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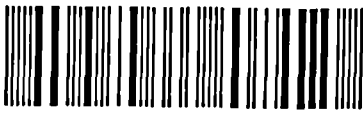
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**AN INVESTIGATION INTO THE GROWTH AND REPRODUCTION
OF THE EARTHWORM *LUMBRICUS TERRESTRIS* L. UNDER
CONTROLLED ENVIRONMENTAL CONDITIONS.**

Submitted by Kevin Richard Butt B.Sc., M.Sc.

To the Open University as a thesis for the degree
of Doctor of Philosophy in the
Faculty of Technology.

May 1990

Authors number - m7023898
Date of submission: 23rd May 1990
Date of award: 26th June 1990

ABSTRACT

Earthworm inoculation for soil amelioration has been shown to be valuable in a range of experiments. At present, inoculation on a large scale is limited by the supply of larger deep-burrowing species of earthworm. This work aimed to assess the feasibility of intensively producing deep-burrowing earthworms for soil amelioration projects. *Lumbricus terrestris*, whose behaviour is well documented, was chosen.

The scientific literature was used to identify points within the life cycle of this earthworm where manipulation of conditions might lead to increased rates of production. Feed quality, environmental temperature, time of year, population density and age of breeding stock were all recognised as important variables. Experiments were performed to identify the optimal conditions for *L.terrestris* reproduction, cocoon development and growth.

Results suggested that reproduction would occur throughout the year and mean annual figures of 37 cocoons per worm were recorded from intensively produced earthworms. Recently matured worms showed greatest levels of cocoon production. As previously reported seasonal variation in reproduction was found even at constant temperature.

Cocoon development was most rapid at a temperature of 20°C, taking 70 days, with a cocoon viability of 83%. Growth from a mean hatchling weight of 53mg to sexual maturity at 5g, took twelve weeks. Growth at constant temperatures of 15 and 20°C was not significantly different.

A synthetic feed, with a carbon to nitrogen ratio of 40:1, created from paper waste and yeast extract, led to greatest recorded figures for both growth and cocoon production.

The results suggest that an intensive production system is technically feasible, and the economic viability needs to be tested.

ACKNOWLEDGEMENTS.

I would like to thank Dick Morris and Jim Frederickson for advice on the technicalities of research, the art of scientific writing and constant support.

My long-suffering parents, Frank and Shirley, who have always offered family support, and never tried to dissuade me from commencing yet another studentship.

Joanne, who has offered emotional support, and gave physical support at the time of my broken leg. (Also to all of my family and friends who lent assistance, particularly during that period.)

The Open University, for giving me the opportunity to carry out this research.

This work was funded for two years by an S.E.R.C. award (C.A.S.E. in conjunction with British Earthworm Technology (B.E.T.*).) The final year was funded by The Open University.

* The Open University took over this position when B.E.T. went into liquidation.

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CHAPTER 1. AN INTRODUCTION.

1.1 Introduction

The benefits of soil conditioning through the activities of earthworms are well established (e. g. Barley & Jennings 1959; Curry 1988; Edwards & Heath 1963; Edwards & Lofty 1980; Rhee 1971; Stockdill 1959). Deep burrowing earthworms increase soil aeration and drainage, while also improving soil crumb structure and enhancing water holding capacity. The incorporation of organic material and its mixing with mineral soil also lead to increased nutrient availability. Certain soils, deficient in earthworm numbers, could probably be improved, both physically and chemically by the addition of specific earthworm types.

Therefore, there exists a potential demand from certain farmers, local authorities, land owners and mineral companies for large numbers of soil dwelling earthworms for use in soil amelioration work. At present this demand could only be partially met by costly and labour intensive field collection techniques. However, an alternative means of supplying the earthworms would be to culture the required species under carefully controlled conditions. Introduction of the worms into the designated areas would follow at an appropriate time and in an appropriate manner. The work described in this thesis may be viewed as the first step towards a programme for the intensive production of deep burrowing, soil dwelling earthworms.

This research will focus on one species of earthworm, *Lumbricus terrestris* (the lob worm, dew worm or nightcrawler). This species was selected because its behaviour in the soil is well documented and in temperate regions it is one of the most potent invertebrates involved in soil amelioration and it is difficult to obtain large quantities of deep burrowing worms. The ability of this species to enhance the soil's physical structure and chemical nature is well documented (e. g. Rhee 1977; Aldag & Graff 1975), and it is described in more detail in Section 2.3. Other large deep burrowing species, such as *Aporrectodea longa*, could have been investigated, but the ability of *L.terrestris* to incorporate surface organic matter into soil, its general robustness (Fitzpatrick *et al* 1987), and the positive results from previous inoculation experiments (Vimmerstedt & Finney 1973; Edwards & Lofty 1980) led to the selection of *L.terrestris*.

This work differed significantly from other studies relating to intensive earthworm culture, as it was concerned with a true soil dwelling earthworm. Previous work, reported in the scientific literature, had almost invariably been associated with worms such as *Eisenia fetida* and *Eudrilus eugeniae*, which do not live in mineral soil, but are only found in materials which have a high organic matter content (section 2.2). The original idea to investigate the intensive cultivation of soil dwelling worms stemmed from knowledge gained by a team of researchers at Rothamsted Experimental Station who required soil dwelling worms for addition to direct drilled experimental sites (Rothamsted Report 1980).

1.2 Aims

At the outset of this work there were two major aims;

1. To investigate the technical feasibility of intensively producing the earthworm *Lumbricus terrestris*.
2. To obtain data in order to more clearly define the parameters of a model for intensive earthworm production.

If favourable, the results obtained would form the basis of pilot scale production trials, and suggest further research areas. Conversely, if the results suggested that commercial production was not feasible, then the questions posed would still have been successfully answered.

1.3 The potential of *Lumbricus terrestris* for intensive culture.

Field collection of *L.terrestris* would appear to be one solution for obtaining large numbers of this earthworm species but it is a very labour intensive process. In America and Canada it is practised, for the fishing bait industry, by snatching the feeding worms from their burrow entrances, under torchlight, during the hours of darkness (Tomlin 1983). Collection by means of a skin irritant, such as dilute formaldehyde, applied to the soil is also possible (Raw 1959) and has been used in advance of inoculation trials (Marfleet 1985). However in both cases, collection can only be achieved where large areas of grassland are available for capture, the particular technique has been mastered and willing, cheap labour can be found.

To supply the necessary earthworms, it would be useful to develop techniques to intensively culture *L.terrestris*. However, Boström (pers.comm.) considers *L.terrestris* to be a difficult species of earthworm to breed, and this process has so far proved to be unsuccessful. Nevertheless, the development of a system for the production of intensively reared earthworms is something regarded by Curry & Cotton (1983) and Brun *et al* (1987), as the key step on which the scope of "biostimulation" projects of earthworm introduction seems likely to depend.

Marfleet (1985) referring to a project at Hillingdon, London, where earthworm inoculation was tried states;

The methods used in this project for harvesting the worms have some drawbacks; they [formalin extraction] are very labour intensive and therefore costly...investigations...into the possibilities of producing these earthworms commercially at an economic cost are very important for establishing the technique of worm inoculation on restored land on a wider basis.

However, in her recent book summarising the literature of scientific earthworm farming, Lofs-Holmin (1985) regards the situation less optimistically;

It [*L.terrestris*] is, however, a slow reproducer and grower and moreover, difficult to cultivate. The species is attractive for its size, up to 30cm - 10g, and is used as fish bait as well as for physiological experiments.

Despite the claim that *L.terrestris* is difficult to culture, it may be possible, by careful manipulation of certain environmental conditions and biotic factors, to shorten stages in the life cycle of this earthworm species (see chapter 3) and develop an intensive production system. This work will initially consider all aspects of the life cycle of *L.terrestris* but will then concentrate on specific phases.

1.4 Thesis Outline

Chapter 2 contains a selected review of earthworm research literature. It introduces aspects of earthworm research which have directly influenced intensive earthworm production for soil amelioration. The chapter is sub-divided into sections which cover areas such as, the types of earthworm which could be investigated and the effects that earthworm activities have on soil structure and fertility. A major section also covers research that has been applied to earthworm inoculation trials, the results of which have indicated that this type of procedure is worth pursuing. Finally chapter 2 examines vermiculture as a science and as an industry. It looks at important discoveries from work

with species which are often cultured commercially and relates these findings to the possible culture of *L.terrestris*.

Chapter 3 describes the life cycle of *L.terrestris* and utilises the published literature to identify specific points where experimental manipulation of environmental and biotic parameters could be beneficial. These potential benefits were visualised as a more rapid rate of production and/or a greater sustainable production. Both environmental parameters and biotic factors were considered as possible variables for experimentation.

The following four chapters are concerned with experimental research. Chapter 4 describes the initial problems associated with the culture of *L.terrestris*, relating to the practicalities involved. The results gained from preliminary experiments described were then used to supplement the published literature and helped identify key areas for the design of further research.

Chapters 5 - 7, focus on three specific phases in the life-cycle of an earthworm. These are reproduction (mating and cocoon production), cocoon incubation and viability (from production to hatching) and hatchling growth to maturity. Chapter 5 investigates the effects of feed type, temperature, and seasonal influence on cocoon production. It also considers the effects of earthworm age and population density on cocoon production. Chapter 6 considers the fate of cocoons once they have been produced. The overall viability of cocoons is examined in terms of when they were produced and the age of the worms from which they were produced. The effects of temperature manipulation on the length of the incubation period are also examined.

The growth of hatchlings to maturity is the subject of chapter 7. Here the emphasis is placed on which feeds promoted most rapid growth, at which temperatures this occurred and whether growth was influenced by the effects of population density. A synthetic feed to enhance worm growth was also developed. The effect of stress in terms of reduction in feed quality and/or a move to a lower temperature environment on the rate of maturation is also assessed.

The sequence of all experiments reported in this thesis is given in Appendix 1. This helps clarify the origin of cocoons and hatchlings produced in the laboratory and used in subsequent experiments.

The final chapter links together all of the experimental findings to suggest the optimal combination of conditions to maximise *L. terrestris* production. From the discussion, conclusions are drawn on the feasibility of an intensive production system. Finally further areas of research are suggested. These relate to improvements on the system devised and to applications of this system.

CHAPTER 2. A SELECTED LITERATURE REVIEW.

2.1 Scope of Literature

This chapter is not meant to be an exhaustive review of the broad spectrum of earthworm research literature. It is designed to focus on a number of key areas on which this thesis is based, and serves to introduce the major research findings. More detailed analyses and discussions of relevant research areas are more appropriately contained in subsequent chapters. This selected review of literature will concentrate on the following areas;

Earthworm types,

Earthworm and soil interactions,

Inoculation (introduction) experiments,

Vermiculture; a science and industry,

Culture of *L. terrestris*.

The amount of published work relating to earthworm research has grown exponentially over the past two decades, (see Satchell 1985.) These research papers cover a wide spectrum of works, often illustrating the development of research interests through a succession of workers within a group, such as Hartenstein and his colleagues in the U.S.A. (see for example Hartenstein 1983); at a more domestic level, papers have been published which advise householders on how to manage and recycle domestic, organic waste using earthworms in conjunction with a composting process (e. g. Applehof 1988). During the late '70s and early '80s research was dominated by the growth of a "vermiculture industry", (see section 2.5), which focused on *Eisenia fetida* (the brandling). This research formed the basis of the vast majority of papers published at that time.

The life histories of only a few earthworm species have been studied in any detail, but those of *E. fetida* (e.g. Graff 1974; Watanabe & Tsukamoto 1976) and *Eudrilus eugeniae* (Neuhauser *et al* 1979; Reinecke & Viljoen 1988) are very well documented, due to laboratory based work aimed at assessing the potential of both species for the management of organic wastes and simultaneous production of useful by-products (section 2.5). Soil dwelling earthworms have seldom been studied completely. Exceptions include

L. terrestris (Lakhani & Satchell 1970; Lofs-Holmin 1983; Hartenstein & Amico 1983), *Aporrectodea rosea* (Phillipson & Bolton 1977), and *Aporrectodea caliginosa* (Nowak 1975; Mazantseva 1982; Boström & Lofs-Holmin 1986). A summary of literature relating to vermiculture science is provided by Lofs-Holmin (1985).

To help clarify the ways that earthworm research has progressed, a simple diagram has been drawn showing some major paths of development with time, (Figure 2.1.1). This is not seen as an all embracing coverage, but rather as a suggestion of some important areas of earthworm research. A detailed review of the published literature on earthworm research has been given by Edwards & Lofty (1977), and Lee (1985), and a bibliography of research in chronological order by Satchell & Martin (1981; supplemented 1984).

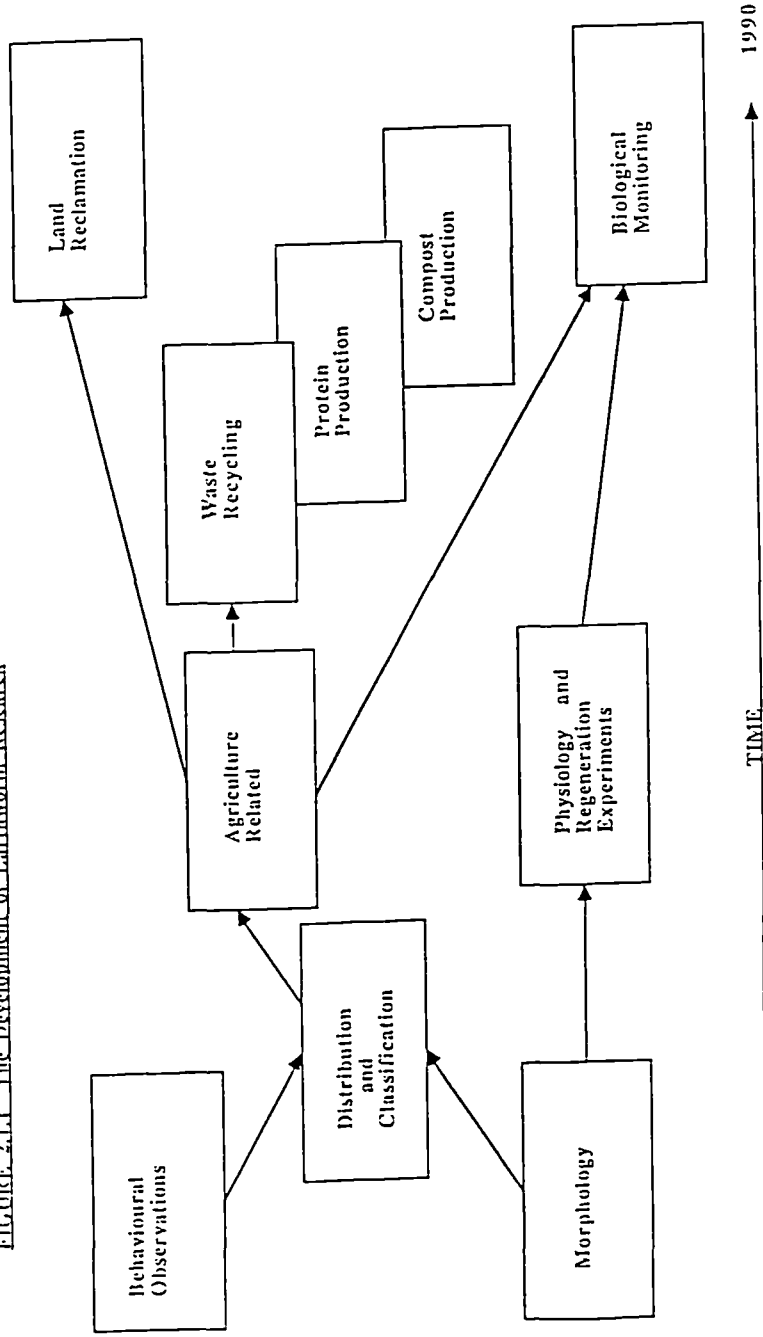
2.2 Earthworm Types

Globally there are approximately three thousand terrestrial earthworm species, of which around thirty are found in Britain (Sims & Gerard 1985). All of these may be sub-divided into three groups by reference to their morphology and behaviour in the soil. These morpho-ecological groupings, described by Bouché (1977) for lumbricids and independently by Lee (1959) for megascolecoids, relate to several factors. These include for example; general size, shape and pigmentation, burrow construction, position in the soil profile, tendency to cast, source of food, and reproductive potential. The classification adopted here is that given by Bouché.

The three types are;

1. "EPIGES" :- These are litter dwelling, have a small body size, tend to possess uniform colouration and exhibit a high reproductive rate. For example, *Eisenia fetida*, *Lumbricus rubellus*. (These are the "muck" worms.)
2. "ENDOGES" :- These create horizontal branching burrows in the organo-mineral layer, have a variable body size and are weakly pigmented. For example, *Allolobophora chlorotica*, *Aporrectodea caliginosa*. (These include many geophagous species.)
3. "ANECIQUES" :- These are deep burrowing, have a large body size, are strongly pigmented, show surface feeding and surface casting behaviour, and exhibit a low reproductive rate in the field. For example, *Aporrectodea longa*, *Lumbricus terrestris*.

FIGURE 2.1.1 The Development of Earthworm Research



Note: Some of the early research areas are still currently active.

From this general outline it is apparent that the three groups will have different effects on the soils in which they are found. Their requirements will exclude some of them from certain types of soil, but all three earthworm types can co-exist and could be encountered in most mature soils.

2.3 Earthworm and soil interactions

All earthworms have certain functions in common. Under natural conditions, they are all responsible for the fragmentation of organic debris, which eventually becomes an integral part of the soil. Earthworm action also stimulates activities of soil dwelling micro-organisms (e. g. Parle 1963) which further degrade the organic material by chemical means (see Appendix 2). Soil structure and composition are affected by most earthworms, but none more so than the aneciques, of which *L.terrestris* is the largest in Britain. It was the earthworm-soil interaction of this species which Darwin (1881) observed and recorded over a century ago. Earthworm research is often thought to have begun in earnest with the observations of this naturalist, as Darwin recognised the direct role of earthworms in incorporating organic matter into the mineral soil and also the way that their castings, thrown up over many years, formed a fresh layer of fine soil on the surface (Darwin's "vegetable mould"). These are but two of the many ways in which earthworms are able, by their normal activities, to improve soil "quality".

As a deep burrower, but surface feeder, *L.terrestris* is one of the most effective animals involved in the improvement of soil quality. By creating burrows through the soil, drainage is assisted (e. g. Rhee 1977), which prevents waterlogging and possible compaction (e. g. Standen *et al* 1982). Soil aeration is also improved by the network of burrows made by this species. When feeding, *L.terrestris* drags surface-lying material, such as dead leaves, into its burrow, (which may extend down to a depth of 2.4m, Edwards & Lofty 1977) and thereby causes a mixing of soil layers and organic material (e. g. Hoogerkamp *et al* 1983). Soil crumb structure is also improved by the ingestion and breakdown of soil particles along with the "food" source, which may then be deposited, at the soil surface in the form of nitrogen rich castings, or within the burrow to form equally rich soil aggregates (e. g. Barley & Jennings 1959). Mucus secreted by the worm helps to bind these particles together which in turn helps to increase the water-holding capacity of the soil. A more detailed survey of these earthworm activities is supplied by Edwards & Lofty (1977) and by Lee (1985).

2.4 Inoculation Experiments

Researchers, some (organic) farmers and gardeners have come to regard earthworms as a vital part of the soil community. This is due to the knowledge of their beneficial activities, outlined in section 2.3. Experiments and observations have further increased the positive status of earthworms in the soil. These may relate directly to agricultural practices, reviewed by Edwards (1983), or be concerned with aspects of land improvement, reviewed by Curry (1988).

Edwards and Lofty (1980) performed a series of experiments concerned with the effects of earthworms on cereal crop production. Root growth, they discovered, was significantly increased ($p < 0.05$) by the inoculation of deep-burrowing earthworm species (*L.terrestris*, *A.longa*), as was crop yield. The effect of inoculating plots with surface dwelling earthworm species (*A.chlorotica*, *A.caliginosa*) was not significantly different from plots containing no worms, but this may have been due to an insufficient inoculation population. Further box experiments revealed that the presence of earthworm burrows led to increased root growth, when compared with artificially constructed burrows. This was not considered to be simply due to the provision of channels for the roots to grow down, but rather the observed effect was most likely to have been caused by enhanced nutrient availability in the castings of the burrow linings, compared to the surrounding soil.

In instances where earthworms are absent from soils, some pertinent observations have been made following their introduction. For example, in parts of New Zealand farmers recognised that grassland around introduced European fruit trees was more productive than surrounding areas. A closer examination revealed the presence of a population of lumbricid worms, which had been introduced with the soil around the tree roots.

Experiments performed by Stockdill (1959), proved that the introduced worms were responsible for the observed increase in productivity. In the absence of worms, a thick, undisturbed mat of dead plant material had accumulated. The peregrine (non-indigenous) worms, mainly *A.caliginosa*, broke down the mat in the immediate area around the trees and the release of nutrients initially led to increased grass production of over 70%. This levelled off at around 30% above former records following the first few years after earthworm introduction, as the stored nutrients became exhausted (Stockdill & Cossens 1966). To further investigate this and increase the scale of inoculation schemes, a machine was developed capable of digging turfs containing earthworms, for relocation elsewhere.

This has proved successful in parts of New Zealand and it was established that complete colonisation of an area with few or no earthworms could be expected from turfs placed at 10m intervals within 6 - 7 years (Stockdill 1982). The expected economic return on earthworm introduction projects of this type was calculated to be more than 300 per cent.

The value of earthworms in soil amelioration has been further documented on other land types. In the Netherlands, where land reclamation is part of the national heritage, the draining of areas formerly flooded by the sea has led to the creation of polders which have subsequently been put into cultivation. In these soils, which may take 50 - 100 years to mature, Rhee (1969, 1971) introduced *A.caliginosa* and *L.terrestris*, under pasture and in orchard soils. The activity of the worms, and that of *A.caliginosa* in particular, was found to increase the rate of soil development. More tree roots grew in worm-inoculated soils than in those without worms, but Rhee did not detect any influence of earthworm activity on grass or fruit yields. The populations of *L.terrestris* declined rapidly after inoculation, suggesting that soil conditions did not favour this species. Unlike Rhee, Hoogerkamp *et al* (1983), investigating similar polder areas did record an increase of grass production caused by earthworm inoculation, but the results obtained were variable. Again *A.caliginosa* was thought to be the most important species of worm, in terms of organic matter incorporation. Rates of population increase and spread of this species were estimated by use of an infra red scanning technique. This measured differences in heat exchange from areas where a surface organic mat was present, or had been removed by earthworms. When earthworms were well established the surface mat disappeared, which resulted in greater heat exchange between the soil and air, and reduced daily fluctuations in temperatures at the soil surface. The mat was ingested and incorporated into the soil within three years of earthworm invasion and a dark A₁ horizon developed within eight to nine years. Better soil conditions positively influenced root growth and distribution. This was reflected in better sward attachment and fewer turfs were detached by cattle grazing. Urine damage on earthworm inoculated plots was less common, and grass yields were on average 10% higher, than on uninoculated plots. However during wet weather some damage by treading and soiling of grass was observed on worm inoculated plots.

Monitoring of earthworm numbers on reclaimed open cast coal mine spoil heaps has been carried out in Wales by Scullion *et al* (1988), and in County Durham by Standen *et al* (1982). Similar work by Vimmerstedt & Finney (1973), has also occurred in the U.S.A.,

where *L. terrestris* was successfully introduced into spoil sites ranging in pH from 3.5 to 7. Worm activity accelerated the incorporation of litter and increased exchangeable cations and available phosphorus. After twelve years populations had become widely dispersed throughout an area of calcareous spoil afforested with *Alnus glutinosa* and *Robinia pseudoacacia*. Numbers and biomass were greater under the *Alnus* cover where the litter was more palatable to *L. terrestris*.

Scullion *et al* (1988) discovered that the bulk storage of top soil during mining operations led to reduced earthworm numbers, and that respreading of this soil compounded this problem. Standen *et al* (1982) suggested that earthworms survived well within the stored heaps but were eliminated by respreading (physically injured or predated). On spoil heaps left to revegetate naturally, rapid development of epige species such as *Lumbricus rubellus* was recorded, where scrub had colonised the site. On more open grass sites anecique species such as *L. terrestris* accumulated, but only after several years. On sites reclaimed from open cast mining and restored to agriculture, earthworm populations were still low after eleven years (Standen *et al* 1982). Vimmerstedt (1983), concluded that populations of *L. terrestris*, although reduced by various mining activities, would eventually recover, if very slowly.

It is certain that the slow recovery of some species of earthworm populations after depletion, can be due to a low reproductive potential (Evans & Guild 1948), but also high mortality caused by unsuitable soil conditions is important (Luff & Hutson 1977). Experimental work by Rushton (1986) revealed that *L. terrestris* was capable of surviving in waterlogged conditions, which were often associated with a lack of vertical fissures in reclaimed open cast mining land, but this species was not capable of penetrating soil with a high bulk density. The inability to burrow through the compacted soil prevented establishment. However, laboratory experiments by Joschko *et al* (1989) showed that individual *L. terrestris* were able to burrow into soil artificially compacted to a pore volume as low as 40% and they were also able to penetrate an artificial "plough pan" deep in the soil. Micro-morphological investigations by these workers revealed that at high levels of compaction the earthworms penetrated by means of eating their way into the soil rather than pushing the particles aside. Under field conditions this would clearly be unsatisfactory if earthworms were introduced on to the soil surface, and not covered, as they would be exposed for lengths of time that would attract predators.

If, in trials, soil physical factors are found to limit the establishment of anecique species, then large scale introduction schemes ought to be delayed, but if food is found to be limiting, the addition of a suitable (waste) organic material, such as sewage sludge or dung, could assist establishment on introduction. Support for this is offered by Satchell & Stone (1977), who examined clay-excavation pits filled with pulverised fuel ash, a waste product of coal-fired electrical power stations. These sites were only colonised by deep burrowing earthworms, including *L. terrestris*, after a dressing of topsoil had been added, and even epige species such as *L. rubellus* and *Lumbricus castaneus* took many years to successfully colonise ash sites where soil was absent.

Other areas where attention has focused on the inoculation of earthworm species are sites reclaimed from peat-digging. These sites present specific problems to soil dwelling organisms such as earthworms as they have a low pH, a high water content and lack oxygen at depth. The soils also have a high carbon to nitrogen ratio and poor quality litter which is usually of low palatability due to the presence of phenols. However, with drainage and reclamation for agriculture, moderate population densities of earthworms can be supported. Guild (1948) found ten earthworm species and population densities from 50 - 100m⁻² in improved hill pastures in peaty soils in Scotland, and Baker (1983) reported fifteen species with densities up to 200m⁻² in established grassland on reclaimed fen peat in central Ireland. Curry & Cotton (1983) surveyed numerous sites in Ireland and determined that population densities of mixed species only exceeded 100m⁻² in sites over twenty-five years old. Litter dwelling, epige species were common in young sites, whereas in the more mature sites typical soil dwelling species were found. Experiments performed by Curry & Cotton (1983) involved burying cubic nylon cages, with edge length 50cm and supplying them with the soil removed for their burial, topped with the turf removed from the site. To half of the cages 4 litres per cage of cattle slurry (10% dry weight) was mixed into the soil, to test the hypothesis that earthworms are food limited in newly reclaimed sites. A mixed population of twenty-two worms, obtained locally, was put into each of these cages. Of the introduced species *A. chlorotica* benefited most from the initial application of cattle slurry and after three years this species provided the greatest contribution to earthworm and cocoon numbers. Earthworm biomass at that time was calculated to be approximately 100g.m⁻², which is within the range found in grassland on mineral soils, although fewer anecique species were present in the reclaimed soil (Cotton & Curry 1980). The same authors, in experiments with similar nylon cages and applying

different fertilizer treatments, found earthworms had no effect on grass production during the first year but yields were 25% greater in the second year and 49% greater in the third year in cages receiving an annual top dressing of cattle slurry, when compared to cages receiving slurry but without added worms.

Marshall (1981) reports that inoculations of *A.caliginosa* and *L.terrestris* in Canadian boreal forest soils were unsuccessful. Three years after inoculation of both species to forty plots, only two plots showed any sign of earthworm activity, and this was limited to within 1m from the point of introduction. Earthworm absence in such areas, thought to be caused by glaciation, was not overcome as easily as the experiences in New Zealand. Marshall (1981) suggests that further work is required to determine why the worms failed to become established, as they should have been able to survive in the soils, even though there were low levels of available nitrogen, since high reserves were present in the organic layers. One likely explanation is that anecic worms were not suitable for the initial inoculum or alternatively that immature worms, cocoons or a mixture of life stages would have had a greater chance of survival.

That deeper working species of earthworm are usually less successful colonisers of newly reclaimed sites is understandable, given the niche that such worms occupy, but inoculation of such species at the earliest possible time is considered essential if they are to begin the process of soil restructuring and rehabilitation (Curry & Cotton 1983). As Satchell (1980) remarks, *L.terrestris* is by nature a "K" strategist (MacArthur & Wilson 1967), and can only behave as a "pioneer" if soil conditions are made more favourable artificially (e. g. by the addition of organic material as feed).

By the introduction of a variety of earthworm types (endoges and aneciques) or worms of different ages (mature worms, hatchlings and cocoons) to suit the given conditions, the upgrading process of waste land may be more rapid and successful.

2.5 Vermiculture; Science and Industry

Over the past forty years craft knowledge of vermiculture has increased. It began with fishermen in the U.S.A. realising that it was easier to raise "brandlings", initially found in compost heaps, than dig for soil dwelling earthworms. This may be seen by reference to Barrett (1949), and Gaddie & Douglas (1975a,b). The works of Gaddie & Douglas are designed to popularise earthworm rearing and promote the "vermiculture industry". By

reading such books, many individuals were encouraged to start their own earthworm "farms", often with good intentions, as markets for bait did exist, but some unscrupulous entrepreneurs also exploited the situation. Although based on sound research, the pyramid selling of stock and buy-back schemes that quickly spread could not sustain itself. A rather scathing review of this period is provided by Satchell (Unpub.).

Research into the science of vermiculture has grown over the past fifteen years. It was realised that epige species could be utilised to help manage organic waste materials, such as sludges at waste water plants. In the U.S.A. Hartenstein and colleagues (reviewed in Hartenstein 1983) examined in detail much of the life history of *E.fetida* and other potentially useful worms whilst tackling this problem. During this type of work extensive knowledge relating to intensive earthworm culture was gained. A detailed review is given by Lofs-Holmin (1985), in which she summarises the research aspects of vermiculture.

Pilot schemes to manage sludges at waste-water plants were set up based on the scientific knowledge gained. These included the Lufkin Project based in Texas (Green & Penton 1981). Initially this was successful and was viewed as competitive with other contemporary sludge management practices. However, the project was later beset by engineering, biological and economic difficulties. These culminated in the death of all of the worms which was attributed to an unidentified source of contamination (Satchell pers. comm).

In Britain, a team led by Edwards, at Rothamsted Experimental Station, concentrated on utilising *E.fetida* to manage agricultural waste materials (see Edwards 1988). It was initially believed that the earthworm protein produced could provide a cheap alternative for animal feeds such as fish meal. However, the economics of the process, covered by Foote and Fieldson (1982) and Fieldson (1988), revealed that the worm-worked material rather than the worms themselves, presented the greater source of potential revenue. This material was found to be an ideal base for horticultural potting compost (Knight 1987; Edwards & Burrows 1988). At present little research is occurring in this area, but the potential future of vermiculture is discussed by Sabine (1988). He suggests that vermiculture has a real future as both a science and an industry. On the scientific side, research will utilise earthworms as indicator species in biological monitoring programmes (e. g. Edwards 1984; Lofs-Holmin & Boström 1988). Also with current agricultural trends towards minimum-tillage of soils, with the rise in popularity of organic farming methods

(e. g. Marland 1989), and with increasing concern for all factors relevant to soil erosion, the role of earthworms in both the biological and physical aspects of pedogenesis is assuming greater significance (Stockdill 1982).

In the U.K. scientific vermiculture research funding has ceased and one medium sized company created to exploit the field of earthworm/compost production, British Earthworm Technology (the industrial collaborator on this project), ceased trading in 1988. Details of this period are provided by Knight (1989).

Following research into vermiculture and dissemination of information, earthworm producers, with names such as; "Turning Worms", "Wonder Worm" and "Lady Muck" still remain. They regularly advertise their products in agricultural publications, and sell either worms as fishing bait, the worm-worked material as compost, or both. However, in all of these cases, soil dwelling earthworms are not produced. Most of these small companies produce "muck" worms such as *E.fetida*, which are of no value in soil upgrading schemes, as they have little effect on mineral soil. If introduced to areas without adequate organic material they will die (Lee 1985).

2.6 Culture of L.terrestris

Since the pioneering work of Evans & Guild (1948), many authors have investigated the growth and reproductive biology of Lumbricid species (see Lee 1985). However, as with Evans & Guild, studies concerning *L.terrestris* have often formed a very minor part of such works. This was almost certainly due to the low reproductive potential of this species, compared with other earthworms which can provide results more rapidly and in greater quantity (Table 2.6.1). Notable exceptions to this, under controlled laboratory conditions, were Meinhardt (1974), Lofs-Holmin (1983) and Hartenstein & Amico (1983). However, even here only Hartenstein & Amico concentrated solely on *L.terrestris*, and looked specifically at growth. Nevertheless the population parameters derived from the experimental work of these authors provide guide-lines for improvement. Details of growth rates and reproductive rates obtained by these authors are given in chapter 3.

It would appear that there is a real demand for intensively produced *L.terrestris* and worms which fill the same niche, particularly for the upgrading of low quality soil, (Curry & Cotton 1983; Brun *et al* 1987). Results from previous researchers suggest that a system of intensive production may not be viable. However, few workers have looked closely into

Table 2.6.1 Biological Data (Maximal Values) For Some Earthworm Species.
(Adapted From Lofis-Holmin 1985).

Species	Start of cocoon production at age (days)	Cocoons per worm per week	Cocoon Incubation period (weeks)	Cocoon hatching success (%)	Hatchlings per cocoon	Optimum temp. (oC)
<i>E. teiida</i>	30	6	3	80	1.6-3.0	20-25
<i>L. rubellus</i>	60	5	-	-	1	15-18
<i>L. terrestris</i>	100	0.7	12-13	-	1	15-18
<i>E. eugoniae</i>	30	11	3	84	1.2-2.8	25-28
<i>D. veneta</i>	40	10	5	80	1	20-25

the problems encountered, and further research appears to be warranted. This work should provide clear insights into the viability of intensive soil earthworm husbandry.

CHAPTER 3. THE IDENTIFICATION OF KEY RESEARCH AREAS.

3.1 Area of Study.

The scope of a programme concerning the intensive production of *Lumbricus terrestris* was potentially very large. Many avenues were possible but a realistic approach was essential in order to obtain reliable data within the study period. To have initiated any work relating to production on a large scale would almost certainly have proved unrewarding, as the range of parameters affecting growth and reproduction would have proved difficult to monitor and vary in a controlled manner.

The programme of study devised was viewed as an appropriate starting point in this research area. Experiments were envisaged to monitor the production of *L.terrestris* under carefully controlled, laboratory conditions. These small-scale pot experiments were considered essential groundwork, in order to establish required husbandry conditions. The existing literature was used as a guide, to identify the parameters having the greatest effect on the growth and reproduction of earthworms and that of *L.terrestris* in particular. These were to be investigated in most detail.

Before examining these areas further, a closer look at the life cycle of *L.terrestris* was desirable.

3.2 Life-cycle of Lumbricus terrestris.

Like all earthworms, *L.terrestris* is hermaphrodite, (see Reynolds 1974), but as classically described by, for example, Marshall & Williams (1972), sperm transfer must occur between copulating adult pairs for viable cocoon production, (Evans & Guild 1947; Michon 1954). During formation, the cocoon (an albuminous secretion of the clitellum) is provided with several ova from the cocoon-producing parent and sperm, stored after mating, from the other copulating worm. Normally only one ovum develops successfully, (Evans & Guild 1948), although in other species such as *Eisenia fetida* (Tsukamoto & Watanabe 1977), and *Bimastos tumidus* (Vail 1974), multiple hatchling production is common. Meinhardt (1974), reports that *L.terrestris* cocoons are produced at approximately ten day intervals until the supply of sperm stored by the worm is fully depleted, when further mating is necessary. Cocoon development takes twelve to thirteen weeks (Meinhardt 1974). On hatching, the young worms weigh approximately 50mg and

are 25mm in length (Sims & Gerard 1985); there is no larval stage. In Britain growth to maturity is usually attained within one year, (Evans & Guild 1948; Lakhani & Satchell 1970), but it may take up to fifteen months in Scandinavia, (Nordström 1976). It is thought that the clitellate condition is not reached until a weight of 3g has been obtained, (Lofs-Holmin 1983). Weights up to 11g have been recorded, with known individuals living for six years under artificially controlled conditions, (Satchell 1967). Growth virtually ceases after three years, (Lakhani & Satchell 1970). The complete life-cycle has been illustrated diagrammatically in Figure 3.2.1. One area, relating to the maintenance of the clitellate condition and whether this can be regained, if lost, is not fully documented. (It is signified on the figure by a dashed line). Michon (1954) reports that the loss of the swollen clitellum by *Dendrobaena subrubicunda* signifies the onset of senescence and death.

3.3 Areas for investigation.

From the life cycle diagram it was clear that there were several points at which the growth and reproduction of *L. terrestris* could be influenced by environmental factors. These points of intervention/manipulation were in;

1. Cocoon production;
2. Cocoon development to hatching;
3. Growth to maturity, following emergence from the cocoon.

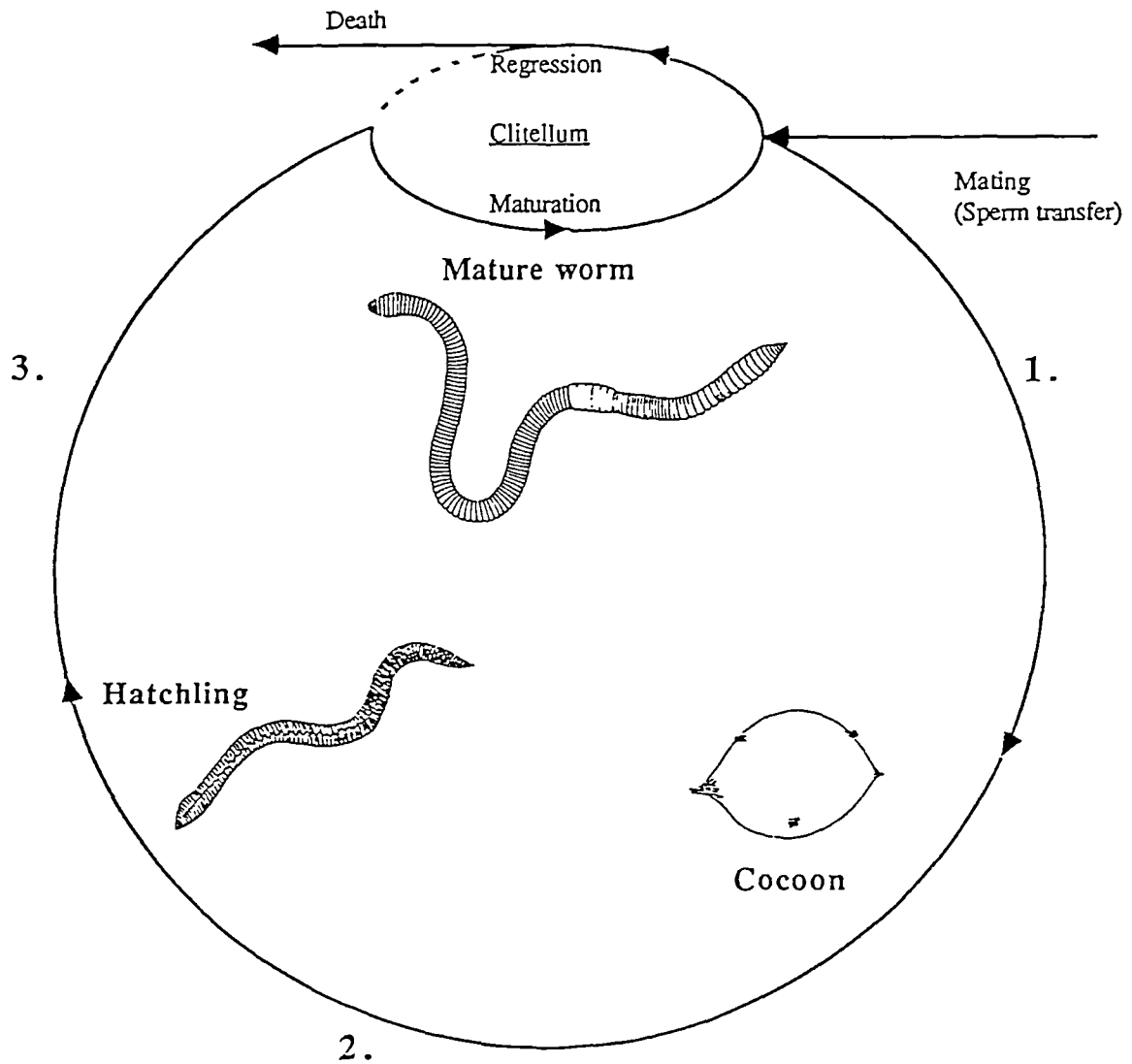
It was felt that the number of hatchlings that emerged from each cocoon was probably beyond environmental influence and possibly controlled genetically.

A very basic question related to the potential ability of *L. terrestris* to survive and reproduce under artificial conditions. The whole success of the research depended on this. Nowak (1975), had laboratory cultures of *Aporrectodea caliginosa* reproducing throughout the year at 13°C, as did Evans & Guild (1948) with *Lumbricus rubellus*. However, the literature suggests that *L. terrestris* is a seasonal breeder (Satchell 1963) and very few workers have kept this species in breeding condition in the laboratory over any length of time, (see Evans & Guild 1947, 1948; Meinhardt 1974). Gates (1961) reports that adult *L. terrestris* have been observed in reproductive condition from March to December, under natural conditions. This suggested that year round breeding was possible.

Figure 3.2.1

18a

A stylised life cycle of Lumbricus terrestris.



Points of Manipulation.

1. Cocoon Production
2. Cocoon Development
3. Growth to Maturity

3.4 Parameters affecting growth and reproduction of earthworms.

The number of parameters that can affect the lives of earthworms is vast, and only those from the literature with the potential to have a pronounced affect were considered here. For example, Reinecke & Kriel (1981), consider important such factors as; population density, inter-specific competition and the influence of ageing of the breeding stock, to obtain an overall picture of the reproductive capacity of *E.fetida*. However, this is not an exhaustive list and other factors may be of more or less importance depending on the species under investigation.

For ease of description, factors considered important to *L.terrestris*, during each stage of the life-cycle, have been classified in artificially contrived groupings. These relate to the ease of experimental variation, or the degree to which the literature suggests experimentation would prove profitable. These groupings apply specifically to *L.terrestris*.

