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STUDIES IN THE GENERAL ECOLOGY, PHYSIOLOGY AND BIOENERGETICS OF WOODLAND LUMBRICIDAE

by P.J. Bolton B.Sc. (Hons) (Dunelm)

in the Department of Zoology, University of Durham,

being a thesis presented in candidature for

the degree of Doctor of Philosophy.

Durham

May, 1969

- 3 OCT 1969

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CONTENTS

	Page
Acknowledgments	ii
Table of Contents	iii
General Introduction	1
PART I	
I The Field Study Site	
1. General site description	6
2. The grid area	8
3. Vegetation	
(i) Trees and shrubs	9
(ii) Ground vegetation	10
4. Soil structure	13
II Lumbricidae of the Field Study Site	
1. Identification and general taxonomy	16
2. Species list	20
3. General ecology of the Lumbricidae at Wynyard	
(i) Introduction	22
(ii) Habitat preferences and niche relationships	23
III Physical Factors of the Soil Environment	
1. Water table and soil moisture content	38
2. Temperature and 'seasons'	44
3. Soil pH	55
PART II	
I The Population Survey	
1. Sampling technique	

Page

(i) Lumbricid extraction methods - a short review 62

(ii) The sampling method used in the present study 65

(iii) Treatment of samples and specimens 70

2. Results of the population survey

(i) Allolobophora rosea (Sav.) f. typica 73

(ii) The total lumbricid population 90

II Secondary Production of Allolobophora rosea (Sav.) f. typica

1. Growth studies in the field

(i) Growth of individual animals 107

(ii) Population tissue growth 121

2. Reproduction studies in the field

(i) Rate of cocoon production by individual animals 127

(ii) Cocoon standing crop 139

3. Excretion and exudation

(i) Excretory products 141

(ii) Mucus production 143

4. Survivorship, mortality and population change

(i) Population survivorship and mortality 146

(ii) Change in population standing crop 150

III Studies in the Feeding Biology and Faecal Output of Allolobophora rosea (Sav.) f. typica in relation to other Lumbricidae

Page

1. Burrow formation and feeding 157

2. Food material

(i) General introduction 168

(ii) Soil preference of Allolobophora rosea (Sav.) f. typica 172

(iii) Ingesta particle size and composition of gut contents 177

(iv) Deprivation effects on A.rosea adults 185

(v) Selection of Organic matter 189

(vi) The diet of A.rosea - general conclusions 193

3. Faecal output

(i) General introduction 195

(ii) Weight of gut contents 196

(iii) Faeces collection and population egestion 201

(iv) Rate of gut clearance 208

4. Assimilation of soil organic matter by Allolobophora rosea (Sav.) f. typica 216

IV Studies in the Respiratory Metabolism of Lumbricidae found in the Study Area at Wynyard

1. Comparative studies using the Warburg apparatus

(i) Respiratory quotient 219

(ii) Effect of ambient temperature on the rate of oxygen consumption by Allolobophora rosea (Sav.) f. typica 235

2. The respirometer used in the present study for absolute measures of carbon dioxide output by 245

Page

lumbricids

3. Seasonal variation in lumbricid respiratory metabolism

(i) Respiratory measures at 10°C 253

(ii) Field population respiratory metabolism of Allolobophora rosea (Sav.) f. typica at Wynyard 271

(iii) An estimate of the total lumbricid population respiratory metabolism at Wynyard 278

PART III

I Determination of Calorific Values

1. Introduction 284

2. Methods employed in the present study for calorific content determinations

(i) Bomb calorimetry 288

(ii) Differential thermal analysis 289

3. Calorific values

(i) Lumbricid worms and cocoons 293

(ii) Soil and faecal materials 299

4. The oxycalorific coefficient 306

II Population Energy Budgets for Allolobophora rosea (Sav.) f. typica at Wynyard (1966/67)

1. Introduction 309

2. Calorific components of the population energy budgets 311

Page

3. Assimilation and secondary production efficiencies 314

4. Discussion 316

PART IV

General Discussion 322

Summary of Results 329

Appendices 1 to 6

References

GENERAL INTRODUCTION

Studies on the ecology of lumbricids have mainly been concerned with the effects of this invertebrate group on the formation and fertility of agricultural soils. This voluminous literature was excellently reviewed by Satchell (1958); other reviews on the subject have been given by Stephenson (1930), Barley (1961), Tembe and Dubash (1961) and Kevan (1962) - to name but a few. Satchell (1960) summarises by stating that lumbricid activity increases the number of water-stable aggregates, total soil pore space and capacity, soil aeration and the stability of soil structure as a whole. Lumbricids increase soil base-exchange capacity and enrich its nutrient status by re-cycling the materials drawn from the topsoil by plants and leached out by precipitation. Hopp and Slater (1948, 1949) demonstrated the beneficial effects on plant growth of including lumbricids in the culture soil. Evans and Guild (1947a, 1948a) indicated the deleterious effects of arable farming on lumbricid populations by the lowering of soil temperatures and moisture content. The beneficial effects of lumbricids in promoting soil fertility are no longer in question and worms have in fact been marketed for this purpose. However, it is also generally acknowledged that terrestrial plants can grow successfully in soils completely

lacking in lumbricid worms. Soil fertility is a function of the combined activities of the soil biotic community. Although primary production can be enhanced by mechanical tillage and the addition of fertilizers, there is an increasing conviction amongst soil biologists that world problems of food supply will only be solved by the application of techniques derived from a better understanding of the functional relationships occurring in soil, and other, biotic communities. One of the most basic functional relationships in biotic communities - and one which is directly related to human problems of efficient food utilization - is the pattern of energy flow through community food webs. The present studies are concerned with this aspect of soil community structure.

Hutchinson and Kamel (1956) showed that sterile soil was more rapidly colonised by an important group of decomposers (soil fungi) in the presence of lumbricids; Barley (from Satchell, 1960) showed that the introduction of lumbricids increased total soil oxygen consumption by twice the amount consumed by the worms themselves; Byzova (1965, 1966) showed that lumbricid species with known differences in mode of life and habitat preference had different rates of metabolism and different metabolic responses to changing environmental conditions. There is a paucity of knowledge on the detailed ecological relationships of individual lumbricid species and an even greater

lack of information on lumbricid metabolism. The examples quoted above illustrate the importance and inseparability of general lumbricid ecology and physiology in the analysis of soil metabolic activity. These factors were therefore necessarily studied in combination with lumbricid ecological energetics, the primary object of the present investigations. Satchell (1958), in concluding his review, stated that 'ecological studies have reached the stage where physiological research into earthworm (lumbricid) metabolism .... is needed. An integration of future ecological and physiological research is essential for an understanding of the relationship between earthworms and soil fertility'.

The study of energy flow through natural communities obviously entails measurements in situations where civilised mankind has exerted minimal interference. Such situations are becoming extremely rare in Britain though the site used in the present studies (see p.7) may be considered a close approximation to the ideal requirements. These studies of lumbricid general ecology, physiology and ecological energetics were undertaken as part of a general investigation - by myself and my associates - into energy flow relations of the decomposer food web in soils and litter of a deciduous woodland. As Murchie (1958) points out, the role of lumbricids in forest soils is poorly understood and the results of studies in agricultural soils are often of limited applicability to forest conditions.

The field study site and major factors of the soil physical environment were studied in detail. The observed habitat preferences and niche relationships of lumbricid species in the study area were simultaneously assessed in relation to previous information for other situations. A soil sampling tool was devised which allowed quantitative estimation of the maximum proportion of the lumbricid population. The total population estimates were subdivided into species components.

Growth, reproduction and apparent mortality aspects of secondary production by the dominant topsoil lumbricid species were quantitavely assessed for the field population. The feeding biology of the same species was investigated in relation to similar considerations for other lumbricid species. The respiratory metabolism of four lumbricid species was studied in relation to ratios of gaseous exchange and the rate of gaseous carbon dioxide output under simulated field conditions. A new respirometer was devised for the purpose of determining the latter. All the above estimates were made at intervals throughout the year. Oxygen consumption rate was studied, in the dominant topsoil species, as a function of acclimatisation and ambient temperatures. Annual field population respiratory metabolism was computed for this species - and approximately estimated for the total lumbricid population (defined above).

Information drawn from the results of calorific content

determinations, performed during these studies, allowed the construction of population energy budgets for the dominant topsoil lumbricid species. Efficiencies of food utilization were also estimated for this species.

After substantiating a general relation between logarithmic rates of respiratory metabolism and secondary production in poikilotherms, the function was used to approximately estimate annual secondary production by the total lumbricid population (defined above) in the study area.

The thesis is concluded with a general discussion and a summary of the results obtained during these studies.

PART I

I The Field Study Site

1. General Site Description

The site chosen for this investigation was a forestry-managed belt of alder/birch woodland, Normal National Grid Reference NZ 420480, at Wynyard, near Sedgefield, Co. Durham. The site was known locally as the Newton Hanzard and was situated on the section of the Londonderry estate which was preserved and developed jointly by the Forestry Commission and the gamekeepers employed by Lord Londonderry. The area was 68 metres above sea level and flat except for a steep-side valley which meandered along the course of a small stream. The stream did not pass close to the area chosen for study. The study area, approximately 120 x 180 metres in extent, was bordered on the eastern and southern sides by a ride and sparse birch woodland with areas of alder; the northern side was bounded by a young birch plantation, whilst the western boundary was formed by arable and pasture land.

The majority of the birch trees of the study area were between twenty and fifty years old, though one tree - dead at the time of examination - was found to have been 120 years old (Hughes, pers. comm.). Three generations of naturally regenerating alder were present in the area, though thinning and coppicing of alder was thought to have occurred

at some early stage - in contrast to the completely natural status of the birch woodland development. In 'A History of the County Palatine of Durham' (1855) a reference, dated 1600, to the Wynyard region as being 'so fruitfull of soyle, and pleasant of situation, soe beautified and adorned with woods and groves, as noe lands in that part of the countrie be comparable to them' indicates that the area has been wooded for some considerable time. Management by the Forestry Commission since 1953 did not involve any interference with the vegetation of the study area, though the region had been beaten for game shooting purposes for an unknown period.

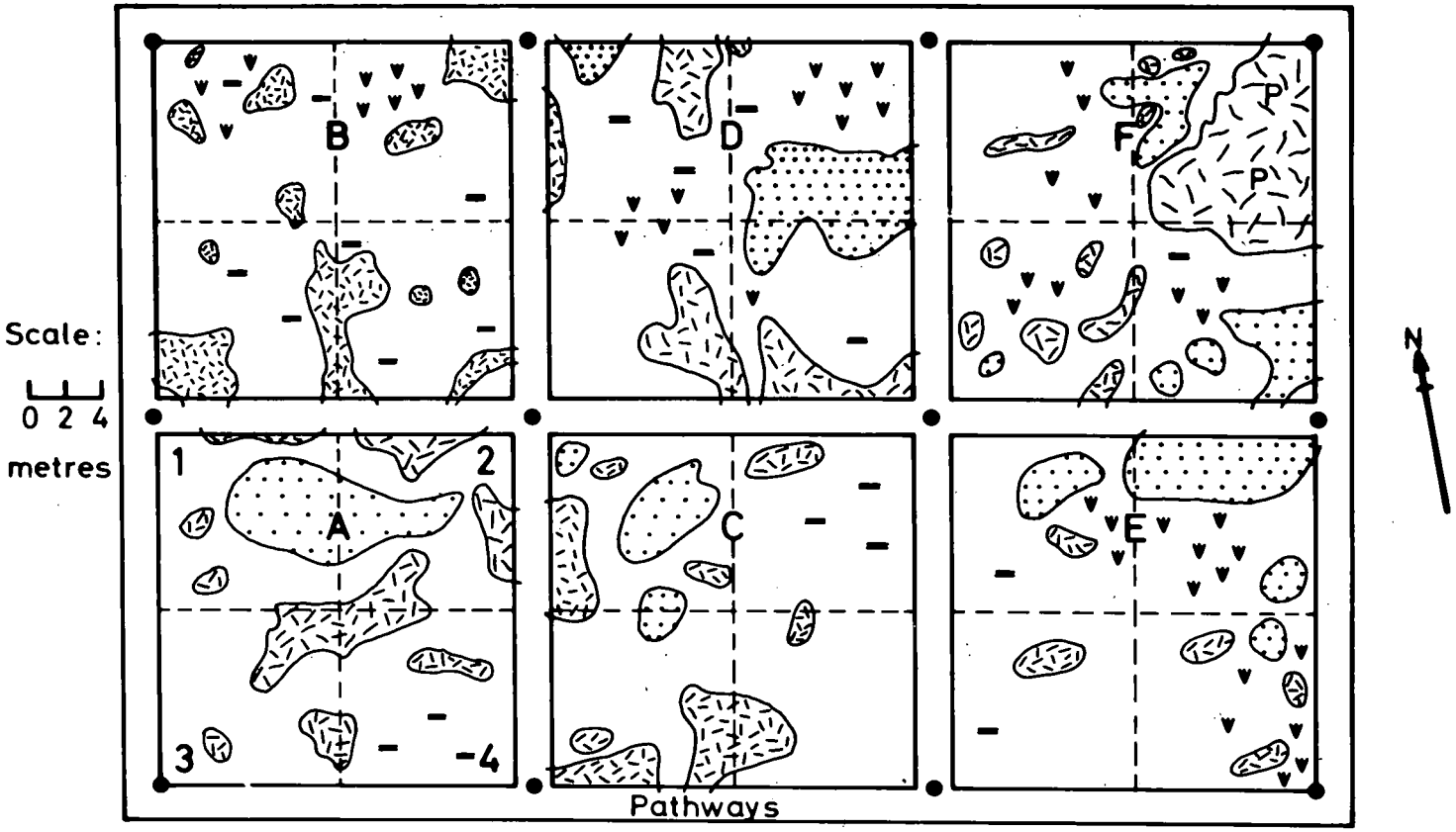
The climate of the region was of the typical low altitude temperature type with minimum air temperatures in winter of approximately 0 to 5^{o}C and maximum summer temperatures of about 20 to 25^{o}C. Precipitation was moderately high throughout the year. By the classification of Taylor and Pohlen (1962), the study area was 'imperfectly or somewhat poorly drained.'

2. The Grid Area

Preliminary sampling within the mixed deciduous woodland indicated an irregular distribution of a variety of Lumbricidae, presumably due to the complex pattern of soil and vegetational types within the ecosystem. Since there were no obvious habitat boundaries, it was decided to delineate the populations for study by means of an arbitrarily defined grid area. A location was chosen well within the woodland belt, to avoid perimeter effects due to the access road and surrounding areas of arable land, low moor-type grassland and the young birch plantation.

Using a prismatic compass, a metre tape and marker stakes the grid area as marked out in the form shown in Fig. 1. The grid consisted of six 20 x 20 metre squares - A, B, C, D, E and F in Fig. 1 - separated from each other and the surrounding areas by pathways two metres in width. The pathways allowed maximum access to the six sampling squares with minimum habitat disturbance. Each of the six squares was divided into four 10 x 10 metre quadrats (numbered 1, 2, 3 and 4 as shown for square A in Fig. 1) for sampling purposes.

Fig. 1. The Newton Hanzard Grid Area. (For explanation see text)



Key: = Ferns dominant; = Bracken; = Bramble dominant;

= Grasses dominant; = Bramble and Ferns equally mixed.

Unmarked areas: Mixed vegetation, mainly Bramble. ● = Water Table borehole

3. Vegetation

A plant species list for the Newton Hanzard grid area is given in appendix 1. Common names are given with this list to which reference may be made to determine the systematic names for species mentioned by common name in the course of this work.

(i) Trees and Shrubs

Appendix 1 includes a species list of all trees and shrubs present in the grid area but, apart from the dominant alder and birch, only the occasional dog rose and sycamore attained sufficient size to affect the adjacent litter and soil formation. For simplicity, the positions of the individual trees are not shown on the vegetation map given in Fig. 1. The area was densely wooded, with trees spaced at approximately 0.5 to 3 metres apart. There were two major cover zones: one dominated by alder, a zone which included the whole of square A, the most southerly parts of B3 and B4, the whole of C1, C3 and C4, part of C2 and parts of E1, E3 and E4; the remaining area - north of the line which passes through B3, B4, C2, E1, E3 and E4 - formed the zone dominated by birch. Dominance was here assessed according to amount of leaf cover and the consequent effects on ground vegetation, rather than by numbers of trees, since the broader-leafed alder tree was found to have a more pronounced effect on ground vegetation and soil type than a birch tree of

similar age.

(ii) Ground Vegetation

For the purposes of this study the ground vegetation was split into three major categories; these were named, according to the dominant vegetational type in each: 'bramble', 'ferns' and 'grasses'. The species composition of these arbitrary categories was seen to differ in the two major tree cover zones. Beneath alder the 'bramble' ground layer consisted of a luxuriant growth of mosses and leafy liverworts, with sparse occurrence of grasses - mainly creeping soft-grass and rough-stalked meadow-grass. Beneath birch the 'bramble' ground layer was dominated by grasses characteristic of the birch zone: mainly tufted hair-grass and common bent-grass.

The 'ferns' beneath alder were exclusively male fern and soft shield-fern, having - with the exception of the limited areas of particularly high fern density - a rich ground layer composed of various forbs and mosses. Beneath the birch the male fern and soft shield-fern had a ground layer mainly composed of birch zone grasses. In F2 the 'ferns' category consisted of an extensive pure stand of bracken.

Whilst tufted hair-grass and common bent-grass occurred occasionally in the alder zone where creeping soft-grass and, to a lesser extent, rough-stalked meadow-grass were common, these two former species - together

with some oat-grass in square B - assumed complete dominance of the 'grasses' category beneath birch. The 'grasses' in the birch zone were much more extensive than beneath alder where individual plants were more dispersed. Fig. 1 shows the spatial distribution of the major vegetation categories within the grid area in November, 1965 - at the commencement of the present study - and Plates 1, 3, 5, and 2, 4, 6, show various regions within the grid area in the summer of 1967 and the winter of 1967/68, respectively, No significant change in the vegetation distribution was observed during the course of this work.

