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Food quality affects production of *Lumbricus terrestris* (L.) under controlled environmental conditions

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ABSTRACT

Birch leaves and horse manure were used to determine the effects of food quality on growth and reproduction of laboratory-reared *Lumbricus terrestris*. Animals grew to maturity within 6 months but attained a significantly ($p < 0.001$) larger adult size with manure (6.17 g) versus leaves (4.20 g). Cocoon production by recently-mated adults maintained in isolation, fed with birch leaves or horse manure, resulted in 4.53 and 3.84 cocoons ind.$^{-1}$ month^{-1} respectively, with an initial hatchability of 86%, falling to zero after 18 months. Re-mating of these known individuals permitted long term monitoring of reproductive output (to 30 months). For the whole experimental period, overall hatchability of the 2010 cocoons produced was 44.4%. Median incubation time of those cocoons that hatched within accepted norms (less than 5 months at 15 °C) was 103 days and was not influenced by adult food type. A proportion (35.5%) of cocoons took in excess of 12 months to hatch. Adult mortality was minimal (25%) during the long term experiment but abnormal cocoon production was recorded after 2 years. Overall results demonstrate that food quality can have a significant influence on somatic and reproductive production of *L. terrestris* and these data may aid construction of production models for this earthworm in ecosystems with contrasting food quality.

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1. Introduction

Laboratory-based research over four years on growth and reproduction of the earthworm *Lumbricus terrestris* (Linnaeus, 1758) is the focus of this paper. Much is known of this animal, but this work was specifically undertaken to obtain data for use within a research or even commercial setting when considering the type of food material for mass earthworm production. Additionally, the data generated was thought to be of value for population model construction and specifically sought to offer information to the area of forestry research, where soil organisms are now given more prominence e.g. when ex-agricultural land is converted to practices such as short rotation forestry (e.g. LTS, 2006).

L. terrestris, an amphimictic, oligochaete earthworm is found naturally across Eurasia (e.g. Satchell, 1967) and has been introduced by Man across most temperate regions of the world (e.g. Frelich et al., 2006). This species is of enormous ecological interest due to its activities in the soil which include: Collection and burial of leaf litter from the soil surface (Wright, 1972) with Satchell (1967) calculating that in a deciduous forest this may amount to 3000 kg ha^{-1} over 3 months; Provision of deep, near vertical burrow systems (e.g. Jégou et al., 2000; Shipitalo and Butt, 1999) which may extend more than 1 m and assist water infiltration through surface-opening macropores, prevent potential surface soil erosion at the surface, even if acting as a preferential flow pathway for dissolved nutrients and suspended particles; and assist with soil aeration. Butt and Nuutinen (2005) reviewed these and other ecosystem services provided by this earthworm, which is designated as an ecosystem engineer (Lavelle et al., 1997). Due to the ecological behaviours already mentioned and other specific biological activities, such as mating on the soil surface, *L. terrestris* has received enormous exposure in the scientific literature, even though some of these behaviours are not necessarily typical of the Lumbricidae (Sims and Gerard, 1999).

Growth and reproduction of *L. terrestris* has been reported in field (e.g. Daniel, 1992; Lakhani and Satchell, 1970) and laboratory-based investigations (e.g. Berry and Jordan, 2001; Butt et al., 1992; Daniel et al., 1996; Hartenstein and Amico, 1983; Löfs-Holmin, 1983) and a combination of the two (e.g. Whalen and Parmelee, 1999). This fundamental information, particularly related to abiotic environmental conditions such as temperature and soil moisture, is of great value and has been utilised in the construction

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of population models for this earthworm (e.g. Pelosi et al., 2008). Experimental work, undertaken in the laboratory but designed to match field conditions, has utilised selected plant material such as willow litter (Curry and Bolger, 1984) or dandelion leaves (Daniel, 1991) as food. Other researchers have fed this species a variety of organic wastes, stemming from the seminal work of Evans and Guild (1948) with rotted animal manures providing basic biological data, to more recent research focussed on potential mass rearing for soil restoration (e.g. Butt et al., 1992). Developments from the latter have meant, over recent years, that some researchers now regard manures as a standard food for *L. terrestris* growth and reproduction investigations (e.g. Berry and Jordan, 2001; Lowe and Butt, 2005). However, recent publications appear to demonstrate that plant debris, in the form of fallen tree leaves, is perfectly acceptable for use (e.g. Grigoropoulou et al., 2008) and may be seen as a return to use of more naturally occurring materials for earthworm growth and reproduction studies.

Measurement of reproductive output (cocoons per earthworm per given time period) may be conducted with field-collected animals (e.g. Curry and Bolger, 1984) perhaps over relatively short periods, or with animals kept together in experiments for a number of years (e.g. Butt et al., 1992), or even from more closely monitored individuals. For example, Butt and Nuutinen, (1998) recorded *L. terrestris* cocoon production from animals that had been observed (video-recorded at the soil surface under infra-red light) following a single mating event (Nuutinen and Butt, 1997). Subsequently, viable cocoon production was recorded from isolated animals.