Manipulation thought profitable (3.4.1)

By experimental manipulation of the parameters in this grouping, it was believed that results would show enhanced growth and/or reproductive output. These included feed type, environmental temperature and population density.

Manipulation possible, but unwarranted (3.4.2)

The literature suggested that other parameters were less worthy of experimental manipulation. These have been included in this grouping and further sub-divided into two sections;

Constant within bounds (3.4.2a.)

Previous documented experimentation has shown that certain factors do affect earthworm growth and reproduction but levels acceptable to *L.terrestris* have been established. These include figures for soil moisture content, and soil type. To vary these experimentally would, under some treatments, provide sub-optimal conditions and almost certainly only confirm previous findings. More importantly valuable resources such as time would be wasted.

Difficult to control/Little marked effect. (3.4.2b.)

Certain manipulations have only a small effect on *L. terrestris*, so variation would lead to little improvement in biological performance. e. g. the pH of soil within wide margins. The effects of another parameter, the amount of daylight, would prove difficult to control under frequently monitored experimental conditions.

3.4.1 Manipulation thought profitable.

3.4.1.1 Feed

The literature suggests that the type of food supplied to earthworms is of vital importance to their growth and reproduction. However, before embarking on a description of likely options it is pertinent to mention that the diet of worms still remains something of a contentious issue. Some authors believe that earthworms do not derive their nourishment from the material they ingest *per se* but rather from the micro-organisms that feed upon that material. Murchie (1960), found that it was not the absolute amount of organic material per unit volume of soil that was critical to the growth of *Bimastos zeteki*, rather the essential ingredients for growth had to be available in some form which the animal could use and might require substantial modification by other biotic elements before utilisation was possible. There exists a vast literature concerning earthworm feeding and micro-organisms. A summary of this is provided in Appendix 2, as it was not the aim of this thesis to address the question directly. A comprehensive coverage is provided by Edwards & Fletcher (1988).

For the past forty years it has been accepted that lumbricids show an increase in both cocoon production and growth rate when fed nitrogen-rich as compared with nitrogen-poor food (Evans & Guild 1948). These authors recorded the cocoon production of *Allolobophora chlorotica* and *Lumbricus castaneus* using nine types of organic matter in three groupings; originally undecayed (two straw treatments and cut grass fodder), partially decayed (animal droppings and peat) and well decayed (sewage sludge and farmyard manure (FYM)). The partially decayed types of organic matter were shown to give high rates of cocoon production. However, as with Lofs-Holmin (1983) and Andersen (1983) who fed *A. caliginosa* with FYM and with pulverized straw respectively, no physical or chemical analyses of the organic materials were given. In contrast more recent works, including those of Boström & Lofs-Holmin (1986) and Hartenstein & Amico

(1983), were more detailed and included the necessary analyses of the feeds utilised, so that an attempt could be made to relate production to feed quality. Boström & Lofs-Holmin reported that food particle size was one of the most important factors affecting the growth of juvenile *A.caliginosa*. Hartenstein & Amico demonstrated that production of biomass in *L.terrestris* was greater in a substrate of activated sludge and loam relative to activated sludge and cellulose. A net yield of *L.terrestris* was not obtained where cellulose was used with less than 2.4% nitrogen present. However, the level of nitrogen in itself may be less critical than the carbon to nitrogen ratio (C:N) of the feed. If this ratio is large, then there may be problems associated with nitrogen extraction for tissue production. Neuhauser *et al* (1980a) demonstrated that populations of *Eisenia fetida* gained weight maximally with C:N ratios which ranged from 9:1 to 38:1 and less rapidly at higher ratios. They also showed that soil was important for the growth of *E.fetida*. Worms fed horse manure had weights about a third greater after three months in the presence soil than in the absence of soil. These workers attributed these differences directly to an increase in tissue production despite not voiding the worms prior to weighing.

Maximum growth rates for *L.terrestris* of $60 \text{ mg.g}^{-1}.\text{day}^{-1}$, have been obtained by Hartenstein & Amico (1983), under laboratory conditions, in soil amended with sewage sludge. These figures are greater than field data of $10 \text{ mg.g}^{-1}.\text{day}^{-1}$ in mineral soil under mixed woodland (Lakhani & Satchell 1970), $6.6 \text{ mg.g}^{-1}.\text{day}^{-1}$ in reclaimed peat grassland (Curry 1988), or $4.5 \text{ mg.g}^{-1}.\text{day}^{-1}$ in peat with *Salix* litter (Curry & Bolger 1984). The quality of feed would certainly have contributed to the observed differences in these figures.

3.4.1.2 Temperature

Another major influence on earthworm growth is temperature, as evidenced by the time taken by *L.terrestris* to reach maturity in Britain (within twelve months) as compared with Scandinavia (up to fifteen months). This may only be circumstantial, but documented evidence from laboratory studies also supports this, (Satchell 1967; Lee 1985).

By increasing the temperature at which certain Lumbricids are cultured, various workers have shown that a reduction in maturation time can be achieved. For example, for *A.chlorotica* this time was 29 to 42 weeks in a cold cellar (Evans & Guild 1948), 17 to 19 weeks at 15°C (Graff 1953), and as little as 6.5 weeks at 28°C , (Michon 1954). Similar

trends in reduced maturation time were found by Tsukamoto & Watanabe (1977), with *E.fetida* (between the temperatures of 10 and 25°C) and Lofs-Holmin (1983), with *Octolasion cyaneum* (between 5 and 15°C). Hartenstein & Amico (1983), also found weight increases in *L.terrestris* to be maximal between temperatures of 15 and 25°C.

Most laboratory work with *L.terrestris* has so far been conducted at around 15°C, (Meinhardt 1973; Lofs-Holmin 1983; Hartenstein & Amico 1983), although Tomlin (1977), considered 11 to 13°C to be a high temperature at which to keep this species of earthworm. Satchell (1963), suggests natural populations of *L.terrestris* are usually found within the temperature range of 5 to 15°C.

Lofs-Holmin (1983), showed that an increase from 15 to 20°C did not increase the growth rate of *O.cyaneum*, but it did marginally reduce the time of maturation. Fitzpatrick *et al* (1987), revealed that earthworms [*L.terrestris*] cultured at 10 and 15°C appeared considerably more robust than those at either 5 or 20°C, especially after a period of several weeks. Worms at 20°C were least robust. These observations accord with the metabolic rate-temperature relationship they recorded, indicating almost complete metabolic homeostasis for 10 - 15°C. Daugbjerg, (1988), testing moisture and temperature preferences of several earthworms found a stenothermal preference of 10°C for *L.terrestris* which was in accordance with Satchell (1967), who concluded that this is the optimum temperature for *L.terrestris* activity. Lee (1985), reports thermal optima in the 10 - 15°C range for natural populations of *Lumbricidae* in Europe. Wolf (1938), and Hogben & Kirk (1944), give the upper lethal temperature limit for *L.terrestris* in the range 27 to 29°C.

Work with several Lumbricid species including *A.chlorotica*, (Gerard 1960), *E.fetida*, (Gerard 1960; Tsukamoto & Watanabe 1977), and the Eudrilid worm *Eudrilus eugeniae* (Neuhauser *et al* 1979), have shown a relationship between incubation temperature and the time taken for cocoons to hatch. By increasing the temperature in the range 10 to 25°C, most authors obtained a reduction in developmental time. However, Tsukamoto & Watanabe (1977) also observed a decrease in cocoon viability, measured as hatching success, with greater temperatures.

3.4.1.3 Population Density

The density at which earthworms are maintained has also been shown to affect their growth and reproductive output. In the past *L.terrestris* has been cultured in various sizes of vessel, with most workers finding that more space was required by this than any other species of earthworm. Evans & Guild (1948) kept three individuals in five pint pots (2.85 litres). Lofs-Holmin (1983), found that 20 litre boxes were necessary for fifteen breeding worms, after finding that a 1 litre pot was not large enough for six to ten individuals, but would accommodate these numbers of *A.caliginosa* or *O.cyaneum*. Hartenstein & Amico (1983), found 1.4 litre glass bowls (15cm deep) would maintain eight immature *L.terrestris* growing steadily, but not reproducing. Tomlin (1977), kept thirty to forty individuals in 18 litre vessels and suggested that mature *L.terrestris* required a soil depth of at least 30cm for successful culture. If this was not supplied he suggested that;

"it [*L.terrestris*] will attempt to leave the soil and container. If it is unable to escape the shallow soil, it lies on the surface and dies."

This is disputed by Lofty (Pers.comm.), who suggested that this species will breed successfully in soil only 7 - 8cm deep and does not necessarily need a permanent burrow. The amount of soil necessary for culturing this earthworm would critically influence the success of an intensive production programme.

The information in this section suggests that the maximum biological capacity of earthworm growth (and reproduction) is seldom manifested in the field. Under laboratory conditions, Meinhardt (1974), Lofs-Holmin (1983) and Hartenstein & Amico (1983) were able to increase growth rate, when compared with figures obtained for natural populations. Overall the duration of the pre-reproductive phase in Lumbricids is strongly influenced by environmental factors (Lee 1985). Experiments involving variation of temperature, feed and population density will determine by how much this is so.

3.4.2 Manipulation possible, but unwarranted;

3.4.2a Constant within bounds

3.4.2a.1 Water (soil moisture)

The water content of soil is of major importance in regulating lumbricid occurrence, activity, and behaviour, (Satchell 1967; Lee 1985). It is closely linked with temperature,

but optimal conditions appear to lie within 25 - 30% of wet soil mass, depending on soil type (Tomlin 1977; Lofs-Holmin 1983). Ideally this level should be maintained at all times. Too little water would lead to drying soil and desiccation of earthworms, whereas too much would cause waterlogging and an avoidance response. In the latter case the worms would move upwards in the soil to keep clear of the underlying water and thus reduce the volume available to them. Ideally, the containers in which the worms are kept would satisfy several requirements. They would be; water-tight, covered, have air holes, and be easily accessible. This would prevent water loss by leaching or excessive evaporation, allow air to circulate, and provide an opportunity for frequent inspection so that further water could be applied. However, it is impossible to satisfy all of these criteria simultaneously. Gerard (1960), conducted experiments with earthworms in Kilner jars, in an effort to obtain optimal soil moisture levels, linked to temperature, for *A.chlorotica*. His data did reveal conditions under which the worms became inactive or died, but these sealed environments were far from realistic, and unsuitable for scaling up, if successful.

In general, the active life of earthworms is restricted to periods when soils contain enough water to support plant growth, (Lee 1985). This is in the range of pF 2.0 - 2.4, a measure of the free energy of water held in a soil (matric potential), which relates to the energy required to extract water from soil, rather than as a moisture percentage on a dry-weight basis. However, *L.terrestris* can remain active when pF values exceed 4.3 in the 0 - 5cm soil layer (Nordström & Rundgren 1972a), but as this species lives in deep burrows it only needs to be exposed to surface soil conditions for short periods, when emerging to feed.

3.4.2a.2 Soil

Soil might seem an obvious essential for earthworm husbandry, but this is not always so. Many epige species included in the "muck" worm category, such as *E.fetida*, actually live within the organic substance that they are ingesting. *L.terrestris* does not fall into this category, but a soil requirement is still worthy of consideration, as large quantities of soil have previously been used in the culture of this species, e. g. Ashby (1976); Tomlin (1977); Lofs-Holmin (1983). It would be a great saving, in terms of space, time and possibly finance if *L.terrestris* could be encouraged to grow in the absence of soil. However, the results of Hartenstein & Amico (1983), suggest that soil is necessary for growth of this species.

The type of soil used for the intensive cultivation of *L.terrestris* must therefore satisfy several criteria: It should;

- (a) be acceptable to *L.terrestris*,
- (b) be of a reasonably constant composition,
- (c) be free from any earthworm material, predators, and parasites of *L.terrestris*,
- (d) contain low amounts of organic material (to permit feeding experiments).

To satisfy the above, two alternatives were possible, either; create an artificial soil, (e. g. Meinhardt 1974; Edwards 1984), or obtain an "acceptable" soil and sterilise it (Lakhani & Satchell 1970; Lofs-Holmin 1983). The former may be ideal but would have been a very labour intensive process and only feasible on a small scale. The sterilisation process may have had some adverse effects in terms of killing microbial biomass, but it was thought essential. Meinhardt (1973), reports that *A.longa* died when introduced to recently steamed soil, but *L.terrestris* was not affected in this way.

L.terrestris is most abundant in England in light loamy soils (Lofty 1974). It is clear that this earthworm, unlike some Lumbricids, needs more than an organic substrate in which to survive. Hartenstein & Amico (1983), suggest that it requires a high ratio of mineral soil to organic matter in its diet in order to extract nutrients efficiently.

3.4.2b Difficult to control/Little marked effect.

Some parameters, essential to the very survival of certain earthworms have less effect on *L.terrestris*. A short note on some of these is, nevertheless, worthy of mention.

3.4.2b.1 pH and ionic conductivity

Satchell (1955), considered that *L.terrestris* was not very sensitive to soil pH and that this species was "ubiquitous", found in soils ranging from pH 3.5 - 7.0. Variation of this parameter would therefore appear to be of little interest unless the ultimate destination of worms produced was known to lie outside of this range and an acclimation process was thought necessary. However, for *E.fetida*, a worm which lives within its food, this would not be true and this species would certainly be affected by processes such as ageing and composting when ionic levels would be altered, (e.g. Knight 1987).

Ramsay (1949) showed that as the osmotic potential of the external medium increased, so did the osmotic potential of the coelomic fluid in *L. terrestris*. The highest experimental concentration applied externally was 1.45% NaCl which caused the coelomic fluid to reach a concentration of 1.6% NaCl. Although these results were obtained very artificially, if they can be related to growth in the presence of organic wastes, they would suggest that earthworms have the ability to osmotically regulate their internal salt concentrations even at very high external osmotic pressures. This would indicate that deaths caused by an osmotic loss of water would occur only at very high conductivities.

Conductivity and pH are very closely linked and considered difficult to control in a system containing an organic material and soil. Alteration of one must affect the other. However, the soil in which *L. terrestris* lives would act as a buffer against such changes if, for example, leaching did not occur. Although pH does, at its extremes, influence the distribution of this species, it is not a major factor thought to affect growth and reproduction and will not therefore be varied experimentally.

3.4.2b.2 Light

There is documentary evidence to suggest that light plays a major part in the behaviour of *L. terrestris*. This species may live in darkness, but feeding and mating take place at the soil surface. Both of these behaviours occur under cover of darkness, and will even be suspended if a very bright moon is in the sky, (Gerard 1960). Therefore light sensors must be present, but this was not under scrutiny, as experimental worms were to be kept in conditions of constant darkness, which if anything, would encourage feeding and reproduction.

The aspect of seasonality reported to affect reproduction (Satchell 1963), could perhaps be attributed to some form of day-length influence. However, in terms of earthworm evolution, which is likely to have taken place in a woodland habitat, the amount of light reaching the soil surface would have been relatively small. Coupled to the worms' life style below the soil surface it is unlikely that a response to day-length has evolved. A more likely influence is that of temperature, which worms would experience through the gradual change of the soil in general, or more markedly by the rapid changes at the soil surface. The way that worms have evolved specific responses to counter a drop in temperature seems to support this.

During adverse conditions such as drought or extremes of cold, *L.terrestris* can readily move to deeper soil through its more or less permanent vertical burrow system (Edwards & Lofty 1977). Other earthworm species may enter an inactive state of quiescence or diapause. During this period the worms stop feeding and construct a mucus-lined chamber in which they coil themselves tightly. Michon (1954), claims that red pigmented worms, which include many species of the genus *Lumbricus*, do not go into diapause, but Edwards & Lofty doubt this. Gerard (1960), reports that *L.terrestris* never becomes quiescent inside spherical mucus-lined cells.

Other parameters could have been included in this section, but it was felt that the major environmental influences on the lives of earthworms had been covered. The aspect of time, in terms of seasonality and age of earthworms, will be examined later.

A summary of the important parameters discussed above and how they may affect different stages of the life cycle of *L.terrestris* is given in Table 3.4.

Table 3.4.1 A summary of key parameters that affect the life of *L. terrestris*.

Life Stage	Temperature	Feed	Density	Water	Soil
Cocoon Production	Y	Y	Y	E	E
Cocoon Development	Y	N	N	E	N
Growth to Maturity	Y	Y	Y	E	E

Y = Affects life stage; N = Little or no effect ; E = Essential to life stage.

The parameters that affect the mature worms during cocoon production and younger worms during growth to maturity appear, from the literature, to be very similar. Only by experimentation would it be possible to confirm this.

Cocoon development appears to be a more simply manipulated stage in the life cycle as food is not necessary and there appear to be no adverse effects of population density. However, this meant that fewer improvements on the natural rate of development could be expected.

3.5 Objectives determined from the literature.

At this point two broad objectives were determined;

1. To discover if *L.terrestris* could be maintained and encouraged to reproduce under artificial conditions,
2. To discover, if manipulation of the parameters, food, temperature and population density, at three stages of the life cycle (cocoon production, cocoon development and growth to maturity), would increase production of *L.terrestris*.

Preliminary experiments were designed to provide initial indications of ways to achieve these objectives.

CHAPTER 4. PRACTICAL CONSIDERATIONS AND PRELIMINARY EXPERIMENTS

4.1 Practical Considerations.

Before undertaking any experimental work it was essential to consider what was practical within the confines of the existing laboratory and field facilities. Many aspects of earthworm culture could potentially be varied, singularly or in combination, but there was obviously a practical limit on the number of such variations that could be examined. To obtain an indication of what was feasible, initial examinations concentrated on equipment, feed sources, methods of feeding and methods of recording data.

4.1.1 Experimental Animals.

These were obtained by the formalin expellant method (Raw 1959), and used to form a stock of animals in the worm-beds at the Open University experimental site, (Knight 1987). These beds were supplied with the chosen soil, (section 4.1.4). The worms were not, at first, subjected to experimental treatments, to allow equilibration to their given conditions. All worms used in these experiments were collected from permanent pasture close to the river Ouzel in the grounds of the Open University. Later experiments involved the use of offspring from these worms.

Collection of worms on a small scale is not difficult, given optimal conditions of soil moisture. However, identification is less simple. Of the dozen or so species collected, adult recognition is relatively straightforward, (Sims & Gerard 1985), particularly when only one species is to be retained for experimental purposes, but the juvenile worms are much more difficult to distinguish. Particular problems occur within the Genus *Lumbricus*, where the smaller specimens of *L. terrestris* and *L. rubellus* are very similar (Snider & Snider 1988).

4.1.2 Recording of Earthworm Data.

Only a limited number of observations may be made relating to earthworm growth and reproduction. Growth could be monitored in several ways, but previous workers have found that with long term experiments, live weight values are of most use. Estimates of length can be difficult to obtain due to the worms ability to contract and relax its circular and longitudinal muscles. Meinhardt (1973), constructed a jig in which she restrained her

worms for measurement of length but it proved far from satisfactory. Nordström & Rundgren (1972b), suggest the use of a volumetric measure, "biovolume", which avoids the errors inherent in measuring length alone. However, the calculation of such a parameter seems unnecessarily complex and was only applied by the authors to preserved specimens. Live weight was therefore used as a measure of worm size throughout this work. In all instances before weighing, worms were washed in distilled water and carefully blotted dry, in the manner of Phillipson & Bolton (1977). Care was necessary when handling earthworms to prevent the exudation of coelomic fluid, an escape response. For some experiments, worms were kept in moist containers free from soil and food for twenty-four hours before weighing, to allow voiding of gut contents. This process is usually complete well within this time, only eight hours were needed by *L. terrestris* at 25°C, (Hartenstein & Amico 1983). Observations relating to the reproductive condition of the worms were also made at the time of weighing. This was a simple visual assessment based on the presence or absence of tubercula pubertatis and clitellum. By assigning levels of development similar to those of Gerard (1960), a sequence of maturation from immature to fully reproductive was recorded. Actual reproductive output in the form of cocoons could only be assessed by searching the medium in which adult worms had been kept. Mortality was also recorded simply by counting the number of worms present and comparing it with the number at the previous sampling.

4.1.3 Experimental Vessels.

Initially the size of vessels to be used was determined by trial and error. This did not necessarily provide an ideal choice but did rule out many unsuitable contenders. From the literature, temperature had been identified as an important regulator of worm growth, so experiments at controlled temperatures were needed. As incubator space was limited, it was desirable to use the smallest possible containers in order to secure reasonable replication. Space is always expensive so use of a minimum was desirable. Smaller vessels were also more easily handled than larger ones. Their disadvantage was that they failed to recreate anything like a natural environment. i. e. the amount of area around the edges was large in relation to the volume. These "edge effects" were undesirable and could alter worm performance compared to that in a large vessel or in undisturbed soil.

Using the information of Evans (1947), that earthworms of the genus *Lumbricus* do not burrow extensively so long as an adequate food supply is present on the surface, plus the

observation by Lofty (pers. comm.) that adult *L. terrestris* do not need a deep burrow in which to live, a brief trial was undertaken with a sample vessel which had a capacity of 2.5 litres with an 18cm depth. With the addition of four air holes of 3mm diameter in the removable lid, four adult worms were added above two litres of soil and excess organic material as feed. With a mean weight of 7g per worm, this gave 14g live weight of worms per litre of soil, which was within the carrying capacity of 22g per litre, recorded for *L. terrestris* by Hartenstein & Amico (1983). After a month the contents were examined. The worms had survived and produced cocoons, so the containers were considered suitable for further experimental use. An added advantage was that the containers were transparent, permitting a view of the worms' activities below the soil surface.

For experiments concerned with the growth of smaller *L. terrestris*, proportionally smaller pots were appropriate. These were obtained in two sizes, 300 and 600ml, with respective diameters of 100 and 125mm. Once again a tight-sealing removable lid was utilised which was provided with 10 holes of 1mm diameter made with a mounted needle. Early growth trials showed that worms up to 7g in weight could live within the larger of the two pots. These three types of containers were taken as standards and used throughout all of the described experiments.

4.1.4 Soil.

A single large quantity of top soil was essential. This was obtained from D.A. Bird, Bugbrooke, Northampton, following an inspection of prospective soils in stock, samples of which were taken away for analysis. The chosen soil had an ash content of 880g.Kg⁻¹, and a pH of 7.5. Identification and hand texturing following the ADAS method, (MAFF 1984), classified it as a loamy sand with 50 - 70% sand and 15% clay. This was considered acceptable for this work as *L. terrestris* is reported to be most abundant in England in light loamy soils (Lofty 1974).

Before use the soil was sieved through a 12.5mm mesh sieve, to remove large stones, and then sterilised. This involved heating the soil to at least 70°C, which killed any living worm material present (Meinhardt 1974), and any potential predators, (M^cLeod 1954). The soil was allowed to cool naturally in a covered container and then wetted to a moisture content of 20 - 25% for experimental use. At this moisture level the soil was wet enough not to adhere to worms' bodies, but dry enough not to coat the worms with a

muddy film. As a supply of several tonnes was obtained at the outset, the same source of soil was available throughout this work. Details relating to soil analyses are included in Appendix 3.

4.1.5 Feed Type.

Earthworms can derive their nutrition from a variety of organic materials (e. g. Edwards 1988) but to some extent this will be determined by the morpho-ecological group in which they are found (section 2.2). *L. terrestris*, an anecique earthworm, is reported to obtain nutrients by the ingestion and breakdown of organic materials which it drags down from the soil surface (Edwards & Lofty 1977). In these experiments it was therefore important to obtain feeds which would satisfy this criterion, however, other considerations were necessary.

In an intensive system, large quantities of feed would be required in order to produce a large biomass of earthworms. The passage of food through an earthworm gut is relatively rapid, eight hours for *L. terrestris* at 25°C, (Hartenstein & Amico 1983). Once the food had passed through the earthworm gut it would rapidly become mixed with the soil and eventually soil and food would become a substance which could not be easily separated. On an experimental scale, this would mean that once applied the food would have a finite life and would have to be discarded along with the soil when a change was necessary, for example, if cocoons needed to be found. On a production scale it would be important that fresh feed could be added to the mix without the mixture becoming toxic to the earthworms. Replenishment of food would need to be either little and often or a large amount given at longer intervals. The discussions in section 3.4.1.1, meant that a non-toxic organic substance, ideally with a carbon to nitrogen ratio in the range 10 - 35:1 (Neuhauser *et al* 1980a) and capable of supporting microbial growth, would be required. These requirements could have been met by several sources but the most obvious, for long term economic reasons, appeared to be the waste products from agricultural or non-toxic industrial processes.

Many factors influenced the choice of organic materials to be used as feeds in these experiments. They may be broadly defined as follows.

1. Availability:- A simple practical consideration.
2. Cost:- It was most desirable to use materials which were considered to be wastes from agricultural, industrial or domestic processes, so that cost was minimal, ideally only incurred during transport.
3. Consistency:- The material had to be available in a constant nature, or (at the experimental stage) in sufficient quantities to allow frozen storage.
4. Diversity:- As wide a range as possible needed to be included, perhaps to cover the three "classes" described by Evans & Guild (1948) (section 3.4.1.1).
5. Tradition:- Substances which have previously been (successfully) utilised as earthworm feeds.

Having decided, to use waste materials, it was necessary to specify exactly which types to use. Four feed materials were initially chosen for this series of experimentation. They were sewage sludge, separated cattle solids, wheat straw, and paper waste. This range was considered large enough for an initial experiment, with the idea that eventually it would be reduced in number to those which provided the best results in terms of growth and reproductive output, although other possibilities were not ruled out at this stage. Some of the characteristics of these materials and reasons for choosing them are given below. More details relating to physical and chemical properties are given in Appendix 4.

1. Sewage sludge

This was obtained from Towcester sewage works, which serves a rural area with little industry surrounding it. The sludge was therefore low in heavy metals, which could otherwise have harmed the earthworms (e. g. Ireland 1983). It was obtained in a belt-pressed, dewatered form with a total solids content of 25%. This material, with a carbon to nitrogen ratio of 15:1, had a high level of microbial activity, as indicated by a respirometry technique (Appendix 5). Similar materials have been used extensively in the growth of earthworm species (for example see Hartenstein & Hartenstein 1981).

2. Separated cattle solids (SCS)

These are cattle slurry from which the liquid has been removed (details relating to the advantages of this process are given in appendix 7). They were obtained from AFRC Engineering, Silsoe, where the separation process occurred. Traditionally bullock droppings or farmyard manure (FYM) have been used for worm growth studies (Evans & Guild 1948), but more recently separated cattle solids (SCS) have been tried, particularly at Rothamsted Experimental Station, (e. g. Edwards & Burrows 1988). SCS have a carbon to nitrogen ratio of 16:1 and were found to have a relatively high degree of microbial activity (see section 5.3.1). They have no apparent ill effects on some earthworms such as *E.fetida*, but must be allowed to "age" by loss of volatilised ammonia or they can be harmful to soil dwelling earthworms, (Knight 1987).

3. Wheat straw

This is a major waste in the East of England, (Staniforth 1982). The potential yield in England and Wales exceeds 12 million tonnes per annum, and on many farms surplus straw is an increasing problem, (MAFF 1986). (It will become more of a problem when straw burning in the field is banned in 1992.) It represents a complete contrast to the previously mentioned wastes, lacking microbial activity and any processing via the gut of an animal, it also has a higher cellulose and lignin content with a carbon to nitrogen ratio of 55:1. Before use it was milled to reduce its particle size.

4. Paper waste

Sometimes referred to as "recoverable fibre" in the paper making industry, this was the fourth waste material chosen. It is obtained by separating the solid fraction from the liquid effluent produced during paper manufacture. Large quantities are generated, one large paper mill, U.K. Paper of Sittingborne, Kent, produces 15000 wet tonnes per annum from a single mill, and this is normally landfilled. This waste has a high carbon to nitrogen ratio (93:1) but because of both mechanical and chemical processing its carbon should be more easily degraded compared to wheat straw. It has a low level of measured microbial activity, (see section 7.3.3). Some previous attempts had been made to grow certain species of earthworm on this waste, (Hand et al 1988; Edwards & Burrows 1988; Satchell & Dawkins Unpub.). Two sources were obtained for these experiments, one locally from Hemel Hempstead and the other from the above mill in Kent, where a previous contact

existed. A neutral sulphite, semi-chemical pulping process (Waldemeyer 1974) is used at both mills.

The four waste materials chosen therefore provided a diverse array which covered several important areas; pre-processing by an animal, level of microbial activity, cellulose and lignin content, carbon to nitrogen ratio and bulk density. However, all were readily available, of a consistent nature, had some history relating to earthworm studies and involved no significant, additional processing prior to use.

4.1.6 Feed Application.

Two distinct methods of feed application are available to vermiculturists. These are total mixing with the soil (e. g. Lofs-Holmin 1983) or surface application (e. g. Evans & Guild 1948). The relative merits of these alternatives depend upon the species of earthworm under scrutiny. Mixing prevents the food from drying out and reduces contamination with surface dwelling fungi, but Lofs-Holmin found that the number of cocoons produced decreased with the age of such a medium. As *L. terrestris* is a litter feeding earthworm, known to drag food down from the soil surface, applying food at the surface seemed appropriate. It also permitted a simple visual assessment of the amount of food still remaining so that more could be applied as necessary. The use of experimental vessels with tight seals prevented major water loss, but occasional checks were made and a spray of water added where necessary.

4.1.7 Sampling, food and soil replacement

Under natural conditions the food available to worms would be found within a worms length of their burrow entrances. This supply would be changing continually, for instance, as dead vegetation fell to the ground. No such natural replenishment could occur with sealed vessels, so the replacement of food, or at least the addition of new material was of some importance. For this reason replenishment of food was considered at sampling times. Decisions relating to food replacement hinged on several factors; i. the amount that remained at sampling, ii. the condition of the remaining food, (some feeds deteriorate with time), iii. the nature of the experiment. (If different feeds were used they were to be presented at the same rate independent of their condition).

Replacement of soil in the experimental containers with a fresh batch was avoided as far as possible, but was necessary under certain conditions. If, for example, the soil and waste became mixed and unsuitable for worm habitation, resulting in deaths, or if cocoons needed to be recovered and counted to provide an indication of the reproductive output of the worms, the soil had to be changed. The sampling period was varied depending on the size of worms, the information required, the amount of work involved and in early experiments, a degree of trial and error.

4.2 Preliminary Experiments.

This section presents details of some initial experimental work. It was conducted in order to develop experimental techniques to obtain preliminary data on some key environmental parameters affecting earthworm growth and, to a lesser extent, reproduction.

Three experimental programmes were carried out;

- A multi-variable investigation relating to the growth of immature *Lumbricus terrestris*,
- The growth of hatchling *Lumbricus terrestris* with a variety of feeds,
- The effect of temperature on cocoon development.

4.2.1 A multi-variable investigation relating to the growth of immature Lumbricus terrestris.

Introduction.

This experiment aimed to examine the effects of varying several of the parameters identified from the literature, which affect the growth of *L.terrestris*. These included;

- i. The type of organic material from which this earthworm is thought to derive its nutrition, (be it directly or indirectly),
- ii. The population density,
- iii. The temperature at which this earthworm is maintained.

As a long term study, the work was also designed to examine if it was possible to maintain worms in breeding condition throughout the year, following maturation under the chosen experimental system.

Materials and Methods.

All experimental work refers to growth of earthworms in the previously described plastic vessels with a capacity of 2.5 litres. Each was supplied with 2Kg of moistened sterilised soil (approximately 2 litres) and covered with an excess of the given organic material. The

worms had been kept in stock for several weeks in wormbeds at the Open University in a mixture of soil and separated cattle solids. Before use the worms were washed in water, blotted dry and then weighed. Each individual weight was recorded. Sorting removed any unwanted worms so that only immature, apparently healthy, *L. terrestris* were used. Worms were randomly selected for each vessel. Immature worms were chosen to facilitate monitoring of growth, as no reproductive activity could initially occur. A mean weight of 2g per worm was recorded.

Four types of organic material were used; i. separated cattle solids (SCS), ii. sewage sludge (SS), iii. wheat straw (WS), iv. paper waste (PW) from Kent. The sewage sludge was collected, as required, from the sewage treatment works, the SCS and paper waste were obtained in batches and stored in a cool outside store at 5 - 20°C. The straw, obtained locally, was kept in bale form and milled to pass a 2mm mesh as required. During the course of this experiment a range of chemical and physical analyses were conducted on these wastes, in order to try and relate properties to performance. The properties of each waste are recorded in Appendix 4 and the analytical procedures are described in Appendix 6.

Either four or eight worms were included in each culture vessel. A greater range of densities per vessel would have been preferred, but this was precluded by the constraints of sampling time. To investigate the effects of temperature three locations were chosen.

These were;

- (A). an external site sheltered from direct sunlight or rain. The vessels here were supported above the ground and insulated with straw to prevent freezing.
- (B). an incubator, with a constant temperature of 20°C. (Most work with *L. terrestris* had been conducted at around 15°C, (Lofs-Holmin 1985).
- (C). an internal, darkened storeroom, with only minor fluctuations in temperature.

During the course of the experiment, a Grant automatic temperature recorder was incorporated at sites "A" and "C" to assess the range of air temperatures experienced. At site "A" the range was 7 - 30°C and at site "C" it was 15 - 25°C.

With three replicates per treatment, this gave a total of 432 worms in 72 vessels.

i. e.

Feeds.....[SCS, SS, PW, WS]

Worms per pot....[4 , 8] x 3

Temperatures....[A , B , C]

Each vessel was given a unique set of code letters, comprising details of the waste, (S.C.S., S.S., P.W., W.S.), number of worms, (4, 8), location, (A = Outside, B = Incubator, C = Internal Store room.), and replicates, (I, II, and III). This made identification very simple. For example, SCS.4.B.I, was the first of three replicates, containing four worms, under separated cattle solids, in the incubator at 20°C.

Several culture vessels containing either four or eight worms were also maintained at 20°C, but no organic waste was applied to the soil. Apart from this, they were treated in exactly the same way as the other experimental vessels. The worm weights obtained were not included in any data analysis but were used to assess the effect of the presence of organic material.

Sampling.

All vessels were sampled every six weeks. This involved the removal of organic waste, soil and worms from the vessel. The soil, after moistening if necessary, was returned to the vessel and reused. The waste was also replaced and replenished as necessary. The earthworms were weighed as previously described. These weights plus the number of worms still alive and their reproductive condition were recorded. The "growth unit" was considered to be the total biomass of worms per culture vessel, not individual worms, as they could not be identified as such. Any dead worms were replaced with live immature individuals kept in stock but any vessel with less than its full complement of worms was not included in the statistical analyses. All worms were replaced on the surface of the vessel containing soil and feed, moistened with a spray of water and allowed to burrow down.

At least one major inspection occurred mid-sampling to ensure that ample organic material was present and that the soil was not too dry. Prior to the first sampling of six weeks, a random selection of vessels was examined (after three weeks) to assess the need for more frequent sampling. All of the vessels were maintained over three sampling periods (18 weeks), but following this time, selected vessels were retained for over one year. These vessels were the ones which contained worms fed with paper waste at 20°C, and were monitored to assess the carrying capacity of the system.

Data analysis.

Relative growth rates ($\text{mg.g}^{-1}.\text{day}^{-1}$) for complete worm units, i. e. vessels with a full complement of either four or eight worms, were calculated after each of the three sampling periods. Each set of data was analysed separately using an analysis of variance. Thus the normal practise of not combining repeated measures in a multivariate analysis of variance was followed (Hand & Taylor 1987).

The data presented is from the second sampling period as this was where the highest level of survivorship gave the largest data set ($n = 60$). This factorial analysis of variance has been handled in the manner described by Parker (1973). The levels of one variable have been treated as "treatments" and the levels of others as "blocks". The data can then yield meaningful conclusions relating to both main effects (variation in individual factors) and to interaction.

Results.

From an analysis of variance, the results of Table 4.2.1.1 show a significant difference between the growth rates of worms fed on the four types of waste material ($p < 0.001$). Of the four feeds examined in the presence of soil, paper waste appeared to be the best contender as a source of organic material for earthworm growth, although sewage sludge growth rates were also always positive. At site "C", mean growth rates of 6.71 and 3.07 $\text{mg.g}^{-1}.\text{day}^{-1}$ were recorded for paper waste and sewage sludge respectively. Further investigation was necessary, as the two feeds were very different in nature. The sewage sludge was acidic with a pH of 5.8, compared with near neutral for the paper waste. Half of the total solids content of the paper was cellulose, three times that of the sewage, whereas, the amount of Kjeldahl Nitrogen recorded was over five times greater in sewage sludge than in paper waste. This gave calculated C:N ratios of 93:1 for paper and 15:1 for

Table 4.2.1.1 The effects of feed type and temperature on the growth of immature *Lumbricus terrestris*

Mean growth rates at both densities (mg.g⁻¹.day⁻¹).

Treatment	Separated cattle solids	Sewage sludge	Paper waste	Wheat straw
Site "A" (Outside)	-4.93 (a)	0.42 (b)	9.95 (c)	-1.11 (b)
Site "B" (20°C)	-3.31 (a)	0.12 (b)	5.29 (c)	-0.84 (a b)
Site "C" (Inside)	0.41 (a)	3.07 (b)	6.71 (c)	1.56 (a b)

Means in the same row followed by the same letter are not significantly different ($p > 0.05$).

Sources of Variation	Degrees of Freedom	Mean Squares	F
Feeds	3	286.81	30.17 ***
Temperatures	2	44.54	4.68 *
Interaction (Feed and temp.)	6	13.18	1.39 n.s.
Density	1	17.40	1.83 n.s.
Error	47	9.51	

The different types of feed had a significant effect on growth rates ($p < 0.001$).

The different temperature regimes also had a significant effect on growth rates ($p < 0.05$).

sewage sludge. The level of microbial activity was also much greater in the sewage sludge, as revealed by a respirometry technique (appendix 5). Further details from the analysis of these and the other waste materials used as feeds, can be found in Appendix 4. Sewage sludge and paper waste were similar in that they both contained a high proportion of water which was not readily lost. At all sites an increase in live weight was recorded with these two feeds which was not the case for wheat straw or separated cattle solids.

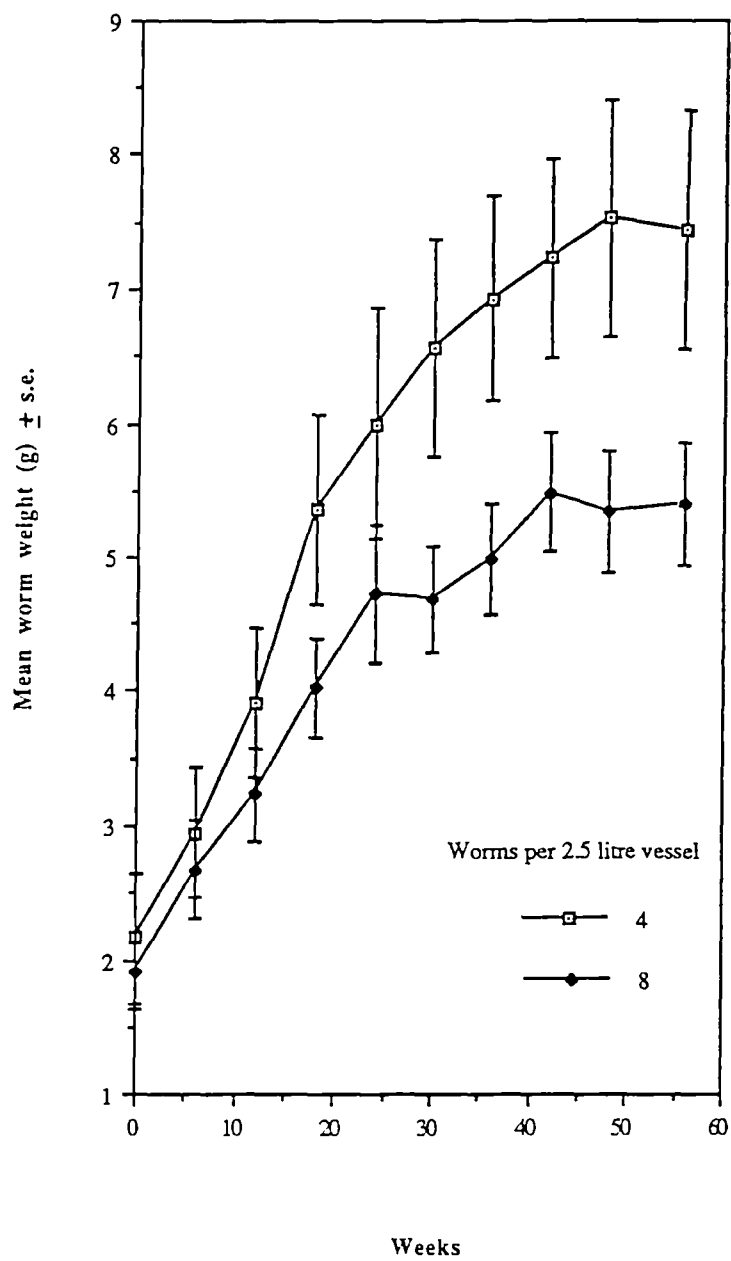
The wheat straw contained little water and the separated cattle solids were also found to dry out very rapidly. This physical factor may therefore have influenced the environmental conditions in the vessels sufficiently to account for some of the differences observed. The cattle solids used in this experiment were not considered to be typical of this material as they were obtained some weeks after separation and had "aged" considerably.

The analysis of variance applied to the results indicated that there was also a significant effect of different temperature regimes on growth ($p < 0.01$). Best growth figures for all feeds were obtained at site "C" (the indoor store), although the greatest figure for paper waste ($9.95\text{mg.g}^{-1}\cdot\text{day}^{-1}$) was recorded at site "A" (outside). Further experiments using more closely controlled conditions were needed to confirm the exact nature of temperature effects on growth. There was no significant interaction between feed type and temperature regime.

The vessels containing four or eight worms showed no significant differences in growth rate, but this can possibly be explained in terms of vessel size. As this experiment began with immature worms, it is conceivable that the carrying capacity of some vessels had not been reached after two sampling periods (twelve weeks), when the analysis was applied. The largest mass of worms finally recorded (after eighteen weeks) was 35g in 2.5 litres. However, in some vessels, notably those containing sewage sludge or paper waste, a dichotomy in worm growth was sometimes seen in the vessels with higher population densities. Some worms grew large and matured whilst others remained small or failed to mature. This suggests scramble competition, (Nicholson 1954), for food or space.

Figure 4.2.1.1, drawn from a limited number of vessels (P.W. 4 & 8, B, I - III), shows the effect of population density over a longer period of time. When the initial experimental programme was terminated, these vessels were maintained at 20°C , as they still contained the original experimental worms. Figure 4.2.1.1 demonstrates density dependent growth.