Apart from the areas of complete dominance shown for the 'bramble' category in Fig. 1, the bramble plant itself occurred over most of the grid area - dominance not being attributed in the unmarked areas of Fig. 1 due to the close association and interspersion of other plants, including male fern, soft shield-fern, various grasses and numerous forbs. Rose-bay willow-herb occurred in all the grid squares, but was noted to be particularly common under birch in squares B, E and F. However, the distribution of this species was generally sparse so that soil and litter formation was not appreciably affected. Dog's mercury occurred commonly in E3 and occasionally in C4, to the notable exclusion of bramble. The distribution of dog's mercury is not shown in Fig. 1 since the soil formation associated with this species was indistinguishable

Plate 1. Vegetation in summer (centre of square C, viewed from C4)

Plate 2. Vegetation in winter (centre of square C, viewed from C4)

Plate 3. Vegetation in summer (centre of square D, viewed from D2)

Plate 4. Vegetation in winter (centre of square D, viewed from D2)

Plate 5. Vegetation in summer (centre of square F, viewed from F2)

Plate 6. Vegetation in winter (centre of square F, viewed from F2)

from that under 'bramble'.

4. Soil Structure

Magnesian limestone forms the rock layer beneath the thick deposit of boulder clay which is characteristic of the region. To determine the extent of soil structure variation over the grid area, pits were dug to a depth of approximately one metre to expose the soil profiles beneath major vegational types. Plates 7, 8 and 9 show the soil profiles beneath 'bramble', 'grasses' and bracken, respectively. Although bracken is not extensive over the grid area, it was the only 'ferns' type which was found to affect the soil profile and is included for this reason. The commonest ferns - Male fern and Soft Shield-fern - were usually associated with bramble and even where dominant they were associated with soils of the 'bramble' type.

Using the classification of Taylor and Pohlen (1962), the illustrated profiles may be described as follows:

'Bramble' Soil Profile (Alder Tree Cover)

Layer		Depth	Comments
O_1 or L	:	0 to 0/1.5 cm.;	depth varies according to season. Plant debris.
O_2 (F and H)	:	0/1.5 cm. to 1.5 cm.;	Variable depth, never extensive.
A_1	:	1.5 cm. to 14/16 cm.;	Dark clay loam.
A_2	:	ABSENT	
A_3 and B_1	:	14/16 cm. to 20/22 cm;	Transitional.

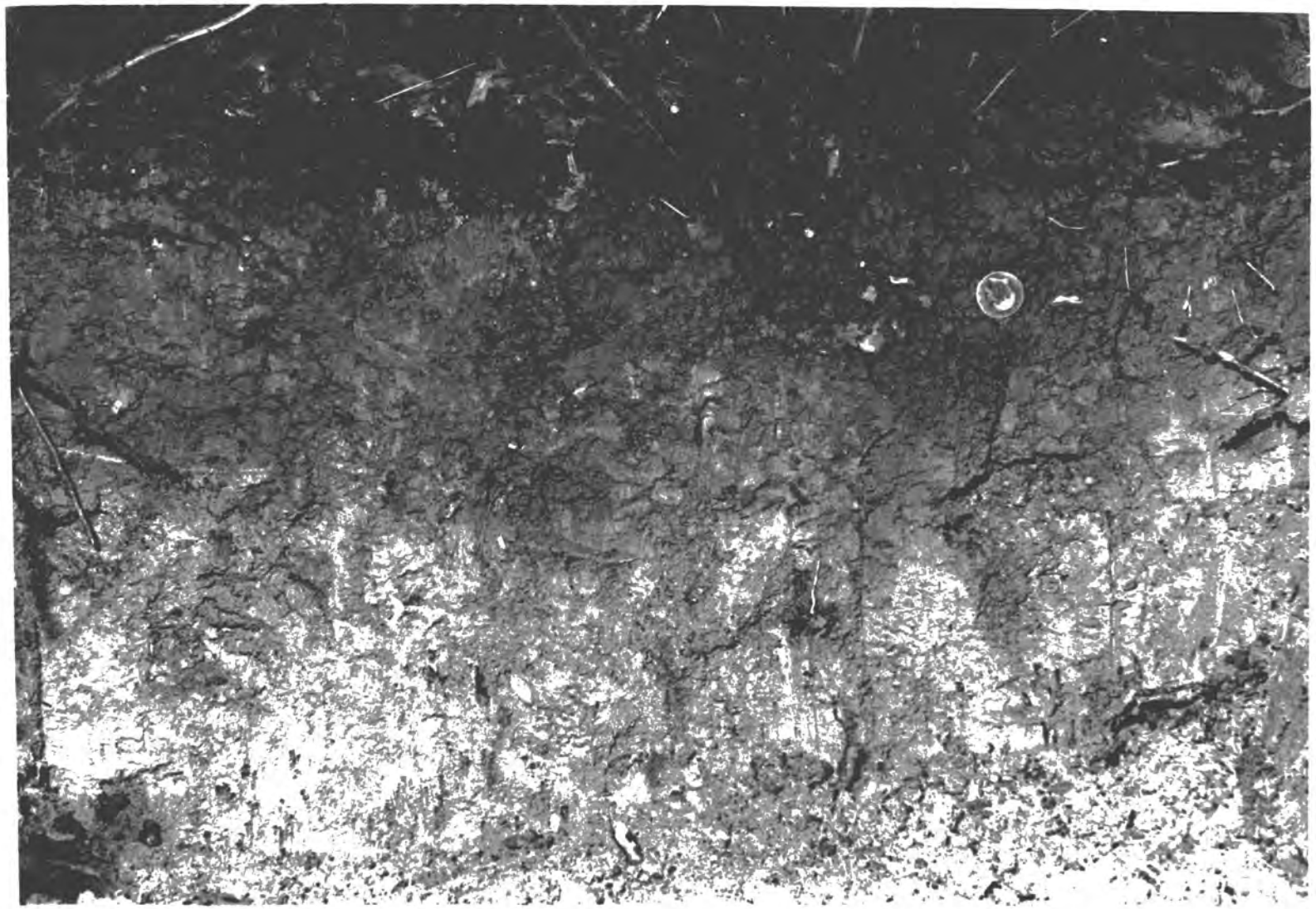


Plate 7. Soil profile beneath 'Bramble'

Plate 8. Soil profile beneath 'Grasses'

Plate 9. Soil profile beneath Bracken

Layer	Depth	Comments
B_2	: 20/22 cm. ;	Gleyed Boulder Clay.

'Grasses' Soil Profile (Birch Tree Cover)

Layer	Depth	Comments
O_1 and O_2	: 0 to 4/7 cm.,	Dead grass material and sparse Birch debris.
A_1	: 4/7 cm. to 14/16 cm.;	Lighter-coloured clay soil.
A_2	: ABSENT	
A_3 and B_1	: 14/16 cm. to 25/30 cm.;	Transitional.
B_2	: 25/30 cm. ;	Heavily gleyed Boulder Clay.

Bracken Soil Profile (Birch Tree Cover)

Layer	Depth	Comments
O_1	: 0 to 4 cm.;	Dead fronds of Bracken.
O_2	: 4 cm. to 9/12 cm.;	Slowly decomposing fronds.
A_1	: ABSENT	
A_2	: ABSENT	
A_3 and B_1	: ABSENT	
B_2	: 9/12 cm. ;	Heavily gleyed Boulder Clay.

Depths were measured from the surface of maximum litter cover.

The soil profile beneath 'bramble' under Birch tree cover was similar to that under Alder, though the A_1 layer was a lighter-coloured soil of higher clay content. Better soil aeration under Birch was indicated by the replacement

of mosses and liverworts (the dominant 'bramble' ground layer) by various grasses.

II Lumbricidae of the Field Study Site

1. Identification and general taxonomy

Mature specimens, preserved in 4% formalin, were identified with the aid of the new edition of the Linnean Society key to the Lumbricidae (Gerard, 1964). The nomenclature and spelling of names found in this key have been used in the present work. Gerard incorporated many changes, based on recent advances in lumbricid taxonomy, into the new edition but the most significant in relation to the present study is the transfer of Eisenia rosea (Savigny) f. typica to the genus Allolobophora (Muldal, 1952a; Omodeo, 1956). Allolobophora rosea was the most abundant species found in the grid area.

The Lumbricidae are said to comprise 220 species, of which 201 are endemic and 19 cosmopolitan (Muldal, 1952a). This is probably a highly conservative estimate to judge by the situation in southern France where a considerable number of species have yet to be described (Bouché, pers. comm.). The Lumbricidae of the British Isles, impoverished by periods of glaciation, include 27 recorded species (Gerard, 1964); 16 of these are widely distributed, peregrine species, the remaining 11 species occurring in specialised, restricted habitats though only four species are thought to be endemic. Most of the species found at Wynyard were forms of widespread occurrence throughout the British Isles and quite readily identified. One species however,

Bimastos muldali Omodeo, was a rare form not previously recorded, to the author's knowledge, in the north of England. Apart from the general characteristics given by Gerard for this species, the feature noted by Muldal (Muldal, 1952b) - the grooved ventral surface near the posterior end - was utilised in making the final identification. This species was found in a similar situation to the 'moist clay in forest ground' habitat described by Muldal.

To the lumbricid taxonomist the identification of preserved mature species in order to compile a comprehensive species list, with appropriate notes on the collection site, might be considered sufficient to complete a systematic survey of the Lumbricidae at Wynyard. For an ecologist the taxonomic problems are more complex: identification of immature specimens is vital and for most purposes animals must be identified alive. The identification of living adults can soon be achieved by study of fresh material prior to its preservation and detailed taxonomic investigation. The identification of immature lumbricids has received little attention from taxonomists, the ecologist being required to familiarise himself with the species present in a given area, by examination of adult specimens, and subsequently become aquainted with the immature material by a process of elimination. The usual procedure followed has been

similar to that for adults, i.e. examination of preserved material in conjunction with observations on living animals. In the present study it was found more reliable to concentrate attention on the living animals, division into species being achieved by a combination of:

(a) Comparison of external characters with those of adult animals,

(b) Assessment of the population size of immature 'types' in relation to the estimated abundance of adults,

(c) Isolation of adults of a particular species in laboratory cultures, with examination of immature animals produced by reproduction.

Procedure (b) was of value for only the most abundant species, being complicated by specific reproductive and mortality rates. Procedure (c) provided conclusive evidence for species separation and was essential when considering immature animals of (i) small pigmented species, and (ii) A. rosea and A. caliginosa (Sav.). The laboratory culture vessels used were aluminium tanks (length: 60 cm; width: 38 cm; depth 27 cm.) with a 4 cm. 'lip' and perforated polythene cover to prevent escape. Soil and litter, from the typical habitat for the species under investigation, was cleared of lumbricid worms and cocoons by freezing at -20°C and subsequently placed in the culture ranks to a depth of 10 cm. A small

quantity of soil and litter, from which lumbricid worms and cocoons had been removed by careful hand-sorting, was then placed in the appropriate tank, which was incubated at 10°C for at least two months. Considerable numbers of adult specimens of the required species were then added to the culture medium which was kept at 10°C. The cultures were dampened with de-chlorinated tap water, according to the water content of the soil type in the field, and examined at intervals for the presence of immature lumbricid worms. Litter in the tanks containing pigmented, surface-active species was supplemented at intervals by small quantities of hand-sorted litter from the field.

A key to the immature lumbricids of Moor House Nature Reserve, N.R.80, has been published (Svendsen 1955). Since such 'local' keys may eventually facilitate the production of a general key to the immature British Lumbricidae, an artificial key to the immature lumbricids of Wynyard Alnus/Betula woodland is presented in Appendix 2. This key is based on the external features of living specimens; the use of a hand-lens or binocular microscope may be necessary in some cases.

2. Species list

As will be seen from the quantitative estimates of population size (p.102), the soil and litter of the Alnus/Betula woodland at Wynyard supports a relatively poor lumbricid population in terms of absolute numbers of individuals, when compared with more neutral mull soils. However, a wide variety of species was found to occur in the region, probably due to the complexity of habitats available.

The fourteen species found on or near the field study site were:-

Genus: Allolobophora Eisen, 1874		
	caliginosa (Savigny)	B.M.*
	chlorotica (Savigny)	B.M.(*)
	rosea (Savigny) f. typica	B.M.*
	and f. macedonia	B.M.
Genus: Bimastos Moore, 1893		
	eiseni (Levinsen)	B.M.
	muldali Omodeo	B.M.(*)
Genus: Dendrobaena Eisen, 1874		
	mammalis (Savigny)	(*)
	octaedra (Savigny)	B.M. *
	rubida (Savigny) f. subrubicunda (Eisen)	B.M. *
	and f. tenuis (Eisen)	B.M.

Genus: Eiseniella Michaelsen, 1900

tetraedra (Savigny) f. typica	B.M.	*

Genus: Lumbricus Linnaeus, 1758

castaneus (Savigny)	B.M.	*
rubellus Hoffmeister	B.M.	
terrestris Linnaeus		*

Genus: Octolasion Oerley, 1885

cyaneum (Savigny)	B.M.	*
lacteum (Oerley)		

B.M. = Checked and confirmed by the British Museum (Natural History), London.

* = Occurring commonly or frequently within the grid area.

(*) = Occurring rarely within the grid area.

Typical specimens of five of these species are by request deposited at the British Museum and their B.M. numbers are listed in Appendix 3. These species are:-

Allolobophora rosea (Savigny) f. macedonia (Rosa)

Bimastos eiseni (Levinsen)

Bimastos muldali Omodeo

Dendrobaena rubida (Savigny) f. tenuis (Eisen)

and Octolasion cyaneum (Savigny)

3. General Ecology of the Lumbricidae at Wynyard.

(i) Introduction

The earliest workers in the field of lumbricid ecology tended to ignore the variety of species, with widely differing habits, included in the category 'earthworms'. Even the work of Darwin (1881) is thought to have included observations on species other than *Lumbricus terrestris* L. As shown by the review of Svendsen (1955a), much work was done on the effects of particular physical and chemical factors on single species, usually *L. terrestris* or *Eisenia foetida* (Sav.).

More recently, increasing attention has been given to the habitat preferences and niche relationships of the various species of Lumbricidae. Most work has been done with a view to assessing the effects of lumbricids on soil fertility (reviewed by Satchell, 1958). Bornebusch (1930) recorded the habitat preferences of lumbricids found in woodlands of Denmark; Perel (1964) studied the distribution of Lumbricidae in the 'taiga', 'sub-zone' and 'steppe' forests of the European U.S.S.R. Similar studies have been made on pasture-land soils in S.E. Scotland (Guild, 1951) and on Pennine moorland (Svendsen, 1955a). Satchell (1955a, 1960) has used the experimental approach, in conjunction with field measurements, to investigate the pH tolerance of a number of lumbricid species; Laverack (1960) and Satchell and Lowe (1967) have, by experimental

analysis, done much to elucidate the selective feeding behaviour and related physiology of L. terrestris L. and Allolobophora longa Ude. Gerard (1960) described the level specificity and burrow formation of various lumbricid species living in pasture-land soil, subsequently studying the depth and activity of these species in relation to field measurements of soil temperature and water content (Gerard, 1963, 1967).

Obviously, it is difficult to generalise when considering the results of previous ecological studies, therefore relevant information is incorporated into the following species by species account of the habitat preferences and niche relationships of the eight principal lumbricid species found at Wynyard. In this account the author's observations at Wynyard are initially presented, in note form, followed by information from other sources for the same species in various situations.

(ii) Habitat Preferences and Niche Relationships

a. 'Pigmented' Species

Dendrobaena octaedra (Sav.)

A surface active species, occurring most commonly in the litter and raw humus of mor-type soils under Sycamore and Birch. Occasionally found in the litter and surface loam of mull-type soils under Birch and Alder.

Bornebusch (1930) called D. octaedra 'the most frugal of all European species'; both Guild (1951) and Satchell (1955a) described the species as 'acid tolerant'.

Svendsen (1955a) found it occasionally on all soil types, but typical of the most acid conditions. Perel (1964) found it the commonest lumbricid in natural forests of the 'taiga'.

The active, surface dwelling mode of life adopted by D. octaedra was thought to account for the high respiration rate, with strong dependence on body weight and a marked sensitivity to external oxygen concentration, found in this species (Byzova, 1965, 1966).

Dendrobaena rubida (Sav.) f. subrubicunda (Eisen)

A surface dwelling species found in various media rich in decaying organic matter, and usually with a high water content (see Table 18, &p229). Usually found to penetrate the surface loam of mull-type soils and often seen to aggregate in the decomposing 'litter wads' at the entrance to burrows of L. terrestris.

D. rubida f. subrubicunda was found to aggregate in fresh molehills of almost pure clay; this behaviour may reflect some mineral requirement of this species. In laboratory culture, D. rubida f. subrubicunda only survived in the presence of surface soil or heavily decomposed litter of fine texture.

The habitat of this species at Wynyard was in accordance with the generally acknowledged requirement of a rich organic medium. Satchell (1955a) described D. rubida f. subrubicunda as 'ubiquitous' in acid tolerance.

In addition to its normally terrestrial habit (Gerard, 1964), the species is found in compost heaps and occurs commonly in sewage percolating filters (Solbé, pers. comm.). Roots (1956a) showed that D. rubida f. subrubicunda was one of several normally terrestrial species which could live under water in the laboratory, often for considerable periods (31 to 50 weeks) if soil was present in the medium.

Lumbricus castaneus (Sav).

A surface active species typical of the surface soil and litter of mull-type soils and grassy sward.