1.1. Aims and objectives

The overall aim of this work was to evaluate *L. terrestris* production (here defined as growth and reproductive output) within a controlled environment. This permitted the following objectives to be addressed: 1) Measurement of growth from hatchling stage to maturity with 2 basic food stocks (manure and leaves); 2) Measurement of cocoon production using the same food materials; 3) Monitoring of viable cocoon production from mated animals, subsequently maintained in isolation; 4) Evaluation of tagging earthworms as a technique for tracking individuals in dynamic experiments. Addressing these objectives stemmed from a number of standpoints: Parallel research in forest ecosystems was beginning at the outset of this work and the value of a selected species of tree leaf for earthworm growth and reproduction, in direct comparison with a proven food source (manure) was desired; this information would then also be available for earthworm population modelling, such as that described by Pelosi et al. (2008); and potentially assist in future development of earthworm biostimulation studies (e.g. Butt et al., 1992).

2. Materials and methods

Two separate experiments were conducted to examine growth and reproduction of *L. terrestris*. The earthworms used were bred from adult stock collected in 2006 by mustard extraction (Butt, 2000), from a mixed deciduous woodland, south of Preston (53° 40′ 33″N, 02° 48′ 54″W), see Grigoropoulou and Butt (2010) for a full site description. Each experiment used a standard sterilised loamy soil (Boughton Loam, UK) with a moisture content of 25%. Opaque, 0.75 L (depth 0.1 m) plastic vessels (Lakeland Plastics, UK) were provisioned with 0.6 L of soil and surface fed with the appropriate food material. Vessels were closed with lids, perforated with a mounted needle to permit circulation of air and maintained at 15 °C (Butt, 1991) in temperature-controlled incubators.

2.1. Growth

This comparatively short term experiment (28 weeks), examined growth of hatchling *L. terrestris* collected individually from laboratory-produced cocoons and incubated at 15 °C (Butt, 1991). On emergence, each hatchling was kept separately in a sealed pot of water at 5 °C until the required number (20) was assembled. For this experiment, a single hatchling (mean mass 60 mg) was placed in each of 20 vessels and food material was applied at the soil surface. Two food treatments were used: silver birch (*Betula pendula*) leaves, collected from a single tree; or horse manure, obtained from the stable of a single horse (Table 1). Leaves from birch were selected as these have been demonstrated as one of the most palatable tree species to earthworms, in terms of both nitrogen and carbohydrate content (Satchell, 1967; Wittich, 1953). In addition, birch is one tree species now used in short rotation forestry (e.g. LTS, 2006) a practice of biomass production, with a duration or growth between traditional forest growth and short rotation coppice. On collection, birch leaves were air-dried and stored until required. The horse manure was pre-treated by drying to 103 °C and re-wetted to make it more acceptable to the hatchling earthworms (Lowe and Butt, 2005). Obtaining these materials from very specific locations meant that consistency of supply could be ensured and e.g. no detrimental effects of unknown horse medicaments would impact upon the long term experiment. Each feedstock was crushed to pass through a 2 mm sieve pre-feeding, as particle size is known to influence earthworm growth (Boström and Löfs-Holmin, 1986).

The experiment was begun in August 2007 and vessels were examined every month, at which point contents were removed, mass of the earthworms and their developmental stage was determined and then each returned to its vessel. The experiment was terminated when all surviving earthworms were recorded as clitellate.

2.2. Reproduction

L. terrestris (mean mass 6.93 g) were grown to maturity in isolation under conditions similar to those described above, from laboratory-produced hatchlings fed with a mixture of crushed birch leaves and dried, crumbled horse manure (1:1). This was to ensure that no pre-conditioning, with respect to food, occurred prior to experimentation. In August 2007, sixteen of these recently matured, virgin animals were randomly assigned in 8 pairs, with each pair housed in a 0.75 L vessel. Four pairs were fed with birch leaves and the other four with horse manure *ad libitum*. After one month, the vessels were inspected and each individual earthworm was re-housed in its own individual coded 0.75 L vessel, fed as before but having had one month for reproductive activity with a partner. Soil from the vacated vessels was wet-sieved through a series of 6.7, 4.0 and 2.8 mm meshes for collection of cocoons (Butt et al., 1994). Any cocoons located were cleaned of adhering soil, had masses determined and were then incubated on moistened filter papers in Petri dishes at 15 °C (Butt, 1991). At monthly intervals thereafter, for a period of 30 months, each vessel was

Table 1
Selected chemical constituents of food materials and soil used in growth and reproduction experiments with *Lumbricus terrestris* (nd − not determined).

Material	pH	OM content (%)	C:N	Nitrate (mg/kg)	P (mg/kg)	K (mg/kg)	Mg (mg/kg)
Fallen birch leaves	6.07	88	21:1	66.6	3418	6063	1719
Green birch leaves	5.25	93	22:1	2566	2933	8574	1341
Horse manure	7.01	90	26:1	449.4	4634	8572	2004
Loamy soil	7.73	5.5	nd	115.5	25.6	117.9	242