FIGURE 4.2.1.1 EARTHWORM GROWTH AT TWO DENSITIES WITH PAPER WASTE AS FEED



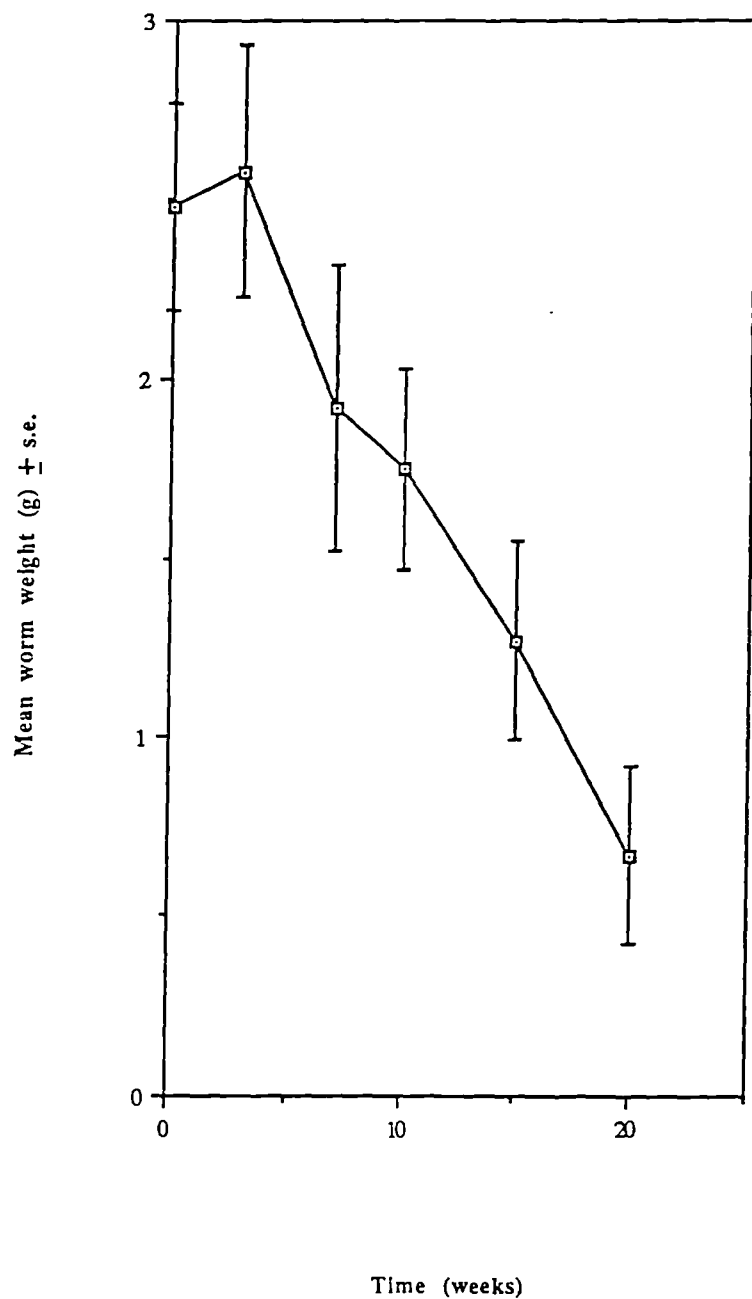
The carrying capacity of the vessels is in the region of 40g live weight of earthworms. This is equivalent to 16 - 20g live weight per litre. Hartenstein and Amico (1983), found that the carrying capacity for *L.terrestris* was 22g live weight per litre in soil and horse manure, but these results were for immature worms.

During the time when the effects of population density were being monitored, cocoon production by worms in vessels of both densities was also recorded. This meant that reproduction of this species was possible in vessels of only 2.5 litres, and experiments aimed at assessing this activity in more detail were planned (chapter 5).

The decline in weight of worms kept in vessels containing only soil showed that the presence of added organic material was important for promoting worm growth. All lost weight rapidly after an initial increase during the first six weeks (Figure 4.2.1.2). The initial weight gain was attributed to a limited amount of organic material in the soil which was soon exhausted.

As noted above, worms were replaced if they died. However, throughout the period of eighteen weeks, no deaths were recorded in some vessels. Thirty of the seventy-two vessels still contained the original worms. Of these, fourteen were those vessel supplied with paper waste. This was by far the most successful medium for maintaining worms, as the only mortality of worms appeared to be due to the heating effect of unavoidable, direct sunlight at site "A", where temperatures as high as 30°C were recorded.

FIGURE 4.2.1.2 WEIGHT CHANGE OF WORMS PROVIDED WITH SOIL ONLY (NO ORGANIC FEED)



4.2.2 Hatchling Growth.

Introduction.

This experiment aimed to examine the conditions necessary for the successful growth of hatchling worms. It ran concurrently with the experiments on the growth of larger worms. Once again, it was seen as a preliminary examination of an area which would become important in an intensive production programme.

As before, growth was measured in terms of increase in live weight, which could then be converted to absolute or relative growth rates. To monitor the growth of small worms, they should ideally be bred in the laboratory to remove doubts concerning age or, even more seriously, species. Initially hatchlings from cocoons of known origin (produced by field-collected adults) were used. This experiment involved monitoring their growth following emergence from the cocoons. As the work progressed experience led to modifications in technique.

The work is probably best viewed as a time series showing the steps taken at each modification;

- (a) Initial monitoring in soil, water and separated cattle solids (maintenance).
- (b) Experimenting with different feeds.
- (c) Expansion, employing field-collected "hatchlings".
- (d) Specific experiments, involving paper waste and soil.

Stages (a, b and c) were used to gain experience in the handling of hatchlings and to design stage (d). The first three stages are therefore reported only briefly.

(a) Initial monitoring

Materials and Methods.

Upon hatching, sixteen worms were retained in petri dishes and a mixture of soil and water was added to the moist filter paper already present. After several days separated cattle solids were added in small quantities. After about one week the worms were

individually transferred to small, 300ml, plastic vessels containing soil with a topping of separated cattle solids. Weights were recorded weekly.

Results.

After two months, growth curves were drawn for several individual worms. The maximum relative growth rate recorded was $30 \text{ mg.g}^{-1}.\text{day}^{-1}$. This occurred after replenishment of separated cattle solids and suggested previous starvation.

When compared with the results of Lofs-Holmin (1983), it was apparent that the growth rates obtained in these experiments were much lower (Figure 4.2.2.1). The mixture of soil and separated cattle solids was not proving to be a suitable medium for growth. Therefore further experiments, testing different feeds were begun.

(b) Experiments with different feeds.

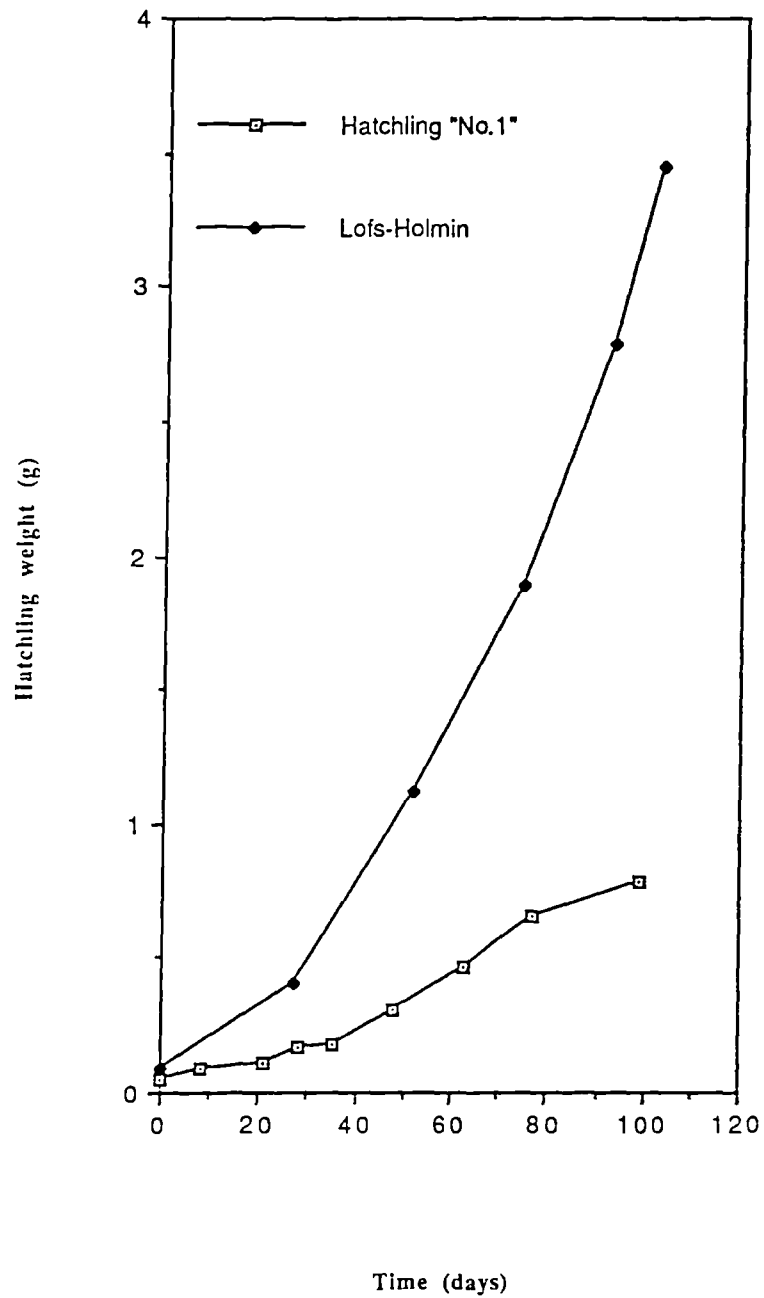
Materials and Methods.

A variety of different feeds were tested for their ability to support weight gain of juvenile *L.terrestris*. Those chosen were similar to the feeds used for growth of larger worms, separated cattle solids, paper waste and sewage sludge. The dry nature of straw prevented its inclusion. Each feed was tried either in the presence or absence of soil. All were placed in 300ml pots and kept at either 15 or 20°C, with one replicate of each treatment. The worms used here were the sixteen of known age previously used in (a), plus eight others which had subsequently hatched. Their weights were within a range of 0.06 - 0.28g.

Results.

The first major finding was that the worms kept in several of the pots were dead, when first inspected, after only one week. All of those that died weighed less than 0.2g. The only worms to survive were those in paper waste or paper waste and soil. All of these had shown an increase in weight. The worms which survived in the paper or paper and soil mixture showed absolute growth rates of between $10 \text{ and } 30 \text{ mg.day}^{-1}.\text{worm}^{-1}$. In the presence of soil the worms grew faster than those in the paper alone. Supplying fresh food proved to be very important as growth appeared to be limited prior to this. Relative growth rates immediately after a change of food were in the range of $25 - 75 \text{ mg.g}^{-1}.\text{day}^{-1}$.

FIGURE 4.2.2.1 HATCHLING GROWTH DURING "INITIAL MONITORING"
COMPARED WITH THAT OF LOFS-HOLMIN (1983)



(c) Experiments with field-collected "hatchlings".

Materials and Methods.

Due to an initial lack of cocoons, the number of hatchlings was small and prevented the desired amount of replication. To overcome this, small *L. terrestris* were obtained by the formalin expellant method and treated as in (b), with three replicates per treatment. Weights were recorded on collection and then every week thereafter. Soil and feed were changed as necessary, after every two or three weeks.

Results.

As before, the same mortality was recorded with a few minor exceptions. All of the worms fed paper waste with or without soil survived. However, some of the worms in sewage sludge and soil also survived. These worms were larger than some of the others, weighing more than 0.5g. Once again absolute growth rates were often higher when soil was present. Figures of 5 - 30 mg.day⁻¹.worm⁻¹. were recorded.

(d) Experiments with paper waste.

Materials and Methods.

After consideration of the results from (b & c), an experiment was set up to investigate the suitability of paper waste as a feed for worm growth. Again field-collected immature worms of a similar size were used. To investigate the suitability of different paper wastes, the one previously described, from U.K. Paper, Kent, plus another obtained more locally (Nash Mills, Hemel Hempstead), were used. The feeds were presented to the worms either with or without soil, under two temperature regimes of a constant 15 or 20°C. Four replicates were prepared for each treatment. Individual worms were put into each 300ml pot. These were sampled every two weeks, to allow sufficient data collection but to keep disturbance to a minimum.

Worm weights were initially recorded with a full gut, but following examination of the first results, the worms were only weighed after they had been allowed to void their gut contents. This was in order to remove any bias caused by the weight of soil particles in the gut. This is something not always practised by other workers. Gut voiding was achieved

by keeping the worms over-night in petri dishes on moist filter paper. An analysis of variance was applied to the relative growth rates obtained.

Results.

An analysis of variance (Table 4.2.2.1) showed that there was a significant difference between growth with the two paper types ($p < 0.01$). The paper waste from U.K. Paper promoted better growth. A significant difference was also found between treatments with soil present or absent ($p < 0.001$). The worms grew better with the paper waste in the presence of soil. There was no significant interaction between paper type and soil presence or absence. The two temperatures investigated showed no significant differences.

The results of Table 4.2.2.2 indicate that voiding of gut contents did not affect the differences in growth rates previously recorded. These differences were not caused by soil particles adding extra weight to those worms grown in paper and soil. Overall the significant differences were similar to the previous analysis with one addition relating to temperature ($p < 0.05$). Higher growth rates were recorded at 20°C for worms with three of the four feed treatments, only Hemel paper alone produced better growth rates at the lower temperature. However, for the latter feed a negative growth rate was recorded at both temperatures.

Discussion.

There were interesting differences between the growth of small hatchling *L. terrestris* and that of larger immature individuals, recorded in section 4.2.1. Probably the most significant were the deaths recorded under all but paper waste for very small worms. This can almost certainly be attributed to the physico-chemical properties of the wastes. Kaplan *et al* (1980), determined that certain conditions such as soluble salts in excess of 0.5% or pH values outside a given range were lethal to *E. fetida*. Similar effects may be occurring here. However, the fact that slightly larger worms, greater than 0.5g, were able to survive in sewage sludge suggests that a physical tolerance is achieved with increased size. This would require further investigation in order to be confirmed.

The presence of soil was shown to lead to significantly greater growth rates compared to worms fed with paper waste alone ($p < 0.001$). However, soil alone did not lead to an increase in weight when provided without an added organic feed.

Table 4.2.2.1 The effects of paper type and soil presence on the growth of hatchling-sized *L. terrestris*

Mean Values for both temperatures (from the second sampling period).

Treatment	U.K. Paper (Kent)		Hemel Paper	
	Only	Plus soil	Only	Plus soil
Growth rate. (mg.g ⁻¹ .day ⁻¹ .)	36.7	55.2	18.2	56.0

Sources of Variation	Degrees of Freedom	Mean Squares	F	
Paper type	1	692.78	9.86	**
Soil	1	6404.02	91.13	***
Interaction (paper type and soil)	2	298.58	4.25	n.s
Temperature	1	183.52	0.11	n.s.
Error	25	702.74		

The presence or absence of soil had a significant effect on hatchling growth ($p < 0.001$).

Paper type had a significant effect on hatchling growth ($p < 0.01$).

The different temperatures tested had no significant effect on hatchling growth.

Table 4.2.2.2 The effects of paper type and soil presence on the growth of hatchling-sized *L. terrestris* after gut-voiding

Mean Growth rate (mg.g⁻¹.day⁻¹.)

Treatment	U.K. Paper (Kent)		Hemel Paper	
	Only	Plus soil	Only	Plus soil
15°C	3.00	5.68	-8.83	7.95
20°C	2.25	28.43	-24.20	12.05

Sources of Variation	Degrees of Freedom	Mean Squares	F	
Paper type	1	591.68	9.24	**
Soil	1	2019.30	31.54	***
Temperature	1	411.845	6.43	*
Error	28	64.032		

The presence or absence of soil had a significant effect on hatchling growth ($p < 0.001$).

The type of paper waste had a significant effect on hatchling growth ($p < 0.01$).

The two temperatures tested had a significant effect on hatchling growth ($p < 0.05$).

The growth results from different types of paper waste indicated that, "paper waste" could not be treated as an homogeneous material. The nature of such wastes will depend upon the quality of paper being produced and the type of processing employed at the particular mill. Before considering the use of any paper waste in a commercial context, thorough chemical and biological testing would need to be employed.

4.2.3 The effect of temperature on cocoon development.

Materials and Methods.

An experiment to look at the effect of temperature on the rate of cocoon development was undertaken with twelve cocoons collected from a 2.5 litre sample vessel containing four mature worms. (The worms had been kept within this vessel for one month.) The cocoons were randomly divided into two batches of six and each cocoon was incubated at either 15 or 20°C. Each was placed individually on a moist filter paper inside a labelled petri dish. The cocoons were then checked daily. The length of incubation *per se* could not be determined, as the cocoons could have been produced up to one month before collection. However it was possible to obtain relative development times.

Results.

The mean time to hatching at 15°C was nineteen days greater than that recorded for cocoons incubated at 20°C. This showed that an increase in temperature within this range led to a decrease in time of development. Further experimentation at these and other temperatures with a larger number of replicates was necessary to obtain a clearer indication of these effects.

4.3 Conclusions

Considering all of the experiments together, a list of initial conclusions is presented.

1. Adult *L. terrestris* can be cultured successfully and will reproduce in vessels as small as 2.5 litres. A large volume of soil is not required.
2. Within 2.5 litre vessels (2 litres of soil), population density appears to have an appreciable effect, groups of 4 worms reached a mean live weight of 7.5g compared with 5.5g for groups of 8 worms. From this the carrying capacity of this system appears to be in the range of 15 - 22g live weight.litre⁻¹.
3. Soil appears to be necessary for successful growth. Significantly greater growth rates ($p < 0.001$) were obtained when paper waste was provided in the presence of soil compared to paper waste alone.
4. In the presence of soil, some forms of paper waste and sewage sludge promote significantly better growth ($p < 0.05$) of 2g worms than either separated cattle solids or milled wheat straw.
5. The temperature regime has a definite effect on worm growth, but the exact nature has yet to be clarified.
6. Survival of worms was greatest with an application of paper waste when compared to that of the other feeds.
7. "Paper waste" is not an homogeneous substance, it may vary from one paper mill to another. Growth with material from U.K. Paper, was significantly greater ($p < 0.01$) than growth with material from Nash mills (Hemel Hempstead).
8. Preferred conditions for hatchlings, including feed type, environmental temperature and soil presence, are very similar to those promoting most rapid growth of larger *L. terrestris*. However, hatchlings are more sensitive to physico-chemical properties of feeds. The survival of hatchling worms was only assured with paper waste.
9. An increase in incubation temperature from 15 to 20°C. reduces the time of cocoon development.

4.4. Clarification of objectives.

At the end of the previous chapter, two general experimental objectives were stated. Following this preliminary work, the key areas relating to an intensive production programme of *L. terrestris* have been identified. The objectives have been sub-divided into three main sections relating to the life cycle stages given in section 3.2.

4.4.1 Cocoon development and hatching.

The major factors found to affect this stage of the life cycle were temperature, moisture, and cocoon viability. The last was mainly determined by the condition of the parental worms, but the first two were vital to cocoon development (or the arresting of it, if cocoon storage was required).

The objectives established were;

- i. To determine the effect of temperature on cocoon development time.
- ii. To establish cocoon viability (hatching success) under different environmental conditions.

Moisture is essential to cocoon development (Table 3.4) so experimental manipulation will be confined to assessing the amount which can be lost by a cocoon and, on rehydration, still lead to successful development.

4.4.2. Growth to maturity.

It was found that the important variables that affected this stage of the life cycle were;

Food and the feeding regime,

The temperature regime,

Population density,

Soil presence.

It was necessary to determine the nature of these effects on growth at the various stages from hatching to maturity. (Although growth does not stop at maturation.) The objectives relating to them were clearly to establish the detailed effects for each.

4.4.3. Reproduction.

The variables that affected this stage of the earthworm life cycle were similar to those that affect growth. The objectives were clearly to determine the effects of these variables on cocoon production.

It was also necessary;

- i. To determine if *L.terrestris* will breed readily in artificial conditions.
- ii. To determine if year-long breeding is possible.
- iii. To determine if earthworm age affects reproduction.

4.4.4. Overall Objective

All of these objectives may be considered together;

To determine the optimal conditions for L.terrestris production in terms of growth and reproduction.

At this point experiments were initiated relating to each stage in the life cycle of *Lumbricus terrestris*, to try and achieve these objectives. The following three chapters concentrate on the three distinct stages of the earthworm life cycle and are presented in the order; reproduction, cocoon development and viability, and growth to maturity.

CHAPTER 5. REPRODUCTION.

5.1 Introduction

This chapter deals with the production of cocoons by mature earthworms (as described in section 3.2). Meinhardt (1974) suggested that cocoons may be produced by *L. terrestris* at any time of year under laboratory conditions, although the production rate for natural populations of earthworms in Europe is greatest in late spring to early summer, with a lesser peak in autumn, and is lowest in winter (Gerard 1967).

The ultimate control on the rate of cocoon production is the evolutionary life strategy adopted by an earthworm species, which will depend on the niche that it has evolved to occupy (section 2.2). Examining the results of Evans & Guild (1948) more closely, Satchell (1967) showed that on average, anecique species, of which *L. terrestris* is an example, produced 3 - 13, endoge species 25 - 27 and epige species 42 - 106 cocoons annually. Satchell related these differences to the probability of cocoon and hatchling survival which is directly affected by the severity of the type of environment occupied by each group. Cocoon production and hatching rates of earthworms are reported to exceed the requirements for maintenance of population size, (Lakhani & Satchell 1970). This counters the higher risk of mortality at an early stage of the life cycle.

Cocoon production by earthworms is not constant throughout their lives. Seasonal variation in cocoon production by *A. rosea* in an English woodland, was explained by Phillipson & Bolton (1977) in terms of temperature variations. Mean soil temperatures, at a depth of 5cm, increased from 6.0 - 12.6°C over the winter to summer period, with an associated increase in cocoon production from 0.02 to 0.53 cocoons per worm per month. A similar decrease was noted as temperatures fell to 9.4°C during the autumn when only 0.13 cocoons per worm per month were recorded.

Evans & Guild (1948) report that *L. rubellus* shows a significant correlation between temperature over the 6 - 16°C range and cocoon production ($p < 0.01$). Also the results of Reinecke & Kriel (1981) support the influence of temperature on reproductive output. Mature *E. fetida* (9 - 15 weeks old) were kept at constant temperatures of 10, 15, 20 and 25°C, with two worms per 5g of cow manure in petri dishes. Cocoon production, measured over 20 - 24 days, increased with increasing temperature. Maximum cocoon

production was 3.4 per worm per week, attained at 25°C, while minimum production of 0.12 cocoons per worm per week was at 10°C. Graff (1974) reported similar high rates of cocoon production for this species at 25°C (3.8 per worm per week).

Earthworm cocoon production can also be influenced by the quality of available food. The seminal study of Evans & Guild (1948) clearly illustrates this. *A.chlorotica* and *L.castaneus* were presented with a variety of feeds in three groupings; well decayed e. g. farmyard manure; partially decayed e. g. bullock droppings; and originally undecayed e. g. straw. Large differences in cocoon production were obtained, with partially decayed materials providing the highest levels of cocoon production, often up to twenty times greater than with some of the other feeds. This suggested that the food source ingested by the worms must have contained sufficient nutrients in an available form that could be assimilated and used for cocoon formation. Nowak (1975) collected earthworms from three areas of Polish pasture; under normal pasture, from an area formerly used as a sheep fold and from an area currently used as a sheep fold. In laboratory cultures, *A.caliginosa* from these three sites produced 26, 35 and 42 cocoons per worm per year respectively. Similar results were obtained from field collected cocoons leading her to conclude that earthworm fecundity was a function of organic matter input into their environment.

Litter and its decomposition products contain little nitrogen and there is some evidence (Lee 1985) that earthworms derive much of their nutritional requirements from the micro-organisms that they ingest with detritus. Overall rates of cocoon production and seasonal variations in these rates may therefore relate more to rates of microbial tissue production on detritus rather than directly to physico-chemical parameters of the soil environment (see Appendix 2).

It has been demonstrated experimentally that population density can influence the rate of cocoon production. Hartenstein *et al* (1979) kept populations of 4, 8, 12 and 16 *E.fetida* in vessels containing a standard volume of 300ml of horse manure with a surface area of 78cm². Maximum cocoon production, attained at an age of 9 - 11 weeks was 5.5, 5.0, 3.4 and 2.4 cocoons per worm per week for 4, 8, 12 and 16 worms per vessel respectively. These rates fell to around 1.5, 1.6, 0.4 and 0.2 per worm per week respectively at the age of 27 weeks. The total number of cocoons produced over the age range 4 - 27 weeks varied from about 70 at a population density of four per vessel to about 26 at a population density of sixteen per vessel. These differences almost certainly relate to scramble

competition for a limited resource, which in this case was most likely to have been food, space or both of these.

It is clear that rates of production can be influenced by a number of parameters, notably temperature, feed and density, or a combination of all three. Experiments performed and reported in this chapter aimed to examine the effect of temperature, feed composition and population density on cocoon production. The age of worms, and seasonal environmental factors can also affect cocoon production, and these factors were also examined.

5.2 Experimental Aims.

- i. To determine the appropriate conditions of;
 - (a) feed,
 - (b) temperature,
 - (c) stocking density, for higher rates of cocoon production.
- ii. To identify inherent seasonal variation in cocoon production.
- iii. To assess the effect of worm age on cocoon production.

5.3 Experimental procedures.

5.3.1 An assessment of the effects of feed material type and sampling frequency on cocoon production.

Materials and Methods.

Practically all experimental work relating to cocoon production was performed using the 2.5 litre vessels described in section 4.1.3. The results of early growth experiments examining the carrying capacity of these vessels (section 4.2.1) suggested that five mature worms was a suitable number for this system. The worms used were collected from the field by formalin extraction.

Initial production experiments were conducted in an indoor storeroom (as described in section 4.2.1) It was thought that undue disturbance of the worms could affect reproductive output, so two series of experimental vessels were set up, differing only in their frequency of sampling, which was either every two weeks or every six weeks. There were five replicates of each feed treatment in each series.

As with the early growth experiments (section 4.2.1) a similar variety of waste materials were tested for their suitability as feeds for the promotion of maximum cocoon production. These were sewage sludge, separated cattle solids (SCS) and paper waste. In addition dried, crumbled and rewetted separated cattle solids (DSCS) and similarly treated paper waste were used as feeds. For DSCS, this treatment was used to remove any excess ammonia, known to kill earthworms (Knight 1987; Bryson Unpub.) and to give a smaller particle size, (80% < 2mm). Smaller particles size had been shown to be more suitable for earthworm growth (Boström & Lofs-Holmin 1986). Problems occurred with the paper waste, which proved very difficult to break up after drying. Each feed was applied, in excess, on the surface of 2Kg of moistened soil.

Cocoon production was monitored from March to August 1988.

Results.

Figure 5.3.1.1 shows the mean cocoon output in cocoons per worm per week for five vessels, each containing five mature worms, fed paper waste, SCS or sewage sludge. For all feed treatments, maximum production was achieved soon after collection and then fell

rapidly over the summer months. Greatest production among the three feeds shown was obtained with sewage sludge, but mortality over the experimental period was six times greater with this feed than with worms fed either with paper waste or with SCS. Loss of reproductive condition, visible in the form of clitellar scarring, was first noticed for a small number of worms at the end of May. By August some worms in all treatments had completely lost their clitellate appearance.

Table 5.3.1.1 contains an analysis of variance which relates to the type of feed given to the mature worms and also the frequency of sampling for cocoons. Sewage sludge was not included in this analysis due to the deaths mentioned above. However, two other categories of feed, dried ground and rewetted paper waste and similarly treated SCS, were compared with each of the wastes in their original conditions. The results suggest that there is a significant difference in cocoon production between feed type ($p < 0.01$). Of the feeds tested, dried ground and rewetted separated cattle solids (DSCS) gave greatest cocoon production. A mean figure of 0.72 cocoons per worm per week was obtained. The frequency of sampling, fortnightly or every six weeks, had no significant effect on cocoon production.

Discussion

The results in figure 5.3.1.1 show a decline in cocoon production over the summer period and support the idea of a seasonal trend as would be expected for field populations. These rates may be greater than those expected under field conditions, as the soil moisture content of the vessels in this experiment was kept reasonably constant throughout. Under field conditions, summer droughts would tend to limit cocoon production. The fall in cocoon production to zero may represent an effect of high temperature in the storeroom, where the worms were kept. The loss of reproductive condition in some worms which accompanied this, may have been due either to high temperature or to a form of fatigue or "reproductive exhaustion", brought about by unnaturally favourable conditions for reproduction.

Worms fed dried separated cattle solids produced the greatest number of cocoons. Previous workers have found similar materials to support greatest cocoon production for various earthworm species (e. g. Evans & Guild 1948; Lofs-Holmin 1983; Edwards 1988;

FIGURE 5.3.1.1 COCOON PRODUCTION WITH THREE FEEDS AT FLUCTUATING TEMPERATURES

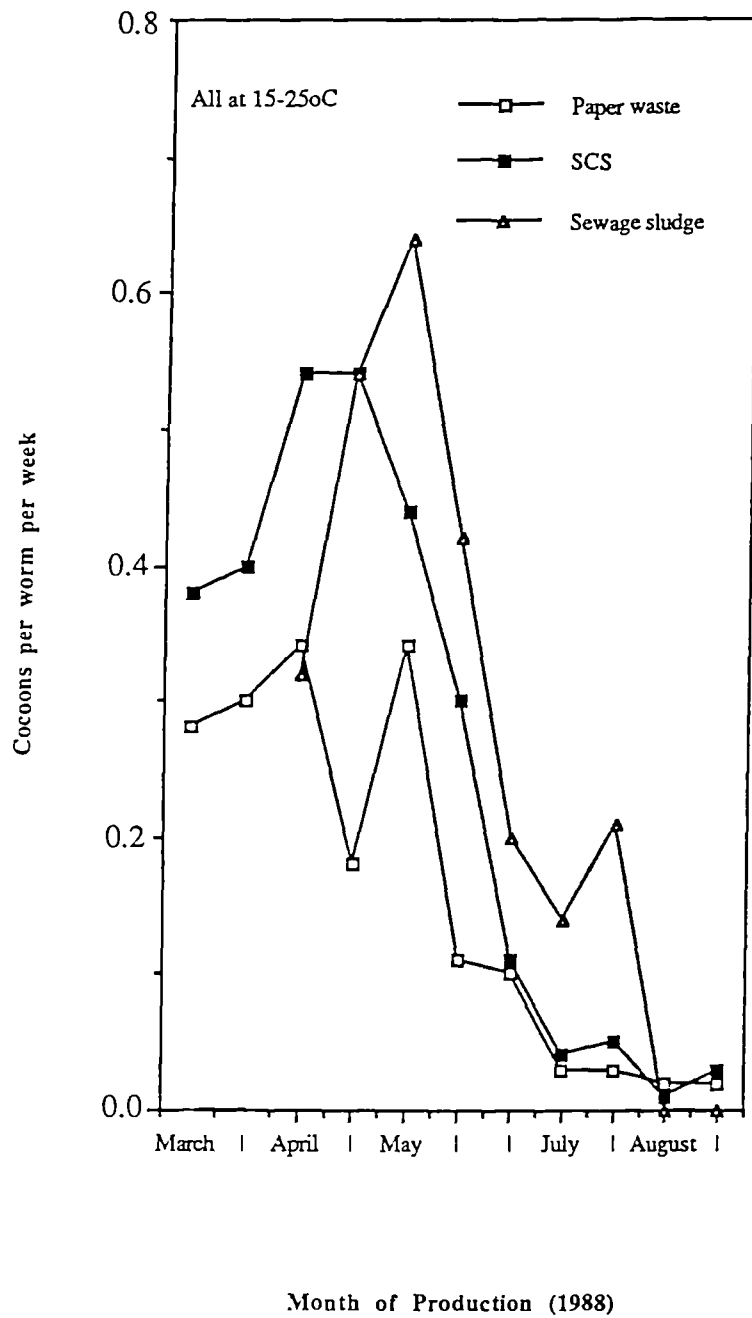


Table 5.3.1.1 The effect of feed type and sampling frequency on cocoon production

Mean figures of Cocoon Production for the first six weeks of sampling.

Feed Type	Paper Waste	Dried Paper Waste	Separated Cattle Solids	Dried Separated Cattle Solids
Cocoons. worm ⁻¹ . week ⁻¹ .	0.43 (a)	0.43 (a)	0.44 (a)	0.72 (b)

Means followed by the same letter are not significantly different ($p > 0.05$).

Sources of Variation	Degrees of Freedom	Mean Squares	F
Feed Type	3	167.73	5.40 **
Sampling frequency	1	120.05	3.87 n.s.
Interaction	3	49.37	1.59 n.s.
Error	28	31.05	

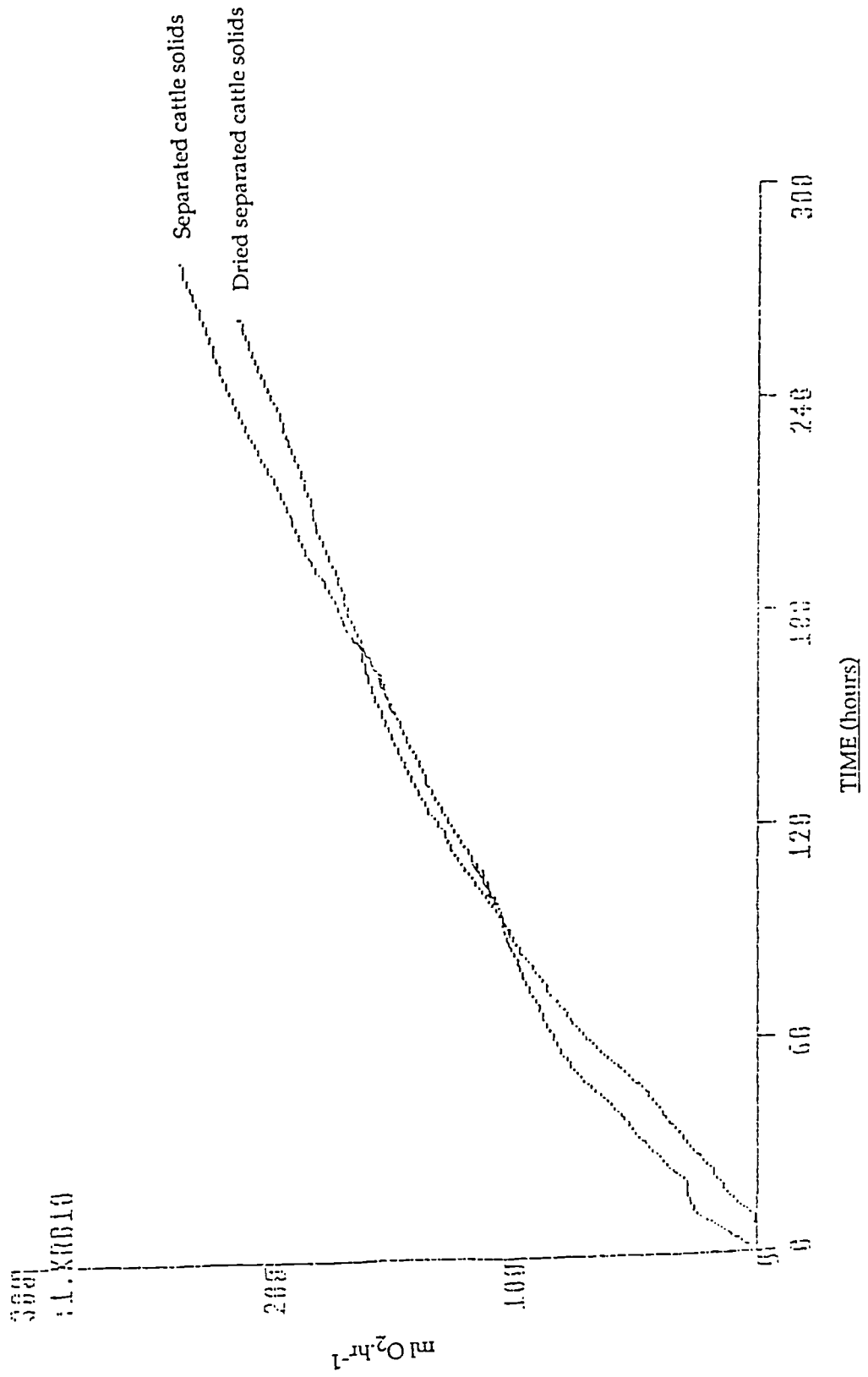
The type of feed has a significant effect on cocoon production. ($p < 0.01$).

Sampling either every two or every six weeks has no significant effect on cocoon production.

Frederickson & Knight 1988). For *L. terrestris* the production rate of 0.72 cocoons per worm per week was equivalent to that of ten days per cocoon given by Meinhardt (1974).

The reduced particle size associated with DSCS, may have benefited the worms directly, but would almost certainly have increased the surface area on which micro-organisms could act. However, results from respirometry studies applied to SCS and DSCS showed that this was not necessarily so. The uptake of oxygen by the micro-organisms on each form of feed, in the presence of soil was very similar (mean values of $0.85 \text{ ml O}_2 \cdot \text{hr}^{-1}$ for SCS and $0.81 \text{ ml O}_2 \cdot \text{hr}^{-1}$ for DSCS at N.T.P., were recorded over a minimum of ten days). A summary of the method (Tribe & Maynard 1989) by which these results were obtained is given in Appendix 5, and an example of respirometry readings is presented in Figure 5.3.1.2. The removal of residual ammonia from the SCS during drying may well have been the major contributory factor in improving its acceptability to the earthworms, thus leading to greater cocoon production.

FIGURE 5.3.1.2 CUMULATIVE OXYGEN CONSUMPTION MEASURED USING A
MAYNARD PROJECTS RESPIROMETER



5.3.2a An assessment of seasonal and temperature effects on cocoon production.

Introduction.

The results of 5.3.1 posed some important questions relating to factors that had influenced cocoon production. Three major factors; seasonality, associated temperature changes, and length of time in captivity required further investigation. As the storeroom in which the vessels of experiment 5.3.1 had been kept was not temperature controlled, seasonal temperature changes could have affected cocoon production. Over the experimental period (March - August 1988) these air temperatures had ranged from 15 - 25°C. It was also possible that some aspect of time in captivity (duration of the experiment) may have influenced cocoon production.

Materials and Methods.

To investigate these effects, a series of experiments was begun in August 1988. It was designed to compare the cocoon production of mature worms over the period of a complete year. Worms were collected at the start of each calendar month and maintained under controlled or semi-controlled temperature environments. Three temperature regimes were chosen for examination. These were; (a) constant 15°C, (b) high fluctuating (indoor; the internal storeroom of 5.3.1) and (c) low fluctuating (outdoor; a covered, unheated building). Both (b) and (c) had air temperature fluctuations recorded as monthly maximum and minimum values. From these readings mid-range values were calculated. These proved to be close to recorded soil temperatures within the experimental vessels. These soil temperatures were recorded periodically at each site during the experiment using an automatic temperature recorder, as described in section 4.2.1. The constant temperature of 15°C was chosen as this was known to be within the optimum range discovered by previous workers (Table 2.6.1).

At the start of each month, soil conditions permitting, a minimum of forty-five healthy, mature *L. terrestris* were obtained by the formalin expellant method (Raw 1959), and after washing they were randomly divided into nine groups of five. These nine groups were then weighed and each group was placed in a 2.5 litre vessel containing 2Kg of moist soil. After burrowing down, they were supplied with excess dried, crumbled and rewetted separated cattle solids (DSCS) as feed. This feed was chosen as it had given the best results for cocoon production in earlier experiments (Table 5.3.1.1). The nine vessels were

then labelled and three were randomly allocated to each temperature regime. Checks for soil moisture content and supply of feed were made after two weeks and sampling was every month. No replacement of worms was possible because it was thought the length of time that the worms had been part of the experiment might be important, and this was to be examined statistically. As time passed the number of experimental vessels grew, towards a theoretical maximum of 108. Collection occurred until July 1989 when twelve monthly samples had been obtained. Mean earthworm weight on collection, over the whole period of the experiment, was 5.3g.

At each sampling, moistened sterilised soil and fresh feed were placed in the vessels. The worms were counted, weighed, and examined for sexual and general condition. The soil taken from each vessel was examined for the presence of cocoons, by the process of wet sieving. i. e. the soil was soaked with water and then carefully hosed through a series of increasingly finer sieves, of mesh sizes, 6.7, 3.35, and 2mm. The largest removed any coarse material, the second collected the majority of cocoons, (their minimum dimensions are given as 4.4 x 3.9 mm; Sims & Gerard 1985), and the smallest retained any unusually small cocoons. Cocoon production in terms of cocoons per worm per month could then be calculated. All cocoons were retained and their development monitored (chapter 6). The experiment was terminated after thirteen months (September 1989).

Statistical analyses were applied to the data to determine the effects of; the different temperature regimes and the month of collection on cocoon production. In some instances it was necessary to analyse sub-samples of the data separately, which is a normal procedure when applying an analysis of variance to repeated measures (for example see Sokal & Rohlf 1981; Hand & Taylor 1987). As the experiment ran for thirteen months, data for complete sets of twelve month cocoon production were only available for worms collected in August and September of 1988. These were therefore utilised to examine annual cocoon production statistically. Cocoon production and monthly mid-range air temperatures were also compared.

To investigate seasonal effects on cocoon production, an analysis of variance was applied to a number of sub-samples from worms collected throughout the year and kept under the three temperature regimes. To negate any effects of captivity (time under experimental conditions), as far as was possible, all worms sampled a given number of months after they were collected have been analysed together. (i. e. these results were not obtained at

the same month of sampling). The treatments examined are therefore the *n*th month of sampling following collection and the temperature regime under which the worms were kept (where *n* is any number of months after collection from 1 to 12). A separate analysis of this type was performed a number of times for different periods after collection. e. g. sampling results for two, three, four, or *n* months after collection. However, only the results for cocoon production two months after collection have been presented as these permit the greatest number of calendar months sampled (eleven) to be included. (The results from sampling worms one month after collection were not included, as it was thought that they might have been unrealistic, due to the affect of the formalin used during collection.)

Results.

The data presented in figures 5.3.2a.1/2/3 shows mean monthly cocoon production with standard error bars, over a twelve month period for the three selected temperature regimes. The graphs are drawn from data obtained from worms collected at the start of August and September 1988. Cocoon production under each temperature regime is then compared with the temperatures experienced.

Cocoon production under constant temperature conditions at 15°C rose from less than one cocoon per worm per month soon after collection (autumn) to a peak in December at around three cocoons per worm per month, where it remained throughout the winter and spring periods. A large fall in production, to one cocoon per worm per month, was recorded from May through to July. Mean cocoon production at 15°C was 25.3 cocoons per worm per year. After twelve months 68% of the worms were still alive and all of these were still fully clitellate.