L. castaneus was one of the few species found to feed on raw leaf litter in laboratory culture. Arthur (1965) has stated that whilst this species can penetrate the soil to a depth of about 10 cm. the burrows are poorly defined, considered by Arthur to be indicative of the feeding habits of this species which 'does not seem to ingest much soil'. With D. rubida f. subrubicunda, it has been found to aggregate in L. terrestris 'litter wads' and fresh molehills of almost pure clay (see D. rubida f. subrubicunda above).

Pickford (1926) and Bornebusch (1930) regarded L. castaneus as typical of mull soils, though Guild (1951) also found it in acid soils in S.E. Scotland and Satchell (1955a) described it as 'ubiquitous' in acid tolerance. Svendsen (1955a) recorded the species only once, beneath dung on a mull area. Byzova (1965) showed a high respiration rate for L. castaneus, strongly dependent on body weight; Byzova regarded this as typical of a soil-

litter dwelling species showing a high degree of muscular activity.

There seems to be some disagreement concerning the soil preference of this species; however, its occurrence in acid soils at Wynyard would suggest that Satchell's conclusions regarding acid tolerance are correct. The restriction of L. castaneus to mull soil habitats in many situations must be due to some limiting factor other than pH.

Lumbricus terrestris L.

A deep-burrowing species found in both mull-type and mor-type soils at Wynyard. Smaller immature specimens typically found nearer to the soil surface. Larger specimens accumulating 'litter wads', composed of leaves, twigs, buds, faecal material and surface soil in a complex decomposing mass, at the entrance to the permanent burrow.

In lighter coloured clay soils, decomposing organic material could be seen to line the upper region of the near-vertical burrow to a depth of about 15 cm. Where the burrow was occupied by a mature individual, this organic layer was usually found to contain numerous cocoons, suggesting a possible 'provisioning' response in soils of low organic content. The common occurrence at Wynyard of cocoon capsules packed with soil suggests that the hatching worms initially feed in the immediate vicinity of the cocoon, faecal material being deposited within the cocoon

capsule. This habit of lining the upper burrow must also increase the efficiency of mor to mull soil conversion.

L. terrestris is exceptional in being a deep-burrowing species which feeds almost exclusively on material collected from the surface of the soil. There have been many studies on the mode of litter-feeding by this species (the more important papers are reviewed by Satchell, 1958), the classical example being that of Darwin (1881) and the most recent work being that by Satchell and Lowe (1967). Since the time of Hensen (1877) it has been known that at certain times of the year the adults of L. terrestris are capable of burrowing to a depth of over two metres, under suitable soil conditions. It is generally agreed that the species is almost purely terrestrial, though as with L. castaneus there seems to be some confusion over the type of soil inhabited. Pickford (1926) and Bornebusch (1930) recorded L. terrestris in mull soils, Guild (1948) found it most numerous on light loam, and its occurrence in garden, arable and pasture-land soil is widely known - though it is not so common in these situations as was once thought, through confusion with the deep-burrowing, surface-casting species Allolobophora longa Ude and A. nocturna Evans. In contrast, Guild (1951) recorded L. terrestris in acid soils and Satchell (1955a) describes it as 'ubiquitous' in acid tolerance. Cernosvitov and Evans (1947) stated that it showed a preference for clayey soil,

though Gerard (1964) simply indicates it to be 'especially abundant in clay soils'. It must be acknowledged that the specialised mode of life adopted by L. terrestris gives the species a certain independence of soil type. It thus occurs in most soils of suitable depth with sufficient available litter, provided the soil acidity is within the generally accepted extremes for lumbricid tolerance (pH 4 to 7).

b. 'Unpigmented' Species

Allolobophora caliginosa (Sav.)

A soil dwelling species typically found in the mull-type topsoil beneath Bramble. Multidirectional, ramifying burrows formed to a depth of about 15 cm. - as against the 7.6 cm given by Gerard, 1960, for a pasture-land population. Rarely occurring in more clayey soils and never in mor-type soils. Immature specimens occurring nearer to the soil surface.

In some regions at Wynyard, where mull-type soils occur extensively, A. caliginosa was predominant in the loamy topsoil, whilst A.rosea retained dominancy in the restricted areas of more clayey soil. In the study area, where more clayey soils occur extensively, A. rosea was predominant throughout the range of topsoil types. It would therefore seem that although A. rosea inhabited a wide range of soil types, and was the fitter species in more clayey soils, A. caliginosa assumed dominance in the mull-

type soils to which it was restricted, provided they were of sufficient extent. This 'critical area' of mull-type soil possibly reflected a late succession change in mull soil conditions which favoured A. caliginosa. During the early stages of mull soil development there may be an element of direct competition in which the more active A. caliginosa gains advantage (for feeding biology see p.168). The assumption of complete dominance by A. caliginosa would seem more adequately explained by a change in the environment. Pickford (1926), Bornebusch (1930), Guild (1951) and Satchell (1955a) all regarded A. caliginosa as intolerant of acid soils. Variation in pH, and the associated effects on soil flora and fauna, was probably the most important factor affecting A. caliginosa distribution at Wynyard. The lower moisture content of the mull-type soils may have enhanced the success of A. caliginosa in mull soil regions. Roots (1956a) found that in the laboratory A. caliginosa had only a limited capacity for survival under water. A. caliginosa f. trapezoides (Duges) has been shown to actively vacate a submerged soil medium (El-Duweini and Ghabbour, 1965a). It must be noted however that this species is known to occur occasionally in the wet soil of river banks (Gerard, 1964), so that high moisture content of the soil is not in itself a barrier to dispersal.

A. caliginosa is most abundant in garden, agricultural and pasture-land soils. In the Stirling district of central

Scotland, Guild (1948) found it to be most numerous in light loam, and in Pennine moorland conditions Svendsen (1955a) found it to be absent from 'poorer soil'. Studying the distribution of this species in soils of New Zealand, Nielson (1951) showed that the population size of A. caliginosa was directly and positively related to the calcium content of the soil. It would, however, be difficult to generalise from this observation since A. caliginosa was almost the sole lumbricid species occurring in the region studied.

A. caliginosa shows a facultative dormancy (gut emptied, worm coiled within a mucus-lined earthen cell) under soil conditions of high temperature and low moisture content (Evans and Guild, 1947a). This inactive state has unfortunately been described as a 'diapause' by many authors, despite lack of evidence to relate earthworm dormancy to this highly specialised condition. The summer dormancy of A. caliginosa is typical of a grassland population, but it was also shown by a high number of A. caliginosa individuals at Wynyard where the soil moisture content was not drastically reduced during the summer. A. caliginosa trapezoides is dominant in the soils of Egypt (El-Duweini and Ghabbour, 1965b) so that temperature alone does not induce inactivity in this form of the species. A laboratory test was carried out to determine the effect of high soil temperature on A. caliginosa specimens from Wynyard: two sets of twelve individuals

(five small immatures, four large immatures and three adults) were kept in separate glass jars filled with mull soil whose moisture content was maintained at a high level. One jar was placed at 15°C and the other at 10°C; the activity of the worms on the outer surface of the soil was checked daily and the condition of all the specimens was assessed after three weeks when the jars were emptied. None of the worms showed any signs of dormancy after three weeks, suggesting that the summer soil temperatures at Wynyard (12-14°C) are not the primary cause of the observed dormancy. It may be concluded that A. caliginosa is highly sensitive to a fall in soil moisture content and summer dormancy is essentially an aestivatory process. It is well known that the drying out of a laboratory soil medium, even at low temperatures, produces dormancy in A. caliginosa, and as a means of avoiding death through desiccation this behaviour is remarkably effective: the author has observed the survival of two large immature specimens of A. caliginosa after more than four months in dry soil, and A. caliginosa trapezoides has been known to survive up to two months under similar conditions, retreating deeper into the soil prior to dormancy (El-Duweini and Ghabbour, 1965a). This Egyptian form of A. caliginosa is said to be capable of penetrating the soil to a depth of about 60 cm. (El-Duweini and Ghabbour, 1964a).

The low respiratory rate, with little dependence on body weight, found for adult A. caliginosa (Byzova, 1965)

can be considered typical of less active soil dwelling lumbricids. Byzova (1966) has also shown this species to be capable of maintaining a constant respiratory rate in conditions of low oxygen tension; this is thought to be an important metabolic adaptation to the soil dwelling habit (see also A. rosea below).

Allolobophora rosea (Sav.) f. typica

The dominant lumbricid species in the topsoil of the study area. Forming multidirectional, ramifying burrows to a depth of 15 cm. and occasionally as deep as 20 to 25 cm. - as against the 7.6 cm. given by Gerard (1960) for a pasture-land population. Newly-hatched individuals occurring mainly in the top 1 to 3 cm. of the soil. Found in all soil types except the clay 'mor' beneath pure stands of Bracken; most abundant in the 'mull'-type soil beneath Bramble. See above for relations with A. caliginosa in different soil types.

Satchell (1955a) regarded A. rosea as intolerant of acid soils, though his parameter for 'intolerance' was absence from soils of pH less than 4.6. At Wynyard, A. rosea was found to be more tolerant of the acid conditions in clayey and mor-type soils than A. caliginosa. A. rosea was not abundant in the pasture-land soils of central Scotland, though it occurred throughout the range of soil types, being most numerous in light loam and silt-clay soils (Guild, 1948). Guild (1951) regarded A. rosea

as intolerant of acid soils in the pastures of S.E. Scotland. Stephenson (1930) gave the habitat preference for A. rosea as 'under rotting leaves in woods.' Perel (1964) recorded the species as dominant in the western regions of wooded steppe forests in the European U.S.S.R. In addition to its occurrence in the loamy and clay soils of pastures, gardens and woodlands, A. rosea has been frequently found on the banks of rivers and lakes in limnic localities (Gerard, 1964). The species was particularly common amongst the roots of Juncus spp. in the waterlogged soil of a low moor area at Wynyard, and whilst the occurrence of A. rosea in this situation may be primarily due to the availability of nutritious material (see p.177) the waterlogged conditions caused no apparent stress on the individuals concerned. Chugunova (1957) showed that A. rosea could survive up to 460 days in a small layer of stagnant water.

Soil conditions of high temperature and low water content induce a facultative dormancy in grassland populations of this species (Evans and Guild, 1947a). Such a dormancy was not observed in the field populations at Wynyard where soil moisture content remains high throughout the year, though it could be induced in the laboratory by drying out the soil medium, even at low temperatures. It seems probable, therefore, that dormancy in A. rosea is a response to extreme dry soil conditions (c.f. A. caliginosa above). A. rosea at Wynyard showed a winter quiescence in which the animals became lethargic,

showed little or no body growth and had a very low respiratory rate - though feeding was continued. This quiescence was almost certainly a direct response to low winter temperatures.

The inactive soil dwelling habit adopted by this species has been said to account for the low respiratory rate, practically independent of body weight and unaffected by oxygen tensions as low as 10%, found in adult specimens of A. rosea (Byzova, 1965, 1966).

Eiseniella tetraedra (Sav.) f. typica

Occurring in the top 1 to 3 cm. of waterlogged soil; found in the whole range of soil types from mull-type soils to pure clay, provided the water content was sufficiently high. An extremely active species; tail fragments easily, presumably protective in allowing the escape of the individual - regeneration of the tail region was found to be rapid, as in most lumbricid species.

Many authors have regarded E. tetraedra as a purely aquatic species. Pickford (1926) described it as 'limnic' at Wicken Fen, Bornebusch (1930) found the species only in swampy situations and Guild (1951) found it in flushes, though he did record it in some numbers from a damp, but not wet, permanent pasture on a sloping hillside in Perthshire. Svendsen (1955a) also found E. tetraedra in stream beds and flushes, with one record of a specimen found in sheep dung. Perel (1964) described the appearance of this species in the wettest areas of boggy spruce forests

in eastern Europe. Gerard (1964) described the species as 'amphibious' (see also Beddard, 1895, and Stephenson, 1930), occurring in wet soil 'mostly covered with water' as well as in purely aquatic habitats. Reynoldson et al. (1955) recorded E. tetraedra from arable land on Bardsey, near Anglesey, and whilst the species undoubtedly requires a high water content in its surroundings it is not restricted to the aquatic situation. However, it must be noted that the ability of E. tetraedra to live totally submerged amongst the weed of ponds and streams allows this species to penetrate an environment rarely exploited by any other member of the British Lumbricidae (A. chlorotica (Sav.) was found amongst the roots of water-plants, at a depth of 2 metres, up to 20 metres from the shore of Lake Windermere (Cernosvitov, 1945), but the species has never been reported as free-living in an aquatic situation).

Byzova (1965) showed E. tetraedra to have a high respiratory rate, strongly dependent on body weight, typical of a highly active surface dwelling species. The restriction of this small species to the surface layer of the soil would seem to substantiate the theory that smaller worms, including small immatures of larger species, are better adapted for surface or shallow 'crevice' movement, whilst larger worms are capable of deeper, true burrowing activities (Arthur, 1965). There are, however, certain notable exceptions to such a general rule: specimens of Helodrilus oculatus Hoffmeister, together with one specimen

of Bimastos muldali Omodeo, were found at a depth of 2.5 to 4.6 metres in a Roman ditch at Verulamium (Dobson and Satchell, 1956) though it was suggested that the original worms might have entered the ditch in the 1st. century A.D. At Wynyard the rare species Bimastos muldali, though usually occurring near the ground surface, has been found at a depth of 30 to 35 cm. in wet clay.

Octolasion cyaneum (Sav.)

A deep burrowing species commonly occurring in almost pure clay beneath grassy sward, as well as in better soils beneath Bramble and Male Fern. Newly hatched individuals living in the top 2.5 to 5 cm. of soil in all soil types though more common in grassy areas. Larger worms living in clay apparently unaffected by the moisture content of the surroundings, being equally common in dry and almost waterlogged conditions. Burrows well-defined, though temporary and multidirectional; unlined and easily distinguishable, in lighter-coloured clay soils, from the vertical shafts of L. terrestris. Form and content of the gut and faeces suggest feeding to be a process involving the passage of large quantities of the surrounding medium through the intestinal tract with the assimilation of all available organic matter - O. cyaneum is one of the lumbricid species in which both cellulase and chitinase are known to occur in the intestine, especially the anterior portion (Tracey, 1951).

O. cyaneum is a widespread species, found in a wide variety of soil types, though it is usually regarded as occurring locally and never in large numbers (Evans and Guild, 1947a; Guild, 1948, 1951; Gerard, 1964). Bornebusch (1930) recorded it from 'humid mull' and the species is thought to prefer moist conditions, though flooding is known to cause evacuation of burrows - individuals being found crawling on the soil surface after heavy rain. Svendsen (1955a) found O. cyaneum to be common in all but the most peaty moorland soils and Satchell (1955a) regarded it as 'ubiquitous' in acid tolerance. In pasture-land the burrow system of adults and large immatures is usually at a depth of about 15 cm. (Gerard, 1960), but at Wynyard the larger burrow systems extend from 5 cm. below the surface to a depth of 30 to 35 cm., and probably deeper during the winter months.

III Physical Factors of the Soil Environment

1. Water Table and Soil Moisture content.

Introduction

Although the moisture content of the soil on the grid area was known to be high for most of the year, it was considered desirable to have a record of any major spatial or temporal variation through the year and also to assess quantitatively the average soil moisture content. The measurement of major variation would allow comparisons with lumbricid population distribution over the grid area throughout the year. It would also provide supporting evidence for the assessment of arbitrary 'seasons'. The quantification of average soil moisture content was required for the reconstruction of field soil conditions during various laboratory experiments - notably those for the investigation of egestion rates in Allolobophora rosea (Sav.) f. typica.

Methods

'Spot' measurements of the depth of the soil water table were made on or about the 15th day of each month for a period of 15 months as follows: twelve holes of approximately 8 cm. diameter were bored to a depth in excess of 1 metre at the positions shown in Fig. 1. A plastic container was placed in the top of each hole to prevent the entry of wind-blown litter, etc., and the depth of the water table from the soil surface was

measured to the nearest inch (2.5 cm.) using a metre rule.

For a six month period - June to November, 1967 - a porous pot tensiometer was available for this work and measurements of 'bramble' topsoil (0 to 15 cm. depth) moisture tension near various boreholes were made at intervals in conjunction with simultaneous measurements of water table depth. These measurements were made not less than 24 h. after any period of precipitation to avoid spurious results due to temporary increases in topsoil moisture content.

