalszych badań laboratoryjnych i terenowych.

Keywords: *L. terrestris*, strategia "bet-hedging", rozwój kokonów, opóźniony wylęg