At the higher fluctuating temperatures (inside), a continuous increase in cocoon production was recorded as monthly mid-range air temperatures fell from 21 to 18°C from August 1988 through to March 1989 and then rose again to 21°C. Cocoon production increased from around one in winter, to above two cocoons per worm per month in May. However, at this point temperatures continued to rise and reached 25°C by July, when cocoon production had fallen to less than 0.5 cocoons per worm per month. During the May to July period, a decline in reproductive and general condition of the worms was also recorded, which in some cases led to death. Only 40% of the worms were alive after a

period of one year, and of those only 75% were clitellate. Mean cocoon production under this temperature regime was 10.1 cocoons per worm per year.

At lower fluctuating temperatures (outside) the shape of the graphs for cocoon production and monthly mid-range temperature appear to show a close relationship. At 1.0 - 1.5 cocoons per worm per month, production was steady throughout the autumn and winter. It rose markedly from April to June and peaked at over three cocoons per worm per month. During this increase in cocoon production temperatures rose from 9.5 to 18°C. During July cocoon production fell by fifty percent, as temperatures climbed above 20°C. Mean cocoon production under this temperature regime was 17.2 cocoons per worm per year. After twelve months of monitoring, 86% of the worms had survived, all were clitellate and all appeared very healthy.

Using the data from worms collected at the start of August and September 1988, an analysis of variance was performed to examine the effects of the three temperature regimes on annual cocoon production. The results of Table 5.3.2a.1 showed that maintaining worms under the three temperature regimes had a significant effect on annual cocoon production ($p < 0.001$). The greatest annual cocoon production of the three temperature regimes examined (25.3 cocoons per worm per year) was obtained from worms kept at a constant 15°C. Under the lower and higher fluctuating temperature regimes, mean annual cocoon production was 17.2 and 10.1 cocoons per worm per year respectively. There was no significant difference between cocoon production for worms collected during the months of August or September.

The results of Table 5.3.2a.2, examining the effects of the three temperature regimes and the month of collection show a significant difference between the temperature treatments for cocoon production from sampling after two months ($p < 0.001$). The highest monthly production rates were at the higher fluctuating temperature (2.06 cocoons per worm), and at a constant 15°C (1.93 cocoons per worm). These mean values were significantly different ($p < 0.05$) from that obtained for the lower fluctuating temperature regime (0.96 cocoons per worm). These results confirmed the importance of the temperature regime on cocoon production, as first identified for annual figures in Table 5.3.2a.1.

FIGURE 5.3.2a.1 COCOON PRODUCTION AT A CONSTANT TEMPERATURE OVER TWELVE MONTHS

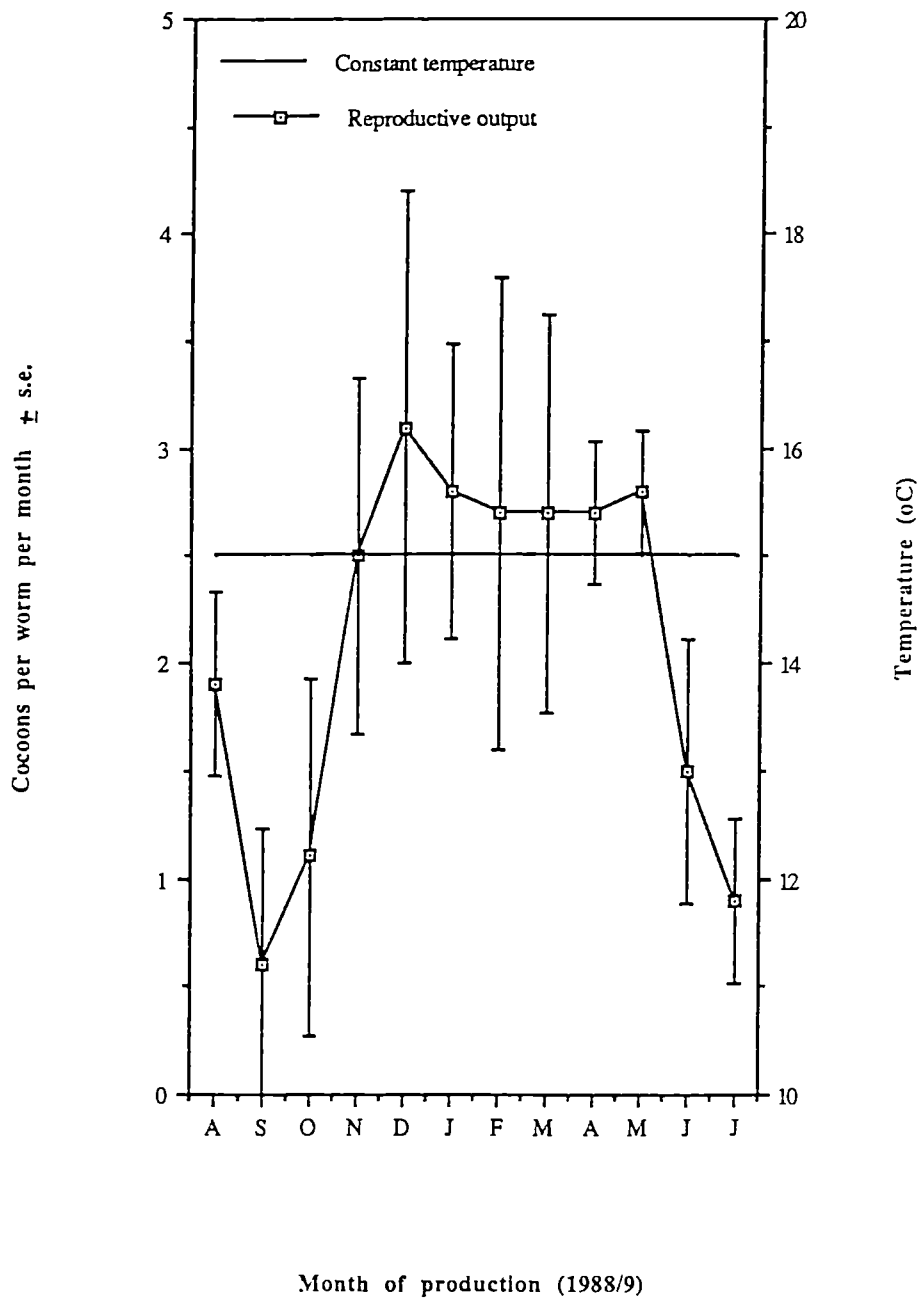


FIGURE 5.3.2a.2 COCOON PRODUCTION AT A HIGH FLUCTUATING TEMPERATURE

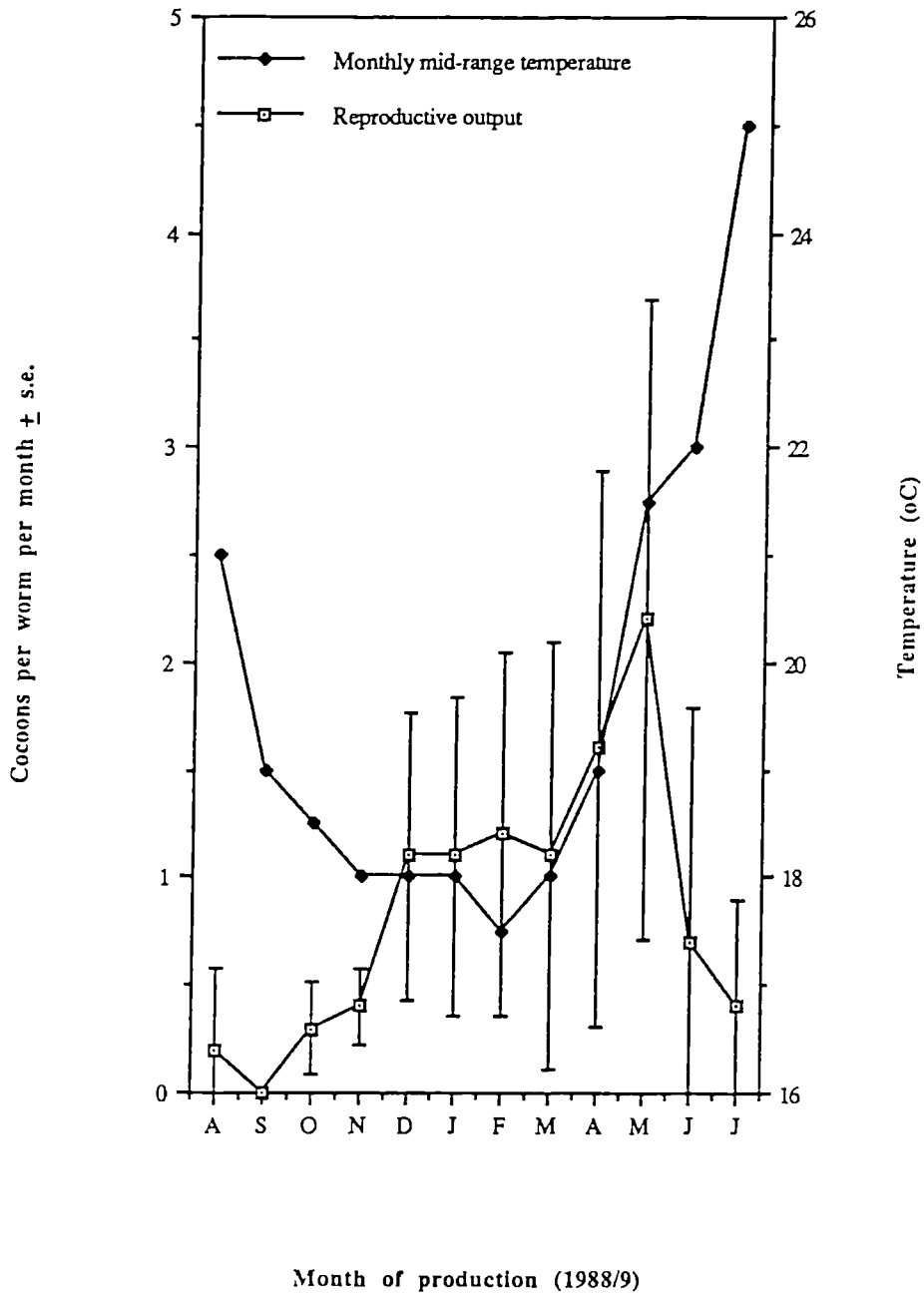


FIGURE 5.3.2a.3 COCOON PRODUCTION AT A LOW FLUCTUATING TEMPERATURE

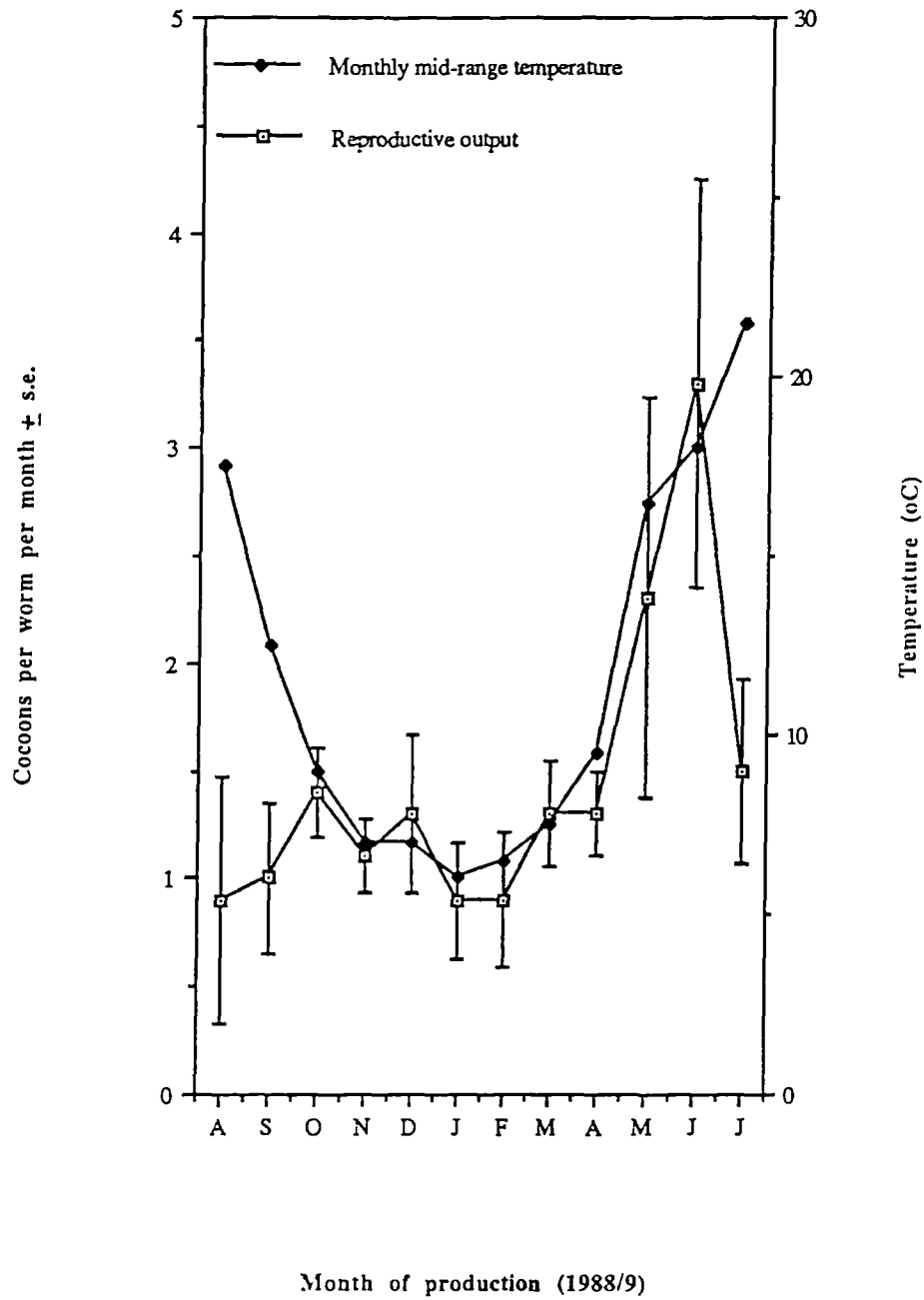


Table 5.3.2a.1 The effects of different temperature regimes on annual cocoon production

Mean Values

Temperature regimes	cocoons.worm ⁻¹ .year ⁻¹ .
Constant 15°C	25.3 (a)
Outside (low)	17.2 (b)
Inside (high)	10.1 (c)

Means followed by the same letter are not significantly different ($p < 0.05$).

Sources of Variation	Degrees of Freedom	Mean Squares	F
Temperature regime	2	356.95	14.73 ***
Month of Collection	1	0.38	0.01 n.s.
Error	13	24.22	

The temperature regimes at which the worms were kept has had a significant effect ($p < 0.001$) on annual cocoon production.

Table 5.3.2a.2 The effect of different temperature regimes and month of collection on cocoon production.

Mean Values

Temperature regime	cocoons.worm ⁻¹ .month ⁻¹ .
Constant 15°C	1.93 (a)
Outside (low)	0.96 (b)
Inside (high)	2.06 (a)

Means followed by the same letter are not significantly different ($p > 0.05$).

Sources of Variation	Degrees of Freedom	Mean Squares	F
Temperature regimes	2	9.590	18.41 ***
Month of collection	10	3.055	5.86 ***
Interaction	20	2.23	4.28 ***
Error	55	0.521	

The temperature regimes under which the worms were kept, the month of collection and the interaction between the two, had a significant effect on cocoon production. ($p < 0.01$).

The month of collection also had a significant effect on cocoon production ($p < 0.001$). As the length of time all worms had been part of the experiment was constant (two months), this result meant that seasonal variation in production was again suggested.

Projected annual results for cocoon production, obtained from monthly derived figures (Table 5.3.2a.2) when compared with actual annual results (Table 5.3.2a.1) suggest that length of time in captivity also has an effect on cocoon production but that this effect is still influenced by the temperature regime under which the earthworms are maintained. It is also likely that time *per se*, rather than time in captivity, may be an important factor in cocoon production. Older worms may produce less cocoons than younger adults. This is more fully explored in section 5.3.3.

Discussion.

A view of cocoon production over a long period of time has seldom been recorded for an anecique species of earthworm. This is due mainly to the nature of their lifestyle (Satchell 1970), the problems associated with maintaining these worms in reproductive condition and the amount of labour involved.

A clear effect of temperature on cocoon production was obtained for both annually (Table 5.3.2a.1) and monthly (Table 5.3.2a.2) calculated results, with the greatest number of cocoons produced at a constant temperature of 15°C for actual annual results. This indicates that temperature plays a large part in determining the reproductive performance of this species, as suggested for *A. rosea* by Phillipson & Bolton (1977).

Cocoon production under all three temperature regimes in these experiments followed broadly similar patterns. All had a cyclic pattern, which rose to a peak and then fell. The differences between cocoon production under the three temperature regimes relate to the size of the peaks and the period during the year at which they occur.

If it is accepted that a minimum temperature is required for cocoon production, and that above a given maximum temperature cocoon production ceases, then an attempt can be made to explain the observed graphs. However, these assumptions are almost certainly an over simplification of what really occurred.

It is proposed that one important factor influencing cocoon production by this species of earthworm is temperature. Temperature limits are known to exist, above and below which *L. terrestris* cannot survive. Between the limits of approximately 0 - 28°C (Wolf 1938) temperature is known to affect behaviour. e. g. when surface soil temperatures rise or fall dramatically, avoidance occurs and this earthworm retreats to deeper burrows. If, as here, this species is kept under sealed experimental conditions, a natural avoidance behaviour is not possible and a reaction to, in this case higher, temperatures must occur. Initially this reaction appeared to take the form of increased cocoon production (this is shown dramatically under the lower fluctuating temperature regime over the months of February to June), but sustaining this rate of production was not possible. This production increase was after mid-range air temperature had increased from 6 - 18°C. Evans & Guild (1948) reported a similar trend in cocoon production for *L. rubellus*, when a four fold increase was recorded as the temperature increased in the range 6 - 16°C.

Lengthy exposure to temperatures above approximately 15°C in these experiments seemed to lead to what has here been termed "reproductive exhaustion", or fatigue by Evans & Guild (1948). The presence of excess food, and acceptable moisture levels are not sufficient to compensate for the raised temperatures experienced. Following such exposures many of the worms which had been in good general and reproductive condition, lost their clitellate appearance and subsequently died. Survival of worms was greatest under lower fluctuating temperature conditions.

It has been demonstrated that *L. terrestris* can reproduce throughout the year, as suggested for this species by Meinhardt (1974), and also for *A. caliginosa* (Nowak 1975). Annual production for *L. terrestris* of twenty-five cocoons has been recorded during this work. At two weeks per cocoon this was less rapid than the figure of ten days per cocoon (Meinhardt 1974) but that higher figure was only reported to occur at limited periods during the year. During the course of this experiment some worms lost reproductive condition. The regression of the clitellum left the worms initially with a scarred appearance, which then gave way to a pre-reproductive appearance, similar to that of maturing worms, but quite different in origin. However, this loss of reproductive condition was frequently linked to loss of general condition shown by weight loss. Also some of the worms lost their pink/purple colouration and became much browner prior to death. With one individual worm, death followed two months after cocoons had last been produced.

The scarred condition of the clitellum and a change in colouration was observed at the monthly sampling mid way between these two periods.

If, as suggested, temperature is important in determining reproductive performance then under constant temperatures it might be thought that cocoon production would remain at a constant level. However, a cyclic trend in reproductive output was recorded which could not be linked to a temperature related seasonal effect, as after collection, the worms were kept in darkness and at a constant temperature throughout the experiment. However, if an internally-controlled biological clock, attuned to the annual cycle is possessed by this earthworm species, proximate factors such as temperature or daylength which could act as cues for the setting of such a mechanism, may have been triggered (as suggested in section 3.4.2b.2). This may have been the result of experiencing a rise in temperature following collection, or it may have been due to light breaks in the constant darkness, caused at times of inspection or sampling.

This "biological clock", if present, must be in tune with the annual seasonal pattern. Similar mechanisms have been identified in other invertebrate and vertebrate organisms (Gwinner 1981) which have been shown to be "set" by daylength or changes in temperature. One major function of circannual rhythms may be to improve the timing precision of seasonal activities in organisms that inhabit environments with pronounced seasonal changes. If these organisms relied solely on external cues, a considerable year to year variability in the timing of their seasonal activities would be expected as a result of the variability in, for example, weather conditions.

The seasonal pattern of production reported for this species by other authors (e. g. Lakhani & Satchell 1970) was seen to occur. Ralph (1957) and Edwards & Lofty (1977) demonstrated circadian rhythms for this species, which continued for more than seven days even after worms were placed in conditions of constant temperature and darkness. The latter also showed seasonal variations of these activity patterns. The seasonal pattern of reproduction demonstrated during this research appears to have an overriding internal control which can however, be influenced by environmental temperatures.

Hence there is a need for lengthy studies of this type if a true description of cocoon production is required. Ideally, an experiment of this nature would have been run for as

long as possible, certainly over a period in excess of two full years to confirm any cyclic patterns of cocoon production and would have included a greater number of treatments.

5.3.2b. A comparison of cocoon production at two constant temperatures.

Introduction.

Due to limited facilities, it was not possible to examine cocoon production in detail at more than one constant temperature (15°C), as in experiment 5.3.2a. However, small scale examination of cocoon production at a constant 20°C was also made. This was to assess cocoon production at temperatures higher than those normally associated with *L. terrestris*. The 20°C temperature was chosen for investigation as early results from the long-term study had suggested that higher fluctuating temperatures as well as a constant 15°C might provide the greatest cocoon production figures.

Materials and Methods.

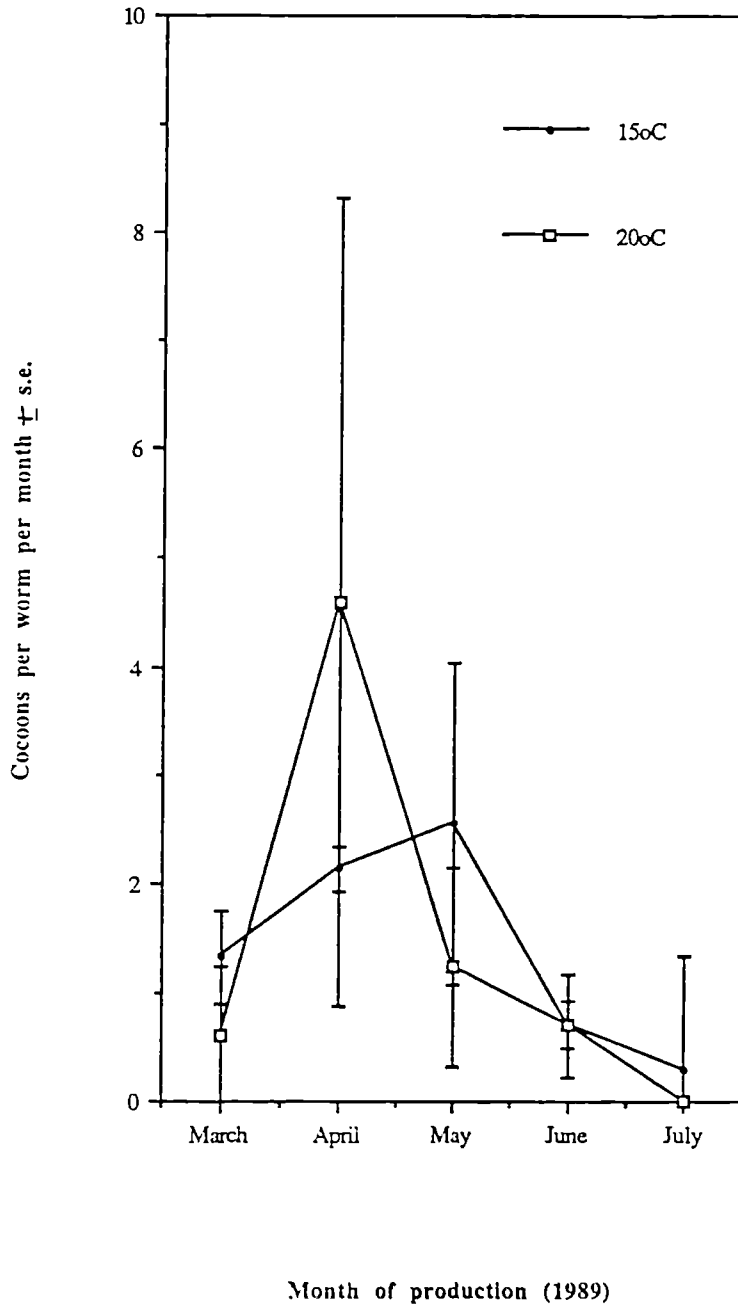
At the beginning of March 1989 three 2.5 litre vessels were set up in the manner of experiment 5.3.2a. These vessels were supplied with 2Kg of moistened, sterilised soil, DSCS as feed and five field-collected, mature *L. terrestris* (mean weight 4.4g) were placed in each and they were incubated at a constant 20°C. The vessels were sampled at the start of each calendar month up to the beginning of September 1989. Figures for five months of cocoon production are presented.

Results.

The results for cocoon production by the worms in these vessels are shown in Figure 5.3.2b.1, and compared with production by worms (from experiment 5.3.2a.) collected at the same time but incubated at 15°C. After two months the production by worms kept at 20°C was very high with a mean figure of 4.6 cocoons per worm per month, more than double that at 15°C. One vessels at 20°C containing five worms, produced thirty-four cocoons in the month of April. At 6.8 cocoons per worm per month, this was the greatest production for this species over a short period recorded so far. However after a period of three months the reproductive rate at 20°C fell dramatically, to a level below that of the worms kept at the lower constant temperature. At the termination of this experiment the worms at 20°C had not produced a single cocoon for two months, many had lost reproductive condition and some had died.

The absolute figures over the five month period for three vessels of each treatment were 82, and 97 cocoons at 15°C and 20°C respectively.

FIGURE 5.3.2b.1 COCOON PRODUCTION WITH DSCS AS FEED
AT TWO CONSTANT TEMPERATURES



Discussion.

On capture, the supply of superior feed, and the greater increase from external, environmental temperatures to 20°C, compared to 15°C, may have triggered the worms to begin rapid cocoon production. However, this may then have led to a form of reproductive exhaustion which might well result if worms reproduce to their maximum potential. No great advantage was found by keeping worms at the higher temperature. If worms were to be used as part of a long term production programme, then the ideal situation would be to encourage a steady continuous cocoon output. These results suggest this would not occur at temperatures much above 15°C.

Alternatively these results may be viewed as a reaction to stressful environmental conditions. Under natural conditions this temperature (20°C) would never be experienced, as this species would simply burrow deeper as the soil surface dried (Gerard 1960). However, this was not possible in a sealed pot. Therefore the worms' large level of cocoon production could be viewed as a possible evolutionary strategy to ensure continued survival by producing cocoons which could resist desiccation and hatch when more suitable conditions returned.

5.3.2c. A comparison of cocoon production between worms given either Dried, crumbled, rewetted Separated Cattle Solids (DSCS) or a synthetic feed.

Introduction.

The choice of DSCS as a feed for reproductively active earthworms was made on the basis of results obtained in earlier experiments (5.3.1). The decision to examine the potential of a synthetic feed for cocoon production was in line with results from growth experiments of *L. terrestris* (section 7.3.2.) running concurrently with later reproductive experiments (section 5.3.2a). It was thought that the synthetic feed with an enhanced nitrogen content, and hence a lower carbon to nitrogen ratio than paper waste alone, could enable enhanced cocoon production.

Materials and Methods.

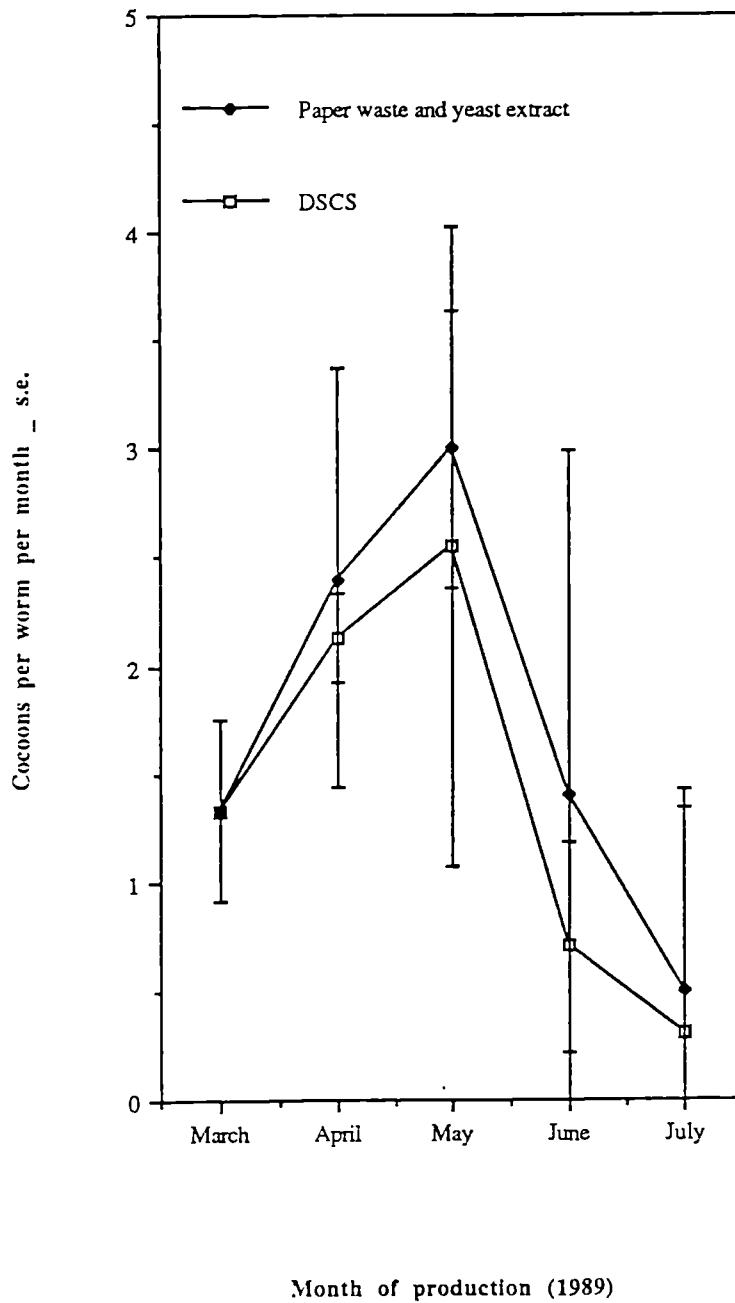
At the beginning of March 1989, three 2.5 litre vessels were set up in the manner of experiment 5.3.2a. They contained sterilised soil but were supplied with a different, synthetically derived feed. This was formulated from paper waste supplemented with yeast extract, in the ratio of 66:1 to give a carbon to nitrogen ratio of approximately 40:1. The reason for using this particular combination is explained fully in section 7.3.2, but basically it had led to enhanced growth of hatchlings.

Five field-collected, mature *L. terrestris* (mean weight 4.9g) were placed in each vessel and these were incubated at a constant 15°C. The vessels were sampled at the start of each calendar month up to the beginning of September 1989. The figures for cocoon production were compared with the production results obtained from worms collected at the same time, and also incubated at 15°C but fed DSCS (section 5.3.2a).

Results.

The results for cocoon production by the worms in these vessels are shown in Figure 5.3.2c.1 and compared with production by worms (from experiment 5.3.2a.) collected at the same time but fed DSCS. Initially cocoon production by worms fed with the synthetic feed was similar to the worms fed DSCS, with a mean figure of 1.33 cocoons per worm per month. However, from one month onwards, cocoon production by worms with the synthetic feed was always greater than that of worms fed DSCS, reaching a peak of three cocoons per worm per month in May.

FIGURE 5.3.2c.1 COCOON PRODUCTION WITH TWO FEEDS AT A CONSTANT 15°C



The absolute figures over the five month period for three vessels of each treatment at 15°C, were 82 and 109 cocoons for DSCS and the synthetic feed respectively.

Discussion.

This small scale experiment showed that this synthetic feed of paper waste and yeast extract was better at promoting cocoon production than DSCS at a constant 15°C. Error bars on figure 5.3.2c.1 indicate that the difference in cocoon production was not significant. However, these results were important and suggest further experimentation would be profitable, particularly as growth figures (chapter 7) suggested that the use of paper waste supplemented with yeast extract as a feed was worth pursuing further.

5.3.3. *The effect of earthworm age on cocoon production.*

Introduction.

With epige species of earthworm it is relatively simple to obtain mature worms of known age through laboratory breeding, as their life cycle is short (Table 2.6.1). However, for anecique species such as *L.terrestris* growth to maturity, following cocoon incubation, takes many months and so most work on reproduction has been concerned with field-collected adults (or sub-adults). Also it has been demonstrated that age following maturation of *E.fetida*, negatively affects reproductive success (Hartenstein *et al* 1979). This experiment aimed to assess the effect of *L.terrestris* age on cocoon production.

Materials and Methods.

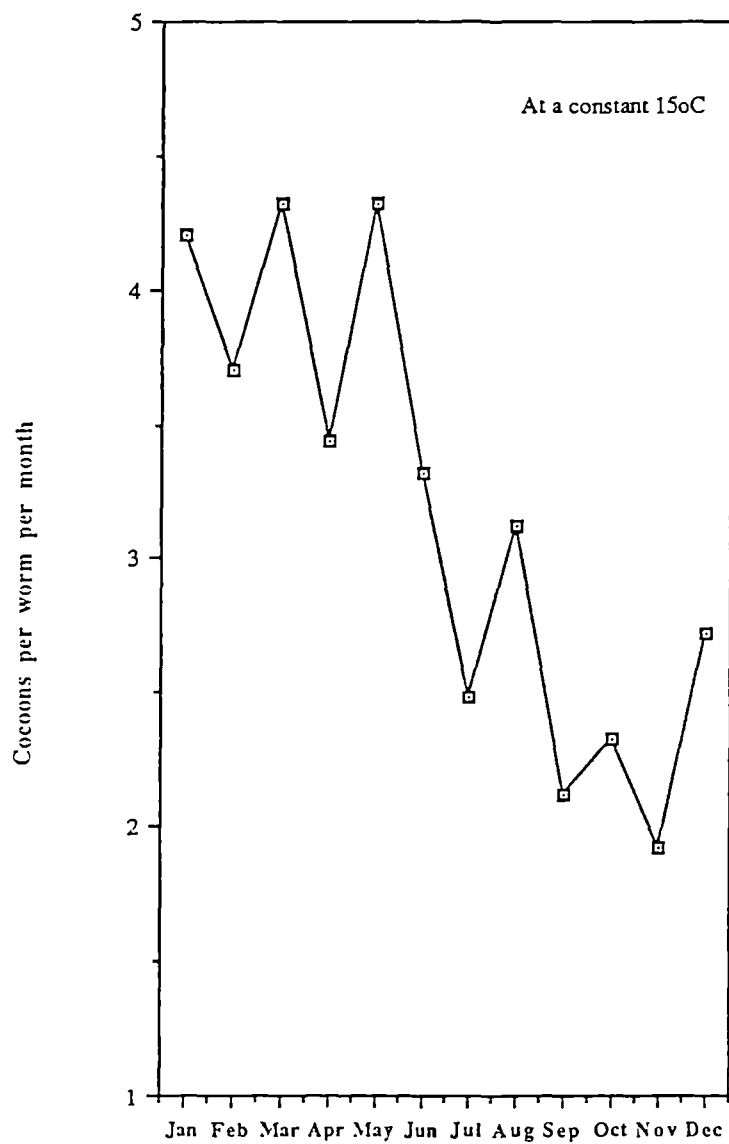
To investigate the effect of age on cocoon production, the first worms hatched and grown to maturity in the laboratory (December 1988) were used in this experiment. Eight worms (mean weight 6.6g) were divided into two groups of four and each group was placed in a 2.5 litre vessel. These vessels were treated exactly as those kept under constant temperature conditions in 5.3.2a. (i. e. they were stored at a constant 15°C, contained sterilised soil and were supplied with Dried, crumbled, rewetted Separated Cattle Solids (DSCS) as feed.) Sampling was monthly with a fortnightly check on soil moisture and feed level. These vessels were monitored over a period of twelve months.

The worms used had been grown to maturity in isolation at 20°C as part of a growth study (section 7.3.2). The move to 15°C was to allow direct comparisons to be made with the worms already involved in the longer term experiment (5.3.2a) and also to meet with the suggested temperature limits (15 - 18°C) for culture of *L.terrestris* outlined in the literature (Lofs-Holmin 1985).

Results.

Cocoon production from recently matured worms was initially very high, at over four cocoons per worm per month but this began to decrease after five months. However, after twelve months it was still greater than two cocoons per worm per month (Figure 5.3.3.1). The mean value obtained for annual production was 36.9 cocoons per worm.

FIGURE 5.3.3.1 COCOON PRODUCTION BY RECENTLY MATURED WORMS WITH DSCS AS FEED



Discussion.

The recently matured worms initially produced over four cocoons per worm per month (during the winter and spring period). This figure is the best sustained cocoon production recorded in these experiments and very similar to the figure given by Evans & Guild (1947) for this species where five worms in a group of three and a pair produced 130 cocoons in seven months, which is equivalent to 3.7 cocoons per worm per month. The months involved in the Evans & Guild study are not given, but the rate was reported to be much slower at the start of the seven month period when only ten cocoons were produced by five worms in the first two months. These worms were also newly matured as they were previously unmated, which therefore lends support to the idea of cocoon production decreasing with age following maturation. It has been demonstrated for another earthworm species (*E.fetida*) that age following maturation, negatively affects reproductive success (Hartenstein *et al* 1979).

The mean value of 36.9 cocoons per worm per year was almost fifty percent greater than the figure of 25.3 cocoons per year from similarly treated, field-collected worms of unknown age (Table 5.3.2a.1). However, as the system under which the larger figure was obtained was continuously disturbed, in order to find cocoons, it is possible that production in an undisturbed system could potentially be greater.

If, as previously suggested, temperature is important in determining reproductive performance then under constant temperatures it might be thought that cocoon production would remain at a constant level. However, a downward trend in reproductive output was seen which could not be linked to a seasonal effect, as the worms were kept in darkness and at constant temperature throughout the experiment and not field-collected and brought into the laboratory at intervals. However, if an internally-controlled biological clock, attuned to the annual cycle is possessed by this species, proximate factors such as temperature or daylength which could act as cues for the setting of it, may have been triggered (as suggested in section 3.4.2a.2). This may have been the result of experiencing a drop in temperature when the worms were taken from their growth conditions of 20°C following maturation, and housed together at 15°C for reproduction, or it may have been due to light breaks in the constant darkness, caused at times of inspection or sampling, as in section 5.3.2a.1. Continued monitoring will reveal if a cyclic pattern of reproduction emerges.

The effect of formalin on the field-collected worms may be insignificant, but could be another factor which reduced their reproductive output in comparison to the laboratory bred worms. Further work could assess this by recording the cocoon production of mature worms, caught by torchlight whilst feeding outside of their burrows (Tomlin 1983), or field-collected sub-adults could be matured in the laboratory.

5.3.4 A closer inspection of individual reproductive potential.

Materials and Methods.

In order to examine the reproductive output of *L. terrestris* in more detail, this separate experiment was designed to look more closely at viable cocoon production and sperm storage ability. It began in May 1988, ran for six months and involved keeping field-collected (mated) worms in isolation or in pairs, with ten replicates of each. The worms were kept in 600ml pots (section 4.1.3) in the previously mentioned internal storeroom (section 4.2.1) with soil and Dried, crumbled, rewetted, Separated Cattle Solids (DSCS) as feed. The pots were examined every two weeks, at which point the soil was sieved for cocoons and the worms were weighed and monitored for any changes in reproductive condition. The cocoons produced were kept individually, in labelled petri dishes and incubated at 20°C. These were then monitored to determine their viability and to measure the time taken for incubation (see section 6.3.1 for details).

Results.

Mature worms that were kept in isolation following capture produced fertile cocoons up to two months later. This indicated that sperm storage had occurred for that length of time. Results from this experiment also provided a maximum production of five cocoons from a single worm over a two week period i. e. 2.5 cocoons per week, however of the five only two proved to be fertile. Results from other individual worms revealed that *L. terrestris* was capable of producing up to two fertile cocoons per week, (four were obtained from a single worm over a two week period).

By recording the dates on which cocoons hatched, and assuming a constant period of incubation for cocoons produced by the same worms, it was possible to derive another measurement for the frequency of cocoon production. This was found to be 1.6 cocoons per worm per week (n = 7).

However, a more realistic figure, obtained from mean values for the ten individual and ten pairs of worms, over the first two weeks of this experiment would lie in the range of 0.55 - 1.25 cocoons per worm per week.

Discussion.

The production of five cocoons (of which only two were viable), in two weeks was the highest rate recorded in any of the experiments performed. This illustrated the potential for increasing cocoon production. By culturing worms in pairs or in isolation, it was much easier to establish if any worms were not producing cocoons, something difficult to confirm when larger numbers of worms were kept together. *L. terrestris* if kept in isolation after maturation does not produce viable cocoons (Evans & Guild 1948; Michon 1954). The results show that cocoon production could be much greater than that recorded in other experiments (5.3.2a.), but the very nature of isolation precluded further mating and therefore gave rise to very unnatural conditions.

Ideal conditions were not available during this experiment and high temperatures in the storeroom did not assist the worms. The loss of reproductive condition mentioned above was also observed during this experiment.

Keeping pairs of worms in small 600ml containers may also have led to conditions of overcrowding. (Even though this was equivalent to about 17g live weight per litre, the depth was only 5cm.) Again this may have given lower than potential figures for cocoon production and may also account for some of the deaths recorded.

5.3.5 The effects of population density on cocoon production.

Introduction.

Most of the experiments performed to this point had been concerned with the effects of feed type, temperature, season and worm age on cocoon production. It was felt that before experiments concerning density could begin experimental optima for most or some of the previous parameters were required. Density was therefore the final aspect of cocoon production examined during this research work.

Materials and Methods.

The population density at which *E.fetida* are maintained has an effect on the number of cocoons that they produce (Hartenstein *et al* 1979). To investigate this effect with *L.terrestris*, two densities were chosen. These were 2.7 and 5.4 mature worms per litre, equivalent to 16 and 32g live weight per litre respectively. These figures were derived from previous experiments reported in this chapter and were either side of the figure of 22g live weight per litre reported to be the carrying capacity for this species by Hartenstein & Amico (1983). Either ten or twenty mature worms were placed in vessels of 3.75 litres. (These vessels were chosen in preference to the 2.5 litre vessels so that a greater population could be kept together.) Five replicates of each treatment were prepared, each contained 3.75 litres of moistened soil, to a depth of 9cm and each was topped with a synthetic feed comprised of paper waste and yeast extract in the proportion 66:1, to give a carbon to nitrogen ratio of approximately 40:1, (as in experiment 5.3.2c). Initially the vessels were supplied with 500g of feed but this was replenished after one month with a similar amount. It was hoped to maintain the vessels at a constant 15°C but due to a lack of facilities they were kept under conditions which fluctuated by up to 5°C either side of this. The worms used here were field-collected during October and the experiment was begun the following month. Because of time constraints this experiment was only sampled once, after a period of two months had elapsed. On sampling the worms were counted, weighed and the soil in which they were present was searched for cocoons by means of wet sieving (section 5.3.2a).