Results

Fig. 2 shows diagrammatically the level of the soil water table over the grid area, orientated as shown for January, 1967, throughout the year. The contour lines, spaced according to the actual depths measured, demarcate areas of similar water table depth at the time of 'spot' measurement. The mean annual water table depth was found to be 12.5 ins (31.6 cm). For most of the year the water table was within 15 ins. (38 cm), and commonly within 10 ins (25 cm), of the soil surface over the whole grid area. However, there was a fall in the level of the water table during the months of June, July, August and September. The increase in water table depth appeared to originate in the region of A1 and B3, and to spread towards the centre of the grid area, affecting the whole of the grid area to a somewhat lesser degree. There was a regression in August

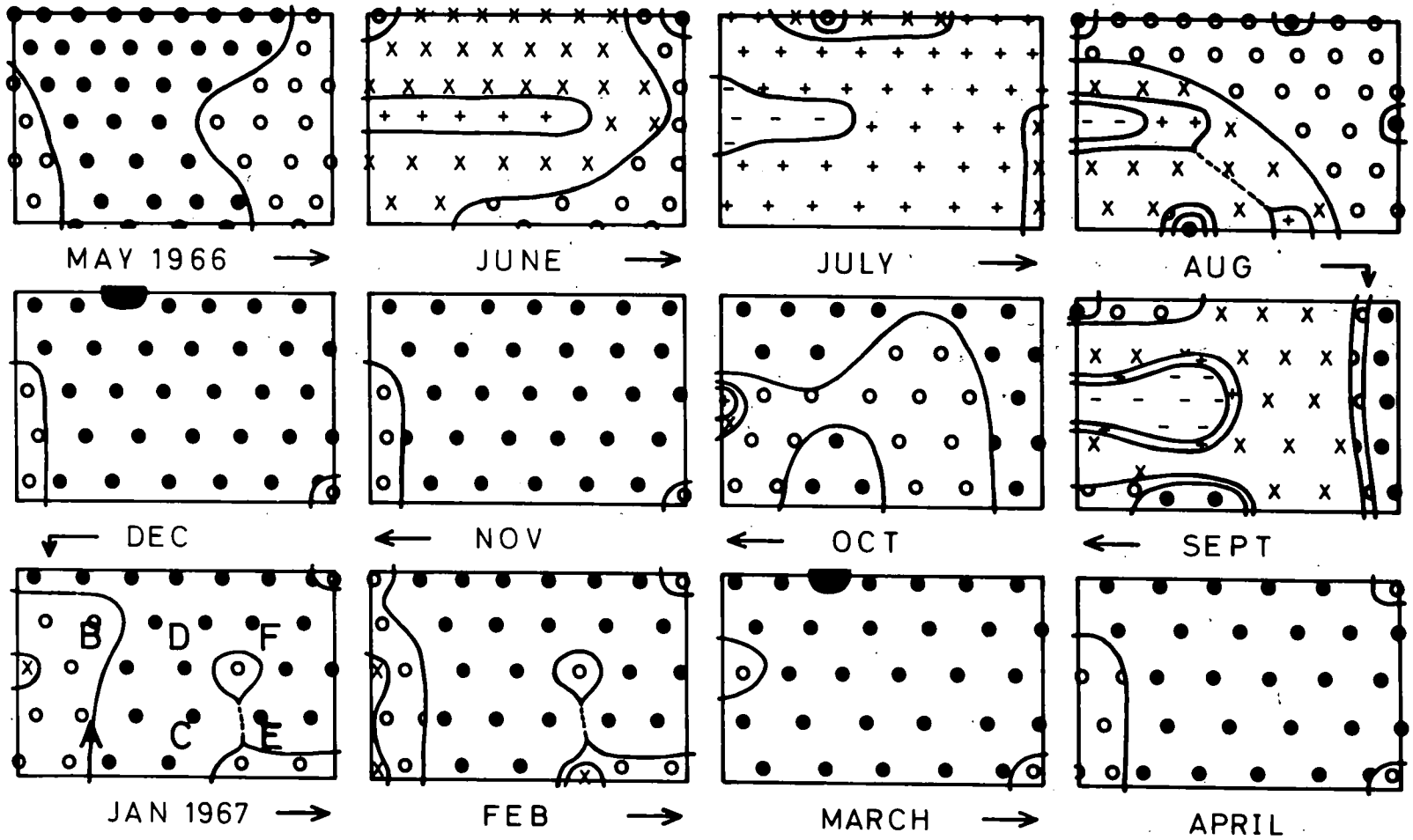


Fig. 2. Water table levels over the grid area at Wynyard (1966/67)

with a resumed increase in depth in September, again from the A1/B3 region. October figures showed a marked rise in the water table level which had assumed a uniformity of depth at a high level in the month of November. The water table remained at a high level until April, 1967 - the last month of measurement - when the situation was very similar to that in May, 1966.

The mean water table depth (over the whole grid area) for each month of measurement is shown, to some extent diagrammatically, in Fig. 3. Apart from a slight fall in January and February, 1967, the mean water table level was remarkably constant throughout the year, except for a sharp fall in June and July, 1966 and sustained low levels in August and September. The relative mean depths in April, May and June were almost identical in 1966 and 1967.

A significant, positive proportionality between topsoil moisture tension and water table depth was indicated by the data shown in Fig. 4.

Discussion

It can be seen from Fig. 2 that the water table level was usually uniform over the whole grid area so that the mean figures given in Fig. 3 are comparable in terms of gross seasonal changes. The similarity between the measurements made in April, May and June, 1966 and those for the same months in 1967 indicates a constancy in the annual cycle. Murchie (1958), studying a southern Michigan

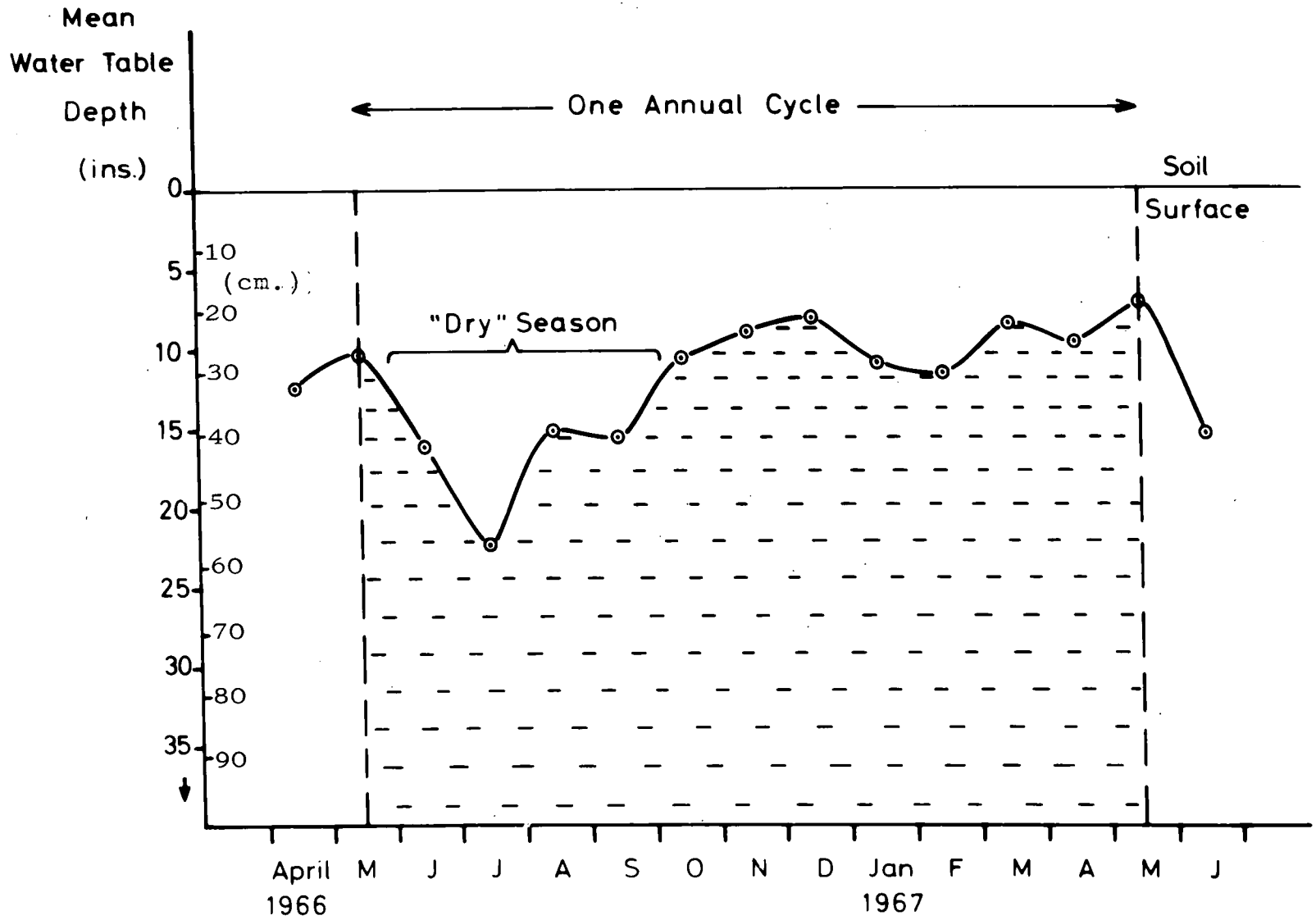


Fig. 3. Annual variation in the mean water table depth at Wynyard.

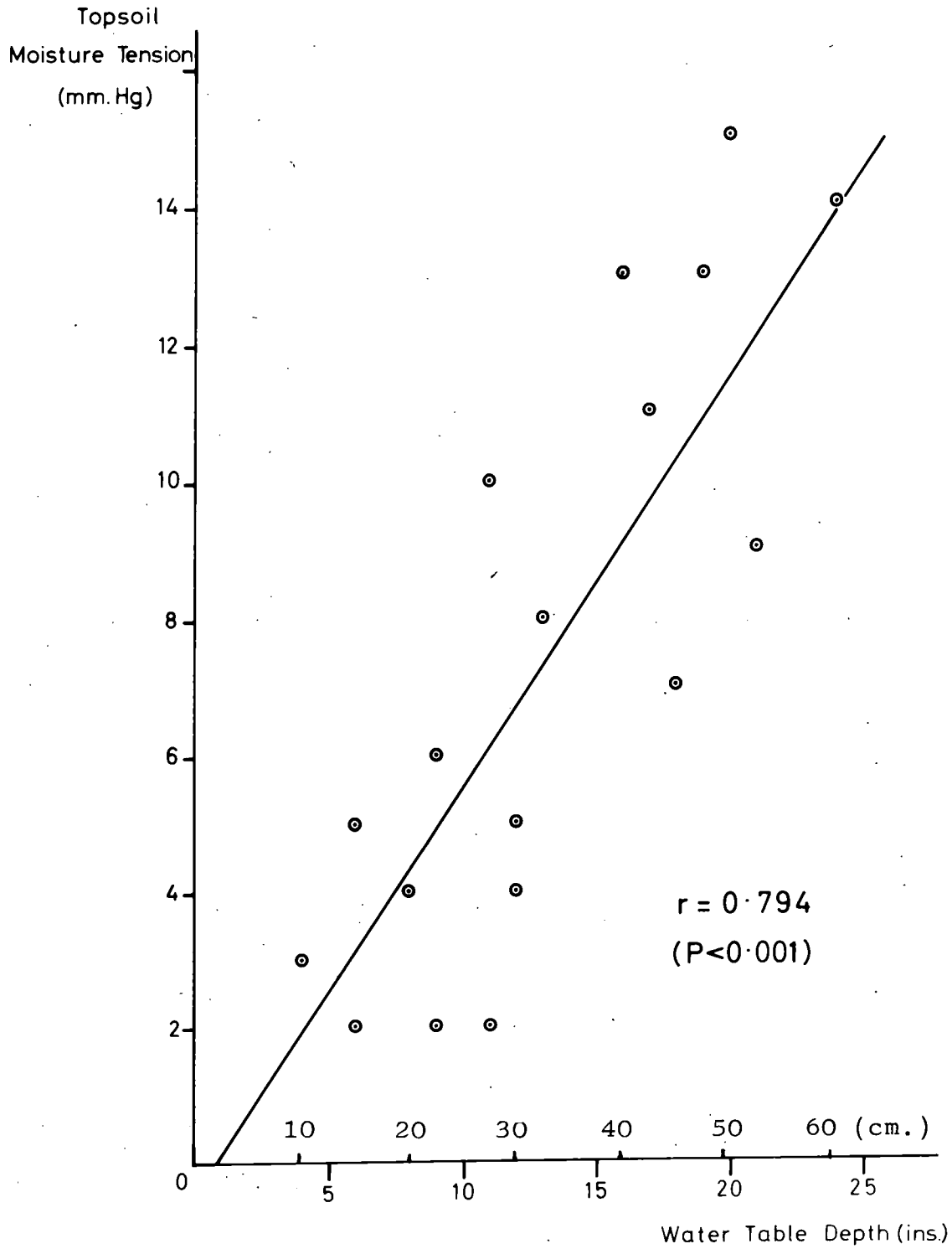


Fig. 4. The relation between water table depth and topsoil moisture tension on the grid area at Wynyard.

upland forest soil inhabited by Allolobophora rosea, found that the moisture content of the soil conformed essentially to two distinct 'seasons'. He noted a constancy of soil moisture content for most of the year with a marked fall in moisture content during the period from late June to late September. The situation at Wynyard seems very similar, with a distinct 'dry' season from June to September, inclusive. This information has been used as supporting evidence for the demarcation of arbitrary seasons in the analysis of the field soil temperature curve (see p.51). It has also been useful in the interpretation of lumbricid population data through the year. Since spatial variation in water table depth was little in evidence, this factor could not be counted as a major variable affecting the spatial distribution of the lumbricid population. The only consistent spatial feature of importance was the initiation of falling water table levels from the region of A1/B3. The only large mole fortress on the grid area occurred in this region and it would seem likely that the water table levels were affected by improved drainage due to mole burrowing activities. In wet soils, such as occur at Wynyard, Satchell (1960) has pointed out that even narrow tunnels drain clear of water at very low suctions; lumbricid tunnels will therefore be particularly important in the drainage and aeration of Wynyard soils in the course

of succession.

The correlation between topsoil moisture content and water table depth was somewhat better than might have been expected - considering the variation possible in surface evaporation (as affected by vegetation and litter cover) and topsoil drainage (affected by both floral and faunal action). The high degree of correlation was probably due to three major factors: the selection of one soil type for measurement, the woodland situation with uniformly high vegetational cover reducing evaporation from the soil surface, and the high level of the water table compared with most terrestrial habitats. From this relationship between topsoil moisture content and water table depth it was possible, using the mean annual water table depth, to designate 'bramble' soil moisture tensions of within 5 to 9 mm. Hg as suitable levels of moisture content for 'bramble' soils used in laboratory media. Soil media at such levels of moisture content were used for purposes of culturing and experimentation concerning Allolobophora rosea, where information collected was intended for application to the field situation. It was not possible to reproduce exact field moisture levels within the sand medium used for respirometry, though the lumbricids were kept in soil taken from the field at the time of animal collection and the sand medium within the respirometer was kept at a high level of moisture content during the period of measurement. Since the moisture content of the soil was also at a high

level for most of the year, the moisture conditions within the respirometer were taken as a close approximation to the field situation.

2. Temperature and 'Seasons'

Introduction

In field studies of individual or population bioenergetics it is essential that the temperature regime within the habitat is known in some considerable detail throughout the year. Such knowledge is required since it is rarely possible to measure all parameters of energy flow under field conditions; laboratory measurements are generally carried out at various constant temperatures to determine the relationship between the variable factor involved and the ambient temperature. When this relationship is known, extrapolation to the field situation is possible by reference to habitat temperature data, providing all other factors are simulated or adequately compensated during the experimental procedure.

Methods

Long-term measurements of field soil temperatures were effected by use of the technique involving saccharose inversion rates. This method was first described by Pallmann, Eichenberger and Hasler (1940) and later modified by Berthet (1960).

The saccharose inversion, according to the equation:

$$\text{Saccharose} + H_2O \longrightarrow \text{fructose} + \text{glucose}$$

proceeds at a rate which is related to the ambient temperature, providing the pH of the solution remains constant. The pH of the reaction was controlled by the use of a buffer solution and a neutral antiseptic material

(formalin) to prevent the activity of micro-organisms. The degree of inversion over a known period was estimated by polarimetry.

The method used in the present study was essentially similar to that outlined in detail by Berthet (1960). It was not found possible to achieve the exact pH value of 1.21 given by Berthet for rapid conversion rates. Therefore the constant (K_x below) used by Berthet was not applicable to solutions used in this work. The problem was solved by placing a set of control portions in rooms of constant temperature for each solution prepared for field use. Thus the constant K_x was recalculated for each set of measurements, ensuring accuracy despite slight differences between the buffered pH values of the solutions used in the course of this study.

Calculation of the temperature of inversion was by means of the following formulae:-

(a) $$K'_T = \frac{1}{t} \cdot \log \frac{a_o - B_o}{a - B_o}$$

where K'_T is the constant of inversion,

t is the period of measurement in days,

a_o is the degree of rotation at t=0,

a is the degree of rotation at time t,

B_o is the degree of rotation at complete inversion ($-9^o10'$)

(b) $$T = \frac{5,854}{K_x - \log K'_T}$$

where T is the absolute temperature ($\theta^{o}C = T-273.2$),

K_x is a constant dependent on the pH of the solution.

In the laboratory, the saccharose and buffer solutions were mixed by vigorous shaking within a measuring cylinder. Portions of the mixture were pipetted into glass tubes of 0.64 cm. internal diameter and 10 to 13 cm. in length. Four bottles were filled with the mixture; one bottle, containing a sample of the original mixture for the calculation of a_o in equation (a) above, was placed immediately in a refrigerator at -20°C; of the remaining three bottles one was placed in a constant temperature room at 5°C, one at 10°C and one at 15°C. The latter three bottles were removed after a known time interval and placed in the refrigerator at -20°C for subsequent use in determining the constant K_x in equation (b) above. The experimental tubes were tightly stoppered with rubber bungs and placed immediately in vacuum flasks containing a calcium chloride and ice mixture at -20°C. Under these conditions the tubes were transported to the field.

Field soil temperatures were measured throughout the year at various depths beneath two distinct vegetational types. There were two measurement sites under alder beneath 'bramble' and two under birch beneath 'grasses'. At each site a pit of approximately one square metre in surface area was dug to a depth of 60cm. One vertical soil face was carefully prepared, preserving the integrity of the soil structure, surface litter and ground vegetation.

Three experimental tubes were horizontally inserted into the soil face of each pit at each of the following depths*:

(a) beneath 'bramble'

0 cm (litter layer - alder and bramble debris),

5 cm (mull-type topsoil),

22 cm (A_3/B_1 transitional horizon), and

50 cm (subsoil - boulder clay);

(b) beneath 'grasses'

0 cm (litter layer - dead grasses),

5 cm (clay topsoil),

22 cm (transitional to boulder clay).

* All depths measured from the soil surface.

Six experimental tubes were thus available from each of the depths specified above for each vegetational type. Five tubes were required to completely fill the polarimeter tube (capacity: 10 ccs.), leaving one spare tube as a replacement in the event of damage to any of the other five sample units.

After the insertion of tubes, each soil face was covered with a nylon mesh sheet and the pit refilled with the original soil. The subsoil was packed down to its original firmness and the topsoil pieces, including surface litter and vegetation, were carefully replaced.