Results.

On sampling it was found that the total number of cocoons produced by the treatments were 144 and 142 for ten and twenty worms per vessel respectively. This corresponded to 1.44 and 0.71 cocoons per worm per month, with a significant difference between these means as revealed by a "t" test ($p < 0.05$). The weight of the worms at the lower population density had increased from a mean figure of 5.95 to 6.71g. At the higher density the increase was less, from 6.19 to 6.35g. None of the worms in the vessels died during the two month period.

Discussion.

The results suggest that cocoon production by *L. terrestris* is inhibited by increased population density. A similar trend has been reported for *E. fetida* by Hartenstein *et al* (1979). The regression equations of these workers showed that cocoon production, at its peak (age 9 - 11 weeks), was over twice as great with eight worms as compared to sixteen worms per 300ml pot. However less of a difference in cocoon production was shown between pots containing either four or eight worms.

The figures for cocoon production obtained during this experiment can be compared directly with those obtained in earlier reproductive experiments as the volume of soil in the vessels here is approximately twice that of the volume of soil used in the smaller 2.5 litre vessels where five mature worms were kept (section 5.3.2a). Given that the temperatures during this experiment most closely resembled those of the higher fluctuating regime of experiment 5.3.2a, cocoon production over the November/December period in the range of 1.0 - 1.5 cocoons per worm per month was very similar at the same population density. These figures may appear low compared with cocoon production from other experiments, but if it is accepted that there is a form of internal control (biological clock mechanism) over reproductive output, then investigations relating to population density ought to be carried out over longer periods of time.

However, reduced cocoon production at the higher population density might be related to less food per individual as feed was applied at the same rate to each of the two density treatments. Evidence from growth figures suggests that the worms at the higher population density were not starved as they did increase in weight, but this increase was less than that recorded for the lower population density.

Clearly this experiment was only the first step in examining the effects of population density on reproduction. Further work with greater control over temperature, a larger experimental period and a larger range of population density treatments is required. Greater emphasis could then be placed on the control over feed rate which could be incorporated as another variable in the experiment. (see section 7.3.4 on population density and growth).

5.4 Conclusions.

Experimental evidence has shown that feed quality has an effect on cocoon production. Initially the best feed for cocoon production was found to be dried, rewetted, separated cattle solids, which was used over a wide range of experiments. However, results for worms collected in March 1989 suggested that a synthetic feed of paper waste supplemented with yeast extract to provide a carbon to nitrogen ratio of 40:1 was equally as good. Further work is required.

Temperature has a marked affect on cocoon production, an increase in temperature initially leads to increased production, but after one to two months at or above 20°C production drops rapidly and may cease altogether. Time of collection from the field can affect production, as the worms will have already been influenced by external temperatures. Cocoon production at a constant temperature of 15°C was found to be 25.3 cocoons per worm per year, while at low and high fluctuating temperatures it was 17.2 and 10.1 cocoons per worm per year respectively.

At a population density of 2.7 mature worms per litre (approximately 16g), which is considered to be within the carrying capacity of such a system, it was found that cocoon production was 1.4 cocoons per worm per month. When the stocking density was doubled to 5.4 mature worms per litre, cocoon production dropped to 0.7 cocoons per worm per month ($p < 0.05$). Further work to determine the optimal density for maximum cocoon production is required.

A seasonal fluctuation in cocoon production was identified, as suggested by previous authors. This fluctuation was detected under all temperature regimes investigated, although the magnitudes differed. There is some debate as to whether an internally controlled biological rhythm is responsible for this. Further work is required under more carefully controlled conditions.

Earthworms of known age, matured in the laboratory, produced 36.9 cocoons per year. This was fifty percent greater than similarly treated field-collected worms. As *L. terrestris* ages cocoon production decreases.

Cocoon production was maintained throughout the whole year.

CHAPTER 6. COCOON DEVELOPMENT AND VIABILITY.

6.1 Introduction

This chapter relates to the second phase in the life cycle of *L. terrestris* as described in section 3.2. It concerns cocoon development, from time of production by the adult worm, until time of hatching.

Soil moisture content and temperature have been shown to be the most important environmental factors that determine successful cocoon development. Workers experimenting with different species of earthworm have found that by increasing the temperature at which cocoons are incubated, a decrease in time of development can be achieved. Gerard (1960) found that at 10°C, the incubation period for cocoons of *A. chlorotica* was 112 days, but this was reduced to 49 days at a temperature of 15°C and further to 36 days at 20°C. Tsukamoto & Watanabe (1977) obtained similar results for *E. fetida* reducing incubation of 86 days at 10°C to only 19 days at 25°C. Working with *A. caliginosa*, Nowak (1975) determined the minimum hatching time in optimum culture conditions to be 1.5 months, which was half the figure for field conditions in Poland. She concluded that those cocoons produced late in the autumn could over-winter, which would bring the incubation time to eight months. The same strategy, adopted by many epige species to survive seasonal desiccation, was recognised by Bouché (1977) and termed "cocoonization". He regarded this as equivalent to encystment in other animal groups.

6.2 Experimental Aims.

- i. To investigate the role of temperature on cocoon development time.
- ii. To determine the viability of cocoons incubated at different temperatures.
- iii. To assess the effect of season of production on cocoon viability.
- iv. To compare the viabilities of laboratory and field produced cocoons.
- v. To assess the influence of parental age on cocoon viability.
- vi. To investigate the effect of desiccation on cocoon viability.

6.3 Experimental Procedures.

6.3.1 The effect of temperature on cocoon development and cocoon viability.

Materials and Methods.

Cocoons were collected at regular periods from soil, in which reproductively active worms had been maintained under laboratory conditions (section 5.3.1). To obtain acceptable data, cocoons needed to be collected as close to the time of production as possible. If the delay was too long, the environmental conditions (particularly temperature) in which the cocoons were produced would begin to influence their development. Various practical considerations meant that a time of 14 days was chosen, as sampling sooner than this had, on previous occasions, led to the discovery of few or no cocoons. On discovery each cocoon was washed in water to remove any soil particles and any loose outer layers, described by Meinhardt (1974), were also removed. The cocoons were dried using tissue paper, weighed (see section 6.4) and placed individually, on a filter paper in a labelled petri dish, which was half filled with distilled water. The petri dishes were then randomly divided into five groups and stored in constant temperature incubators, at either 5, 10, 15, 20 or 25°C. Temperatures greater than this were not tested as the upper lethal limit for *L. terrestris* is given as 27 - 29°C (Section 3.4.1.2). For similar reasons, lower temperatures, were avoided although some cocoons were frozen in order to assess their viability following this procedure. After a period of several weeks these were defrosted and incubated at a range of constant temperatures.

Daily inspections of the cocoons were made to record any signs of hatching or the need for replenishment of water. The 575 cocoons treated individually were kept covered with water and as Roots (1956) described, they were not affected by complete submersion, and hatched successfully. The degree to which cocoons could be dried, rehydrated and still develop successfully was determined separately (section 6.3.6). On hatching the length of the incubation period was recorded, the hatchlings were weighed (see section 6.4) and then used in growth experiments (chapter 7). The total period of cocoon development was determined by adding seven days to the number of days the cocoon was kept at the given constant temperature. This was to account for the collection of cocoons at fourteen day intervals. Any cocoons which had failed to hatch were dissected if the elapsed period of time was greater than twice the mean value then known for that particular temperature.

Results.

Incubation over the range of constant temperatures had a significant effect on cocoon development ($p < 0.001$). The most rapid development was at 20°C, when a mean time of 70 days was required. At 5, 10, 15 and 25°C the mean cocoon incubation periods were 272, 183, 90 and 81 days respectively. The standard error bars on Figure 6.3.1.1 show that there was little variation about these means values. An analysis of variance performed on these figures is presented in Table 6.3.1.1.

All of the cocoons that were frozen and subsequently defrosted, failed to hatch. This happened following incubation at all of the temperatures previously mentioned. Inspection of the cocoon contents revealed that no embryonic development had occurred.

Figure 6.3.1.2 shows the relationship between temperature and the rate of development (development time⁻¹). The equation of this line; $y = 7.5x - 7.5$, had a correlation coefficient (r) = 0.98. Extrapolation of the straight line obtained suggested that development would cease within the cocoon at a temperature of approximately 1°C.

Figure 6.3.1.3 represents cocoon viability at the five constant incubation temperatures. The highest figure for successful hatching (seventy-two percent) was obtained at 10°C. Above this temperature hatching success decreased as temperature increased. At 15, 20 and 25°C, cocoon viability was recorded at 51, 42 and 41 percent respectively. At 5°C the figure was 66 percent.

Figure 6.3.1.4 shows the viability of cocoons obtained from cocoon production experiments during 1988 (section 5.3.1), in terms of the month in which they were produced and regardless of the temperature at which they were incubated. The bar chart drawn suggests that hatching success was greatest for cocoons produced during the early part of the year and fell as the summer months were reached, to climb once more in the autumn.

Discussion.

Meinhardt (1974) reports that cocoon development of *L. terrestris* normally takes 12 - 13 weeks, but this time can vary, mainly in response to soil temperature. In these experiments this period was reduced to ten weeks, at 20°C, a temperature unlikely to be experienced in

FIGURE 6.3.1.1 COCOON INCUBATION PERIOD AT
CONSTANT TEMPERATURES

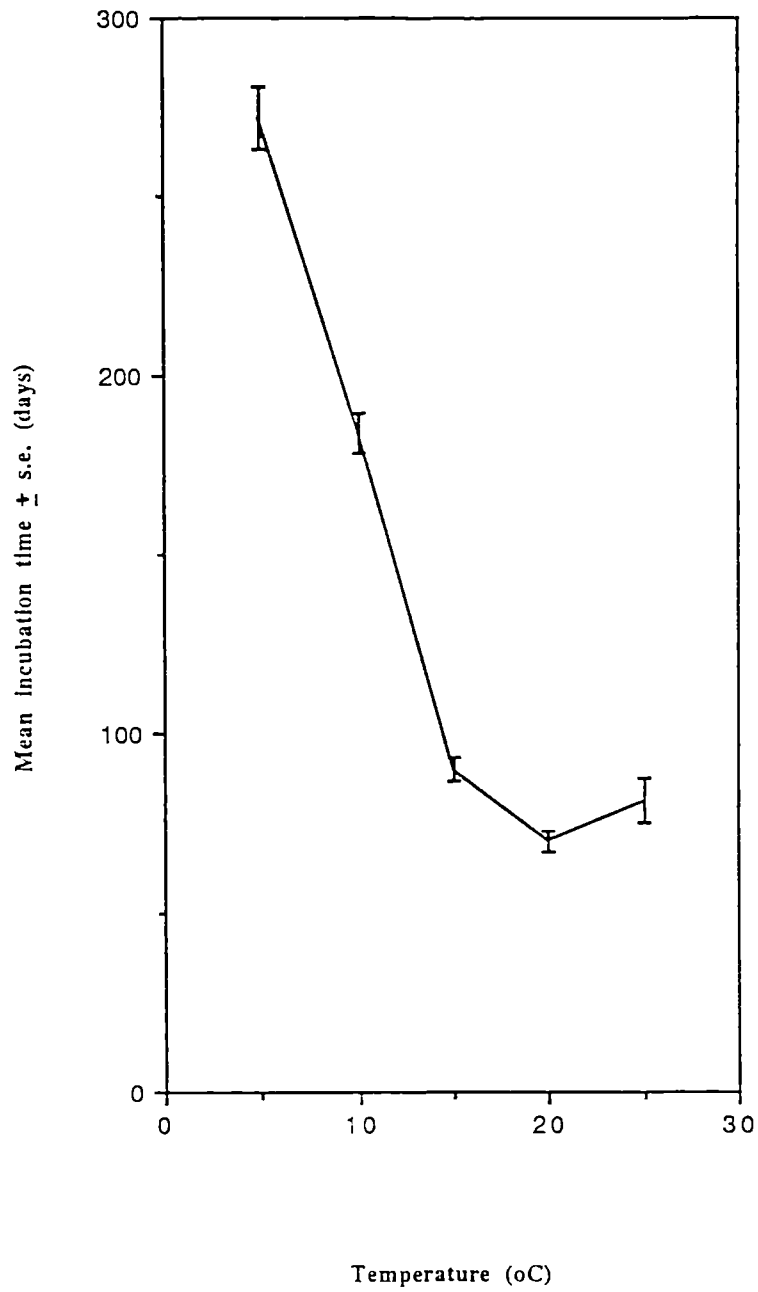


Table 6.3.1.1 The effect of constant temperatures on cocoon developmentMean Values

Temperature (°C)	5	10	15	20	25
Days to hatch	272(a)	183(b)	90(c)	70(d)	81(e)
(n)	10	56	95	66	29

Means followed by the same letter are not significantly different ($p > 0.05$).

Sources of Variation	Degrees of Freedom	Mean Squares	F
Temperature	4	18311.2	66.04 ***
Error	251	277.276	

The constant temperature treatments have a significant effect ($p < 0.001$) on incubation time.

FIGURE 6.3.1.2 COCOON DEVELOPMENT RATE
AND TEMPERATURE

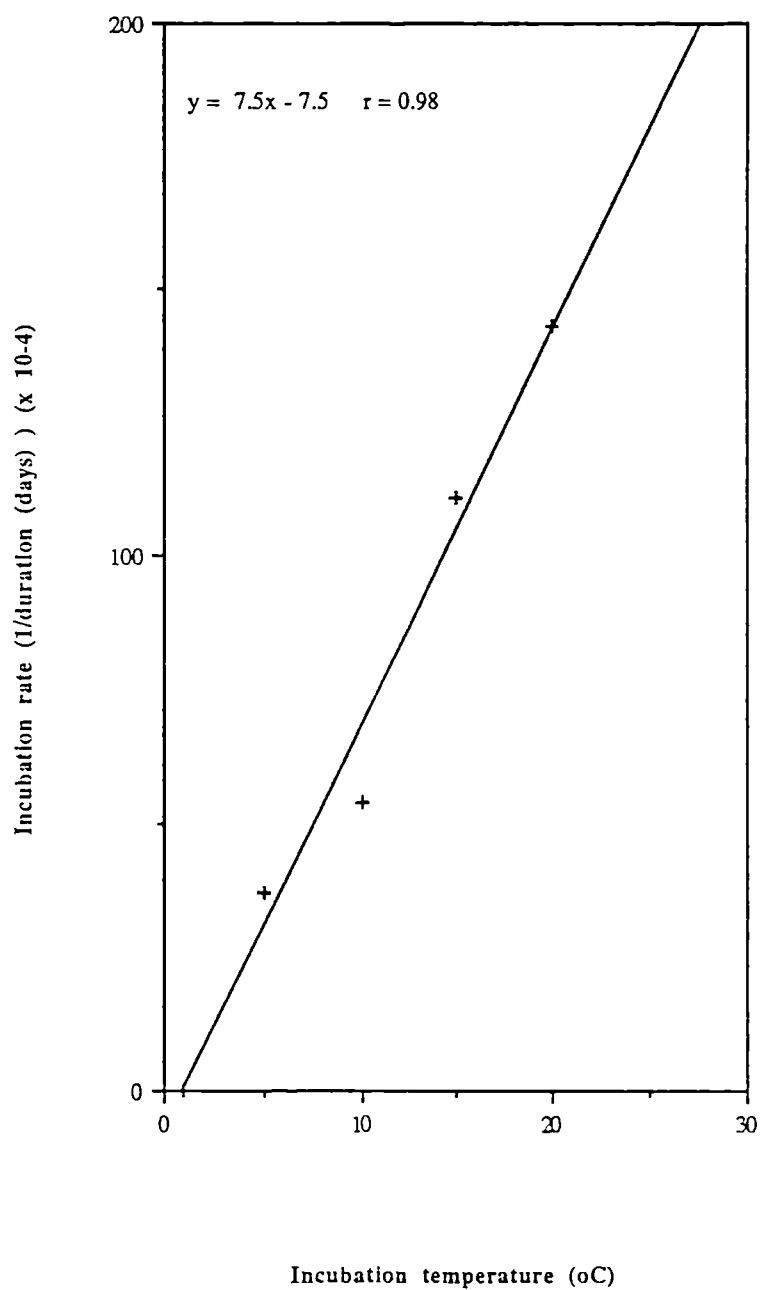


FIGURE 6.3.13 COCOON VIABILITY AT CONSTANT TEMPERATURES

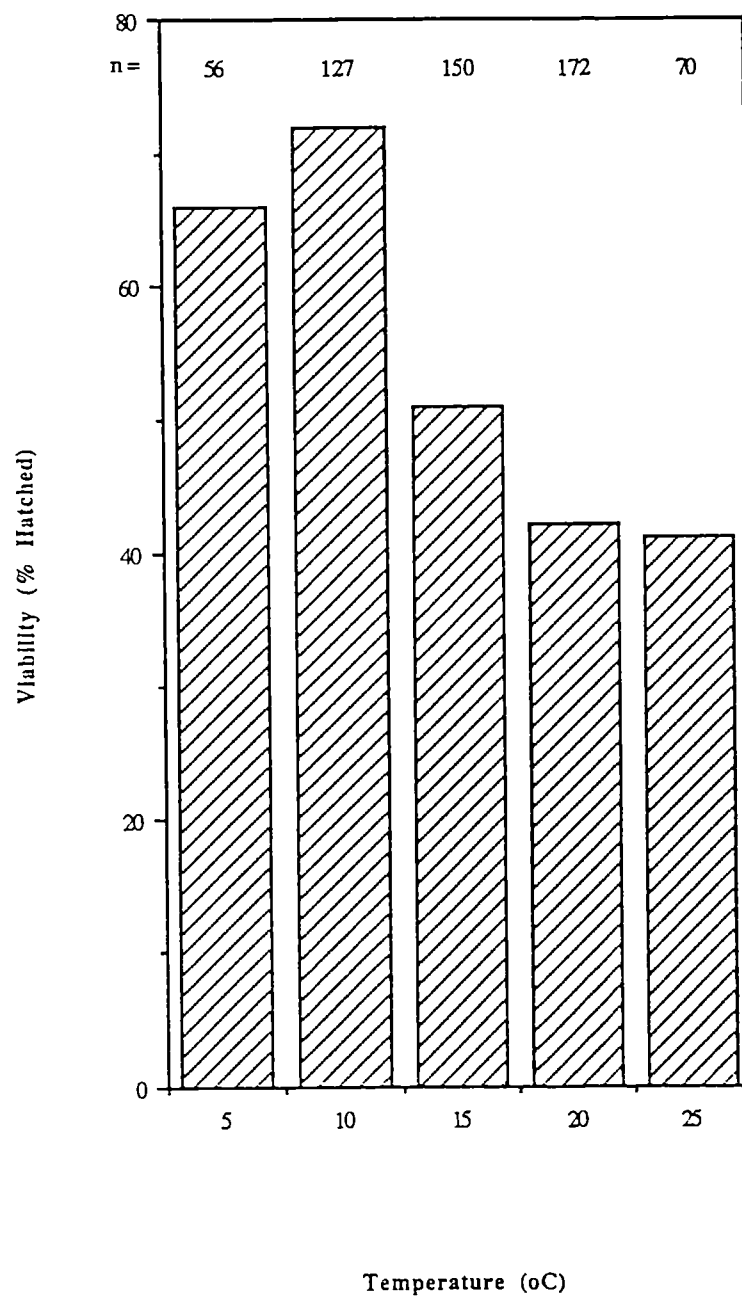
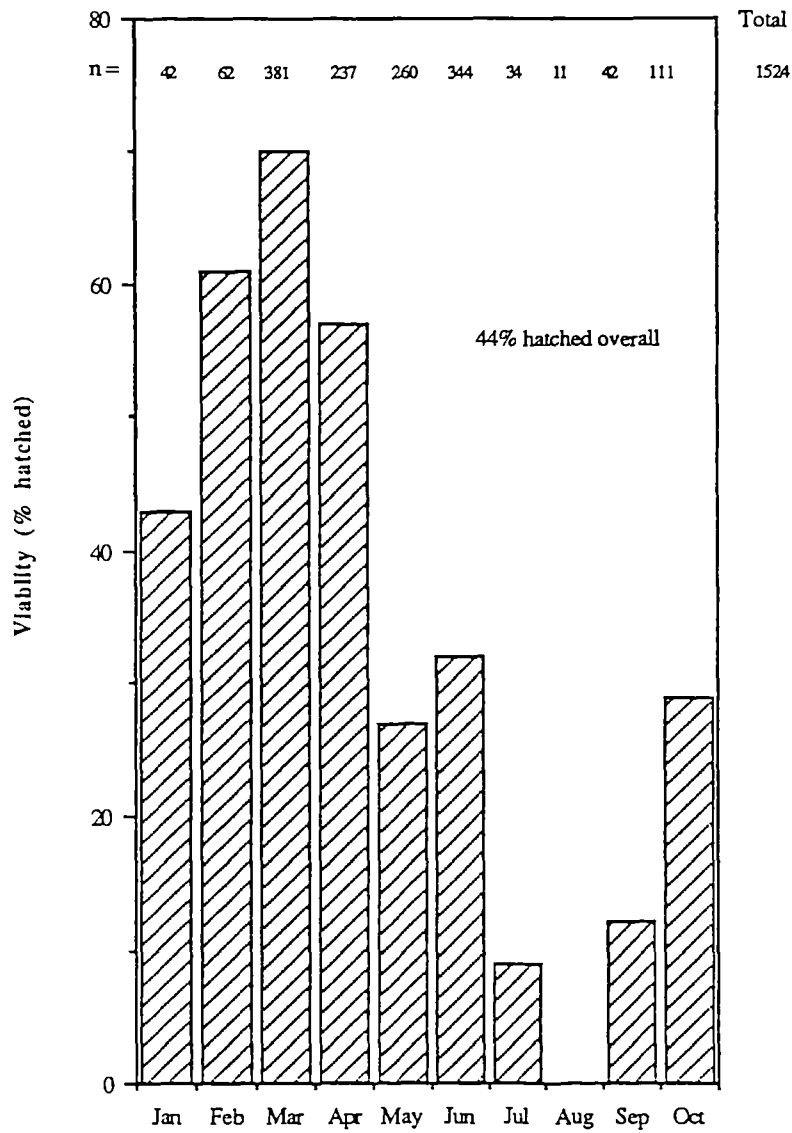


FIGURE 6.3.1.4 SEASONAL VIABILITY OF COCOONS
(INITIAL RESULTS)



Month of production (1988)

the soils of temperate regions (Satchell 1967) where cocoons are usually deposited at a depth of 0 - 20cm (Gerard 1967). A further increase in temperature, to 25°C, led to an increase in mean development time, making a temperature close to 20°C optimal for this species.

Freezing cocoons of *L.terrestris* led to death, most likely from ice crystal formation. Under natural conditions it is unlikely that the soil would freeze to a depth that would affect cocoons.

The inference that cocoons of *L.terrestris* will not develop below a temperature of 1°C is similar to the findings of Tsukamoto & Watanabe (1977) for *E.fetida*. These authors suggest that cocoons of this earthworm do not develop below 6°C. In both species this is almost certainly an evolutionary adaptation for survival in the low temperatures that would be experienced during winter in temperate regions, when emergence of hatchlings would almost certainly be fatal.

The decrease in cocoon viability of *L.terrestris* associated with an increase in temperature was very similar to that reported by Tsukamoto & Watanabe (1977) for *E.fetida*. These authors report a drop in hatchability from 88% at 10°C to 30 - 41% at 25°C.

The seasonal viability of cocoons, represented in figures 6.3.1.4 is derived from worms that had been maintained in an internal storeroom at relatively high temperatures (section 5.3.1). It was interesting to compare this with Figure 5.3.1.1, which represents the production of these same cocoons. A decrease in cocoon production over the May to August period was accompanied by a similar decrease in the viability of the cocoons produced. The seasonal effects on production, higher temperatures, or length of time the worms were in storage had drastically reduced the number of viable cocoons, by as much as 95 percent over the period of May to August. This was further investigated by carrying out a large scale viability assessment of cocoons produced over a twelve month period (section 6.3.3).

6.3.2 Notes on Hatchling number per cocoon

During experiment 6.3.1 an as yet unpublished phenomenon was recorded. Of the cocoons kept in isolated conditions at the five constant temperatures, 326 produced viable offspring. Of these 322 produced a single hatchling, but four cocoons produced multiple offspring, three sets of twins and one of triplets. A mean weight of 25.2mg (n = 6) was recorded for the twins and 15.3mg (n = 3) for the triplets. These were therefore proportionally smaller than single hatchlings which had a mean weight of 53mg (n = 210). Multiple hatchling production accounted for 1.2% of viable production. During later experiments (section 6.3.3) when many cocoons were kept together in the same petri dishes, indirect evidence of further multiple production was discovered. This included more hatchlings present than empty cocoons, small sized hatchlings and in some cases, for example November 1988 production (high fluctuating temperature), more hatchlings produced than cocoons incubated (70 from 53). In one other instance a set of live twins was found on dissecting a cocoon which had failed to hatch but these worms were physically joined at the anterior extremity. These "Siamese" twins were incapable of hatching naturally and if they had not been removed from the cocoon, would have certainly remained unnoticed.

Evans & Guild (1948) reported that one hundred percent of *L. terrestris* cocoons produced only a single hatchling, but the numbers of this species these authors were working with was very small, and only part of a larger study with many earthworm species. The multiple offspring results obtained during this present work, although previously unrecorded, are almost certainly quite natural at around a one percent level, and would only be detected by work, such as this, involving numerous individually treated cocoons.

6.3.3 The effect of seasonal production on Cocoon viability.

Materials and Methods.

To further investigate the seasonal effect on cocoon viability, cocoons were utilised from reproduction experiments (section 5.3.2a), when hundreds of cocoons were obtained on sampling days. These cocoons came from mature worms maintained under three different temperature regimes. Due to limited incubator space and the time involved in individual cocoon processing, groups of cocoons were put into single labelled petri dishes. Records of incubation times were not kept for these cocoons, as there was a period of one month between each collection time. These stocks were monitored in order to provide a measure of seasonal cocoon viability (and also to supply hatchlings for growth experiments). All of the cocoons were incubated at 20°C, as results from 6.3.1 had shown this temperature to induce most rapid hatching. A total of 3285 cocoons were studied in this experiment.

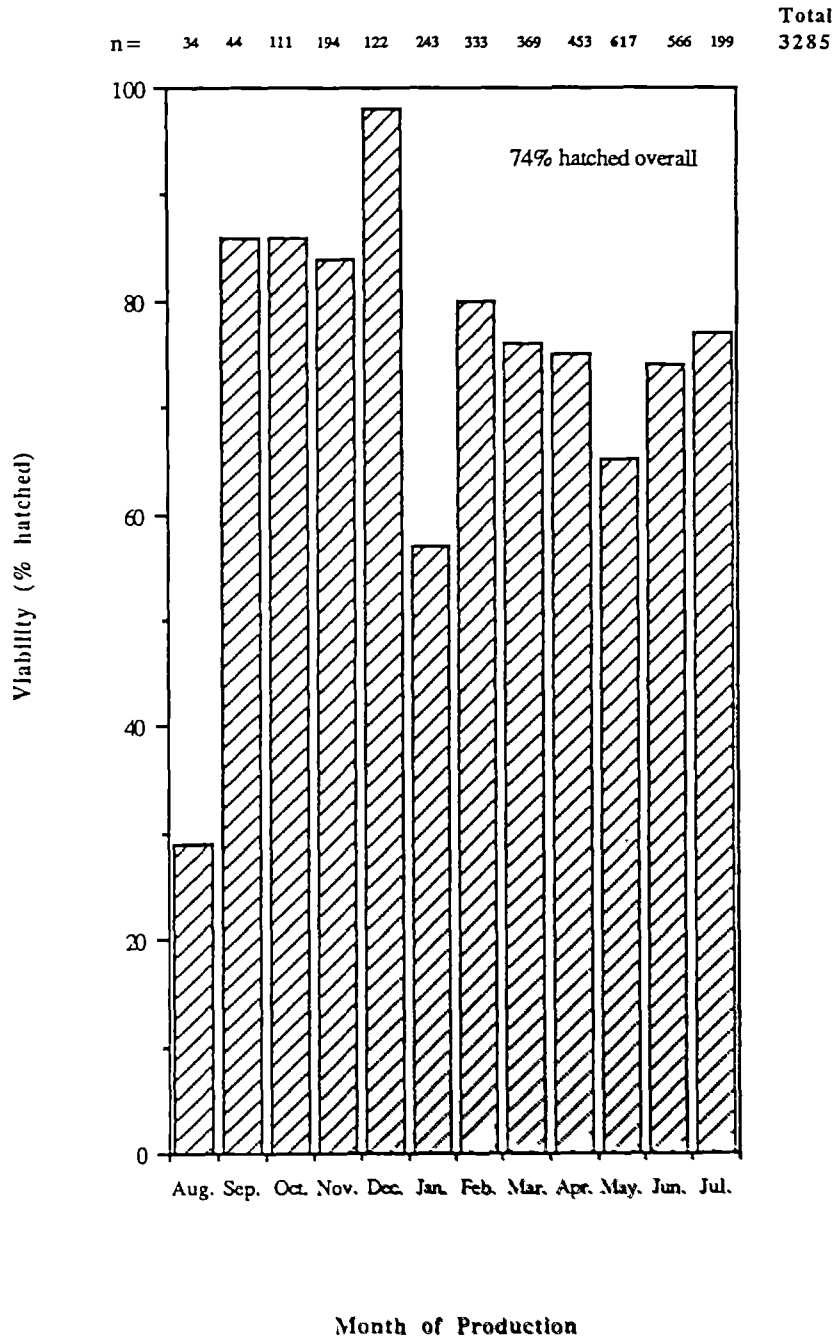
Results.

The viability figures for cocoons produced by field-collected worms from August 1988 to July 1989 are represented in Figure 6.3.3.1. From a total of 3285 cocoons incubated, 2431 successfully hatched to give an annual cocoon viability of seventy-four percent. Only in the months of August and January was this figure below sixty percent. The increasing number incubated each month was a function of seasonal cocoon production and also a growing experimental population of adult worms (section 5.3.2a).

Discussion.

The overall viability of cocoons from field-collected worms, discovered here, (seventy-four percent) was much higher than the forty-four percent found in earlier experiments (Figure 6.3.1.4) and similarly greater compared with those previously recorded for cocoons incubated at 20°C (Figure 6.3.1.3). The viability figures obtained here were considered to have greater validity as they were based on a much larger sample and the worms that produced them were mainly in good reproductive condition. Both cocoon production and cocoon viability in the earlier experiment were thought to have been affected by high temperatures, which may have prevented fertile cocoons from being produced. The very low value of viability recorded for August can be explained because all cocoon producing worms had just been captured, and may have been suffering ill

FIGURE 6.3.3.1 COCOON VIABILITY OVER TWELVE MONTHS (1988/9)



effects from the formalin used to obtain them. The low value for January was caused, in part, by the loss of data relating to cocoons produced under one of the temperature regimes (low fluctuating temperature, outside). An incubator malfunctioned, which froze and therefore killed 51 developing embryos. Over the whole year no consistent pattern of cocoon viability was found.

6.3.4 Viability of cocoons produced in the field.

Materials and Methods.

It was thought that the effect of unnatural environmental conditions (parental capture or laboratory production) might have reduced the viability of cocoons to seventy-four percent, from a higher figure for the field. To assess this, turfs of soil were cut from natural grassland and cocoons were obtained from them. The turfs of 0.25m² and 10 - 15cm deep were obtained every 2 - 3 months during 1989 although the dry soil conditions over the summer months made collection very difficult. In the laboratory, the cocoons were extracted, by the wet sieving method described in section 5.3.2a, and then incubated at the optimal temperature of 20°C determined from previous results.

Results.

The hatching success for the naturally produced cocoons, collected from the field, was seventy-two percent (n = 36 from 50). The month of collection did not affect viability. The low total number of cocoons incubated was due to a low density of cocoons in the area where turfs could be cut.

Discussion.

The production of cocoons under laboratory conditions appeared to have no deleterious effects on hatching success. Field produced cocoons showed a very similar hatching success rate to those produced under artificial conditions in the laboratory and incubated at 20°C (seventy-two and seventy-four percent respectively).

6.3.5 The effect of parental age on cocoon viability

Materials and Methods.

Cocoons obtained from recently matured worms, bred in the laboratory, and therefore of known age (section 5.3.3) were treated in the same manner as cocoons obtained from worms collected from the field. Both sets were incubated at 20°C in batches, in petri dishes, but were kept separate so that their respective viabilities could be compared.

Results.

The viability of the cocoons produced by recently matured worms is shown in Figure 6.3.5.1. From a total of 188 cocoons incubated over a period of twelve months 156 successfully hatched to give an overall viability of eighty-three percent. The figure for any one month was never less than sixty-eight percent. (No figures are given for the months of March and May as the petri dishes containing these cocoons became infected with fungi, which led to total mortality.)

Discussion

The viability of eighty-three percent recorded for cocoons produced by recently matured worms was higher than for field-collected worms (74%) or field-collected cocoons (72%). It could be suggested that the collection technique, i. e. formalin extraction, had reduced the ability of the worms to produce viable cocoons. although this hypothesis was not supported by a similar hatching success for field-collected cocoons and those produced by field-collected worms. These too would have been produced by a variety of worms of different ages. (Lakhani & Satchell (1970) predict the longevity of *L. terrestris* to be between four and eight years.)

This suggested that as worms age, the viability of the cocoons they produce tended to decrease. As the worms from the field were collected randomly, it is likely that they would have represented a range of ages following maturation (possibly up to several years).

It is also possible that the constant temperature of 15°C, at which the recently matured worms were kept, had led to increased viability of cocoons produced. However, by considering separately the viability of cocoons which were also produced at a constant 15°C, by field-collected worms (one of the temperature regimes of experiment 5.3.2a) a

FIGURE 6.3.5.1 VIABILITY OF COCOONS FROM RECENTLY MATURED WORMS

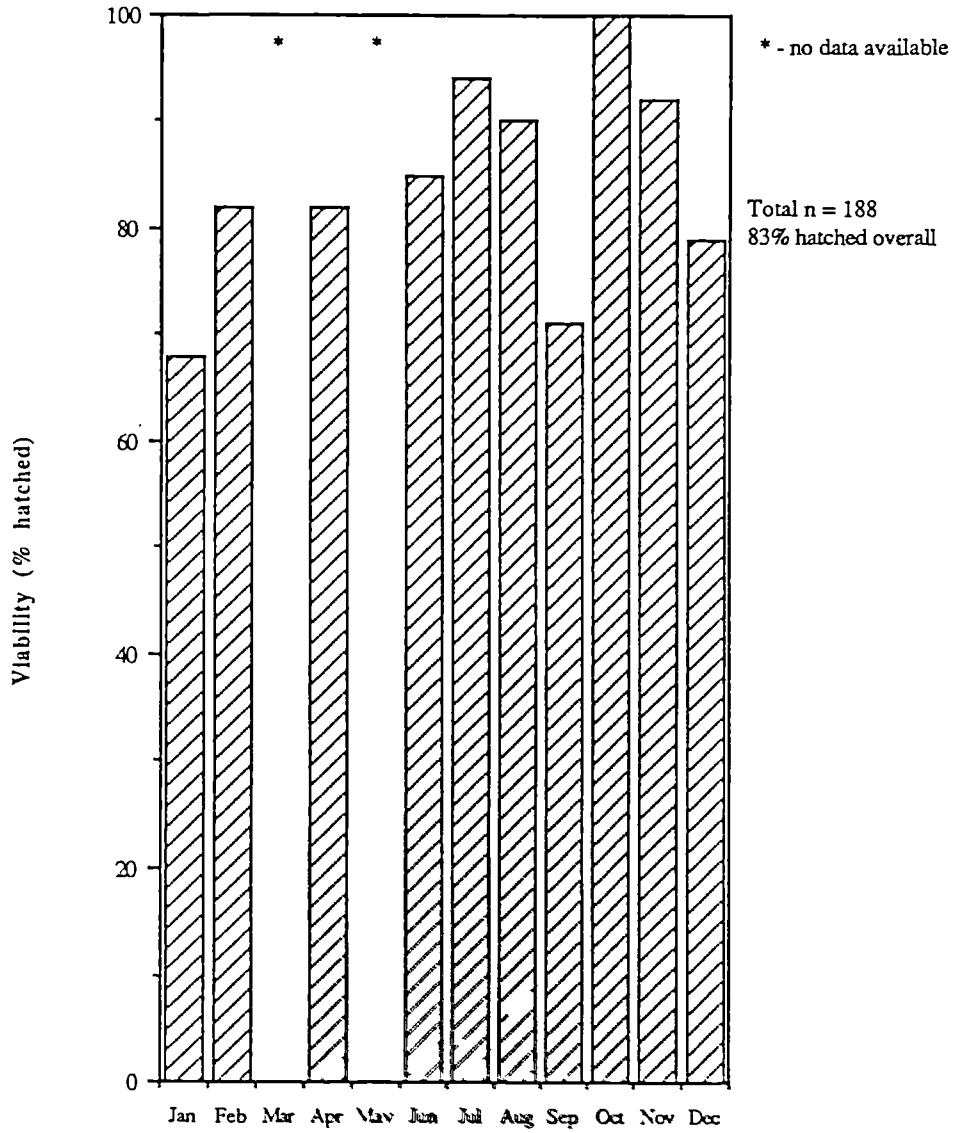


figure of 857 hatchlings from 1148 cocoons gives 74.7%. As this figure is very similar to the overall figure for viability of cocoons from field-collected worms, it does not support the hypothesis that the temperature at which the adult worms are maintained affects cocoon viability.

6.3.6 Desiccation and cocoon viability.

Materials and Methods.

Soil moisture is vital for cocoon development. When it is lacking cocoons will cease to develop. This experiment was designed to assess how much of the water contained within a cocoon could be lost before the embryo inside was killed.

To achieve this, a group of recently produced cocoons were washed free of soil and dried with tissue paper. They were then divided into seven groups of twenty and weighed. These groups were then allowed to dry naturally in the laboratory for varying lengths of time, for a maximum of several hours. The cocoons were then reweighed in their groups, rehydrated and left to incubate at 20°C on filter paper, in labelled petri dishes. One group was oven dried overnight at 105°C to assess the total water content.

Results.

The results from oven drying revealed that cocoons have a water content of seventy-five percent. Using this figure it was possible to calculate the percentage of total water removed from each of the experimental groups. These were 27, 40, 44, 47, 54 and 67 percent. The viability of cocoons in each of these groups was found to be 85, 75, 90, 40, and 30 percent respectively.

Discussion

Desiccation of cocoons, even for a short period of time did appear to reduce hatching success. The greater the amount of desiccation, the lower the success rate. However, even after a loss of two thirds of the water within a cocoon, successful development was still possible in some cases following rehydration. Further work with larger numbers of cocoons and at different levels of desiccation would be required to validate the results obtained here.

6.4 General Observations on the cocoons/hatchlings of L.terrestris.

The live weights of 829 cocoons were recorded during this work and they are represented in figure 6.4.1, subdivided by 5mg intervals. Records ranged from 26 to 93 mg. The cocoon weights were found to be normally distributed about a mean of 61mg with a standard deviation of 11.4.

The cocoon weight distribution illustrated in Figure 6.4.1 was thought to be quite natural, as the size of cocoons produced by earthworms is closely related to adult size. This relationship has been demonstrated by Lavelle (1981) for twenty-six species of both tropical and temperate earthworms. The physical process of cocoon production, by the secretion of a cylinder of albuminous material around the parental clitellum, determines that this must be so.

Figure 6.4.2 represents the relationship between cocoon weight and hatchling weight, based on 130 observations, a significant linear relationship was obtained ($r = 0.92$). Although a greater number of cocoons were weighed, time constraints and the storage of many cocoons together in later work, prevented the weighing of as many hatchlings. Cocoon size was found to be a good indication of the expected hatchling size, given that multiple hatchlings did not develop.

FIGURE 6.4.1 L.TERRESTRIS COCOON WEIGHT DISTRIBUTION

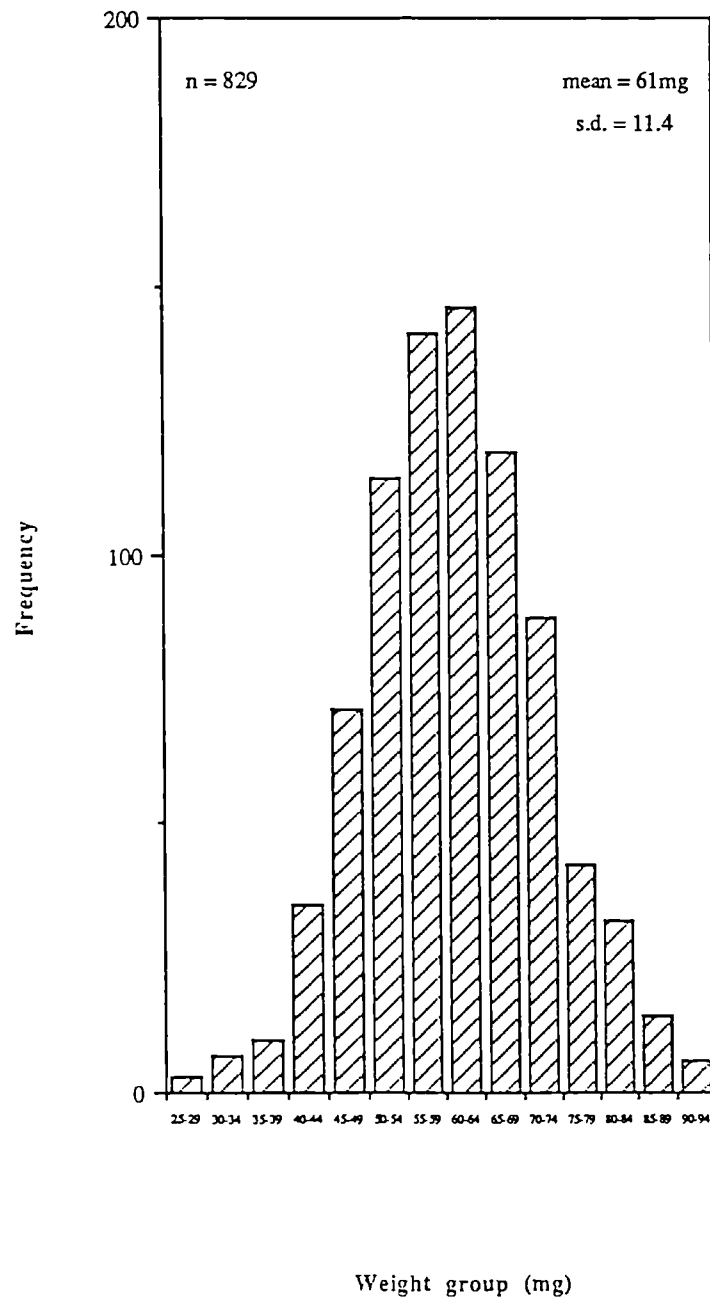
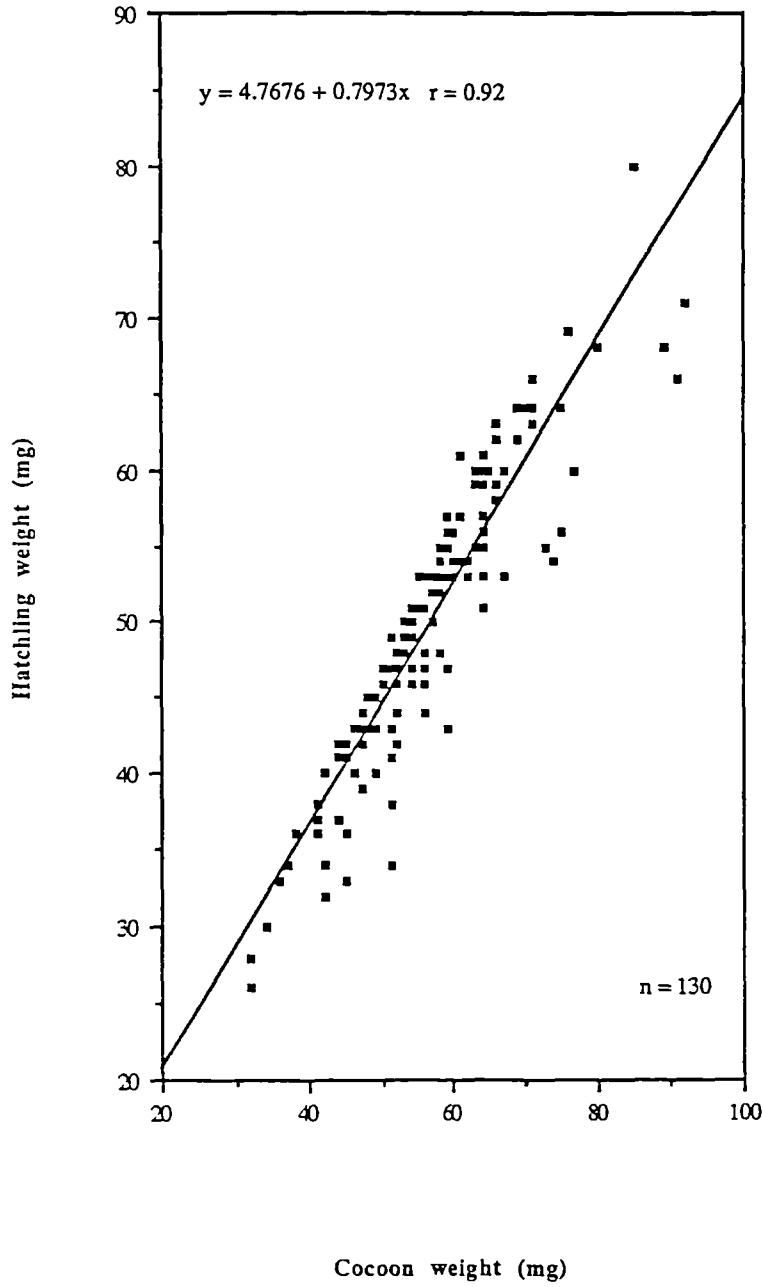


FIGURE 6.4.2 COCOON AND HATCHLING WEIGHT RELATIONSHIP



6.5 Conclusions.

Cocoon incubation was found to be most rapid at a temperature of 20°C (70 days + 2.9). No deleterious effects, in terms of hatching success, were noted at this temperature compared to lower temperatures during later experiments. Cocoon development was calculated to cease at temperatures less than 1°C.

There appeared to be no difference in levels of viability between cocoons produced throughout the year.

Successful development of cocoons and hatching from them was not impaired by the handling techniques employed. Experimental and "natural" hatching success were found to be approximately equal, with seventy-four percent viability.

Cocoons from recently matured adults showed greater viability with an eighty-three percent hatching success.

Freezing cocoons rendered them unviable and high levels of desiccation (> 50%) led to a pronounced decrease in viability.

Cocoon weight was found to be closely related to both adult size and hatchling weight.

CHAPTER 7. GROWTH TO MATURITY.

7.1 Introduction

This chapter covers the phase in the life cycle of *L. terrestris*, (described in section 3.2) from hatchling emergence to the attainment of sexual maturity. This condition is reached when an earthworm is able to mate and produce viable cocoons. i. e. it must be fully clitellate. For *L. terrestris* it is suggested that a minimum weight of 3g is required before the clitellum will develop (Lofs-Holmin 1983), but this condition does not appear to be triggered simply by weight attainment. Hubl (1953) showed that the annual reproductive cycle of *L. terrestris* seemed to be controlled by neurosecretions. Immature worms lacked certain secretory cells in the cerebral ganglia, and if these ganglia were removed from mature worms, the secondary sexual characteristics disappeared and cocoon production ceased.

The natural loss of reproductive condition may indicate the onset of senescence, as Michon (1954) demonstrated for *Dendrobaena subrubicunda*. Gerard (1960) reports that for *A. chlorotica*, *A. caliginosa* and *A. rosea* the clitellate condition may be lost resulting in a "regressed" condition, which resembles quite closely the pre-reproductive condition. This loss may be due to sub-optimal conditions, which could in part, account for seasonal cocoon production. It is thought that the clitellate condition, if lost, may be regained.

7.1.1 The Scope of Earthworm Growth Studies.

The growth of earthworms may be studied in a number of ways. Natural populations can be monitored, worms can be enclosed under semi-natural conditions or they can be maintained in very unnatural, but highly controllable situations. The literature contains examples of all three types of work, and many papers constitute combinations of all three methods.

Population studies under natural conditions, while ideal for obtaining a true indication of certain parameters, such as peak time of hatchling emergence, may not be so helpful for measuring growth rates. Most problems are due to the often unpredictable nature of field work caused by any number of natural agents, but the major difficulty with growth studies is recognition of experimental subjects. Meinhardt (1976) used water soluble food dyes to stain worms after capture for future recognition as part of a mark-recapture study. This

method has been used with considerable success by Mazaud & Bouché (1980) to study earthworm dispersal and mortality rates in french pasture soils.

However, to overcome the loss of worms through migration, predation or some other removal, the use of enclosed, semi-natural conditions is advisable and greatly facilitates the likelihood of recovery. Muslin and nylon bags filled with earth taken from the study site have been used extensively (e. g. Edwards & Heath 1963; Satchell 1963). These may be examined and reburied at the experimental site as often as required. Given adequate feed of the required type, such enclosures can provide valuable information on growth under "natural" conditions without the inherent problems associated with completely natural studies. If however, a measure of optimal, rather than natural growth is required, then the ideal situation is to create carefully controlled conditions for the experimental earthworms. Lofs-Holmin (1983) used such conditions for rearing nine species of soil dwelling earthworms. These were examined for their growth and reproductive potential. The most easily cultured species were to be used experimentally in biological monitoring studies, for testing the toxicity of agricultural chemicals, particularly pesticides. Hartenstein & Amico (1983) also examined optimal conditions for the production of *L. terrestris* and concentrated solely on this species.

The life histories of only a few earthworm species have been studied in any detail. Those of *E. fetida* (e. g. Graff 1974; Watanabe & Tsukamoto 1976) and *Eudrilus eugeniae* (Neuhauser *et al* 1979) are well documented due to laboratory based work aimed at assessing the potential of both species for the management of organic wastes and simultaneous production of useful by-products (section 2.5). Soil dwelling earthworms have seldom been studied completely. Exceptions include *L. terrestris* (Lakhani & Satchell 1970; Lofs-Holmin 1983; Hartenstein & Amico 1983), *A. rosea* (Phillipson & Bolton 1977), and *A. caliginosa* (Nowak 1975; Mazantseva 1982; Boström & Lofs-Holmin 1986). All include useful growth data.

Lakhani & Satchell (1970) constructed growth curves for two populations of *L. terrestris* found under mixed woodland. This they achieved indirectly by the field collection of specimens treated with formalin, over a period in excess of two years. They recorded rapid growth, with short pauses in mid-summer and mid-winter during a worm's first three years. The mean live weight attained after this time was approximately 9.5g, which changed little for the next four years. Few worms survived to seven years. Juvenile

mortality rates were high. The model of Lakhani & Satchell predicted, 20% mortality prior to 120 days, at a weight of 0.3g; 40% prior to 400 days at 1.75g; and 50 - 60% after 600 - 800 days. Laboratory based work by Meinhardt (1974) suggested that juvenile development lasted 130 days with growth complete after a minimum of 200 days, however these figures are not linked to weights so cannot be clearly compared. The data of Lofs-Holmin (1983) for *L. terrestris* suggested the appearance of the clitellum at a weight of 3g after 100 days. These worms were fed farmyard manure (FYM) mixed with soil and kept at a temperature of 15°C.

Phillipson & Bolton (1977) monitored the growth of the geophagous earthworm *A. rosea* by sampling monthly 2 litre pots, filled with soil from the site of earthworm capture, and buried in soil. Escape was prevented by a covering of gauze. From these data they constructed a growth curve for an "average" individual based on bimonthly grouping of arbitrary weight classes. Maximum growth per worm per unit time was in the grouping ranging from 100 - 180mg live weight, corresponding to the age range 1.5 - 2.3 years. The generalised growth curve formed was imperfectly sigmoid up to the four year stage with a final live weight of approximately 260mg. After this point adults showed a negative mean growth rate which suggested that there were a greater proportion of senescent, than actively growing individuals, in this age class.

Nowak (1975) studied the growth of *A. caliginosa* in field culture. After twenty months, worms had reached a mean live weight of 325mg from a mean weight at emergence of 27mg. She distinguished a rapid pre-productive phase of growth to a mean weight of 210 - 260mg, reached after thirteen months, followed by a phase of steadily decreasing growth after attainment of sexual maturity. A third post-productive phase distinguished by Michon (1957) led to a decline in weight as senescence was reached. From laboratory studies of the same species, Boström & Lofs-Holmin (1986) obtained growth to 450mg from hatchlings in 125 days. The worms were grown in isolation and provided with soil and a mix of ground (< 0.2mm) barley roots/straw. Other plant residues proved less successful at promoting growth as did barley residues of larger particle size.

Murchie (1960) during a laboratory based study, provided the North American earthworm, *Bimastos zeteki*, with a series of different organic substances which it would naturally encounter. These included, leaf litters, soil layers and rotting wood material. He discovered significant differences ($p < 0.01$) between weight increase on different

substrates (feed) types, which was attributed to the nutritional quality of the substrate. Greatest weight increases were obtained from worms fed with organic rich, loamy sand.

Lofs-Holmin (1983) cultured *Octolasion cyaneum* at temperatures of 5, 15 and 20°C. She found little difference in the growth rates at the two higher temperatures, where the clitellate condition was attained after 100 days, but at 5°C, growth was much slower and the worms took 200 days to reach maturity. Tsukamoto & Watanabe (1977) kept *E. fetida* under temperature controlled, laboratory conditions in compost- filled petri dishes. The rate of growth was found to be greatly influenced by temperature. After ten weeks, worms which weighed 5mg on hatching, had attained weights of, 27mg at 10°C, 100mg at 15°C, 190mg at 20°C and 650mg at 25°C. These results clearly demonstrate the effect of an increased temperature on the growth of certain earthworms. The point to which a temperature increase may be beneficial, in terms of increased growth rate, must depend on the nature of the species under scrutiny. Loehr *et al* (1984) demonstrated that a temperature of 30°C was detrimental to the growth of *E. fetida* after 14 days, up to which point growth had been equivalent to that at 20 - 25°C. After 70 days, the final weight achieved at a temperature of 30°C was little better than that at 10°C.

As suggested by these results for a variety of earthworm species and outlined in section 3.4, poor quality food and sub-optimal temperatures are known to limit the potential of earthworm growth. In a delimited artificial environment, with low levels of mortality, population density also becomes important as growth continues and the carrying capacity of the system is reached (Neuhauser *et al* 1979). The aims of the experimental work covered in this chapter were primarily to investigate the effects of different temperatures and feeds on the growth of *L. terrestris*. The effect of a range of stocking densities on earthworm growth was then studied and an estimate of the carrying capacity of the growth system was obtained.

7.2 Experimental Aims.

- i. To identify the optimum temperature for maximum growth.
- ii. To identify feed characteristics that maximise growth and to create a synthetic feed to promote enhanced growth.
- iii. To identify the maximum population density (carrying capacity) that can be supported under optimal experimental conditions.
- iv. To investigate the effects of stressful conditions on the rate of maturation.

7.3 Experimental Procedures.

Unless otherwise stated, all worms used in these experiments were produced from cocoons of field-collected mature worms and hatched in the laboratory (chapter 6). Upon hatching the worms were about 25mm in length (Sims & Gerard 1985) with a mean weight of 53mg ($n = 210$). Each worm was treated according to specific experimental procedures described below. Initially most results refer to hatchlings grown in soil supplied with paper waste as a feed, in 300ml plastic containers (section 4.1.3). Later work involved the addition of substances to the paper waste in order to try to enhance growth rates. No attempt was made to vary the particle size of the paper waste as previous experiments had showed that grinding it, when dry, was very difficult (section 5.3.1).

7.3.1 The effect of different temperatures on the Growth and survival of hatchlings.

Materials and Methods.

Initially all hatchlings used in this experiment were obtained from cocoons which had developed at between 15 and 20°C. Upon hatching worms were weighed and then put individually into 300ml pots containing 200g of moist sterilised soil and 50g of paper waste as feed. The latter was chosen for its benign character and the promising growth results from earlier work (section 4.2.1). These pots were then assigned, on a rotational basis, to one of five constant temperatures, chosen at five degree intervals, in the range 5 - 25°C. The reasons for the choice of these temperatures are given in section 3.4.1.2.

At first the worms were weighed after every two weeks, to obtain the greatest amount of data, but on inspection of the results, this was later reduced to monthly sampling. Greater growth rates appeared to occur with less disturbance. Soil and feed were replaced with fresh every four weeks. These experiments were run for a maximum of thirty-six weeks, by which point growth patterns were quite clear.

On completion of an experiment to examine the effect of temperature on cocoon incubation duration (6.3.1), the hatchlings obtained were treated as above but a record of their incubation temperature was also noted. This allowed a comparison to be made between growth rates at a range of constant temperatures, following incubation at a similar range of constant temperatures. i. e. It permitted an investigation into the effect of incubation temperature on subsequent growth. This was thought to be important with intensive production as an ultimate aim, as incubation of cocoons and growth of hatchlings in a production system would, almost certainly, need to take place under similar temperature conditions.

Worms incubated at 10, 15, or 20°C, were allowed to grow at 10, 15, or 20°C, with all combinations examined. Growth at a higher temperature following incubation at 5°C was not examined as this incubation period was very long (approximately nine months). Nor was growth at lower temperatures examined, for cocoons incubated at 25°C, due to the low viability of cocoons at that temperature.

Results.

Figure 7.3.1.1 shows the influence of temperature on the growth of hatchling in soil and given paper waste only as feed. Higher temperatures, in the range 5 to 20°C, led to an increased growth rate. At the highest temperature of 25°C, initial growth was similar to that at 20°C, but after only two to three weeks, high mortality of worms, and weight loss prior to death, was recorded. In Table 7.3.1.1 an analysis of variance compares the weights gained by hatchlings after 20 weeks. The earthworm weights at the four constant temperatures, 5, 10, 15 and 20°C were found to significantly different ($p < 0.001$).

The survival of hatchlings at the various constant temperatures was considered to be of equal importance to recorded growth rates (Figure 7.3.1.2). Only at 25°C was survivorship poor (only eleven percent after 20 weeks). A higher temperature within the range of 5 - 20°C led to only a small decrease in survivorship, a result which was favourable for an intensive production system. At 20°C both rapid growth and a high level of survivorship were recorded. At 20°C survival after 20 weeks was only just below ninety percent.

Figure 7.3.1.3 shows growth curves of hatchlings fed paper waste and grown at one of three temperatures (10, 15 or 20°C), after incubation at either 15 or 20°C. The growth curves described are very similar to those for hatchlings where there was less control over incubation temperature (Figure 7.3.1.1). Hatchlings therefore appeared to show temperature related growth regardless of the temperature at which they had developed as cocoons. Results for worms grown at the same three temperatures after incubation at 10°C produced a very similar set of growth curves, but have not been included in order to add clarity to the figure.

Discussion.

At a temperature of 20°C the highest growth rates for *L.terrestris*, fed on paper waste, were 20 - 30 mg.g⁻¹day⁻¹. Hartenstein & Amico (1983) gave growth rates of 60mg.g⁻¹day⁻¹ for eight worms (31g), in a hemispherical bowl of 1400cm³ with a surface area of 91cm², filled with soil amended with sewage sludge. These figures are much greater than any given for natural populations of *L.terrestris*. Lakhani & Satchell (1970) report rates of 10mg.g⁻¹day⁻¹ in mineral soil under mixed woodland while Curry (1988) gives 6.6mg.g⁻¹day⁻¹ for growth in reclaimed peat grassland. However, comparisons of growth rates of worms from different experiments must be made with caution. Growth rates are not

FIGURE 7.3.1.1 HATCHLING GROWTH AT CONSTANT TEMPERATURES WITH PAPER WASTE AND SOIL

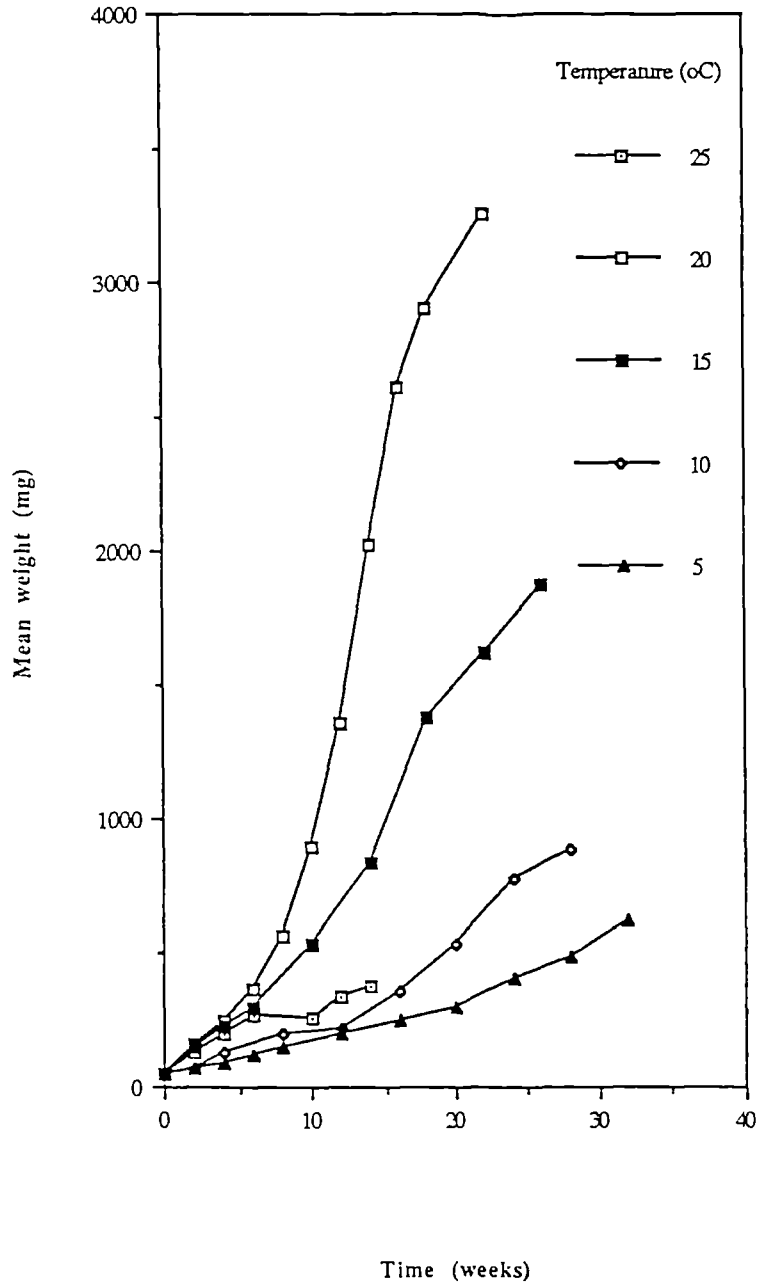


Table 7.3.1.1 The effect of constant temperatures on hatchling growth with paper waste and soil.

Mean Values

Temperature (°C)	5	10	15	20
Weight after 20 weeks (mg)	293 (a)	534 (a)	1619 (b)	3253 (c)
(n)	8	8	4	4

Means followed by the same letter are not significantly different ($p > 0.05$).

Sources of Variation	Degrees of Freedom	Mean Squares	F
Temperature	3	9131923.0	63.38 ***
Error	20	144086.5	

The constant temperature treatments have a significant effect ($p < 0.001$) on size attained by hatchlings after 20 weeks.

FIGURE 7.3.1.2 HATCHLING SURVIVAL WITH PAPER WASTE
AT DIFFERENT TEMPERATURES

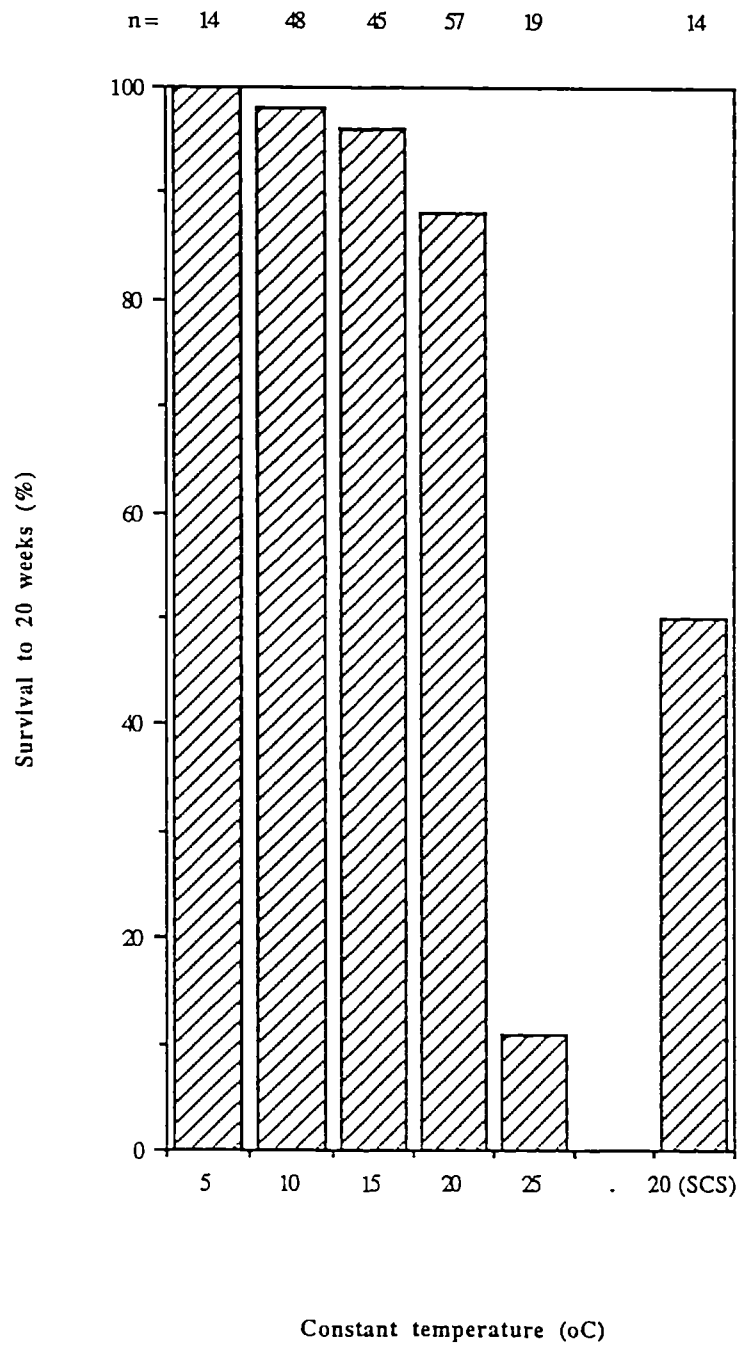
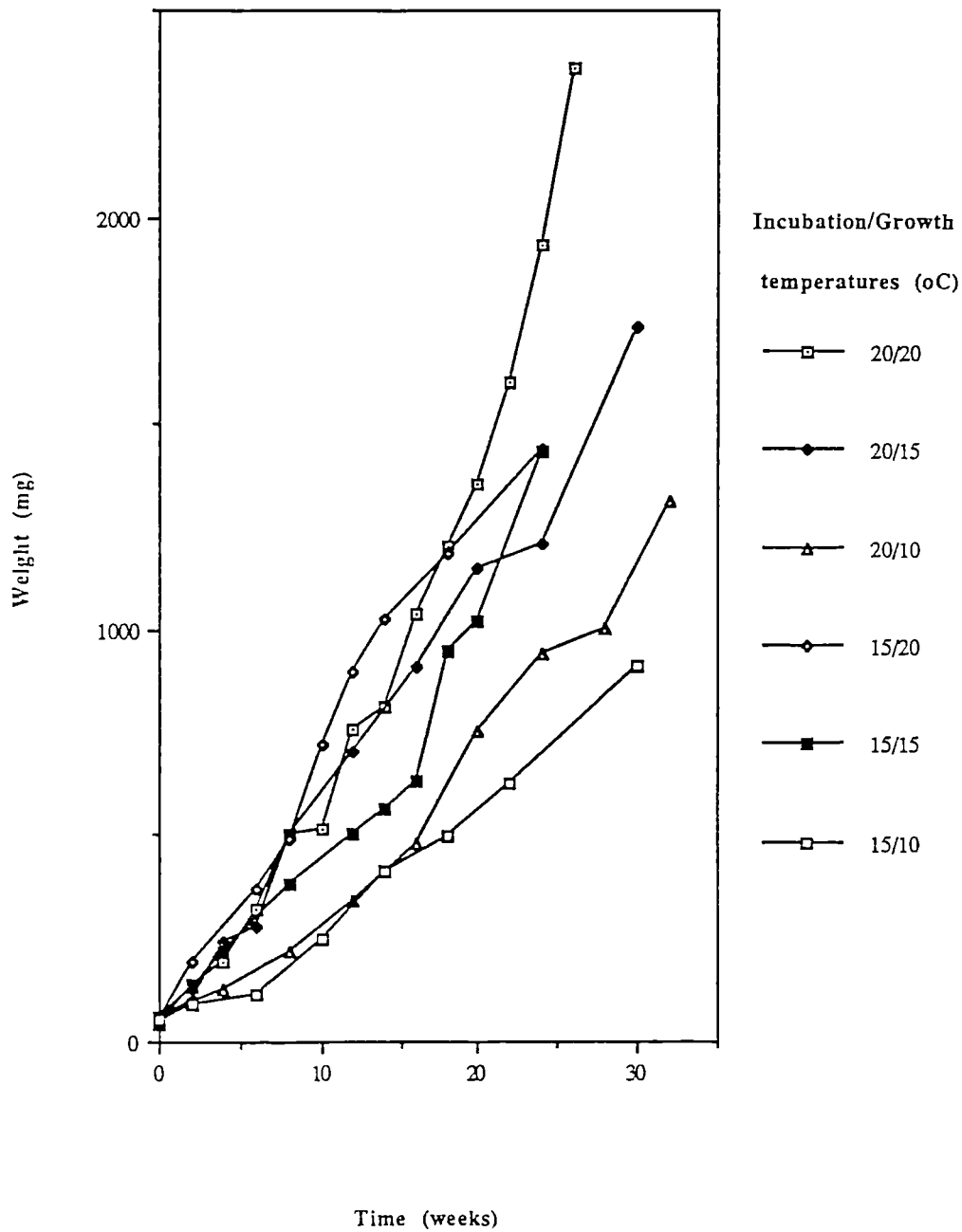


FIGURE 7.3.1.3 TEMPERATURE COMBINATIONS FOR INCUBATION AND GROWTH WITH PAPER WASTE AS FEED



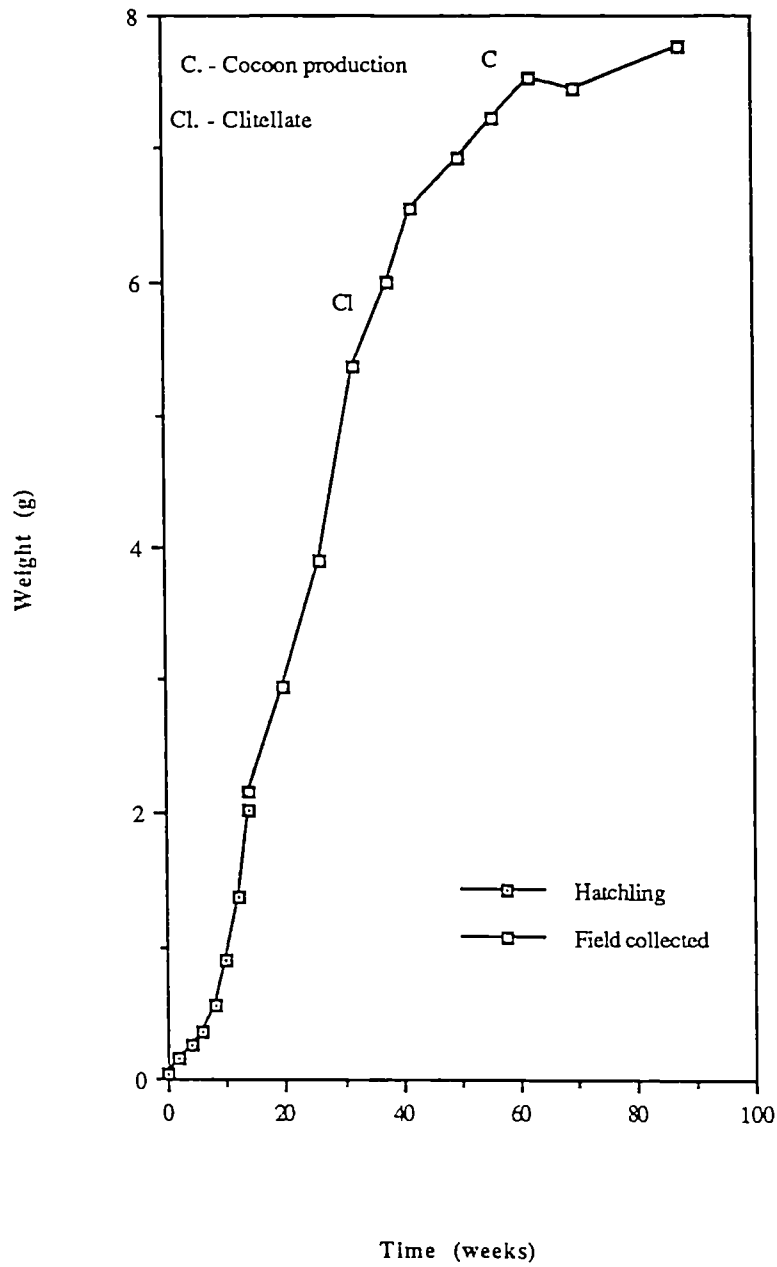
constant throughout life even under ideal conditions, (e. g. see Figure 7.3.1.1). Therefore the point at which the growth increase is examined is very important. Also the amount of time that has elapsed between successive sampling of the same worms can influence the relative figures obtained.

The implications of results from combinations of different incubation temperature and growth temperature were quite straightforward. As subsequent growth was not influenced by the temperature of incubation, a system for promoting maximum production of *L. terrestris* could be focused on growth rather than incubation of cocoons. This was especially so since the temperatures for both incubation and growth appeared optimal at around 20°C. No doubt this is an evolutionary adaptation on the part of the worm in order to be able to withstand adverse conditions within the cocoon stage, and then grow as rapidly as possible given the limits set by environmental temperature.

Increased survival would be expected for laboratory reared worms compared to naturally existing populations, due to removal of environmental pressures liable to lead to mortality. The figure of 20 percent mortality after 120 days, predicted by the model of Lakhani & Satchell (1970) for natural populations, was not reached during this work, under controlled conditions. Mortality over a similar period for hatchlings fed paper waste at temperatures of 20°C and below was reduced to between zero and twelve percent. At 20°C mortality was around ten percent, higher than at 15°C, even though growth rates were greater at the higher temperature. The benefits and costs of this are investigated further in section 7.3.3.

Using the growth data for hatchlings at 20°C and the data from earlier experiments with larger worms (section 4.3.1) a composite growth curve for *L. terrestris* fed on paper waste was constructed (Figure 7.3.1.4). From this an estimated thirty to forty weeks from hatching would be required for the clitellate condition to be reached, at around a weight of 5 - 6g. At this stage in these experiments, this growth curve to maturity was the best obtained for *L. terrestris*.

FIGURE 7.3.1.4 A COMPOSITE GROWTH CURVE WITH PAPER WASTE AS FEED AT 20°C



7.3.2 Growth enhancement by the supply of a synthetic feed.

Introduction.

Earlier experiments (section 4.2.2) had shown that paper waste was an ideal feed for allowing low hatchling mortality and better growth rates ($20 - 30\text{mg}\cdot\text{g}^{-1}\text{day}^{-1}$ at 20°C) than those recorded for natural populations of *L. terrestris*. However, growth with separated cattle solids (SCS) although unsuitable as a feed, due to the high levels of mortality recorded, (fifty percent dead in 20 weeks; Figure 7.3.1.2), did produce growth rates of up to $50\text{mg}\cdot\text{g}^{-1}\text{day}^{-1}$ at 20°C , and a weight of 4g was reached within twelve weeks (Appendix 7). This suggested that the growth rate of worms fed with paper waste could be increased by a supplement to their diet. It was therefore necessary to closely examine the constituents of both feeds, by chemical analyses, in order to determine; a) the cause of high mortality with SCS; b) the nutritional components that promoted comparatively rapid growth rates with SCS; c) the nutritional components that were lacking in paper waste.

The chemical analyses of paper waste and SCS revealed striking differences, particularly in the levels of nitrogen, which resulted in widely differing carbon to nitrogen ratios (appendix 4). The total Kjeldahl nitrogen levels were 2% for SCS but only 0.5% for paper waste. This was reflected in the C:N ratios which were 16:1 for SCS and 93:1 for paper waste, the carbon in the latter consisting mainly of cellulose. As previously mentioned (section 3.4.1.1) if carbon to nitrogen ratios of feeds are high, earthworm growth can be limited (Neuhauser *et al* 1980a), and nitrogen-rich feeds are known to enhance earthworm growth (Evans & Guild 1948; Abbott & Parker 1981).

Materials and Methods.

In order to enhance the growth rates achieved with paper waste to levels approaching those with SCS, but maintaining survival levels, a decision to add nitrogen supplements was made.

A wide variety of nitrogen sources were available, but the two finally chosen were ammonium chloride (an inorganic form), and yeast extract, (an organic form). The reasons for these options are given below. In both cases their ability to support the growth of a

mixed flora of micro-organisms was essential, as it is reported that these promote earthworm growth (Appendix 2).

a. Ammonium chloride (NH_4Cl).

This is utilised by microbiologists in media for the culture of micro-organisms, (Stanier *et al* 1977). In the presence of a carbon source, (here paper waste) and in aerobic conditions, it should support the growth of bacteria and a range of fungi. These, in turn, may well enhance the growth of earthworms.

The amounts of ammonium chloride used were determined by reference to work previously carried out at Rothamsted by Bryson (1983), on the mortality of *Eisenia fetida*. She found an (approximate) critical level of ammonia at $3 - 5\text{mg.g}^{-1}$ of piggery waste. It is difficult to draw direct comparisons to this experimental work for two main reasons. One is the different type of waste material under scrutiny, but more importantly is the difference in nature of the life strategies of the two types of earthworm. It is predicted that *E. fetida* would be able to tolerate greater levels of ammonia as it is often found living in areas such as muck heaps, whereas *L. terrestris* may only feed on nitrogen rich material, and is found in mineral soil (section 2.2).

The results of pilot experiments showed that *L. terrestris* hatchlings could survive for a short period of time (three to four days), with a feed level up to 20mg ammonium chloride per 1g of paper waste. For this reason, and taking into account the altered C:N ratios, a unit value of 10mg.g^{-1} was chosen, supplemented by 5mg.g^{-1} and 20mg.g^{-1} treatments. These figures were later thought to be too high, as the pilot experimental worms were found to be in the soil layer of their pots, showing an avoidance response.

b. Yeast extract.

This material, produced by the autolysis of yeast cells at high temperatures is readily available, cheap and contains a high proportion of nitrogen, usually fifty to eighty percent proteinaceous material, consisting of peptides and amino acids (e. g. Reed 1984). Berry (1982) reports that certain yeasts, for example *Candida utilis*, have been used to produce biomass in sulphite liquor of paper waste.

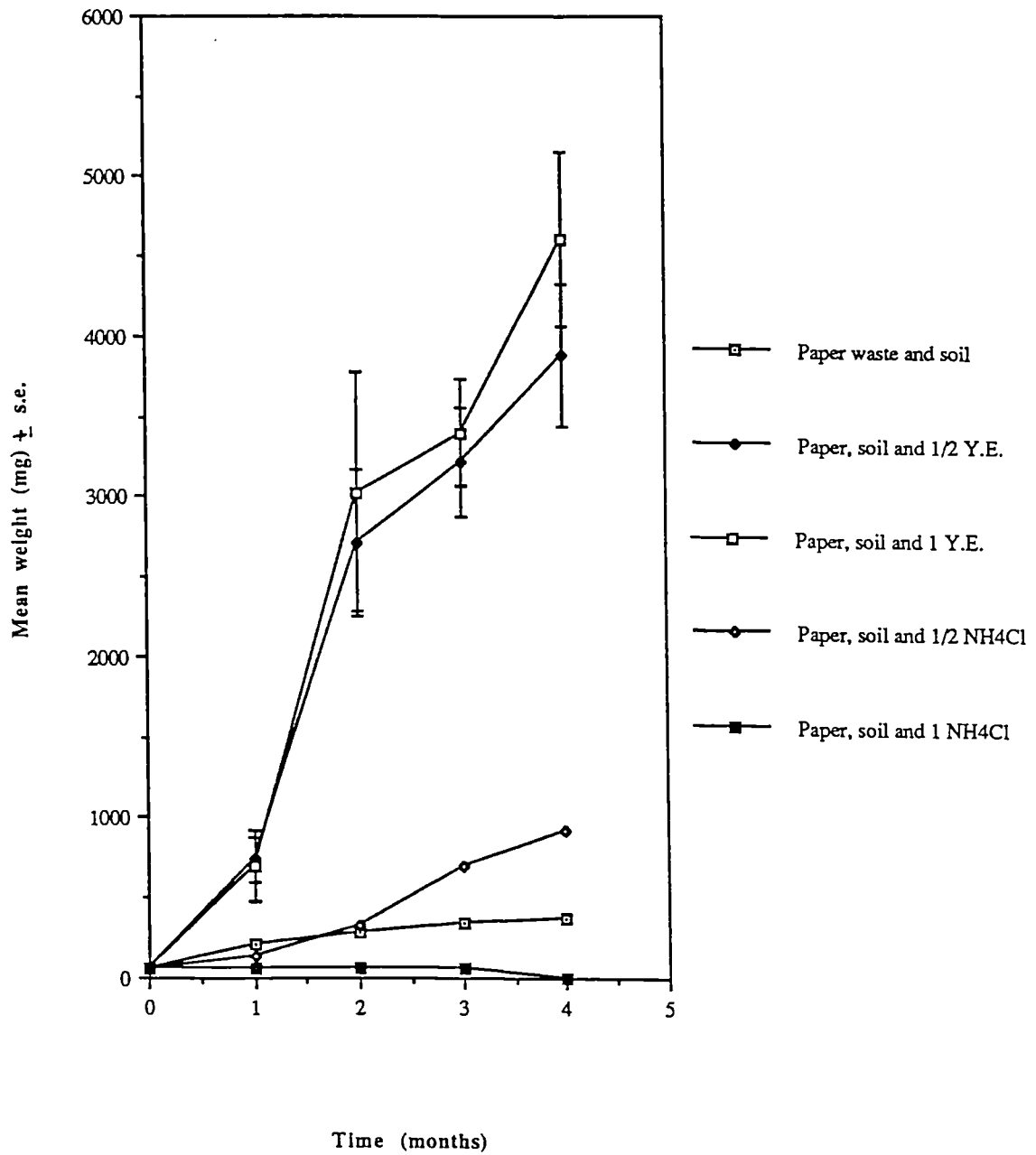
However, yeast extract was chosen in preference to live bakers yeast on the results of pilot studies. Bakers yeast caused a large microbial overgrowth, which was not as apparent using the extract from autolysed yeast cells. Levels of application were governed by equating nitrogen levels to those produced by adding ammonium chloride, here a unit value of 30mg.g^{-1} was chosen, supplemented by 15mg.g^{-1} and 60mg.g^{-1} treatments. Pilot studies showed that levels of application higher than this led to one hundred percent mortality. (The yeast extract was obtained from Sigma Chemical Co., St. Louis; No. Y-4000.)

The amount of nitrogen present in each of these substances was calculated using the Kjeldahl method (MAFF 1981). With these figures it was possible to estimate the amount of each substance required, which on addition to a sample of paper waste, would give a carbon to nitrogen ratio approximately equal to 16:1. This level of application, to 50g of wet paper waste was, 0.5g for ammonium chloride and 1.5g for yeast extract (which reflects the relative amount of nitrogen in each). These two figures were accorded the value of unity (1 NH_4Cl or 1 Y.E.). The same supplements at twice (2 NH_4Cl or 2 Y.E.) and one half (0.5 NH_4Cl or 0.5 Y.E.) of these levels were also added to 50g of paper waste. This gave six supplemented feed treatments in total, with six replicates of each treatment. Each feed was supplied above 200g of moistened soil in a 300ml pot. One hatchling (mean live weight 53mg) was put into each pot. Other treatments, detailed in Table 7.3.2.1, included the use of paper waste and soil as a control, paper waste only, and soil only to verify earlier findings. All were incubated at 20°C , following the results of initial growth experiments (Figure 7.3.1.1).

Results.

With two of the treatments containing yeast extract, (0.5 and 1 Y.E.) growth was greatly increased compared to the paper waste control (Figure 7.3.2.1). A mean weight of 3g per worm was reached within eight weeks, four times faster than previous experiments with paper waste alone. In the case of the lower application five of the six worms survived to four months compared to four worms for the higher application. A "t" test revealed that after four months there was no significant difference between the weights attained ($p > 0.05$). This therefore led to use of the lower level of yeast extract application in subsequent experiments.

FIGURE 7.3.2.1 HATCHLING GROWTH WITH NITROGEN SUPPLEMENTS TO PAPER WASTE



Growth with a supplement of ammonium chloride was only greater than the paper waste control at the lowest level of application. At twice this level (0.25g per 50g paper waste), a net decrease in weight was recorded after one month and none of the worms survived to four months.