The tubes were changed monthly from November to May, inclusive, and every two weeks during the warmer months due to the more rapid inversion rates. The returning tubes were transported back to the laboratory, in vacuum flasks

containing calcium chloride and ice, and placed in the refrigerator at -20°C. They - and the sample of original solution, plus control bottles from constant temperature rooms - were subsequently analysed for rotation by polarimetry.

Results

The relation between mean soil temperature and soil depth beneath 'bramble' and 'grasses' from early August 1966, to early October 1967, inclusive, is shown in Fig. 5. The ordinate values of soil temperature are varied according to the temperature range encountered for each period of measurement, but the abscissa soil depth values are as shown for the final period of measurement: late September/early October 1967. Soil temperatures were almost identical beneath 'bramble' and 'grasses' at all depths. The greatest differences occurred in the winter of 1966/67 and the early spring of 1967. However, the maximum difference between surface temperatures - occurring during March 1967 - was only 0.7°C and the maximum difference between soil temperatures at depth - occurring at a depth of 22 cm. during January 1967 - was 0.4°C. Temperature differences between soils of the two vegetational types were usually much less than these maximum figures, being only of the order of 0.1 to 0.2°C. Greater differences occurred between surface and topsoil temperatures beneath 'grasses' than occurred between these strata beneath 'bramble'. The classical situation of soil temperature being proportional and inversely proportional to soil

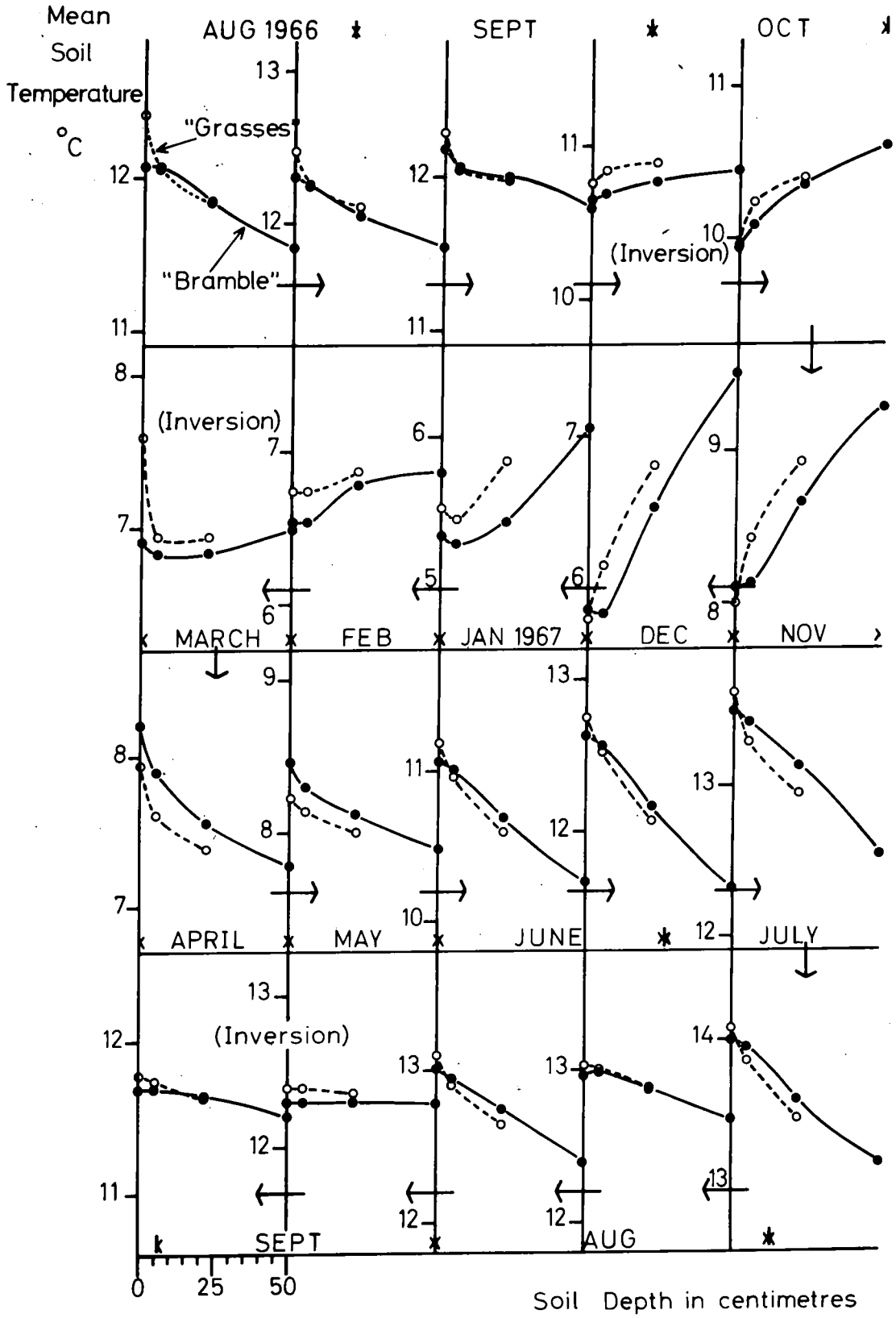


Fig. 5. The relation between soil temperature and soil depth beneath two vegetational types, according to season.

depth in winter and summer, respectively, is shown quite clearly in Fig. 5. The maximum difference between surface temperature and the temperature at 50 cm. depth - occurring in December 1966 - was 1.5°C. Soil temperature inversion beneath both 'bramble' and 'grasses' occurred in September/October 1966, March 1967 and September/October 1967.

Fig. 6 shows the mean field soil temperature curve for the period August/September 1966 to September/October 1967 at a depth of 5 cm. beneath 'bramble'. The annual cycle has been divided into arbitrary seasons and the times of seasonal measurements of lumbricid respiratory quotients are shown. The annual range of mean soil temperatures was 8 or 9°C. Minimum temperatures of about 5°C occurred in January 1967 and maximum temperatures of about 13 to 14°C in July/August 1967. August/September soil temperatures differed by only 0.6 to 0.7°C in the two years of measurement.

Discussion

Since the rate of inversion of saccharose is not directly proportional to ambient temperature, being very slightly biased towards the higher temperatures under varying temperature conditions, the mean temperatures measured were somewhat higher than the arithmetic mean temperatures over each period of investigation. Berthet (1960) has described the saccharose inversion temperature measurement as the 'ecological mean temperature' being more meaningful than the arithmetic mean in terms of biotic

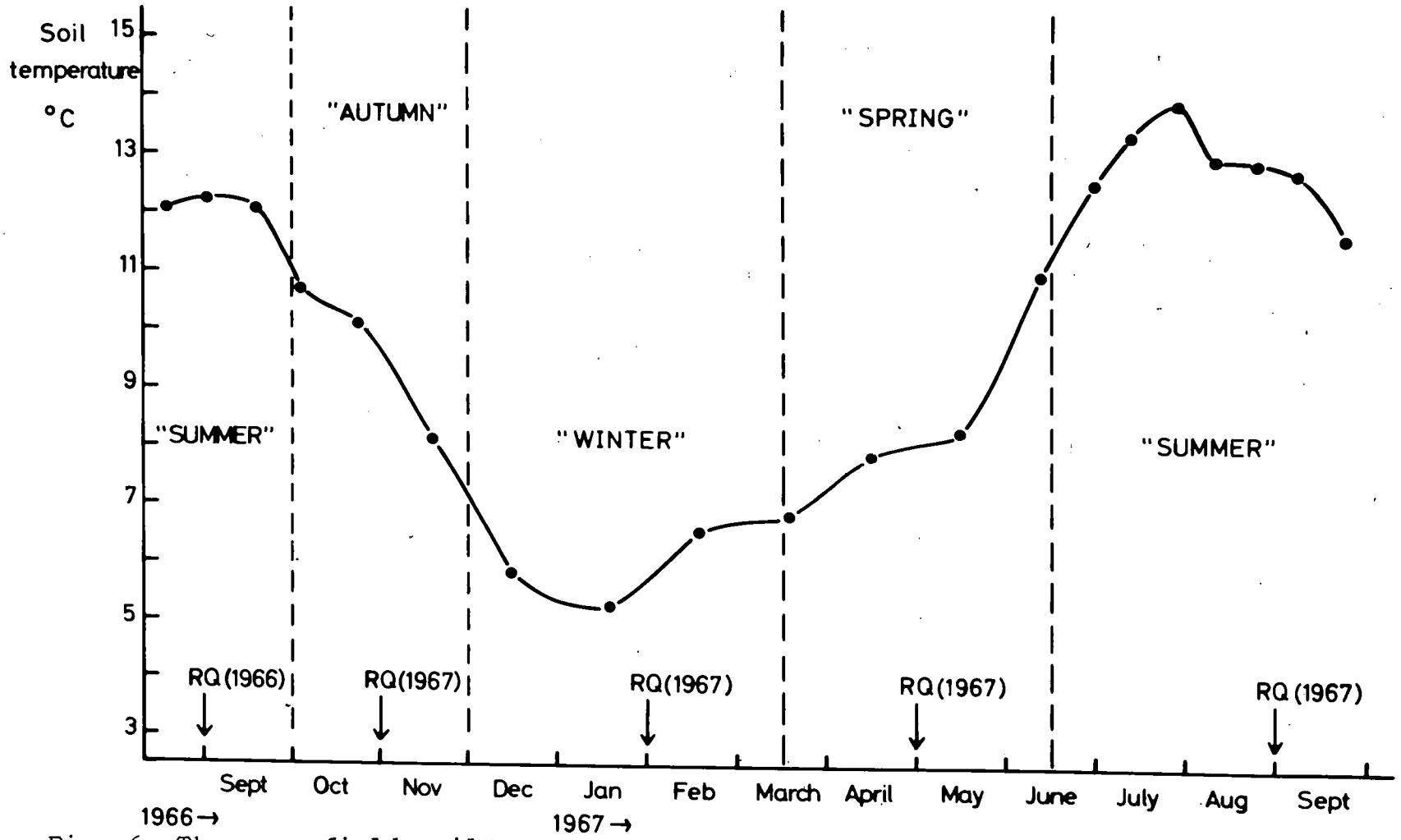


Fig. 6. The mean field soil temperature curve at Wynyard, measured at a depth of 5 cm. beneath 'bramble' vegetation. (See text)

activity. However, the difference involved conforms to a logarithmic relationship with absolute temperature and is only of the order of $1^{o}C$ at an ambient temperature of $20^{o}C$. The differences occurring in the ranges of temperatures measured during the present study would therefore be negligible in terms of the aspects of field population metabolism to which the soil temperature data were applied.

The use of small sample units dispersed through a number of situations with the same depth characteristic served the dual purpose of delineating accurately the depth of measurement and ensuring an average value for a particular depth.

In any particular period, soil temperature differences between 'bramble' and 'grasses' at the same soil depth, shown in Fig. 5, were usually not significant in absolute terms due to errors in the calculation of K_x caused by slight fluctuation in 'constant' temperature room conditions in the course of this work. However, each set of measurements, for a particular period, calculated for a particular solution mixture with a defined K_x value, were significant in relative terms within the set. Thus comparisons of the temperature/depth relation in different periods of measurements, as shown in Fig. 5, are possible, but it would be unrealistic to plot comparatively the soil temperature curves for different depths or the different vegetational types in the form shown in Fig. 6.

Allolobophora rosea (Sav.), the species to which calculations

involving temperature data were applied, was found to prefer the 'bramble' soil type (see pp.77,176) and to be most abundant in the upper levels of the topsoil, usually penetrating the soil to a depth of only 15 cm. Since soil temperature variation between different vegetational types and at different depths was so small - and often insignificant in absolute terms - the mean temperature measurements obtained for soil at 5 cm. depth beneath 'bramble' (shown in Fig. 6) were used for all calculations involving correction for field soil temperature. The arbitrary 'seasons' shown in Fig. 6 were demarcated for the purpose of dividing the annual cycle into its major components with regard to the state of the physical environment. From such divisions parameters of energy flow through the A. rosea population could be better defined on a temporal basis. In view of the complex buffering systems existing in soils at the Wynyard level of succession it was assumed that soil pH variation through the year would be minimal. Therefore the demarcation of 'seasons' was based solely upon soil temperature and the soil moisture content through the year. The level of the soil water table, closely related to topsoil moisture tension beneath the major vegetational type, was shown to be constant for most of the year, the only major variation being an increase in depth during the months of June, July, August and September (see pp.40,41). This period of low soil moisture tension was seen to correspond with the highest measurements of

soil temperature and thus the period from mid-June until the end of September was termed the 'summer' season. The other three seasons, in the absence of soil moisture variation, were demarcated on the basis of the temperature status of the soil: the period of rapidly falling soil temperatures from the beginning of October until the end of November was termed 'autumn'; the period of minimal soil temperatures from the beginning of December until mid-March was termed 'winter'; the period of rising soil temperatures from mid-March until mid-June was termed 'spring'.

The annual range of soil temperatures - 8 or 9°C - is only about half the annual range measured by Healey (1967a) for the soil of an exposed ridge of Welsh moorland over the period May 1962 to June 1963. He recorded maximum differences between temperatures at the soil surface and only 3 cm. soil depth of 4 or 5 °C, during the summer of 1962. These very great differences between the soil temperature regimes at Wynyard and for moorland are undoubtedly due to a number of factors: differences between the year and the altitude of measurement will have a considerable effect, and the use of a method involving measurement of fortnightly or monthly means at Wynyard, as against Healey's use of integrating thermistor units to obtain weekly mean temperatures, will certainly have produced a 'damping' effect on the annual fluctuation of soil temperatures. The maximum and minimum mean daily air temperatures recorded

by the Durham University Observatory over the period August 1966 to October 1967 were 14.9°C, in August 1967, and 3.2°C, in January 1967, respectively. It is therefore probable that the factors outlined above were largely responsible for the difference in annual range between the two soil situations. The somewhat higher mean winter temperatures in Wynyard soil, compared with air temperatures, were probably due to the insulating effect of woodland litter and vegetation. The greater similarity between woodland soil temperatures at various soil depths than those in moorland soil are almost certainly due in part to the insulation and equilibriation effected by dense vegetation - and a high soil water table, since water has about 25 times the thermal conductivity of still air (Russell, 1961). Satchell (1962) measured soil temperatures at 10 cm. depth in a woodland situation during 1960. The tree cover was comprised of coppiced hazel (Corylus sp.), ash (Fraxinus sp.) and sycamore (Acer sp.) and the soil was a deep, base-rich glacial drift overlying Carboniferous limestone. Despite differences in woodland type and the year of measurement, Satchell's annual range of soil temperatures - 3.2 to 13.8°C - shows close agreement with the temperature data for Wynyard.

The importance of soil temperatures to lumbricid activity is outlined by the fact that most species living in a temperate climate have relatively low heat death temperatures. In carefully temperature equated laboratory

experiments Miles (1963a) demonstrated heat death temperatures for Allolobophora terrestris (Sav.) f. longa (Ude) and Eisenia foetida (Sav.) of only 25.7°C and 33.3°C, respectively. Grant (1955a) demonstrated a positive relation between acclimatisation temperature and the heat death temperature in the megascolecid species Pheretima hupeiensis, but recorded heat death temperatures of only 24.7°C and 26.3°C for the lumbricid species E. foetida and A. caliginosa, respectively, after acclimatisation at 22°C. Species adapted to a warm climate are more independent of soil temperature: El-Duweini and Ghabbour (1965) showed Allolobophora caliginosa (Sav.) f. trapezoides (Duges) to have a temperature preference range of 2 to 37°C! Many temperate climate species avoid the desiccatory effects of high temperatures by entering a phase of dormancy within a mucus-lined soil cell. Low temperatures decrease the activity and general metabolism of lumbricids in the manner normal for poikilotherms. Under temperature conditions approaching 0°C a state of quiescence was observed in A. rosea at Wynyard and Murchie (1958) found that whilst this species was undergoing dormancy, due to low soil moisture content, a fall in soil temperature tended to prolong the quiescence. A. rosea at Wynyard did not undergo the period of summer dormancy normally found in grassland populations of this species. The uniformity and stability of soil temperatures at Wynyard were thought to be factors beneficial to the successful establishment of lumbricid populations.

3. Soil pH

Introduction

Hurwitz (1910) and Shohl (1912) were amongst the first workers to investigate the reaction of lumbricids to extreme pH conditions. They found that irritation of Eisenia foetida (Sav.) was proportional to the concentration of hydrogen and hydroxyl ions in the medium. Arrhenius (1921) said that the presence or absence of lumbricids in a soil depended on the pH of the soil. From experiments in Java and California he concluded that lumbricids were extremely sensitive to soil reaction and could only exist in neutral or slightly acid soils (minimum pH : 6). Such a generality of statement might be considered of little value, but it did give rise to a surge of interest in lumbricid pH relations. Moore (1922), Phillips (1923) and Wherry (1924) investigated the minimum level of soil pH under which lumbricids could exist. It was generally concluded that this minimum level was in the region of pH5. Wherry reported the existence of Helodrilus lönnbergi Hoffmeister - a species with exceptionally large calciferous glands - in a root peat soil of pH 4.7 to 4.9. Allee et al (1930) studied the occurrence of lumbricids in soils of pH 5.6 to 8.3. On the basis of population density they defined the optimum field soil pH as lying between pH 7.0 and pH 7.8. In laboratory experiments involving the study of 'avoidance reaction' to filter papers dampened by solutions at various pH values they found that

all the lumbricid species studied - including Allolobophora rosea (Sav.), the commonest species at Wynyard - showed a wide range of tolerance to pH. However, these workers could not correlate species distribution in the field with soil pH differences, concluding that other factors of the soil environment must be more limiting - though the effect of pH on soil micro-organisms may affect overall lumbricid abundance.