At the highest levels of application for ammonium chloride (1g) and yeast extract (3g) per 50g of paper waste, total mortality was recorded at the first sampling period, (one month). Treatments of either soil and yeast extract only or paper waste and yeast extract only, produced similar findings (Table 7.3.2.1).

Both soil only and paper waste only treatments resulted in hatchling weight loss and death of all replicates followed within three to four months.

The increased growth recorded with the two lower treatments of yeast extract (0.5 and 1 Y.E.) with paper waste were both greater than growth recorded with SCS as feed.

The growth rates for worms fed paper waste with soil (control) were lower than identical treatments obtained in previous experiments (Figure 7.3.1.1), but feeds enhanced with the lower levels of yeast extract still promoted much greater growth rates than these previous figures.

Discussion.

After results for growth with soil and paper waste only were analysed, it was apparent that improved growth was possible. The results using separated cattle solids (SCS) as a feed in this work, and previous results from Lofs-Holmin (1983) and Hartenstein & Amico (1983) were found to be superior. This meant that although paper waste alone proved a suitable feed as it had no adverse effects on the worms, it was lacking in certain nutrients required by them. The obvious deficiency, nitrogen (Appendix 4), was confirmed by chemical analyses (Appendix 6) and was amended, leading to increased growth. The improved growth may be compared with the results obtained by Morgan (1988) who added carbohydrates to SCS and recorded increased growth rates with *E.fetida*. However, she did not record increased growth rates with supplements of yeast extract. This could have been due to a simple alteration of carbon to nitrogen ratios to suit the particular earthworm species, or the micro-organisms on which they feed (Appendix 2).

Table 7.3.2.1 Survival and weight increase of hatchlings after one and four months with a variety of feeds.

Treatment	% Survival (1 month)	Mean wt. (mg)	% Survival (4 months)	Mean wt. (mg)
Soil + Paper	100	209	100	373
Soil + Paper +1/2 Yeast Extract	100	738	83	3882
Soil + Paper +1 Yeast Extract	100	694	67	4609
Soil + Paper +2 Yeast Extract	0	-	0	-
Soil + Paper +1/2 NH ₄ Cl	100	129	83	918
Soil + Paper +1 NH ₄ Cl	83	63	0	-
Soil + Paper +2 NH ₄ Cl	0	-	0	-
Soil +1 Yeast Extract	0	-	0	-
Paper +1 Yeast Extract	0	-	0	-
Soil +SCS	50	389	17	3053
Soil only	100	52	0	-
Paper only	100	70	17	15
SCS only	0	-	0	-

SCS = Separated Cattle Solids

(Survival calculated from original total n = 6)

The growth rates recorded for the two lower levels of yeast extract application were as good as those previously recorded for growth with SCS as a feed. However, during the period of eight to twelve weeks, the growth rate with a supplement of yeast extract had decreased sharply. This suggested that the growing worms were in need of a replenishment of feed, which had not until then (twelve weeks) been supplied. Greater growth rates were recorded following this (twelve to sixteen weeks) which confirmed that food quantity had been a limiting factor. This was useful information as it indicated when more feed was required. Growth rates are therefore likely to be enhanced further when worms are fed on demand. In subsequent experiments (section 7.3.3) replacement of feed occurred at either four or eight weeks.

The results of growth with soil only and paper only confirm earlier findings and also those of Hartenstein & Amico (1983). *L.terrestris* needed both soil and feed for satisfactory growth, or in this case any increase in growth at all. It is likely that there are essential trace elements in the soil, although this species also appears to use the soil component as a refuge particularly when recently emerged. Unlike *E.fetida*, for example, *L.terrestris* does not live within its own food.

7.3.3. Closer examination of the effects of a synthetic feed on growth.

Materials and Methods.

The results from the first series of experiments using paper waste supplemented with a nitrogen source (section 7.3.2) prompted a closer examination of the use of yeast extract. The level of application chosen was that of 15mg.g^{-1} of paper waste, i. e. the lower of the two that had resulted in improved earthworm growth (as there had been no significant difference in growth at this and 30mg.g^{-1} of paper waste ($p > 0.05$)). Six replicates of 300ml pots were supplied with 200g of moist soil, topped with 50g of paper waste mixed with 750mg of yeast extract, to give a carbon to nitrogen ratio of 40:1. A single hatchling, mean weight 53mg, was placed in each. Five treatments were set up, these included growth at 10, 15 and 20°C . (This was to verify results from earlier experiments when growth at temperatures of 15 and 20°C showed no significant differences, Table 4.3.2.1; and to assess the survivorship of hatchlings with this feed at different temperatures as it had been done with paper waste only, Figure 7.3.1.2). At the two higher temperatures, 15 and 20°C , one treatment set had the contents of the pots changed every four weeks and another set every eight weeks. Sampling and weighing of worms occurred every four weeks, with an inspection mid-way between.

As hatchlings began to mature, a measure of their reproductive condition was required. For this reason a subjective assessment was devised. This was scored on a scale from zero to six. Zero represented no signs of maturation, six was fully clitellate. Scores of one and two related to appearance and swelling of the tubercula pubertatis respectively. Stages three to six were assigned to development of the clitellum, in quarter phases.

Results.

Figure 7.3.3.1 represents the effects on growth rates of feeding *L.terrestris* with paper waste and a supplement of yeast extract. As shown in Table 7.3.3.1, a significant difference was found for the effect of temperature on growth rate over the first two months ($p < 0.001$). Worms maintained at 20°C initially grew more rapidly than those kept at 15°C . Growth at 10°C was much less than at either of the higher temperatures, as expected from previous experiments, but the rate of growth was twice the rate obtained with paper waste alone at that temperature (Figure 7.3.1.1).

FIGURE 7.3.3.1 EFFECTS OF TEMPERATURE AND FEED REPLENISHMENT RATE ON GROWTH

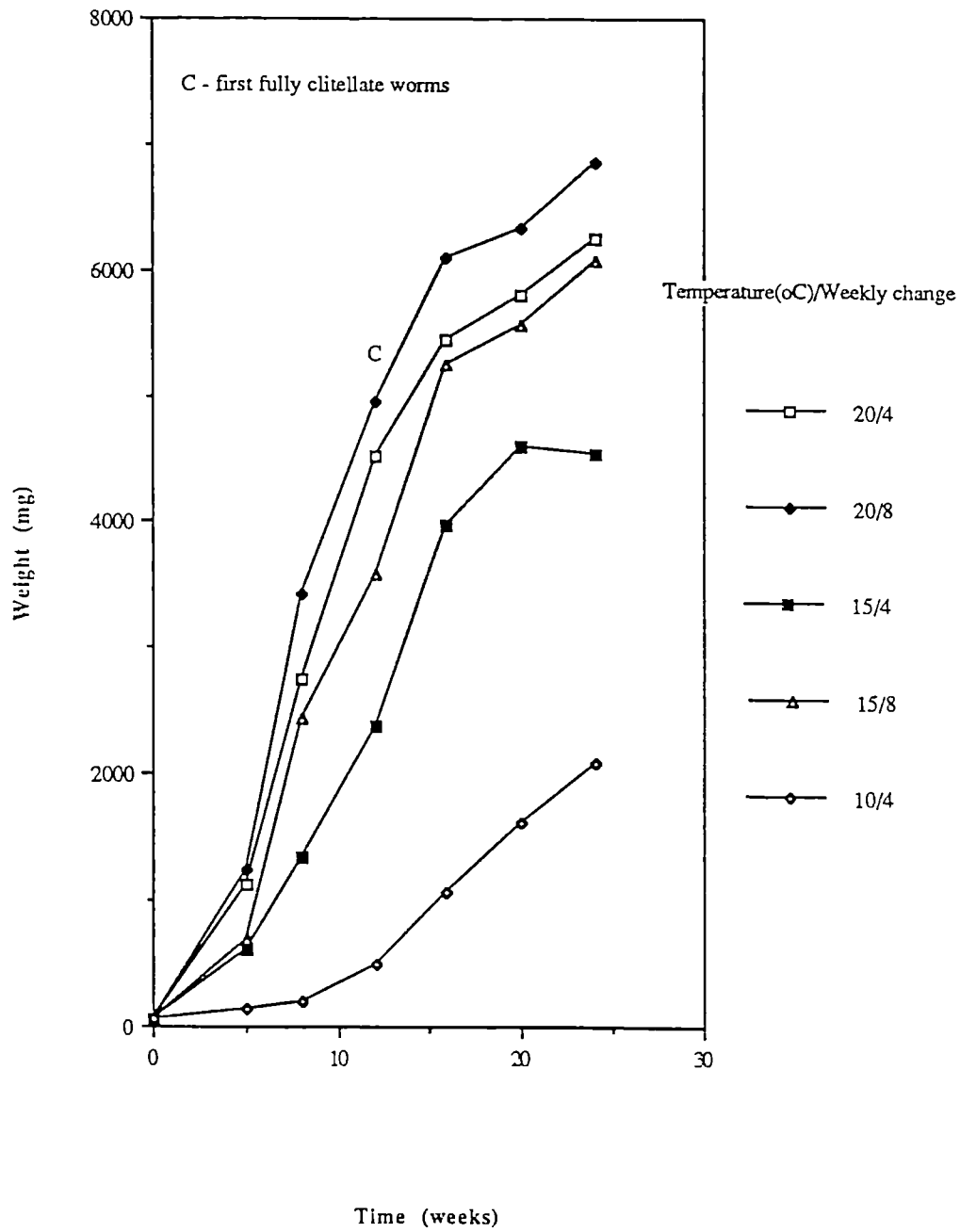


Table 7.3.3.1 Hatchling growth with paper waste and yeast extract as feed at two temperatures and with two rates of feed supply (0 - 8 weeks).

Mean Values (weights after 8 weeks (mg)).

Replenishment of feed (weeks)	4	8
Temperature (°C)		
15	1337	2436
20	2750	3428

Sources of Variation	Degrees of Freedom	Mean Squares	F
Temperature	1	7952930	44.31 ***
Replacement frequency	1	5263996	29.33 ***
Interaction	2	75952	0.42 n.s.
Error	19	179475.89	

Both temperature and the frequency of feed/soil replenishment have had a significant effect on growth over the period of the first eight weeks ($p < 0.001$).

After eight weeks no flattening of the growth curves was recorded (as in 7.3.2.1), which supported the need for a more frequent supply of feed. Examination of the effects on replacement of soil and feed after either four or eight weeks (at the two higher temperatures) showed a significant difference ($p < 0.001$) in the early stages of growth (zero to eight weeks) but less so thereafter. The pots in which the soil and feed were changed less frequently showed better growth rates. After sixteen weeks a flattening of all of the growth curves was recorded.

After four months a "t" test between the two sets of worms which had soil and feed changed every eight weeks (at 15 and 20°C) revealed that there was no significant difference between the weights they had attained ($p > 0.1$).

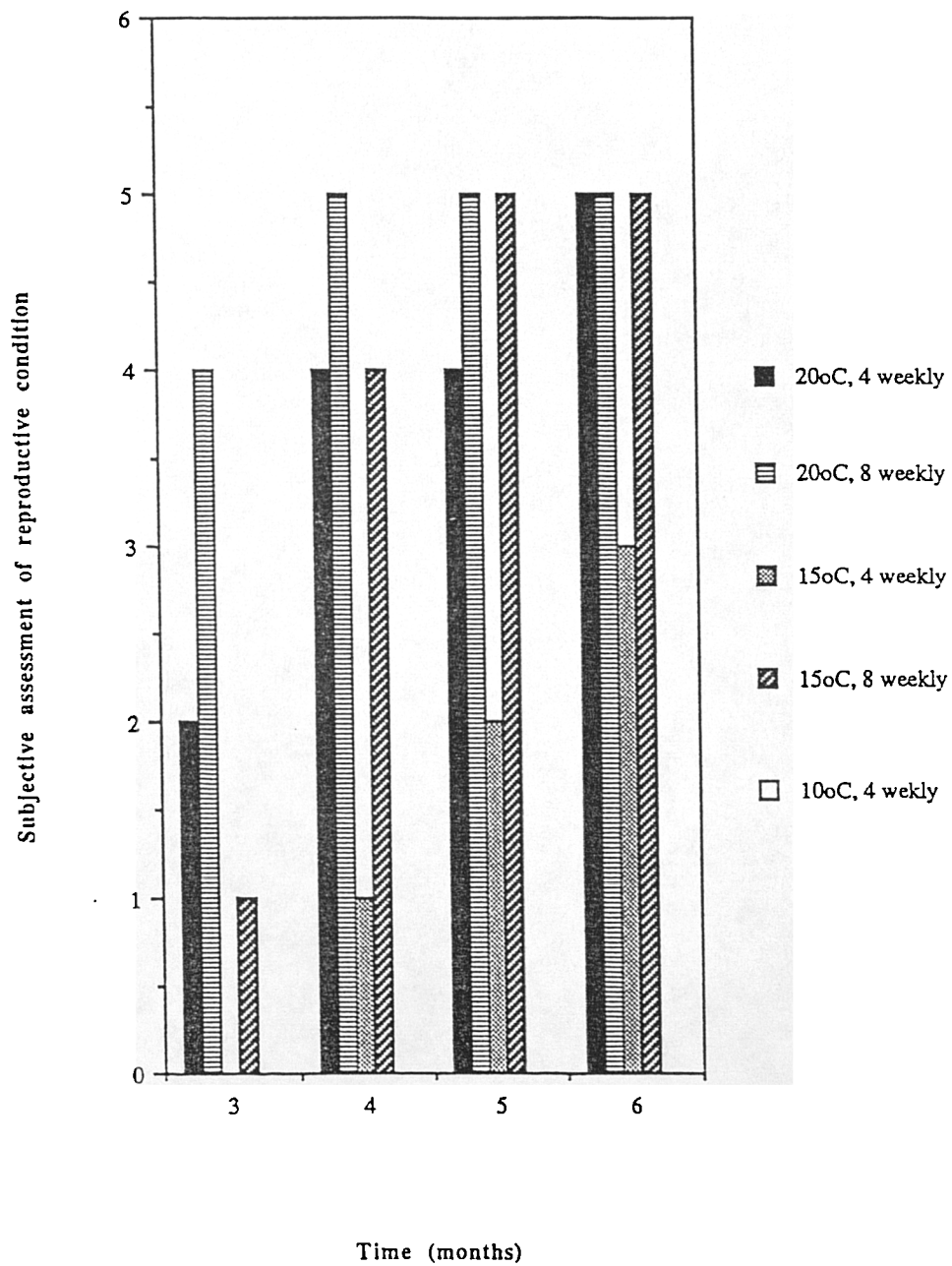
Figure 7.3.3.2 shows a mean subjective assessment of the reproductive condition of the worms in each experimental group. After three months some of the worms were fully mature. Those to reach this condition first were at 20°C, in the group where most rapid growth was recorded. On average sexual maturity was achieved more rapidly where the feed was changed less frequently, at both 15 and 20°C. At 10°C none of the worms showed any sign of developing visible sexual characters, which was not surprising as they had only reached a mean weight of 2g after six months.

Discussion.

The improved growth rate with higher temperatures confirmed the findings of previous experiments. A temperature of 20°C still gave the most rapid growth figures which ranged from 50 - 900 $\text{mg.g}^{-1}.\text{day}^{-1}$. (see discussion section of 7.3.1). The highest figures were recorded over the first month of growth.

As worms grew larger (after eight weeks) a more frequent change of soil and feed had less of an effect on the growth of the worms, which may have been due to the size of the food particles. When newly hatched, the worms could only "work" the feed at a slow rate, which would increase with increased size. A change of feed after four weeks meant that for the worms concerned a fresh start had to be made, whereas those with unchanged feed could continue comminution of the feed. These results supported earlier finding that hatchlings grew better if sampled every four weeks compared to every two weeks.

FIGURE 7.3.3.2 EFFECTS OF TEMPERATURE AND FEED REPLENISHMENT RATE ON MATURATION



After eight weeks no flattening of the growth curves was recorded, as had been the case in the previous experiment (Figure 7.3.2.1), due to the more frequent replacement of feed. The flattening of the curves observed after sixteen weeks may be attributed to the worms, at weights of 5 - 6g, reaching the carrying capacity of the 300ml pots they were grown in, at approximately 20g.litre^{-1} .

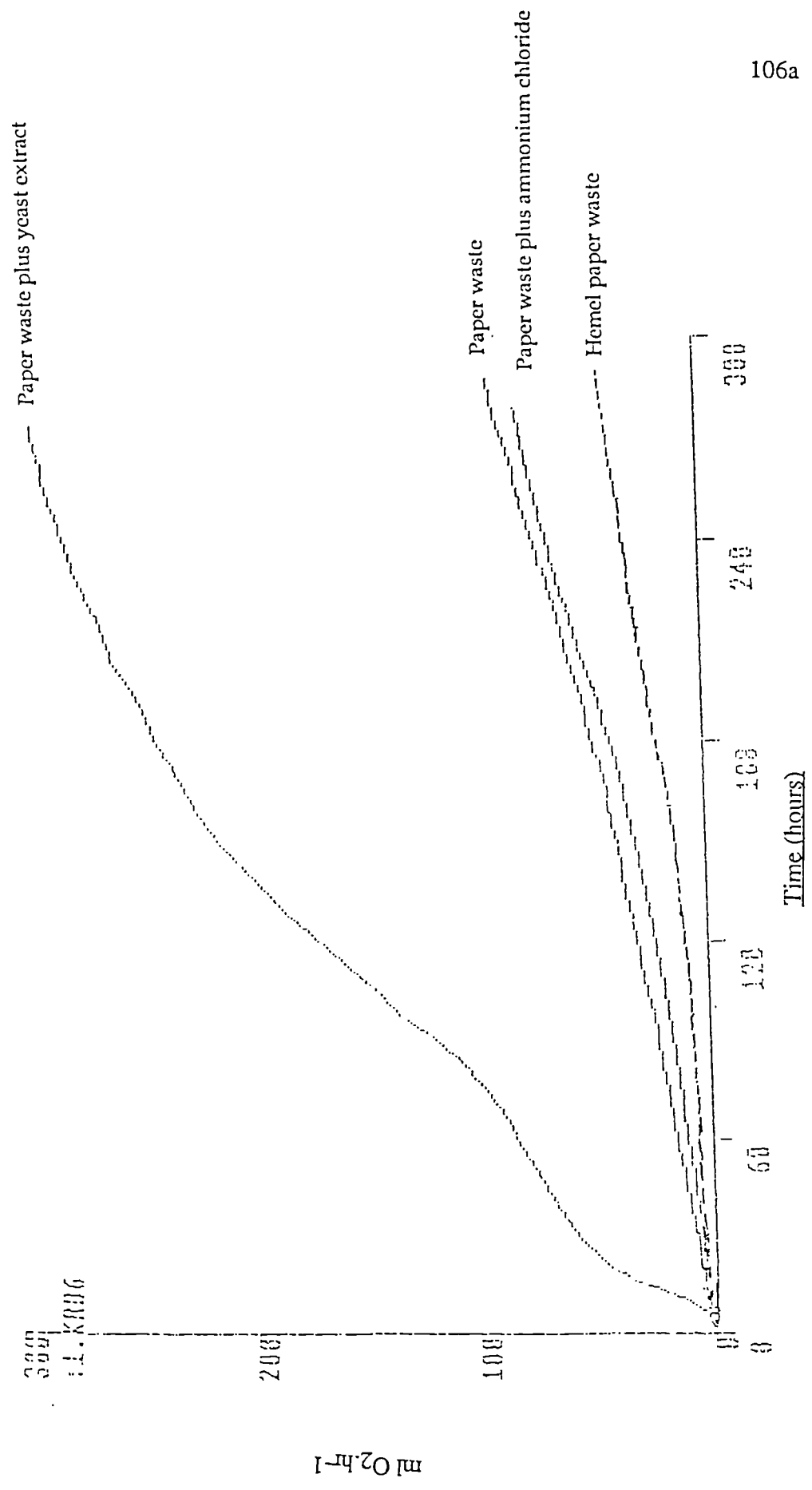
That there was no significant difference between the weights attained after four months by worms at 15 and 20°C was very interesting. If earthworms are to be produced intensively, then the whole life cycle must be considered and not just the rate of growth over the first few weeks. Maintaining a production system at a higher temperature will necessitate increased costs, so this result was considered to be of vital importance.

The important role of micro-organisms on the feed must not be overlooked. Neuhauser et al (1980) and Boström & Lofs-Holmin (1986) have shown that a relationship between increased growth and reduced particle size of feed can be achieved. The former obtained greater growth of *E.fetida* when horse manure was ground from 1.0 to 0.3mm^2 and the latter similar increases with *A.caliginosa* fed on ground barley residues. This increase in surface area would have encouraged greater microbial growth which in some cases may enhance the availability of the carbohydrates and nitrogenous compounds which earthworms utilise (see Appendix 2).

As a supplement to the experimental procedures relating to different earthworm feeds, respirometry values were recorded. These were obtained by using a system developed by Tribe and Maynard (1989) which automatically recorded microbial activity, as oxygen uptake, over periods up to several weeks. All of the feeds utilised were examined in this manner, to assess the level of microbial activity and compare it with the associated earthworm growth figures.

Figure 7.3.3.3 shows readings obtained from a number of feeds which contained paper waste as a constituent. Respirometry readings comparing paper waste with paper waste supplemented with yeast extract showed large differences in levels of microbial activity. These were $1.05\text{ml O}_2.\text{hour}^{-1}$ for paper waste supplemented with yeast extract (15mg.g^{-1} paper waste) compared to $0.3\text{ml O}_2.\text{hour}^{-1}$ for untreated paper waste. A supplement of ammonium chloride (5mg.g^{-1} paper waste) produced readings of $0.27\text{ml O}_2.\text{hour}^{-1}$. All readings at N.T.P. were mean values from a period of at least ten days. Figures for paper

FIGURE 7.3.3.3 CUMULATIVE OXYGEN CONSUMPTION MEASURED USING A
 MAYNARD PROJECTS RESPIROMETER



waste obtained from Hemel Hempstead, used in preliminary experiments (4.2.2), were lower at $0.15\text{ml O}_2\cdot\text{hour}^{-1}$. These can be compared with figures of approximately $0.8\text{ml O}_2\cdot\text{hour}^{-1}$ for both SCS and DSCS (section 5.3.1). The level of microbial activity measured in this way appears to add weight to the suggestion that earthworms derive nourishment either directly or indirectly from certain micro-organisms. Further work would be required looking specifically at this aspect of earthworm nutrition to confirm this.

*7.3.4 The effects of population density on the growth of *L.terrestris*.*

Introduction

Previous experiments discussed in this chapter suggested that growing *L.terrestris* to maturity from hatchling size was possible within a period of twelve weeks. This was achieved by culturing worms in soil with paper waste and a supplement of yeast extract as feed. However, in all cases worms were grown in isolation. No data had been collected relating to the possibility of interaction between individuals cultured together. It is likely that intra-specific competition for food, space or both may occur. Results from preliminary experiments starting with 2g worms, showed that under the conditions then provided (paper waste only as feed) population density did have an effect on the size that the worms eventually reached (see figure 4.2.1.1). This experiment aimed to investigate this further but using a superior feed (paper waste plus yeast extract with a carbon to nitrogen ratio of 40:1) and with a feed replenishment rate that would not limit growth.

Materials and Methods.

To obtain any effects of population density as rapidly as possible, small 300ml pots used in previous growth experiments were also used here. Densities of 1, 2, 4, 8, and 12 worms per pot were chosen. These corresponded to 3.3, 6.6, 13.3, 26.7 and 40 worms per litre respectively. Each pot was supplied with 200g of moistened soil and 50g of paper waste with 750mg of yeast extract. Hatchling worms (mean live weight 53mg) were then assigned randomly to the pots at the given densities. Five replicates of each density were set up and all were incubated at 20°C. The pots were sampled every month, at which time the worms were counted, weighed and returned to their pots. Fresh feed was given to the worms at each sampling but the original soil and some of the "old" feed was left in each pot.

Results.

The growth curves drawn in Figure 7.3.4.1 show that increased population density has a negative effect on individual earthworm growth. With increasing densities in the range of three to forty worms per litre the recorded mean growth rates are lower. Maturation was also more rapid at lower densities. At the two higher densities no worms had shown any signs of developing tubercula pubertatis after five months.

FIGURE 7.3.4.1 THE EFFECT OF POPULATION DENSITY ON GROWTH

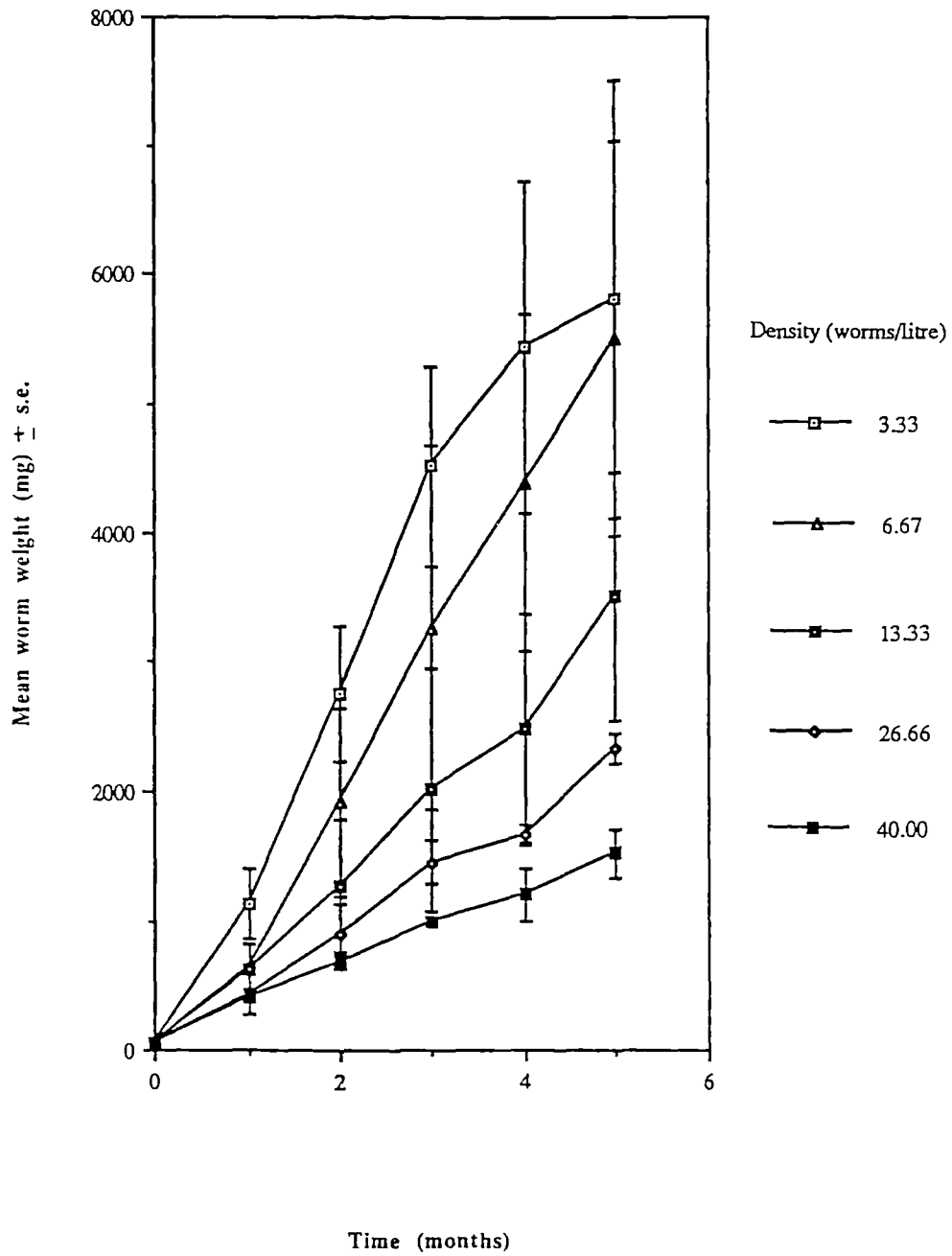


FIGURE 7.3.4.2 TOTAL EARTHWORM MASS AT DIFFERENT DENSITIES

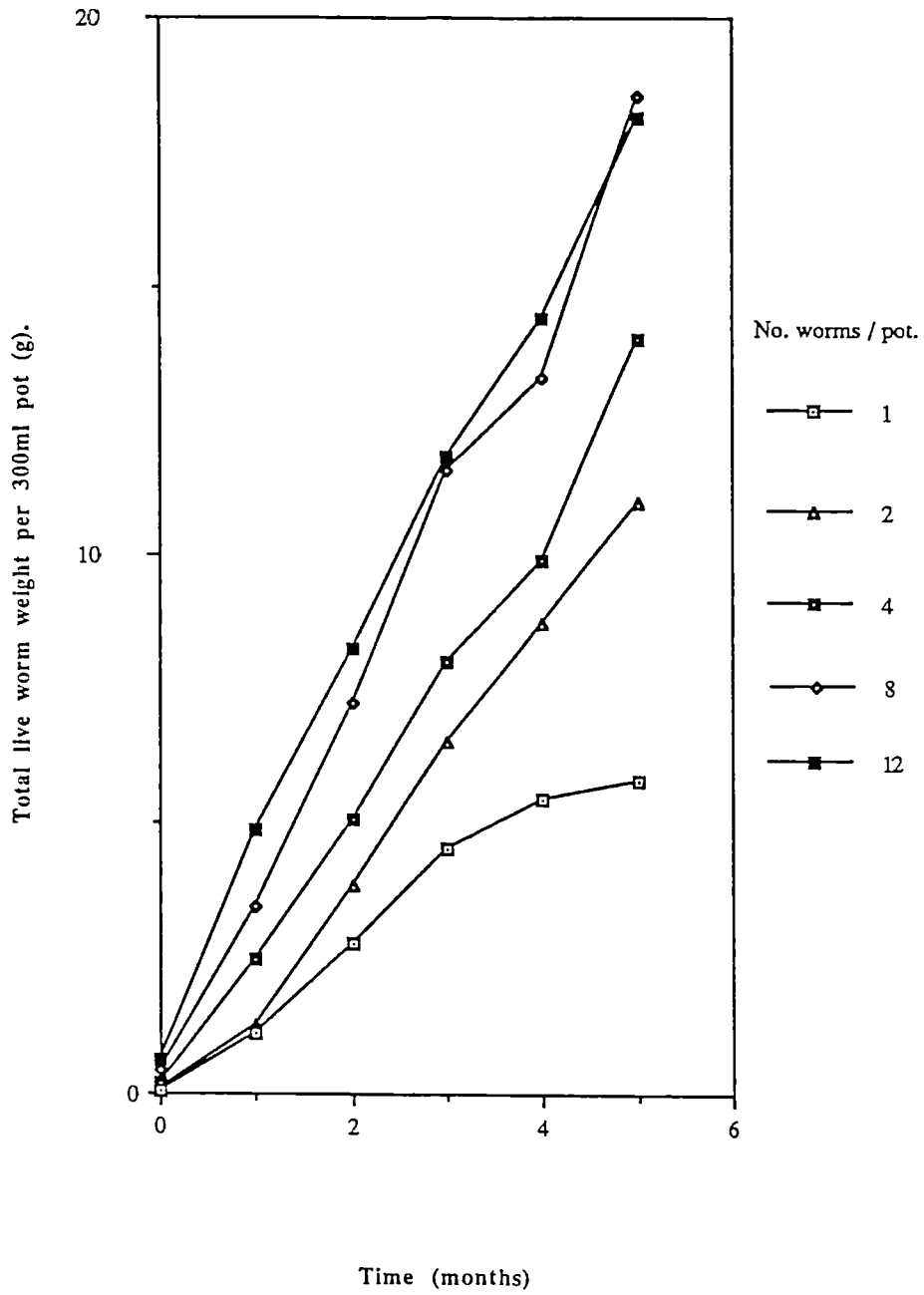


Figure 7.3.4.2 shows the total biomass per pot after a period of five months was 5.8, 11.0, 14.0, 18.4 and 18.1g for 1, 2, 4, 8 and 12 worms respectively. The shape of these graphs show that flattening of the growth curve has not yet occurred with a maximum of 18.4g in 300ml, which is equivalent to 61g.litre⁻¹.

Discussion.

That population density affected growth rates was not unexpected. Neuhauser *et al* (1979) demonstrated that an increase in population density of *E.eugeniae* fed with horse manure led to a decrease in mean growth per worm, as did Neuhauser *et al* (1980b) with *E.fetida*. In any system there is a carrying capacity, above which a sustained increase in biomass is not possible. One factor that could have been operating here was the physical effect of space limitation. *L.terrestris* is reported to utilise a permanent burrow system (Edwards & Lofty 1977), but previous experiments (section 7.3.3) have shown that this species can grow to maturity in a 300ml pot with a depth of only 4cm. However, worms kept individually were often seen to lie in a circular fashion around the base of the pot. When more than one worm was present this would be physically impossible. It may also be that earthworms have an innate avoidance reaction to each other, which is only over-ridden by the necessity to reproduce.

It is often difficult to separate the effects of lack of physical space and feed rate as limiting factors. This is because to add feed at a greater rate not only affords the worms with more potential to ingest and assimilate that feed, but the feed itself also adds to the volume of the experimental system. This is particularly true when worms are younger, and hence smaller, and the feed is bulky in nature. Further experiments are required in which feed rate can be examined as a treatment at a range of population densities.

7.3.5 The effect of Stress on immature earthworms.

Introduction.

From previous growth data the composite figure (7.3.1.4) suggested that with paper waste only as a feed, development of the clitellum was not expected until after thirty weeks. This experiment was devised to try and decrease this time. The rationale was that worms put under stress might mature more rapidly in order to reproduce, if death was imminent. (Cocoons are often able to survive in conditions under which both immature and mature worms will perish.)

Materials and Methods.

This experiment ran concurrently with 7.3.3 and was similar in design. Initially it involved growing twenty-four worms individually in 300ml pots at 20°C, with sterilised soil and yeast extract-supplemented paper waste as feed. When these worms had reached a weight of 4 to 5g (after twelve weeks), groups of six replicates were treated in the following ways; (a) one was moved to 10°C, (b) a second had the feed replaced with paper waste only and (c) a third group had the feed changed to paper waste only and was also moved to 10°C. A control group remained at 20°C with paper waste and yeast extract as feed. All of these treatment groups were sampled monthly, and had both soil and feed replaced with fresh on sampling.

As hatchlings began to mature, a measure of their reproductive condition was required and the same subjective scoring system (zero to six) described in experiment 7.3.3 was utilised.

Results.

Figure 7.3.5.1 shows that prior to the application of "stressful" treatments, all growth, as expected, was much the same. The required weight of 4 to 5g was reached three months after the start of the experiment, at which point the treatments described above were applied. The application of the treatments led in all cases to a decrease in weight compared to the control group. Where the temperature was decreased but yeast extract was retained as feed this occurred within the first month (treatment (a)). A decrease in the other two treatment groups was not observed until after a period of two months, when a greater decrease was seen at the lower temperature (treatment (c)). Table 7.3.5.1 shows

that growth following the application of the treatments was significantly affected by temperature ($p < 0.05$). Growth rates were greater at 20°C compared to 10°C. When worms had grown to this size, the lack of yeast extract had no significant effect on subsequent growth.

The development of sexual characters, tubercula pubertatis and clitellum, for each of the treatments is represented in Figure 7.3.5.2. These show that stressful treatments did nothing to increase the rate of maturation.

Discussion.

The effects of applying stress to worms growing in optimal conditions did not increase the rate at which they matured (Figure 7.3.5.1). Those worms which remained as controls at 20°C and were fed paper waste with yeast extract added, continued to grow steadily and matured most rapidly. Compared to the worms which matured most rapidly in experiment 7.3.3, all of the worms here developed less rapidly.

Applying stressful situations in the form of poor quality feed and/or low temperature condition does not lead to more rapid development to maturity, as might be expected in evolutionary terms, if the animal were in danger of dying. A final effort of cocoon production by worms which were already mature, may be the way that some epige species overcome adverse conditions as cocoons could then remain dormant for the length of the stressful period. *L. terrestris* however, does not appear to follow this strategy.

FIGURE 7.3.5.1 THE EFFECT OF "STRESSFUL" TREATMENTS ON GROWTH

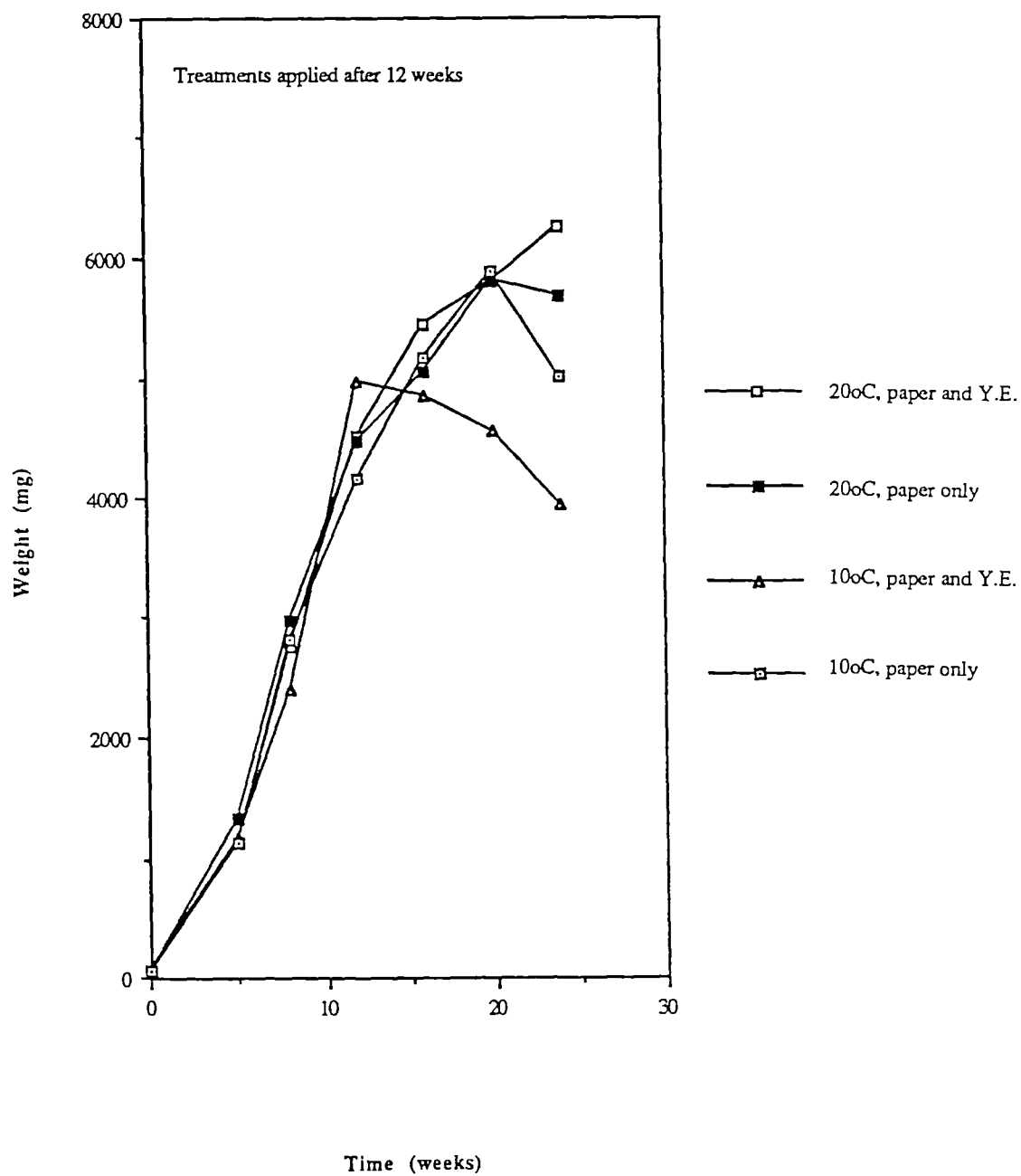


Table 7.3.5.1 The effect of a decrease in temperature and/or application of an inferior feed on maturation.

Mean growth rates (mg. g⁻¹. month⁻¹).

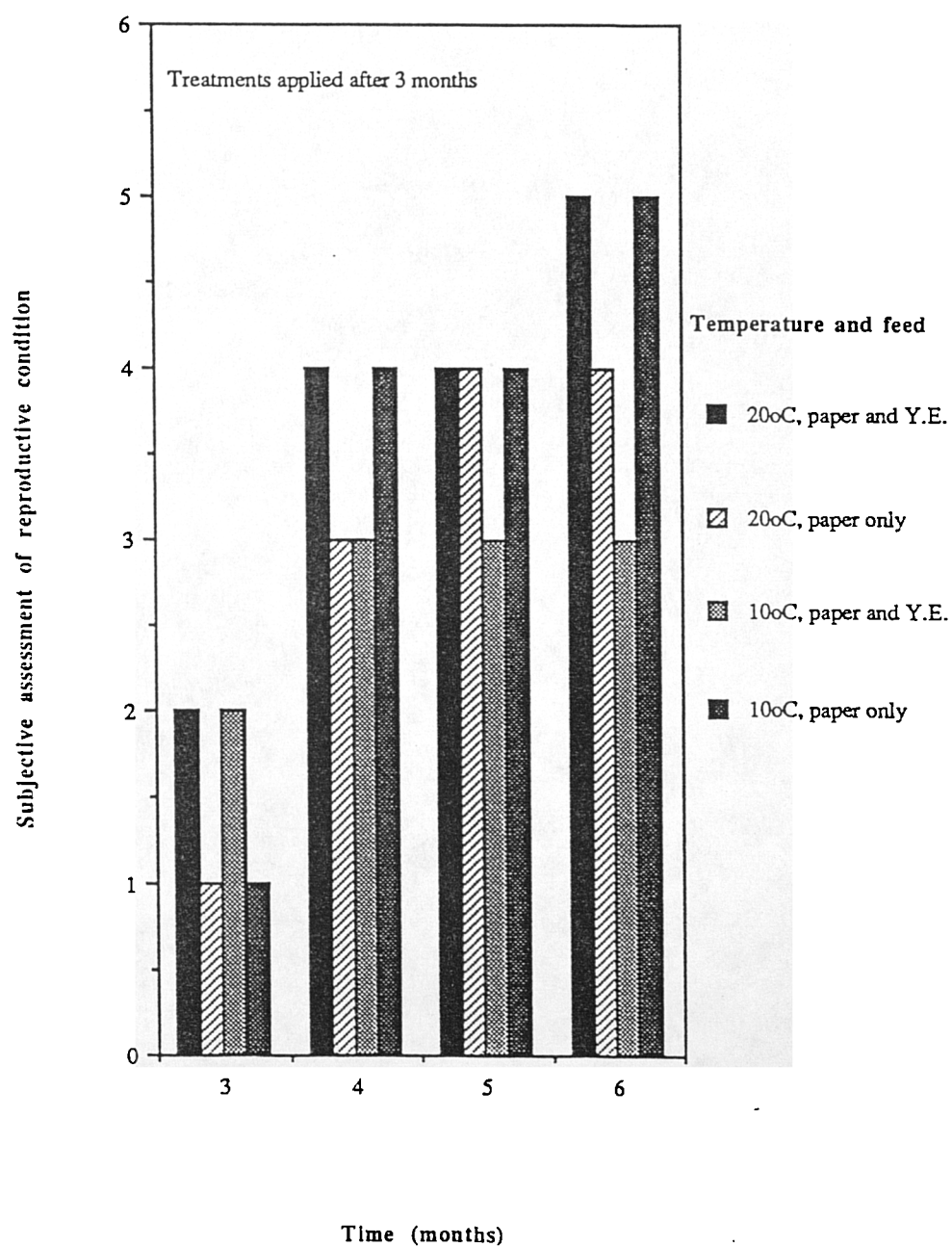
Feed Type	Paper Waste	Paper + Yeast Waste Extract
Temperature (°C)		
10	28.6	-23.1
20	49.5	125.4

Sources of Variation	Degrees of Freedom	Mean Squares	F	
Temperature	1	43023.36	8.18	**
Feed Quality	1	869.82	0.17	n.s.
Interaction	1	24400.89	4.64	*
Error	20	5262.30		

A reduction in temperature significantly affects growth rate. ($p < 0.01$).