In the laboratory experiments of Allee et al, Allolobophora caliginosa (Sav.) f. trapezoides (Duges) showed the widest range of pH tolerance. Bodenheimer (1935) considered the high pH values in Egyptian soils to be responsible for a paucity of lumbricids. El-Duweini and Ghabbour (1964b) found that A. caliginosa trapezoides could tolerate highly alkaline conditions in laboratory experiments and El-Kifl (1958) disagreed with the conclusions of Bodenheimer after studying the distribution of this species in Egyptian soils. Satchell (1955a) showed that pH is not the primary irritant in acid soils since five common species could tolerate solutions containing sulphuric acid to pH values as low as 3.0 before finding them strongly irritating, yet Allolobophora chlorotica (Sav.) would not burrow in soils of pH4.4 and lumbricids were rarely found in soils of pH less than 4.5. Bornebusch (1930) found only the rare occurrence of Dendrobaena octaedra (Sav.) in Danish forest soils of pH 4.3 or less.

Laverack (1961) has shown that lumbricids have sense organs in the body wall which respond directly to stimulation

by acid solutions and oscilloscope recordings of activity in segmental nerves were used to demonstrate inter-species differences in the sensitivity of these sense organs. Using information from various sources (Laverack, 1963) he concluded that the internal pH of lumbricids was well regulated - to the exclusion of variation due to external soil conditions. Despite these conclusions, in the present work it was considered advantageous to carry out laboratory studies under pH conditions which simulated those in the field soil environment as far as possible. It was assumed that field soil pH would vary little over the year due to the action of complex buffering systems existing in soils at this stage of succession. Wherever possible, soils from the study area were used for laboratory cultures and experiments.

To determine the extent of any variation in soil pH over the grid area, and to estimate a mean soil pH value for use in lumbricid respirometry, it was considered desirable to carry out a complete survey of the pH values of the various soil types within the grid area.

Methods

The soil types were categorised according to vegetation cover. For this purpose the ground vegetation was divided into eight types: pure bramble (Br); pure bracken (Bf) or male fern/soft shield-fern (Ff); pure grasses (G); pure rose-bay willow-herb (Rbwh); mixed bramble and male fern/soft shield-fern (Br/Ff); mixed grasses and male fern/soft

shield-fern (G/Gf); mixed bramble and grasses (Br/G); mixed bramble, grasses and male fern/soft shield-fern (Br/G/Ff). Squares A and C were taken as representative of alder tree cover, and squares E and F to be similarly representative of birch tree cover. Eight soil samples, one from each ground vegetation soil type, were taken from each of the four grid squares. Samples were cut to a depth of 7 to 8 cm., after the removal of surface litter and raw humus, and transferred without handling into polythene bags for transport to the laboratory. The pH of the samples was estimated immediately on return to the laboratory, using a Beckman potentiometric meter with a glass electrode at the plastic limit of the soil.

Results

Table 1 shows the pH values determined for the eight soil types, classified according to ground vegetational cover, in the two tree cover zones. No consistent differences in topsoil pH values were observed between different ground vegetational types. Similarly the alder and birch zones showed no significant differences with regard to topsoil pH: the mean value beneath alder was pH 6.23 and beneath birch pH 6.21. The distribution of pH values measured was as follows: pH 5.00 to 5.49 - one sample; pH 5.50 to 5.99 - eight samples; pH 6.00 to 6.49 - fourteen samples; pH 6.50 to 6.99 - seven samples; pH 7.00 to 7.49 - two samples. The data thus conformed to a normal distribution curve about the mean pH value for the

Soil Types according to Ground vegetation:		Br	Ff	G	Rbwh	Br/Ff	G/Ff	Br/G	Br/G/Ff
Alder Tree Cover	Square A	6.50	6.00	5.90	6.00	5.75	6.00	6.20	6.05
	Square C	6.55	5.55	6.75	6.05	6.75	6.15	6.35	7.05
Birch Tree Cover	Square E	6.25	7.00	5.95	5.85	6.85	6.35	6.90	6.00
	Square F	5.85	Bf 5.25	5.65	6.50	6.35	6.35	5.85	6.45

Table 1. Soil pH values beneath various vegetational types (see text).

total number of samples measured. This mean value was found to be pH 6.22 ± 0.07 (St. Error), with standard deviation ± 0.41.

Discussion

The use of a glass electrode with soils at the plastic limit, or in suspension at dilution 1 : 0.5 (e.g. 100 gm. fresh soil to 50 ml. distilled water), was recommended by Krupskiĭ et al (1962) for the measurement of soil pH. They found that greater dilutions did not measure the true acidity of the soil due to varying alkalifying effects on different soil types. Quinhydrone and antimony electrodes were tested but found less reliable than the glass electrode.

The present survey showed a high degree of homogeneity in topsoil pH over the grid area. This was thought to indicate a stability of physico-chemical conditions in the soil due to the complex vegetational pattern over the area. The only vegetational stand of absolute purity was that of bracken in F2 and the pH of this soil (pH 5.25) was the lowest recorded value. Bracken is known to increase the acidity of soil conditions.

In deciding the pH value suitable for buffering the media used in respirometry, it was assumed that lumbricids in the field would tend to avoid the soils of lowest pH value. Thus the mean pH of the soils inhabited by the majority of lumbricid individuals was assumed to be somewhat higher than the mean pH (6.22) calculated. Since the occurrence of soils with a significantly low pH value was

not extensive, this difference was assumed minimal and a pH value of 6.50 was considered suitable for artificial laboratory media. Any errors involved in this estimation of an average pH value encountered by the lumbricid population may be considered negligible - in view of Laverack's conclusions regarding lumbricid physiological independence of external pH conditions.

PART II

I The Population Survey

1. Sampling Technique

(i) Lumbricid Extraction Methods - a short review

As Satchell (1958) pointed out, no single extraction technique has yet been devised which will give complete recovery of all lumbricids from any soil sample of suitably large size. The efficiency of recovery achieved by a particular method is therefore difficult to assess in absolute terms. The principle quantitative extraction techniques used in field studies are the application of the chemical vermifuges potassium permanganate solution (Evans and Guild, 1947a, 1948b; Guild 1948) and formalin (Raw, 1959); the use of electrical discharge as an irritant (Johnstone-Wallace, 1937; Doeksen, 1950; Satchell, 1955b); the use of washing and flotation apparatus, either singly (O'Connor, 1968) or in combination with other methods (Raw, 1960); the mechanical extraction of lumbricids by hand, commonly known as 'hand-sorting' (numerous authors including Hensen, 1877; Pickford, 1926; Bornebusch, 1930; Dreidax, 1931; Kollmansperger, 1934; Nielson, 1951; Svendsen 1955a,b; Murchie, 1958; Raw, 1960; Van Rhee and Nathans, 1961; Nelson and Satchell, 1962; Zicsi, 1962; El-Duweini and Ghabbour, 1965b); the creation of a temperature gradient within a soil block sample unit on the principle of the Baermann funnel (Satchell, 1968).

Most comparative studies have been directed towards the

assessment of the efficiency of 'automatic' methods in relation to that achieved by hand-sorting. Svendsen (1955b) showed that permanganate extraction yielded only a small proportion of the lumbricid population in a moorland soil, whilst Murchie (1958), in studies on Allolobophora rosea (Sav.) said that 'wet sampling' methods were inoperable under soil conditions of high water content, high clay content or with periods of extreme cold or drought. El-Duweini and Ghabbour (1965b), working in heavy clay soils (c.f. Wynyard soils), found permanganate and formalin methods impractical or inefficient for lumbricid sampling. Other objectors to the use of the permanganate method have included Reynoldson (1955), Satchell (1955b) and Boyd (1957). A major disadvantage of the chemical vermifuge technique is the destruction and pollution of the habitat by the necessary addition of chemicals and removal of ground vegetation over quite large areas. Electrical sampling methods are inefficient in their present form due to difficulties in defining the volume of soil samples, since electrical resistance varies between successive soil horizons (Satchell, 1960), and in assessing the degree of irritation on deep-burrowing, inactive or dormant lumbricids. Flotation techniques, originating from the Salt and Hollick apparatus based on the principles outlined by Ladell and Morris (see Kevan, 1962), have been little used due to their cumbersome and somewhat 'untidy' nature. However, with refinements of structure and procedure the flotation apparatus

could produce the most efficient yield in terms of lumbricid numbers. Raw (1960) has shown that washing and flotation methods, compared with hand-sorting, produce a much greater yield of lumbricid numbers in soils with a thick surface mat of vegetation. However, the recovery by hand-sorting in terms of lumbricid biomass - of major importance in studies of ecological energetics - was a much higher percentage, and in soils without a surface mat (such as mainly occur at Wynyard) the recovery by hand-sorting of numbers and biomass was 89% and 95%, respectively, of that found by the more laborious method of washing and magnesium sulphate flotation. Nelson and Satchell (1962) tested the hand-sorting method by introducing known numbers of lumbricids of various sizes and species into blocks of various soil types - previously cleared of lumbricids by freezing at -16°C. The blocks were sorted by experienced operators who had no knowledge of the introduction procedure. The investigators found that large and medium sized worms were not missed in appreciable numbers but there was evidence that quite appreciable losses occurred with individuals which possessed one or more of the factors of small size, dark colouration, immobility and presence in turf rather than soil. These workers also noted, however, that total biomass recovery was practically complete, despite the losses in numbers of lumbricids. Nelson and Satchell outlined the difficulties due to limitations on depth of sample with regard to the recovery of deep-burrowing species,

suggesting the use of a chemical expellent after the removal of topsoil. Such a technique would doubtless improve the recovery of deep-burrowing species, but would be inadvisable for use in a restricted area, such as the grid area at Wynyard, where repeated sampling was carried out over an extended period.

(ii) The Sampling Method used in the Present Study

Introduction

Chemical and electrical methods were considered unsuitable for use in a quantitative study of this type. Heat extraction is a useful technique for recovery of small surface active pigmented worms from matted turf or highly organic surface soil layers, but the method was impracticable with mineral soil samples of high clay content and with a minimum of surface organic matter such as were most common at Wynyard. The hand-sorting technique was adopted in preference to methods involving washing through sieves and/or flotation in magnesium sulphate solution since it was considered desirable to extract the lumbricids with a minimum of damage or contamination which might affect measurements of biomass and calorific content. The soil conditions at Wynyard were considered conducive to the efficient application of the hand-sorting method in the extraction of most lumbricid species for the purpose of biomass estimations. The primary aim of this population investigation was to obtain quantitative biomass data, on a metre square basis, for as many as possible of the

species occurring in the grid area. It was acknowledged that the deep-burrowing species, Lumbricus terrestris L. and Octolasion cyaneum (Sav.), would be underestimated by a 'digging' and hand-sorting method of the type used, but it was hoped that some qualitative information on the abundance and activity of these species through the year would emerge in the course of this work. Such acknowledgments were thought necessary in any work of this type since, as Satchell (1960) has pointed out, no one method is equally suitable for the extraction of all stages of all lumbricid species. Ghilarov (1968) stated that since hand-sorting methods are used more universally in soil ecology they are to be preferred to more exotic extraction techniques due to the facilitation of data comparisons. Whilst this argument has an element of practical significance, the determination of soil fauna population data to the maximum degree of accuracy, by whatever extraction techniques are most suitable for prevailing soil conditions, must remain the only true basis for comparative research in this field of soil ecology.

Sampling Tools

The 'digging' of soil sample units has usually been carried out using a standard gardening spade. This method involves the disturbance of the sample unit for a considerable time prior to removal, so that many subsoil species retire to deeper soil. The area of the sample unit is poorly defined, since the 'vertical' sides are invariably

sloping, and lumbricid worms are often needlessly bisected by random movements of the cutting edge. Svendsen (1955a, b) partially overcame this inefficiency by the use of a spade which was semicircular in cross-section, whilst Zicsi (1962) used a metal square with flat vertical sides; both these implements were pushed into the ground by foot pressure. Zicsi's method was preferable since the area sampled was perfectly defined by the vertical sides of the implement; however, both these sampling tools were unsuitable for use at Wynyard where the heavy clay soil contained a network of tree roots in the surface layers, in contrast to the moorland and arable soils studied by Svendsen and Zicsi, respectively. Plate 10 shows the sampling tool used in the present study. It was essentially a modification of the Zicsi implement, consisting of a square steel base with vertical flat sides and an upper portion for use in lifting the sample unit from the ground. The upper portion, which was detachable, included a horizontal bar for 'rocking' the sample to break loose the base of the soil block. The steel square, measuring 25 cm. x 25 cm. in area x 30 cm. in depth was provided with a sharp cutting edge and two blocks of specially hardened steel, welded to two sides of the upper edge for hammering purposes. To remove a soil sample unit the cutting edge of the steel square was thrust into the ground surface and the square was rapidly hammered into the ground, using a 10 lb. sledge hammer, until the upper edge was level with

Plate 10. The sampling tool

the soil surface. The soil was levered away from the upper edge of the square on the two sides not used for hammering, exposing the holes for attachment of the upper portion. Four metal pegs, two on each side, at the base of the upper portion were slotted into the holes by expanding slightly the upper frame which was then tightened using the spindle and wing nut provided. The sample unit was 'rocked' using the horizontal bar and lifted on to a metal tray where it was expelled from the sampling tool by minimal foot pressure. The soil block was usually removed in its entirety; occasionally pieces of subsoil broke away from the main sample block and these were recovered using a standard gardening spade. For maximum efficiency, the cutting edge of the steel square was sharpened before each monthly sampling occasion and the time for the complete removal of a soil sample unit, measured for each sample unit taken in the field sampling programme, varied between 1.5 and 2.5 min.

The Sampling Procedure

The sample unit size of $^{1}/_{16}$th square metres in area by approximately 30 cm. in depth was chosen in accordance with Zicsi's findings regarding optimal sample unit area and with due regard to the size, abundance and depth of activity of lumbricid individuals to be sampled at Wynyard. Healy (1962) has emphasised the importance of stratified random sampling to ensure a certain degree of even coverage. In the present study the grid area was divided into the six

grid squares: A, B, C, D, E and F, and each square was subdivided into quarters numbered 1, 2, 3 and 4 as shown for square A in Fig. 1 . Each of the quarter-squares was then sampled on a random basis using Snedecor's list of random numbers (Snedecor, 1956).

On or about the first day of each month, over a twelve month period, one soil sample unit was removed from each quarter-square - a total of twenty-four sample units per month. The exact position of each sample unit was decided as follows: the quarter-square was theoretically divided into ten one metre sections, numbered 0 to 9 from west to east, along the north side and similarly divided, with one metre sections numbered 0 to 9 from north to south, on the west side. Random numbers were used to compose a four digital code for each sample to be taken. The first number referred to a particular metre section on the north side of the quarter-square and the second number to a similar section on the west side. By use of a large metal right angle and strings marked at one metre intervals, the metre square which was opposite the two sections, so that the lines joining them were at right angles, was located in the field. The third and fourth code digits, composed only of numbers 1 to 4 inclusive, referred to quarters and $^{1}/16$ths of the metre square, respectively. The quarters of the metre square, and the $^{1}/16$ths within each quarter, were numbered 1, 2, 3 and 4 in the same orientation as was used for the sub-division of the grid squares. Thus the complete four digit

code allowed the isolation of a single $^{1}/_{16}$th square metre sample unit area in the field. If any sample unit area was found to be unsuitable for sampling purposes - e.g. across the base of a tree - then the next available sample unit within the quarter metre square, in a clockwise direction, was taken. Each sample unit, after removal on to the metal tray, was transferred to a suitably labelled, thick polythene sack for transport to the laboratory.

(iii) Treatment of Samples and Specimens

Sample units were stored in a constant temperature room at 10°C. The total sample for each month was hand-sorted over a period of five days; there was considerable variation due to soil type but, on average, each sample unit was sorted in approximately two hours. Each lumbricid individual found was washed in dechlorinated tap water to remove surface soil and detritus and placed, in a labelled glass tube, in a vacuum oven at 60°C. For each sample unit, the soil type, surface vegetation, grid position and time for removal were noted and the extracted lumbricids classified and counted according to species and size class. Worm fragments were counted as 'half' for population numbers assessment. It was assumed that approximately equal numbers of fragments which were greater or less than one 'half-worm' would occur in the complete monthly sample, so that, on balance, the 'half' worms counted would involve negligible error. Lumbricid cocoons were collected and treated as for lumbricid worms, though it was appreciated that the

extraction of cocoons by hand-sorting on this scale would be qualitative for most species.

Worms were usually classified as 'adult' (with a fully formed clitellum) or 'immature' (without a fully formed clitellum). Immatures of A. rosea were later subdivided - according to body weight - into 'small immatures' (without a fully formed clitellum; body fresh weight less than 100 mg.) and 'large immatures' (without a fully formed clitellum; body fresh weight greater than 100 mg.) - c.f. lumbricid age categories of Gerard (1967) and Van Rhee(1967). The relation between fresh and dry weight for A. rosea was determined by fresh-weighing a number of individuals on each sampling occasion, prior to their drying in the vacuum oven at 60°C. The animals, after washing in dechlorinated tap water, were rolled briefly on Whatman's No. 1 filter paper to remove surface water and individually weighed in a stoppered weighing bottle containing a small amount of dechlorinated tap water.

After a period of not less than 24 hrs. in the vacuum oven, all the extracted lumbricid worms and cocoons were cooled in a desiccator and individually dry-weighed on a tared watch-glass. The weights were noted, according to species, size class and soil sample, and the dried material for each species size class was grouped and stored in a desiccator for determinations of calorific content. From the dry weights of the A.rosea individuals previously fresh-weighed, the fresh weight: dry weight relationship

for worms of this species was determined. Biomass figures were calculated from population density, on a metre square basis, and the mean individual body weight for a particular species size class. For calculation of lumbricid biomass in terms of calories, the mean calorific content of A. rosea (three size classes) was estimated seasonally, and other species annually, according to the techniques described in Part III, Section I. Monthly, 'seasonal' (see Fig. 6) and annual mean biomass figures were based on the monthly 'spot' measurements.

Each sorted sample unit was returned to the field and replaced in the same hole from which it had been removed. The position of the returned sample unit was marked with a coloured stake and the area defined by a circle of radius 0.5 metre, with the stake at its centre, was regarded as unsuitable for sampling on any later occasion.

2. Results of the Population Survey

(i) Allolobophora rosea (Sav.) f. typica

The upper limit of the fresh weight size range for small immatures of A. rosea was arbitrarily set at 100 mg. - later found to correspond with changes in growth rate (see pp.111,118). Fig. 7 shows the relation between dry weight and fresh weight for worms of A. rosea. In addition to defining a mean upper limit to the dry weight size range for small immatures (21.2 mg.), this relation provided supplementary evidence for the division of immatures into distinct size classes at about 100 mg. fresh weight. The dry weight increase per unit of fresh weight was greater for immature worms weighing more than 100 mg. fresh weight than for immatures of lower fresh weight. The relations between dry weight and fresh weight for large immatures and adult worms were virtually identical, conforming to the single set of regression coefficients shown in Fig. 7.

Fig. 8 shows the seasonal variation in numbers of each of the A. rosea size classes, measured at monthly intervals (on or about the first day of each month) and calculated on a metre square basis. Adult worms were present in low numbers for most of the year. The highest numbers were recorded in spring (May, 1966, and April, 1967). Large immature numbers showed two peaks in September and December with comparatively low numbers in winter, spring and early summer. Small immatures were present in the field throughout the year, with increased numbers in May,

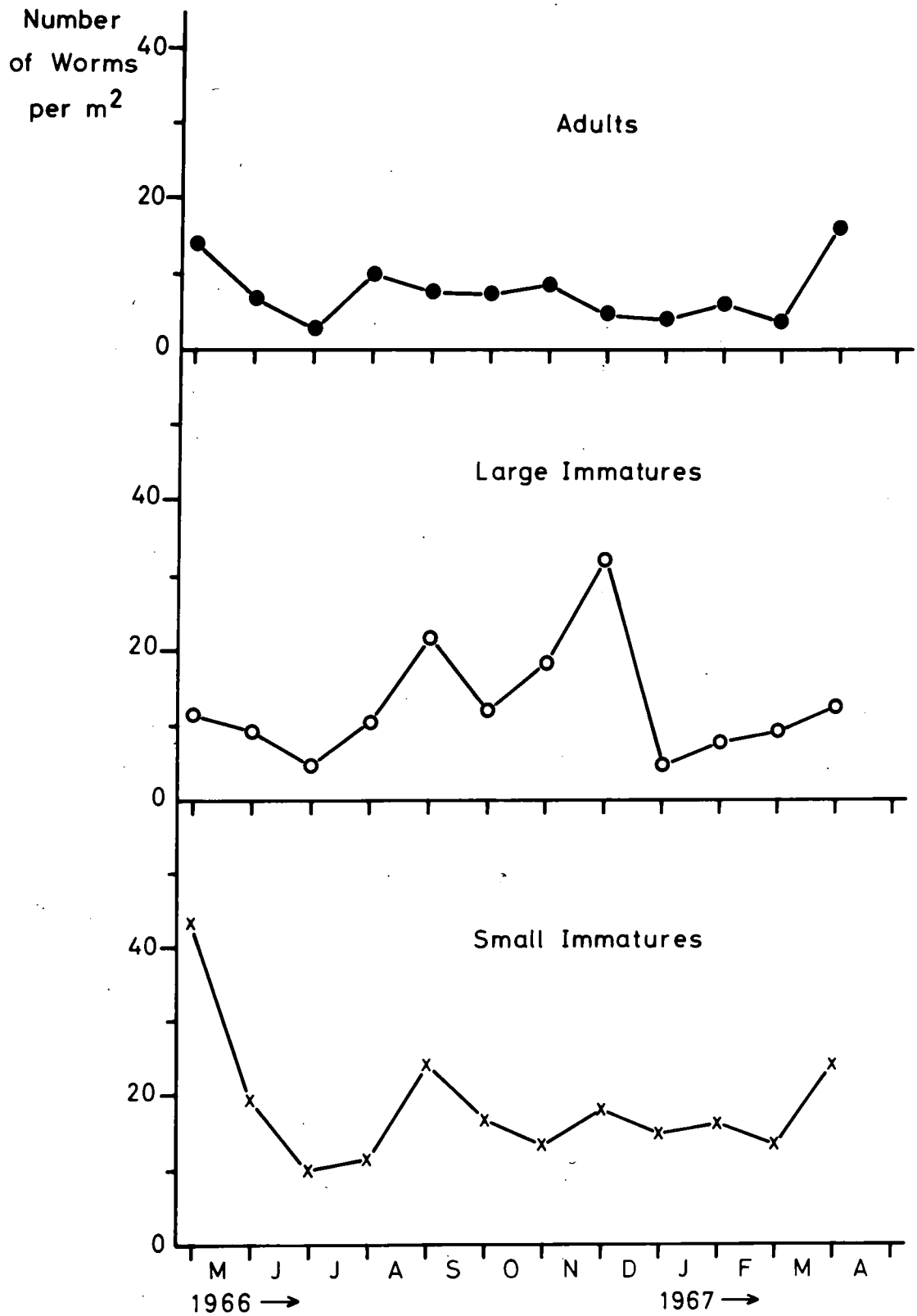


Fig. 8. Population densities of the three major A.rosea size classes through the year.

1966, September, 1966, and April, 1967. Fig. 9 shows the 'Disturbance Index' of Lexis, λ , evaluated for each size class of A. rosea on each monthly sampling occasion (on or about the first day of each month). The monthly index for the total A. rosea population is shown for comparison. For small immatures the index approached unity in mid-summer and late winter; the index was greater than unity at other times of the year, with a peak value in May, 1966, and somewhat increased values in September, 1966, and mid-winter, 1966/67. The index for large immatures showed an approximately constant value for much of the year, with somewhat higher values in autumn and values approaching unity in mid-winter, 1966/67. There was a marked difference between index values obtained for immatures and adults of A. rosea; the index for adult worms approached unity for most of the year, slightly higher values occurring in spring (May, 1966; April, 1967) and in September. The index calculated monthly for the total A. rosea population showed a constant value of approximately 1.85 throughout the year, with the exception of values for May, 1966, and September, 1966, which were notably higher.

Monthly biomass estimates for the three size classes of the A. rosea population are shown in Fig. 10. Biomass is presented as fresh weight, dry weight and Kcalories, all calculated on a metre square basis. Mean numbers of individuals per metre square and mean individual weights were estimated on or about the first day of each calendar

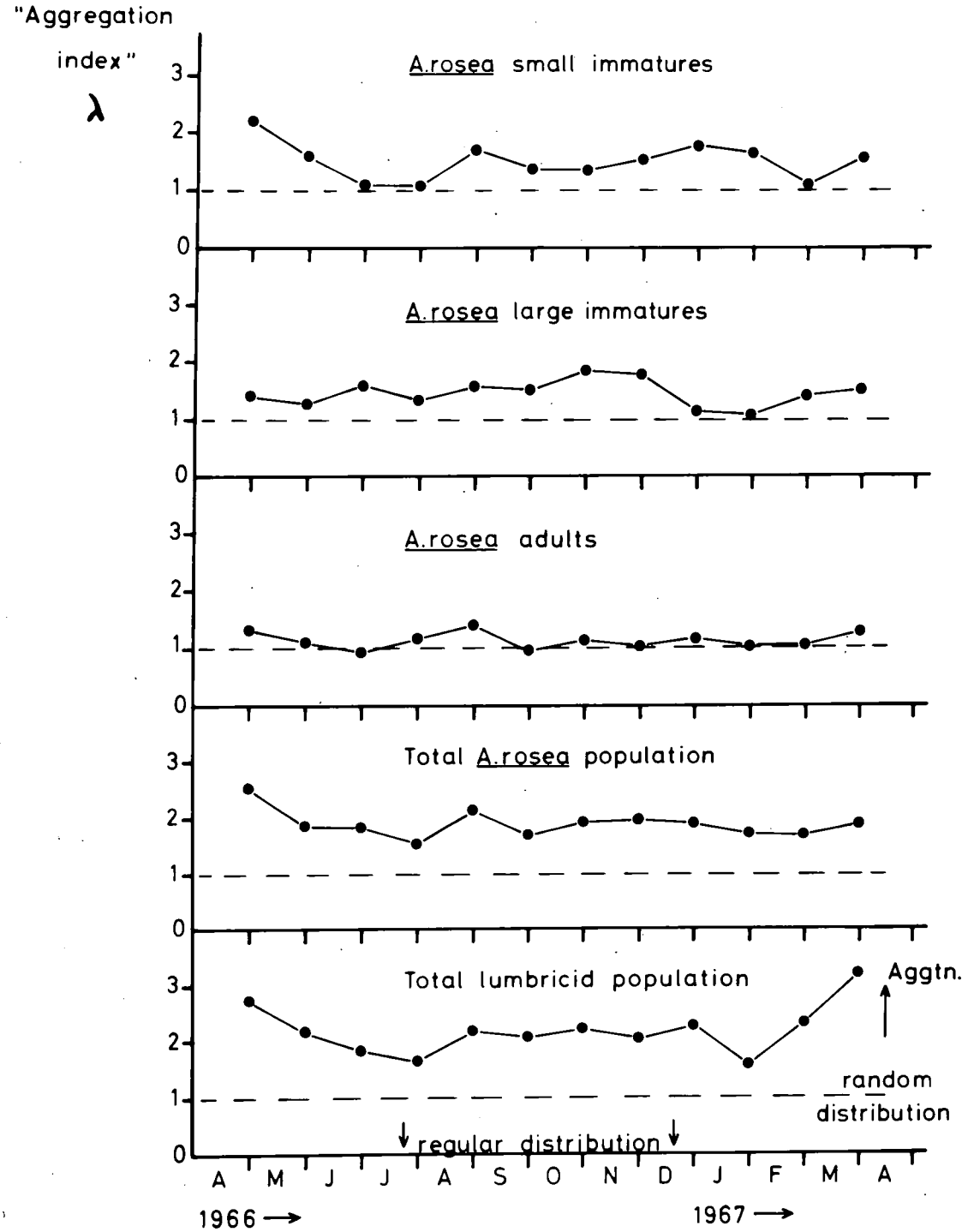


Fig. 9. Seasonal variation in population aggregation indices for the A.rosea and total lumbricid populations at Wynyard (1966/67).

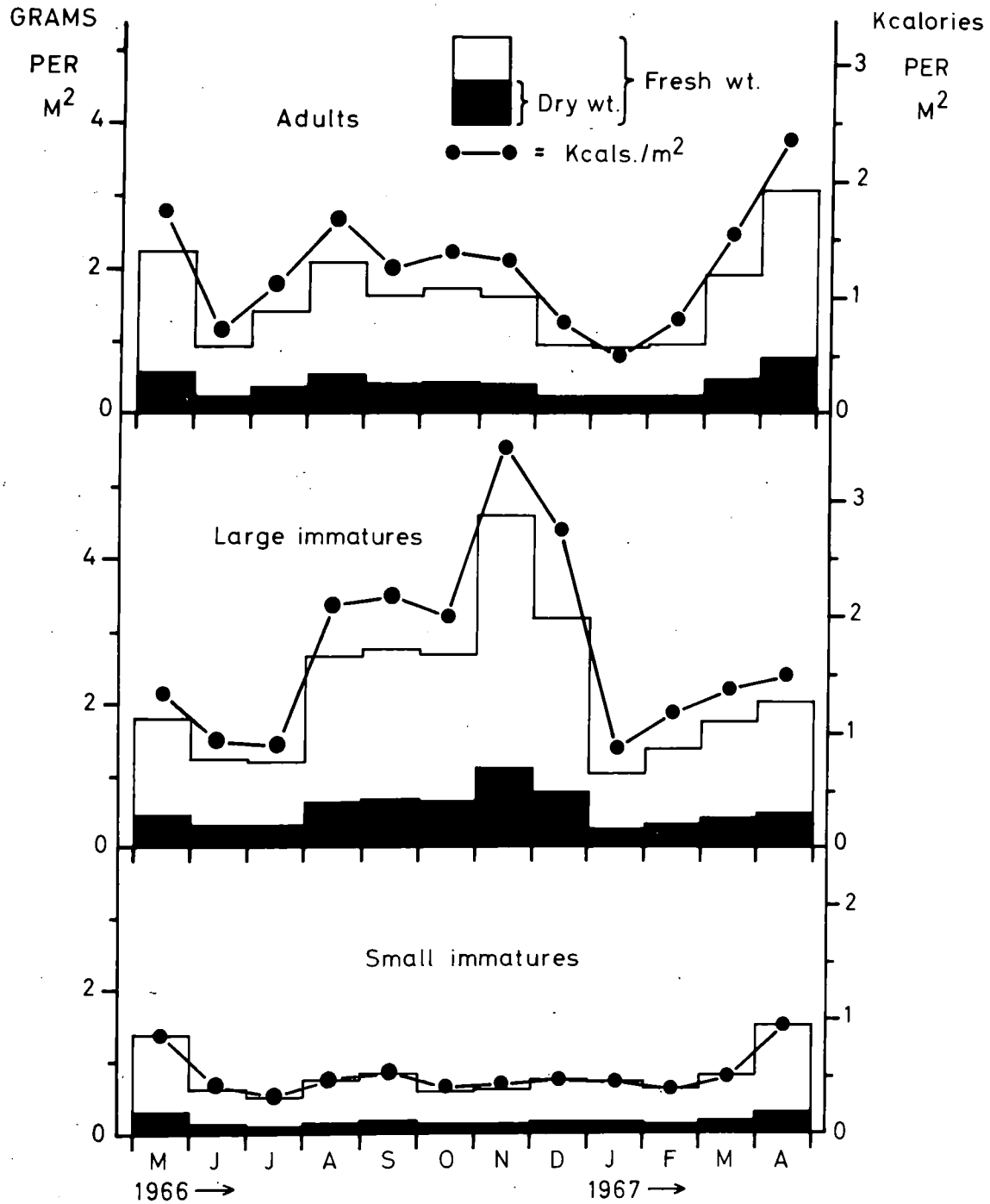
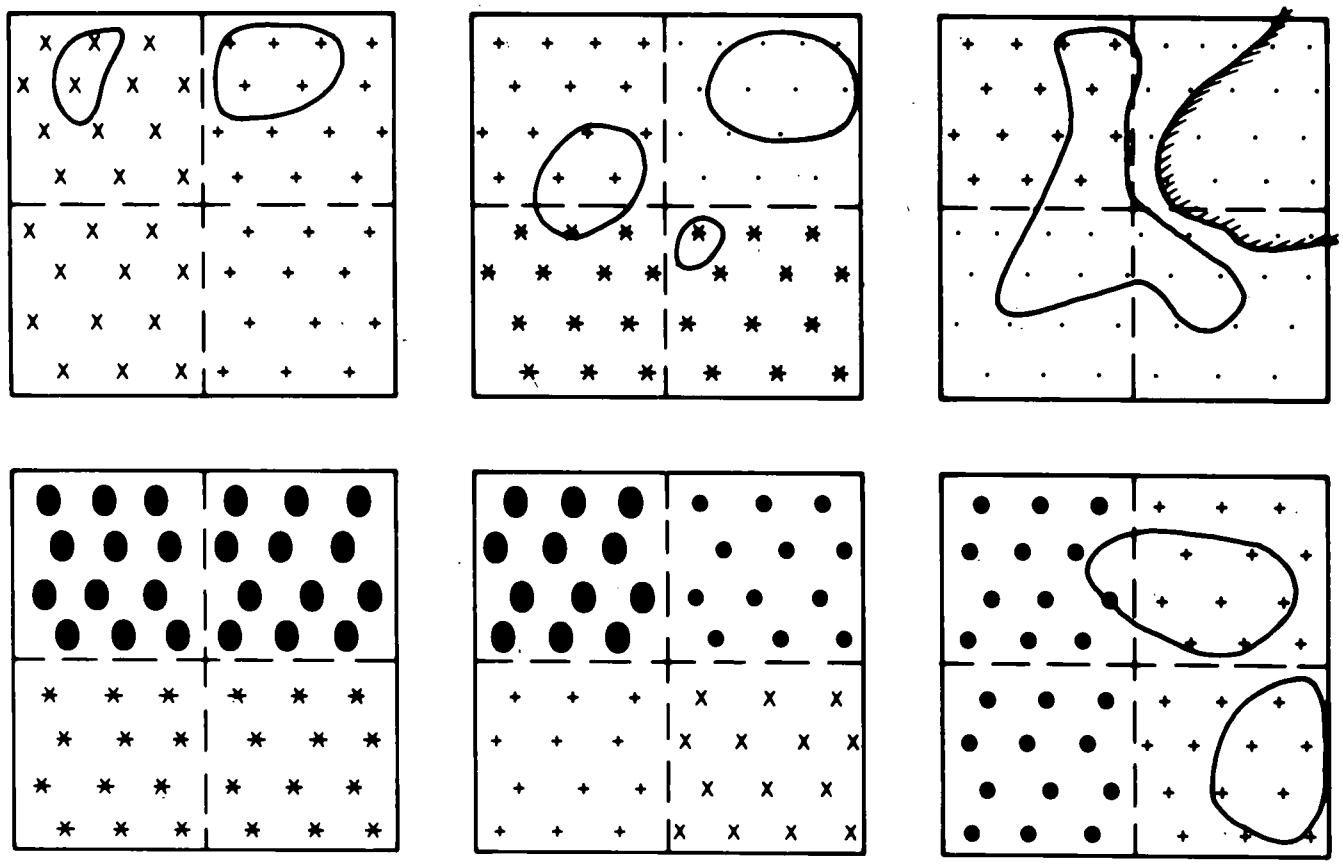


Fig. 10. A.rosea size class population biomass estimates - as fresh weight, dry weight and Kcalories - for the grid area at Wynyard (1966/67).

month. Monthly biomass averages were calculated as averages of consecutive monthly estimates, assuming near constancy of mortality, recruitment and emergence rates. Seasonal estimates of calorific content were used to convert dry weight biomass figures to estimates of Kcalories per metre square. For the purpose of standing crop estimations, it is sufficient to consider the mean monthly biomass figures in Kcalories.