There is also a significant interaction between temperature and feed quality ($p < 0.05$).

FIGURE 7.3.5.2 THE EFFECTS OF "STRESSFUL" TREATMENTS ON MATURATION



7.4 Conclusions.

The most rapid growth rate for hatchling *L.terrestris* recorded during this work was achieved at 20°C. This was with a mineral soil with a twenty-five percent moisture content and a feed of paper waste supplemented with yeast extract to give a carbon to nitrogen ratio of 40:1. However after a period of four months, worms grown at 15°C with the same feed had achieved weights which were not significantly different from worms grown at 20°C ($p > 0.1$).

From a mean weight at hatching of 53mg, growth to 6g was achieved at 20°C, within sixteen weeks, and the fully clitellate condition was reached in twelve weeks at a weight of 5g.

Worms grown in 300ml pots in isolation, at both 15 and 20°C were clitellate 3 - 4 months after hatching. At this point they began to show a reduced growth rate. This was attributed to the carrying capacity of the pots having been reached, at 20g.litre⁻¹.

The optimum temperature for growth of *L.terrestris* was 20°C. Both above and below this temperature growth was impaired. Feed quality can also lead to reduced growth rates within certain biological limits. A feed with a readily available carbon and nitrogen source is essential.

The temperature at which cocoons are incubated does not affect the growth rate of the worms that they produce, within the 10 - 20°C range.

A too frequent change of the growth medium, when the worms are small, can have adverse effects on subsequent growth rates. The figures given above relate to a frequently disturbed system.

Population density was found to affect growth rates for this species. More work is required to discover the optimal feed rate, population density and minimum soil requirements.

Maturation is not hastened by stressing worms which have reached a weight of 4 to 5g.

CHAPTER 8. DISCUSSION AND FUTURE RESEARCH.

8.1 Introduction

This chapter aims to link together all results so far presented and to develop the optimal conditions for an intensive production process for *L.terrestris*. The conclusions offered at the end of the preceding chapters related solely to particular stages in the life cycle of *L.terrestris*. The question of the economics of this process will be discussed briefly as little prominence has been given to this subject so far. This was a deliberate course, although certain experimental options investigated during this work, such as the use of waste materials as feeds, were certainly influenced by a long term economic view.

8.2 Experimentally derived optima.

Temperature

As indicated in section 3.4.1.2 temperature has had a marked effect on all phases of the life cycle. At 20°C cocoon development was most rapid and cocoon viability was not reduced significantly compared to lower temperatures. Growth of hatchlings to maturity was also achieved in the shortest time. Survival of hatchlings to twenty weeks was also over ninety percent certain, at this temperature. At a constant 20°C reproductive output was initially very high but fell to zero after only a few months. Greater annual cocoon production was recorded at a constant 15°C (25 cocoons per worm per year). Fluctuating temperature regimes gave lower annual results, but production was enhanced during periods of increased environmental temperature, particularly in the range 10 - 20°C. This presented two possible options for *L.terrestris* production and temperature control;

i). To maintain worms at 20°C for limited, maximum cocoon production, with associated rapid incubation and growth of hatchlings. Although, this would mean that cocoon production by mature worms would be limited to a few months, after which the adults would lose reproductive condition and possibly die.

or

ii). A compromise could be sought, as cocoon production is a critical stage in the life cycle and reproductively active adults need to be maintained for as long a period as possible. The period of cocoon incubation is significantly greater at 15°C compared to 20°C, but

growth to maturity is not significantly different at these two temperatures. Optimal production for a complete life cycle would probably occur within the range 15 - 20°C.

Feed type

Feed type affects both growth of hatchlings and the rate of cocoon production (Table 3.4). These experiments suggested that a synthetic feed comprising paper waste and a supplement of yeast extract (both waste materials/by products) was superior to single sources of feed tested. During both the growth stage and the reproductive stage, this synthetic feed gave most rapid weight increase and highest levels of cocoon production. Growth with this feed was superior to separated cattle solids, which it was initially created to mirror in terms of carbon to nitrogen ratio. With the synthetic feed, growth rates from hatching to maturity were greater than any published data for *L.terrestris* (see Figure 8.2.1).

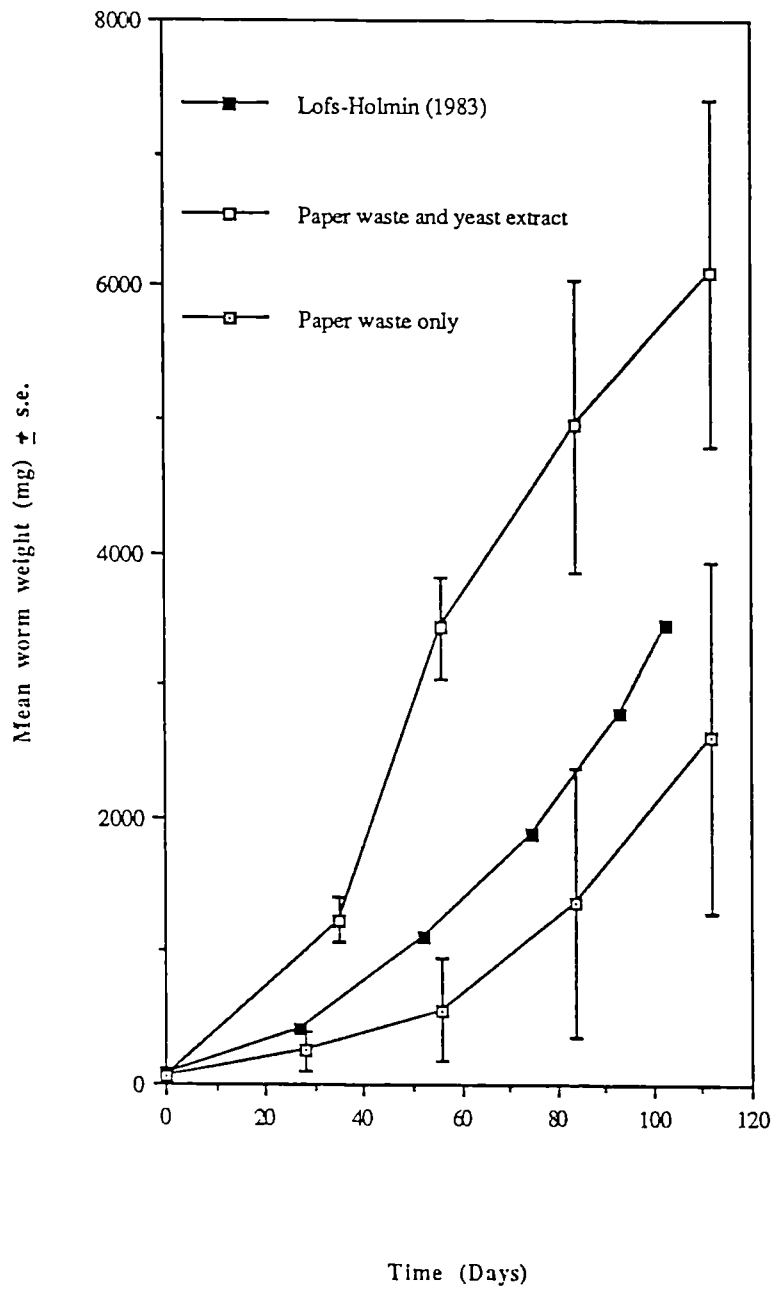
Population Density

This has a marked effect on both cocoon production and growth to maturity (Table 3.4). An optimal figure has yet to be established as experiments in this area were only initiated after other optima had been established, to permit the best utilisation of available worm stocks. (Experiments with high population densities and sufficient replicates would have required many hatchlings.) Individual growth rates were reduced at increased population densities, but this may have been due to feed limitation, as the greater number of worms may have actually made nutrient uptake easier by greater comminution of the feed. Optimal growth rates were at around 20g live weight.litre⁻¹ as worms matured within three months. At greater population densities maturation took longer but this may relate to an insufficient feed rate.

Cocoon production was also affected by population density. Reproduction was greater at a lower (16g.litre⁻¹) rather than a higher population density (32g.litre⁻¹), but more experiments will be required.

The effect of feed rate was not clearly established as it was not examined experimentally. It may be possible to keep a greater number of earthworms in a smaller volume of soil if regular, frequent feeding is practised.

FIGURE 8.2.1 GROWTH WITH PAPER WASTE (ALONE AND SUPPLEMENTED) AND BEST DOCUMENTED GROWTH



Earthworm age

This aspect would appear to have a marked effect on reproductive output. The rate of cocoon production by recently matured worms was found to be almost 1.5 times greater over an annual period compared to field-collected earthworms of unknown age. This suggests that culturing worms for reproduction would provide a greater cocoon output than from collected adults. Any extra costs incurred "in growing worms on", financial or otherwise, would need to be compared with enhanced cocoon production.

Seasonal effects

It might be expected that if worms are kept under controlled conditions, the effects of seasonal changes ought to be minimal. Both cocoon development and growth to maturity appear to be unaffected by this factor. However, cocoon production appeared to cycle with an annual periodicity. Even under constant temperature conditions and under conditions of total darkness an inbuilt biological mechanism was apparently able to keep track of the annual cycle. This, if uncontrollable by external influences will restrict the potential cocoon production of this species. However, most of the experiments from which these results were obtained were not designed specifically to monitor this phenomenon. Much greater care over temperature and light conditions, for example on sampling, would be required so that environmental cues were not experienced by the worms. This requires further experimentation. These effects also suggest that breeding worms for cocoon production is essential as field-collected, mature worms would have already entered the annual reproductive cycle.

Selective breeding/Forcing

Attempts at selective breeding from the twin worms obtained from a small percentage of cocoons failed to produce a breeding line of twins. This was not totally unexpected as the occurrence of twins/triplets was probably both genetically and environmentally controlled. If genetically controlled, then more than one gene might be involved and assessment would require a large number of experiments with a larger number of hatchlings and careful control over matings. If environmentally controlled then experimental manipulation of temperature, for example, might be worth trying. However, on a production basis this is unlikely to be profitable as it has been shown that the hatchling produced would be proportionally smaller in size than single hatchlings, which means that

they would be disadvantaged during the first stages of their growth. It is almost certain that the manipulations necessary, if possible, would not warrant the results obtained.

Attempts to bring about a more rapid maturation of 3 - 4g worms, by the application of stressful conditions, failed in comparison to the provision of optimal conditions. There is no evidence to suggest that this is worthwhile with *L.terrestris*. It was initially tried as immature worms fed with paper waste only, had taken up to ten months to mature, but later results suggested that a lack of suitable feed was responsible for the delay to both growth and maturation.

8.3 An Intensive Production System

A wealth of information relating to the production of *L.terrestris* under controlled conditions has been gained during this research work. The following information can now be provided relating to the aims and objectives set out at the end of chapters one, three, four, five, six and seven.

It appears that the intensive production of *L.terrestris* is technically feasible, and data relating to specific population parameters are now available. Reproduction can be encouraged to occur throughout the year, even if an as yet unspecified seasonal influence is present. Recently matured worms are more fecund than field-collected adults which may have been mature for an unknown length of time. Cocoons produced by newly matured worms also have a greater viability, at around eighty-three percent, compared with seventy-four percent for cocoons produced by field-collected worms or from field-collected cocoons. Both observations suggest that the ageing process affects both cocoon production and cocoon viability. Cocoon development time can be reduced from twelve and a half weeks at 15°C to ten weeks at 20°C. Growth to maturity can be reduced to twelve weeks, by which stage the hatchling worms had increased from 53mg on emergence to a mean weight of 4.5g. In total a complete *L.terrestris* life cycle could be achieved within 22 weeks, allowing two generations per year.

In the past production units employed by vermiculturists have always tended to be of a large size. This had many advantages when raising worms such as *Eisenia fetida*, but it also led to problems which are possibly more important here. These included the risk of infection which could have catastrophic effects on earthworm populations. (e. g. during the Lufkin project, Satchell (pers.comm.) (section 2.5). Also the aim of this work was to

produce worms in an environment that was conducive to growth and reproduction with soil and feed present. On inoculation into the soil, worms would be least disturbed and hence have a greater chance of survival and establishment, if they remained in their original growth medium. To this end the unit would best be of a size that could easily be handled and transported to the site of inoculation. The time of inoculation would be governed partially by environmental conditions (season) and by the time when the inoculation unit contained a population of worms at its carrying capacity.

For these reasons it is proposed that the optimal unit for production would have a volume of less than ten litres and possibly as little as five litres. For reasons of storage and transport the type of container used would also need to be light, cheap and possibly disposable. Preliminary work has already begun on the development of this idea and a patent application has been filed. This technique, using an "inoculation unit" has many advantages over existing earthworm inoculation practices.

8.4 An intensive production model.

Population parameters of *L.terrestris* covering all of the life cycle stages are given in Table 8.4.1. A comparison is made of the information present in the literature prior to this work and data gained during this research programme. In most categories, as would be expected, an improvement has been shown on field data but also on previously recorded laboratory results.

The parameters relating to all aspects of *L.terrestris* production can be linked together to provide a first attempt at defining a population model for an intensive system, even though the data derived throughout this work is based on a frequently disturbed (sampled) system, and therefore greater production might be expected with less disturbance. A production system would naturally depend on many factors yet to be determined, such as density tolerance, interaction between adult and juvenile worms and control over seasonal cocoon production. The length of development of each culture unit would, in some instances, prevent the need to consider some of these factors, if for example, field inoculation occurred prior to any hatchling emergence. Data from Table 8.4.1 has been used to create Figure 8.4.1. This represents unrestricted growth and reproduction at greatest recorded levels. No attempt has been made to include a carrying capacity or rates of mortality into the model but further development will do so.

Table 8.4.1 Population parameters of *L. terrestris*: published and recently discovered.

Popn. Parameter	Published Data	Field collected (This work)	Laboratory bred (This work)
Annual cocoon production (c/w/y)	-	25.3	36.9
Cocoon Incubation (weeks)	12-13 (M)	10	-
Cocoon Viability (%)	-	74	83
Hatchlings per cocoon	1 (E&G) (Mi)	1 *	1 *
Juvenile mortality (%) (20 weeks)	20 (L&S)	-	0-10
Growth to maturity (days)	100 (LH)	-	84
Mortality (%) (1st year)	40 (L&S)	-	10
Carrying capacity (g/l)	22 (H&A)	20	20
Soil Requirement (depth cm)	15 (H&A)	9	9

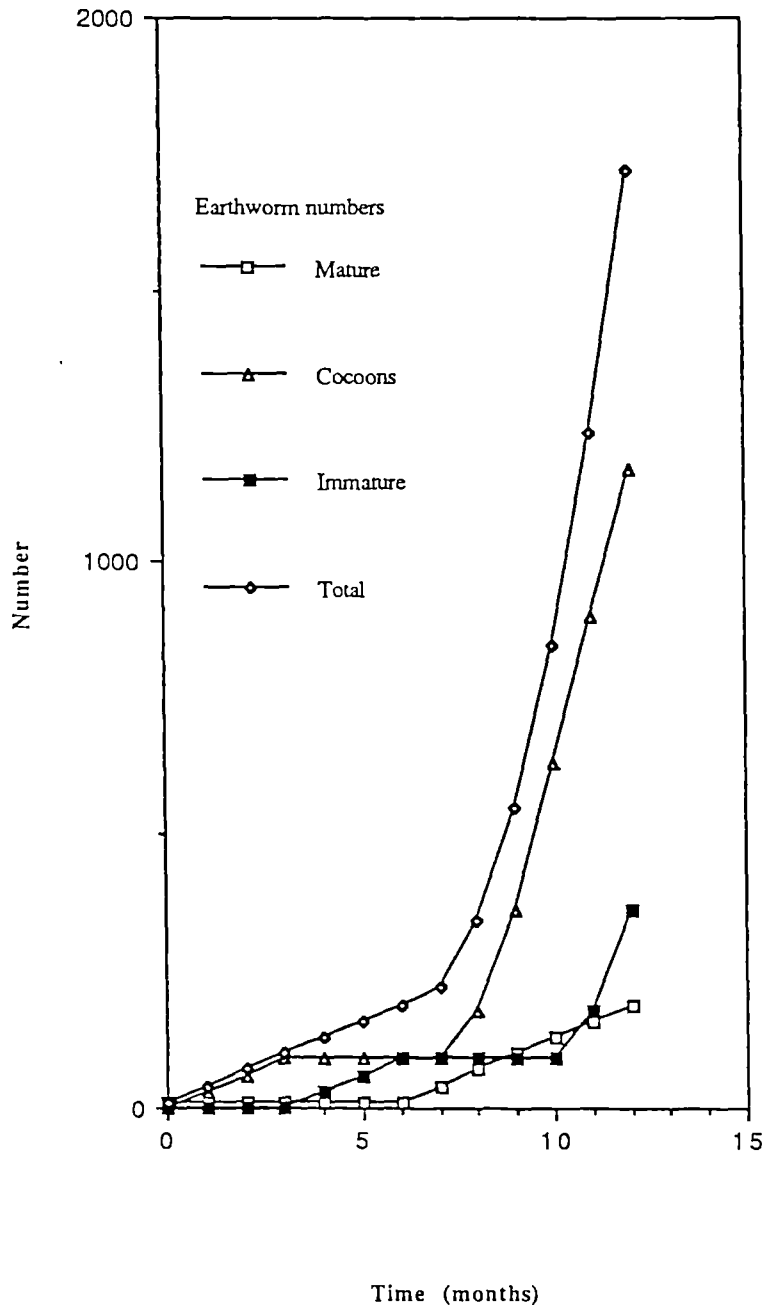
(* - Approximately 1% of cocoons produce more than one hatchling)

Abbreviated references. (All results laboratory based except L&S).

E&G - Evan & Guild (1947);
LH - Lofs-Holmin (1982);
M - Meinhardt (1974);

H&A - Hartenstein & Amico (1983);
L&S - Lakani & Satchell (1970);
Mi - Michon (1954).

FIGURE 8.4.1 UNRESTRICTED POPULATION GROWTH
FROM EXPERIMENTALLY DERIVED DATA



8.5 Economics and Potential markets.

There does appear to be a real market for soil dwelling earthworms. Following television and press publicity, the Open University received enquiries for the supply of *L.terrestris* from several sources. The annual requirements ranged from hundreds, for environmental monitoring experiments, to tens of thousands for landscape reclamation. In all cases realistic costs were envisaged by the enquirers.

The technical feasibility of intensive *L.terrestris* production has been demonstrated on a laboratory scale. Larger scale research is now required to verify optimal experimental findings, produce worms for inoculation, carry out this procedure and monitor the sites after inoculation. Costs of the whole process can only then be realistically considered. An economic assessment would warrant a separate research programme in itself.

8.6 Future research

This research has identified certain areas of earthworm intensive production that require further research. These have been sub-divided into three categories.

1. Further basic research to try and improve upon growth and reproduction of *L.terrestris*. More detailed density experiments are required, which could look more closely at carrying capacity. The effects of seasonal variation in cocoon production also warrant closer examination.
 - 1a. To put into practice all of the findings from this research as a whole. This would either validate or refute models constructed from growth and reproductive data.
2. Development of the "inoculation unit" technique, to assess the applications of intensively produced earthworms. Monitoring would include, for example;
 - i. The effects of the worms on degraded soils,
 - ii. Rate of spread from point of inoculation,
 - iii. Survival of inoculated worms.
3. Experiments to intensively culture surface dwelling earthworm species, e. g. *Aporrectodea caliginosa*, shown to complement the actions of deep burrowing species, of

which *L.terrestris* is an example. This could lead to growing a mixture of species together to provide a "complete package" within an inoculation unit for soil amelioration projects.

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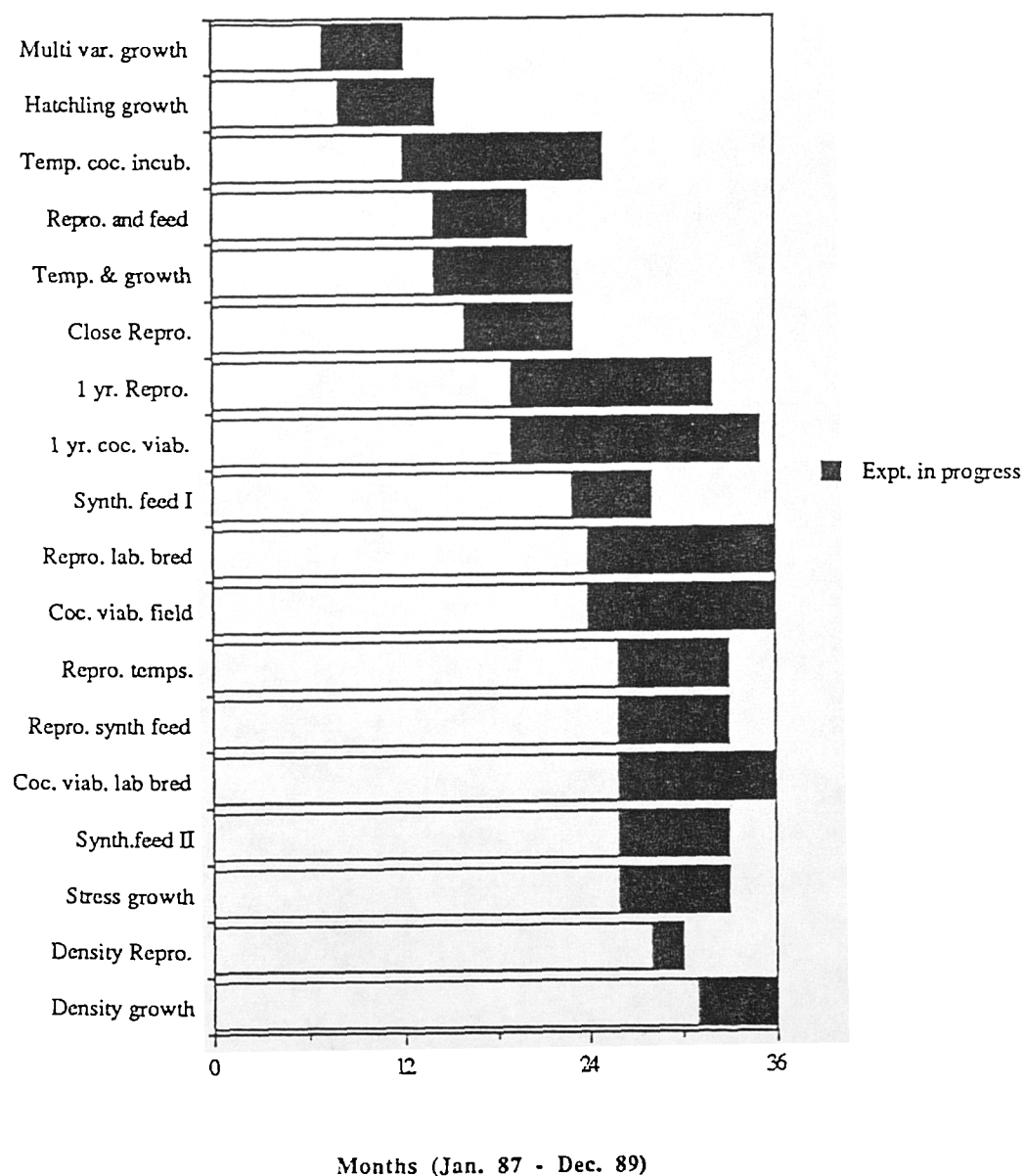
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Appendix 1. Development of experimental work.

This chart illustrates the sequence and duration of the experiments reported in this thesis.



APPENDIX 2: EARTHWORMS AND MICRO-ORGANISMS.

It is generally agreed that the species of micro-organisms found in the soil/organic material in which an earthworm lives are similar to the species found in the earthworm gut. This is not surprising as earthworms ingest large quantities of material from their environment. For example, on comparing the micro-organisms present in the alimentary canal and in the castings of *L. terrestris*, Bassalik (1913) found over fifty species but these were common to both sources. Similar results have since been obtained by Parle (1963) and Pearce (1978). According to Edwards & Fletcher (1988) this is positive circumstantial evidence that earthworms depend upon micro-organisms for food. If micro-organisms were used to digest food in some symbiotic relationship, then the gut species would be likely to differ from those in the surrounding soil.

Many authors report an increase in the numbers of micro-organisms during their passage through the earthworm gut. Parle (1963) reported increases up to one thousand fold of actinomycetes and bacteria in the digestive tracts of *L. terrestris*, *A. caliginosa* and *A. longa*. Supplementary evidence comes from reports of increased numbers of micro-organisms in earthworm casts, which the worms must have consumed, compared to the surrounding soil (e.g. Atlavinyte *et al* 1971). However, not all micro-organisms increase in number during passage through the earthworm gut, Parle (1963) found that yeast and fungi did not proliferate. Dash *et al* (1979) reported on the digestion of various microfungi species in the gut of the tropical earthworm *Drawida calebi*. Species that produced antibiotics, such as *Penicillium* sp. and *Aspergillus* spp., or possessed thick walled spores were not digested.

Conclusive experimental evidence relating to earthworms feeding on micro-organisms is limited. That which exists can be divided into two categories, simple experiments feeding inoculated discs to worms and more sophisticated experiments under more carefully controlled conditions.

Wright (1972) fed *L. terrestris* on discs of apple leaves and found that they consumed 35% more of the treatment inoculated with the bacterium *Pseudomonas aeruginosa* than uninoculated leaf discs. However, similar experiments using filter paper discs, by Cooke & Luxton (1980), produced conflicting results. *L. terrestris* did not feed preferentially on bacteria-inoculated discs, but when the bacteria inoculation was substituted by two species

of fungi (*Mucorhiemalis* and *Penicillium* sp.) the consumption of inoculated discs was greater than that of uninoculated discs. Later experiments (Cooke 1983), found that *L. terrestris* preferred certain species of fungi over others. Pearce (1978) confirmed that earthworms were attracted to fungi, he found that a significant component of the food of six lumbricid species were fungi and algae.

Morgan (1988) suggests that many of the experiments reported in the scientific literature relating to earthworms feeding on micro-organisms must be viewed with caution. Mixed populations of micro-organisms are often, if not always, present on materials offered to earthworms in feeding experiments. If an inoculation of a particular species of micro-organisms is then added, it will initially swamp the resident populations, but within a short time a state of equilibrium will be reached between the resident and the introduced species. Therefore any results attributed directly to the addition of a single species may be misleading as they will relate to a mixed population of microflora. To overcome this, Morgan (1988) used worms which had been treated with antibiotics to make them externally and internally microbe-free, as well as experimenting in sterile conditions. Her aim was to establish if the earthworm *Eisenia fetida* depended on the microflora in organic wastes for nutrition. This was done by adding worms to cattle waste to assess the effects on the component populations of the resident microflora, and by assessing the growth of worms fed on simple nutrients and pure cell cultures of micro-organisms in natural and synthetic environments. Results showed that by adding worms a reduction in the bacterial and fungal populations resulted. This could have been due to their consumption by the worms or to competition between them for resources. Rapid worm growth correlated with periods of high earthworm numbers, but this was only under conditions of high worm densities in undrained, unfed dishes. Addition of simple nutrients to cattle waste resulted in weight increases greater than in non-supplemented controls. Worms showed greatest increases in the presence of carbohydrates (< 0.1% w/w) which suggested that either worms can utilise carbohydrates or that the presence of carbohydrates stimulates other organisms on which the worms feed. Other less successful nutrients tested including liver digest and yeast extract.

Experiments with pure cultures of micro-organisms, isolated from cattle waste, showed weight increases in the presence of four species of fungi but weight loss and death with other organisms. Overall these experiments suggest that *E. fetida* can utilise both micro-

organisms and simple nutrients (directly or indirectly) for growth, but more work is required. Direct evidence for *L.terrestris* is even less well documented.

APPENDIX 3 : DETAILS OF SOIL ANALYSIS.

Top soil obtained from, D.A.Bird, Camp Hill, Bugbrooke, Northampton. Before use the soil was passed, through a 12mm sieve to remove larger particles, and then sterilised by heating to at least 70°C in an electrically powered soil steriliser.

Identification and hand texturing.(ADAS)

The soil was identified in accordance with the ADAS leaflet (895), "Soil Texture" (MAFF 1984) It was predominantly rough and gritty, stained fingers and was difficult to roll into a ball. It was therefore a loamy sand with 50 - 70% sand and 15% clay.

Kjeldahl-Nitrogen.	Total Carbon.
2.24g./Kg.	3.1 %
Extractable Potassium.	Extractable Phosphorus.
550-600 mg/l.	3.65 mg/l.
Total Ash Content.	pH
881 g/Kg.	7.5

The type of soil used in these experiments had to satisfy several criteria. It had to;

- (a) be acceptable to *L.terrestris*,
- (b) be free from *L.terrestris* or any other earthworm material,
- (c) be free from predators or parasites of *L.terrestris*,
- (d) contain low amounts of organic material,
- (e) have a reasonably constant composition.

From the analyses, (a) was satisfied as Lofty (1974) states that *L.terrestris* is most abundant in England in light loamy soils.

Both (b) and (c) were achieved by steam sterilisation of the soil before use and (d) was acceptable, see above. Bulk purchase and thorough mixing before use, ensured (e).

It is clear that *L.terrestris*, unlike some Lumbricids, needs more than an organic substrate in which to survive. Hartenstein & Amico (1983) suggest that *L.terrestris* requires a high ratio of mineral soil to organic matter in its diet in order to extract nutrients efficiently.

Appendix 4. Feed material properties (chemical and physical).

Waste	Separated cattle solids	Sewage sludge	Paper waste(Kent)	Wheat Straw	Paper waste(Hemel)
% Total Solids	27.1	25.5	19.6	91.7	25.1
Vol. Solids (% of T.S.)	66.5	74.4	78.0	91.1	55.8
Neutral Detergent Fibre (%)	72.4	63.8	84.1	81.6	84.8
Neut. Det. Sol. Mat (%)	26.6	36.2	15.9	18.4	15.2
Acid Detergent Fibre (%)	54.1	32.2	71.2	51.9	79.1
Hemicellulose (NDF-ADF %)	18.3	31.6	11.9	29.7	5.0
Cellulose (%)	15.7	15.5	49.1	40.9	60.2
Lignin (%)	13.4	10.0	12.0	8.6	6.1
Ash (%)	25.0	6.7	10.2	2.4	12.3
Total Kjel. Nitrogen (%)	2.0	2.7	0.5	0.9	0.2
Crude Protein (TKN x 6.25)	12.5	16.9	3.1	5.6	1.3
% Oxidisable Organic C.	23.7	30.0	35.1	37.5	21.0
Total Carbon (OOC x 1.33)	31.5	39.9	46.7	49.9	27.9
C:N ratio	16:1	15:1	93:1	55:1	140:1
pH	9.1	5.8	7.1	7.5	7.3

APPENDIX 5: RESPIROMETRY NOTES.

Recording of microbial activity associated with the waste materials used as feeds was achieved using an automatic electrolytic respirometry system. This system was obtained from Maynard Projects of Cambridge. (Tribe & Maynard 1989).

The respirometer allows continuous measurement and automatic recording of oxygen uptake by micro-organisms from adequately replicated "soil" samples of moderate size over long periods of time. Records can be made for indefinite periods of time at intervals of from minutes to days. The volume of the standard respirometry measure 50ml in volume.

A volume of soil is enclosed with a volume of air in a sample unit, together with a vessel of sodium hydroxide, and the unit is connected, through a U-tube containing acid copper sulphate electrolyte, to a compensator unit of approximately equal air volume. This system, the respirometry unit, should be maintained at constant temperature. A platinum anode wire is placed just above the surface of the electrolyte in the sample limb of the U-Tube, and a copper cathode is submerged in the electrolyte. As the soil organisms respire, carbon dioxide is evolved and collected in the sodium hydroxide solution. Oxygen is taken up and causes a reduction in pressure in the air of the sample unit, and the air in the compensator unit pushes the electrolyte up the sample limb into contact with the anode. A current flows, oxygen is generated at the anode and copper is deposited on the cathode. When pressure is again equalised, contact is broken at the anode and the current ceases to flow. The cycle repeats as respiration continues. The current is measured by a four channel multiplexed ammeter inside an electronic control unit and recorded by a BBC Microcomputer with its associated hardware. Carbon dioxide collected in the alkali may be determined by titration at selected intervals, thus enabling the calculation of respiratory quotients.

Examples of computer print-outs from the respirometer are provided as Figures 5.3.1.2 and 7.3.3.3. The information on them is explained within the relevant text.

APPENDIX 6: DESCRIPTION OF ANALYTICAL PROCEDURES.

The MAFF book *The Analysis of Agricultural Materials* (1981), was used as a reference for the determination of; Kjeldahl-nitrogen, pH, extractable phosphorus and potassium content. A modified Walkley-Black method was followed for the determination of Oxidisable organic carbon, as described by Hesse (1971). The van Soest method was followed for fibre analysis as described in Goering & van Soest (1970), but slight modifications were made (Knight 1987).

Forage Fibre Analysis.

All samples referred to were oven-dried and ground to pass a 1mm mesh.

1. Neutral Detergent Fibre.(NDF)

- i. 100ml of 30% (30g/l) Sodium lauryl (dodecyl) sulphate solution. Buffered to a pH of 7.0. (Not found to be necessary). Two-three drops of silicone antifoaming agent.
- ii. 0.5g sample boiled for one hour in 250ml round-bottomed flask fitted with a reflux condenser. (Setting 6 after coming to the boil).
- iii. Filtered through a tared, sintered glass crucible using a vacuum pump. Rinsed twice with hot (90-100°C) water, then twice with acetone, breaking up the fibrous mat with a glass rod. Oven-dry for eight hours or overnight.
- iv. Weigh crucible after cooling in a desiccator.
- v. Ash residue in the crucible for three hours at 500°C in furnace. Cool in desiccator. Reweigh.
- vi. Report findings of recovered NDF as % of sample, = cell wall constituents (hemicellulose, cellulose and lignin).
- vii. Estimate cell soluble material by subtracting this value from 100. Ash content = ash insoluble in neutral-detergent.

2. Acid Detergent Fibre. (ADF)

- i. 100ml of sulphuric acid-cetyltrimethylammonium bromide (CTAB) solution and two-three drops of octan-2-ol in 250ml flask. CTAB sol. made by dissolving 28ml of conc. sulphuric acid in 1 litre of water = 0.5 M. Then dissolving 10g of CTAB in this. No filtering necessary.
- ii. Reflux, as above, for 1 hour. Octan-2-ol prevents excessive foaming.
- iii. Filter through a tared, sintered glass crucible, as above, using a vacuum pump. Rinse twice with hot (90-100°C) water, then twice with acetone, breaking up the fibrous mat with a glass rod. Oven-dry for eight hours or overnight.
- iv. Weigh when cool, then calculate ADF.
- v. Retain the contents of the crucible for further analyses.

3. Cellulose.

- i. Determined by treating the residue from above (ADF) with 72% sulphuric acid. Cover the contents of the crucible with cooled (15°C) H_2SO_4 and stir with a glass rod to a smooth paste, breaking all lumps. Fill crucible about half full and stir. Refill with acid at hourly intervals as acid drains away. (A 50ml beaker is a useful drainage vessel). Crucibles do not need to be kept full at all times. Three additions suffice.
- ii. After three hours, filter off as much acid as possible with vacuum, then wash contents with hot water until free from acid. Take care to rinse stirring rod.
- iii. Oven-dry crucible and weigh. Loss is an estimate of cellulose content.
- iv. Retain residue.

4. Lignin and ash (silica).

- i. Ignite crucible, from above, in a muffle furnace at 500°C for three hours. Allow to cool, then reweigh.
- ii. Loss is an estimate of acid detergent lignin content. Residue is acid insoluble ash (mainly silica).

APPENDIX 7 : GROWTH OF HATCHLINGS WITH SCS AND SCS DETAILS.

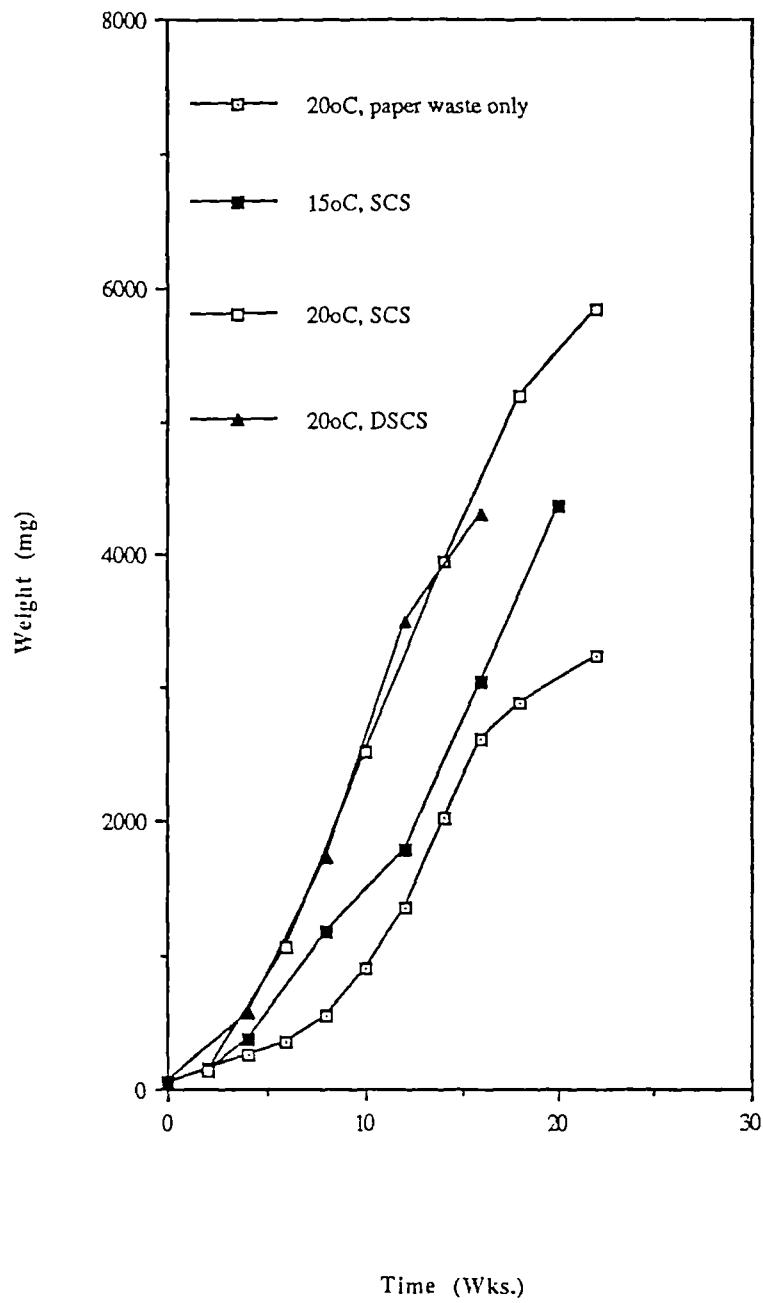
Growth of hatchlings with SCS.

The following figure (p.140) depicts growth curves for hatchlings fed with separated cattle solids (SCS) at two temperatures, 15 & 20°C, dried SCS (DSCS) at 20°C, plus the best growth obtained with paper waste only from Figure 7.3.1.1. All three growth rates with cattle solids were greater than growth with paper waste alone. With SCS an increase from 15 to 20°C led to a greater rate of growth, but the attainment of sexual maturity occurred no sooner at the higher temperature. Drying of the solids did not lead to a greater growth rate, but did increase survival compared with the fresh SCS which had a high ammonia content (section 4.2.2). Although growth rate is much greater with SCS as a feed, compared with paper waste, the mortality of hatchlings previously reported was confirmed (Figure 7.3.1.2). Growth rates with paper waste and yeast extract were greater than any of the cattle solid feeds.

Discussion.

With SCS growth rate was better than that for paper waste only as feed, up to $50\text{mg}\cdot\text{g}^{-1}\text{day}^{-1}$. The survival of hatchlings with SCS was poor. Increased survival would be expected for laboratory reared worms compared to naturally existing populations, due to removal of environmental pressures liable to lead to mortality. The use of SCS did not support this as many of the hatchlings were rapidly killed in the relatively confined conditions of growth, most likely by the high ammonia levels. The figure of 20% mortality after 120 days, predicted by the model of Lakhani & Satchell (1970) for natural populations, was greatly exceeded by the 50% mortality of SCS-fed worms over a similar time (figure 7.3.1.2). Conversely, with paper waste as feed and in controlled conditions, mortality over a similar period from hatching, below 20°C, was reduced to 0 - 10%.

HATCHLING GROWTH WITH SCS AND OTHER SELECTED FEEDS.



SCS DETAILS

With the increase in housed cattle systems, collection of manure as slurry without the use of bedding material has become more popular (MAFF 1987). This has the disadvantage that the waste product cannot be stacked as is the tradition with farmyard manure. In its untreated form cattle slurry presents some problems to the farmer; the large semi-liquid volume must be retained to prevent water pollution, and the resulting smell following field application may be a nuisance, (Fieldson 1988). However, by separation, the farmer can be provided with two potentially useful products. The solids can be treated by the action of worms such as *E.fetida*, and used profitably as a base for potting compost when mixed with peat. The liquid fraction can be more cheaply applied to the fields or carried away by tanker in the absence of the potential blockages caused by the fibrous solids. On one farm (Bore Place) at Edenbridge, Kent, the liquid fraction is also sold in bulk as a seed carrying agent for propagation on motorway embankments and in smaller quantities to garden centres as an organic fertilizer.

Raw slurry, unlike SCS is reported to be harmful to some earthworms, including *Lumbricus terrestris* and *Aporrectodea longa*, when applied to fields (Andersen 1980). It fills their burrows and either causes drowning or drives them to the surface, where they are easily preyed upon by birds.