For most of the year, small immature worms showed a constant, but small, biomass; it was only in the spring months of April and May that this size class assumed biomass proportions comparable to those for larger worms. Large immature worms formed the bulk of the standing crop during the late summer, autumn and early winter, with relatively lower values at other times. A well-defined peak in large immature biomass occurred in November/ December, 1966, subsequent to the high values of the previous three months and prior to the minimal values in mid-winter. Adult biomass was at a maximum in the spring months of April and May. A low value was obtained in June, 1966, prior to the moderately high standing crop observed over the summer and autumn periods. There was a marked fall in adult biomass over the early winter months of December and January, followed by a sharp rise in February and March towards the attainment of maximum standing crop in April, 1967, Seasonal and annual mean biomass figures for A. rosea are shown in Table 4 (p.95).

To construct Fig. 11 the total number of A. rosea worms collected during the sampling programme was sub-divided and summated according to the quarter grid square of collection. Since A. rosea is a relatively inactive, soil-dwelling species, this presentation was taken as accurately representative of the spatial distribution of the A. rosea population over the grid area. Comparison with the vegetation map for the area (Fig. 1) shows an association between low A. rosea densities and the areas of 'grasses' and bracken dominance which are delineated in Fig. 11. The bramble plant was seen to occur extensively in areas of maximum A. rosea population density, suggesting a positive relationship between the bramble plant and A. rosea distributions. Since the vegetational type occurring on each soil sample unit was recorded, it was possible to divide the total number of A. rosea extracted from each monthly sample into those taken from sample units with and without associated bramble. From the proportion of the total sample in which bramble occurred the expected numbers of A. rosea were estimated and a chi square analysis was performed, as shown in Table 2. The numbers of A. rosea found under vegetation including bramble was seen to be significantly higher than expected in nearly every month. The difference showed a tendency to be less significant in May and June (though still yielding 98% and 99% confidence, respectively) and was not significant in October and February. All other chi square values yielded a statistical confidence of 99.9%.



KEY to numbers of A.rosea collected and two relevant vegetational types:

= 0 to 10 = 21 to 30 = 41 to 50 = "Grasses" dominant

= 11 to 20 = 31 to 40 = 71 to 80 = Bracken

Fig. 11. Total numbers of A.rosea worms collected in each of the grid quarter-squares during the population survey. The spacial distribution of two relevant vegetational types is shown.

Month:	May	June	July	Aug	Sept	Oct	Nov	Dec	Jan	Feb	March	April
No. A. rosea under vegtn. with Bramble	83	43	25	42	67	51	59	80	30	42	36	47
No. A. rosea under vegtn. without Bramble	23	12	1	6	14	3	2	5	7	3	5	32
% of samples with Bramble	66.7	58.3	62.5	62.5	62.5	87.5	75.0	79.2	50.0	83.3	54.2	37.5
Expected no. under Bramble	71	32	16	30	51	47	46	67	19	37	22	30
Expected no. under vegtn. without Bramble	35	23	10	18	30	7	15	18	18	8	19	49
χ^2	6.14	9.04	13.16	12.80	13.55	2.63	14.94	11.91	13.09	3.80	19.23	15.53
P <	.02	.01	.001	.001	.001	.20*	.001	.001	.001	.10*	.001	.001

* = Not significant at the 95% level.

Table 2. Occurrence of A.rosea under vegetation with and without Bramble.

Discussion

In the population survey the relation between fresh weight and dry weight was used solely for determining the upper limit of the small immature dry weight size range. However, these calculated regression coefficients were used extensively in the course of this work for inter-conversion of individual fresh and dry weights. The lower slope of the regression line for small immatures suggests that worms of this size incorporate a component of higher water content than the equivalent in larger worms. Since body tissue is unlikely to vary significantly in composition, the gut contents may be the causal factor involved. Small immatures of this species - in common with small immatures of many soil-dwelling species - are known to inhabit the more organic upper layers of the topsoil, burrowing deeper into the mineral soil as their size increases. It might therefore be postulated that the lower slope of the small immature regression of dry weight on fresh weight was due to a more organic gut content in these worms. It should be noted that the difference in slopes could be explained by a relatively larger gut volume in larger worms (Satchell, 1968), but this was thought unlikely to produce a distinct change in the fresh weight/ dry weight relation.

Due to the displacement caused by the lower slope of the small immature dry weight/fresh weight regression line, the large immature and adult relation between dry and

fresh weights is not a direct proportionality - incorporating an appreciable negative constant factor. It should be noted that as a consequence of this factor, the percentage water content of these worms is not a constant - varying from 89.1% for a worm of fresh weight 100 mg. to, for example, 73.7% for a worm weighing 300 mg. fresh weight. Grant (1955b) estimated the water content of A. caliginosa (Sav.) to be 85.0% of total fresh weight. Such an estimate for a soil-dwelling species may be viewed with suspicion. A similar estimate of 70.7% water for O. cyaneum (Sav.), measured by Bouché (1967), may be less in error for this larger species - see Fig. 25 below which shows the relation between gut content weight and total body fresh weight for O. cyaneum. Calculations of percentage water content for surface-active pigmented species might be expected to yield more constant values, but the percentage water content of L. terrestris L., as calculated by Durchon and Lafon (1951), French et al (1957) and Bouché (1967) may include some error for worms without fully voided guts since larger worms of this species are known to ingest soil in addition to the litter material which forms the diet of the smaller immature stages.

The A. rosea population was shown to vary, both in numbers and biomass, through the year. In contrast to many invertebrate populations, the A. rosea population showed at least similar, and often greater, fluctuations in biomass to those shown in population numbers. This

was attributed to the relative constancy of mean A. rosea population individual weight throughout the year, due to a continuous emergence of young worms. The division of the A. rosea population into its three component size classes facilitated the analysis of temporal variations, though such divisions were criticised by Murchie (1958). He considered a classification into juvenile, immature and mature individuals unjustified since individuals mature at various sizes and regression in sexual activity may render adults and immatures indistinguishable. It was shown during this study that precocious adults show equivalent growth rates and cocoon production to normally developing individuals so that Murchie's first objection would seem of little practical importance. Adult regression is a more disturbing phenomenon, on which there is little information. In 1898, Foot recorded the disappearance of North American 'earthworm' clitella during the coldest months (Stephenson, 1930), though Pickford (1926) said that in this country clitellate individuals of a number of species - including A. rosea - were found throughout the year. In the present study it was found that in laboratory media regression of A. rosea adults occurred only under conditions of (a) high soil temperature (15^{o}C) over long periods (3 to 4 months) (b) low soil moisture content sufficient to induce dormancy or (c) extensive soil pollution. At Wynyard only the first of these situations is relevant, and since the maximum soil temperatures of

approximately 14°C were only of comparatively short duration any regression effects must be limited. Field growth and mortality data (see pp.111/112,148) for A. rosea at Wynyard showed no evidence of regression so that, for the practical purposes of this study, the effect can be assumed negligible.

The occurrence of high numbers of small immatures in spring and late summer was undoubtedly due to increased emergence from cocoons. The decline in small immature numbers in early summer probably reflected a high mortality due to higher temperatures and falling moisture content in the surface layers. The period from September until December was characterised by increased numbers of large immatures. This period is centred on a time exactly $1\frac{1}{2}$ years after the peak small immature emergence of the previous year and the late summer and early autumn decline in small immature numbers was probably affected by the recruitment of larger individuals into the large immature size class (see p.119). High soil temperatures during September may also have caused losses of small immatures due to mortality.

The late summer and autumn rise in large immature numbers was interrupted by a fall in numbers during the month of September. There was no corresponding rise in adult numbers so the decline must be assumed due to mortality caused by high soil temperatures and/or mole predation. The marked fall in large immature numbers during December was most probably due directly to mole predation (see below).

Murchie (1958) found that numbers of A. rosea clitellate individuals in a southern Michigan population increased in May and June but were very low at other times of the year. At Wynyard a similar spring peak was observed and must be assumed due to increased large immature recruitment. Such recruitment is not directly indicated by the large immature population numbers at this time, though the steady rise in large immature numbers over the period from January until April, 1967, suggests continuous small immature recruitment which may have masked maturation effects. The decrease in adult numbers in May/June was attributed to mole predation.

Thus the overall pattern of variation in A. rosea population numbers through the year resolves into an approximately constant level with three periods of peak numbers: a spring peak in April/May caused by high numbers of adults and small immatures, a September peak caused by large immatures and small immatures, and a November/December peak due solely to high numbers of large immatures. The validity of this analysis is supported by the observations of Evans and Guild (1948a) who studied seasonal variation in total population numbers of A. rosea in old grassland, of deciduous woodland origin, at Rothamsted. They also found three population peaks, two of which corresponded exactly with the April/May and September peaks at Wynyard and the third, in October/November, was sufficiently close to the large immature peak at Wynyard to be regarded as

equivalent. Evans and Guild, using the permanganate expellent method, did not extract A. rosea during the period from May/June to July/August. They realised the limitations of the expellent method and, in accordance with the qualitative nature of the study, simply recorded the species as 'inactive'. The summer dormancy found in grassland populations of A. rosea was not observed at Wynyard, presumably due to sufficiently high summer levels of soil moisture content in the woodland situation.

It will be noted that mean figures only are used in the presentation of lumbricid population data in the present study. It became obvious at an early stage in this work that the lumbricid populations at Wynyard were of the aggregated type, and the aggregation of A. rosea individuals on a large scale is clearly shown in Fig. 11. This was not surprising since aggregation is known to be a feature of lumbricid populations in most natural situations (Guild 1952a, b; Satchell 1955a; Svendsen 1957; etc.). Aggregated population data can be transformed logarithmically for the purposes of statistical analysis (Debauch, 1962; Southwood, 1966) but such manipulation of the data was considered unnecessary in the present work since only the arithmetic mean monthly values were required for the computation of mean annual biomass and energy flow parameters. The arithmetic means obtained by the random sampling technique were considered valid since selective and random sampling of aggregated populations have been

shown to produce similar frequency distributions (O'Connor, 1968b). Debauche (1962) recommended the use of the 'Disturbance Index' of Lexis, λ , as an index of aggregation since it is dependent on the number of aggregates in the population and on the density of individuals within these aggregates. Whilst accepting the arithmetic mean values for monthly population estimates, it was considered desirable to assess the degree of A. rosea population aggregation through the year by use of the Lexis index.

The small immatures were seen to be aggregated for most of the year, maximum aggregation occurring at the peak emergence period. This suggests two possibilities: either the adults became aggregated during periods of maximum cocoon deposition or those cocoons deposited in 'less suitable' soils failed to develop. Since the adults were more aggregated in September - a possible time for the deposition of over-wintering cocoons (see p.139) - the first alternative does have some credibility. However, the second explanation seems more likely since in the present study cocoons were shown to be sensitive to anoxic soil conditions such as might occur during periods of waterlogging (see p.132). Since the water table level at Wynyard was so high for most of the year, such waterlogging was inevitable in certain regions. During the period of minimum small immature numbers during July the individuals were found to be more randomly dispersed.

Large immatures were also aggregated for most of the year, with maximum aggregation at the time of peak numbers

in November and December. This factor was thought to support the suggestion of mole predation to explain the rapid decline in large immature numbers, since predation by moles would probably be most effective on an aggregated lumbricid population (see Appendix 6). The near random distribution of this size class in January and February was considered evidential of extensive aggregation destruction by moles.

The approximately constant, near random distribution of the A. rosea adults could be due either to similar recruitment rates from the large immatures in all areas due to the increased aggregation mortality postulated above, or to a greater degree of dispersive activity in this size class. Whichever of these explanations is correct, the conclusion remains that the high degree of aggregation shown by the monthly Lexis index for the total A. rosea population was due solely to the immature stages.

As previously stated, biomass variation was essentially similar to that found for population numbers. The use of monthly mean figures tended to smooth the data, eliminating minor fluctuations due to chance. Owing to their high individual weight, the adults contributed much more to the biomass than their numbers might suggest. The contribution of the large immatures to total population biomass was maximal. This was due to comparatively low small immature mortality, followed by rapid growth in the large immature phase (see pp.147 ,118). The high mortality rate in larger worms was

responsible for the depression of adult biomass.

The correlation of lumbricid population distribution with variation in environmental factors has received considerable attention from workers in the field of lumbricid ecology. Kollmansperger (1934) showed aggregation of various lumbricid species to be associated with patches of wetter soil, and Guild (1952a) considered soil moisture the most important limiting factor on hill land in Boghall Glen, Midlothian. However, Satchell (1963) - for L. terrestris - found no statistically significant relation between soil moisture content and population estimates obtained using a formalin expellent technique. Since water table depth, and associated soil moisture tension, showed no consistent spatial variation at Wynyard, it was not possible to attempt the correlation of A. rosea population density with soil moisture content. Murchie (1958) failed to correlate A. rosea distribution with soil pH, water retaining capacity, organic content or clay content - results which illustrate the lack of knowledge on factors determining distribution in this species. Guild (1951) suggested high soil acidity to be responsible for decreases in lumbricid numbers and species, and Satchell (1955a) considered pH to be an important limiting factor on lumbricid distribution. Soil pH variation at Wynyard was within such a narrow range that it was not possible to test this hypothesis. Satchell (1955a) showed correlations between the density of a grassland population of L. castaneus (Sav.) and various

environmental factors including soil pH, calcium content of the soil, abundance of Leguminosae in the surface vegetation and numbers of Staphylinid beetles in the soil and litter. However, he concluded that these factors were insufficient to explain local aggregations within L. castaneus and A.rosea populations. He suggested that 'family' groupings were the primary cause of aggregation in these species. In L. castaneus such groupings were said to result from a high rate of cocoon production with rapid maturation of immature worms (shown by Evans and Guild, 1948b), and in A. rosea, with a low cocoon production rate, the effect was said to be due to 'several seasons in which conditions have continued to favour the aggregating effect of reproduction more than the randomising effects of mortality and dispersion'. As in the present study, the aggregations found were largely due to concentrations of immature worms, adults being more dispersed. Svendsen (1957) did not support this theory of 'family' groupings since, in moorland soil, aggregations were mainly composed of mature individuals. Svendsen considered lumbricids sufficiently active for aggregation to develop by slower movement of individuals through more favourable localities.

Much work has been devoted to the correlation of lumbricid distribution and population density with soil type (Bodenheimer, 1935; Saussey, 1956; Lee, 1959; Kühnelt, 1961; Volz, 1962). Guild (1951, 1952b) stated that lumbricids show aggregation by both species and numbers

according to local soil, and associated vegetation, conditions. The 'family grouping' and 'local soil and vegetation' theories are generally recognised as the two major approaches to the problem of lumbricid aggregation.

In the present study, A. rosea population density was shown to be lowest in the 'grasses' and bracken areas. These areasoccurred exclusively in the birch tree zone and were associated with poorer soils of high clay content. On a more local scale, individuals of A. rosea were shown to occur more commonly beneath vegetation including bramble, than beneath other vegetational types. This was assumed due to the mull-type soils which were associated with bramble. Thus A. rosea aggregation seemed primarily due to soil and vegetation type, whether considered on a broad scale or in more local proportions. However, the restriction of aggregation to immature individuals suggests that the life cycle of this species affects population distribution. 'Family' groupings according to the chance effects of climatic conditions seem extremely unlikely and the true explanation of A. rosea aggregation must incorporate both life cycle factors and the influence of soil and vegetation. In the author's opinion A. rosea aggregation at Wynyard was due to either a positive taxis of adult worms, prior to cocoon deposition, towards favourable soil types, or random deposition of cocoons with high cocoon mortality in unfavourable soils. From the low value for the adult aggregation index throughout the year and the moderately

high values estimated for cocoon mortality (see p.150), the 'random deposition' theory according to classical concepts of natural selection would seem the more likely explanation of A. rosea aggregation. The 'taxis' theory might be more applicable to populations of surface active pigmented species where only immatures show aggregation (e.g. the L. castaneus study by Satchell, 1955a), though experimental evidence would be required to substantiate such a proposal. It may be significant that L. terrestris has been shown to possess prostomial chemosensory organs (Laverack, 1960; Satchell and Lowe, 1967).

The close association between the abundance of A. rosea individuals and the occurrence of bramble in the soil vegetation cover (see also soil preference experiments, p.172) was considered sufficient basis for the use of 'bramble' soil in field studies of production and in laboratory experiments for the investigation of A. rosea ingestion, assimilation and egestion (see Part II, Section III).

(ii) The Total Lumbricid Population

Fig. 12 shows the extent of variation in total lumbricid population numbers and biomass, in Kcalories, per metre square through the year. The total lumbricid population referred to in this section is that which was available to the sampling device used in the present study, i.e. the lumbricids occupying the top 30 cm. of the soil. The section of the deep-burrowing species (O. cyaneum (Sav.) and L. terrestris L.) populations occurring below 30 cm. depth is therefore not included and the effects of this source of error are discussed below (see p.104).

Both total lumbricid density and biomass showed three peak values in April/May, August/September and early December. These parameters of population size were numerically low in May/June and September/October, and minimal in mid-winter.

The mean lumbricid population density over the year of study was 101.69 worms per metre square - equivalent to approximately 400,000 worms per acre. In Fig. 13 total lumbricid density is divided into its component species populations through the year. Since the single species data were insufficient for quantitative examination in most cases, the total numbers of worms collected on each sampling occasion - from 24 samples, each $^{1}/_{16}$th metre square in area - were plotted accumulatively, by species, against the time of sampling. Pigmented and unpigmented species are treated separately for ease of comparison of these lumbricid

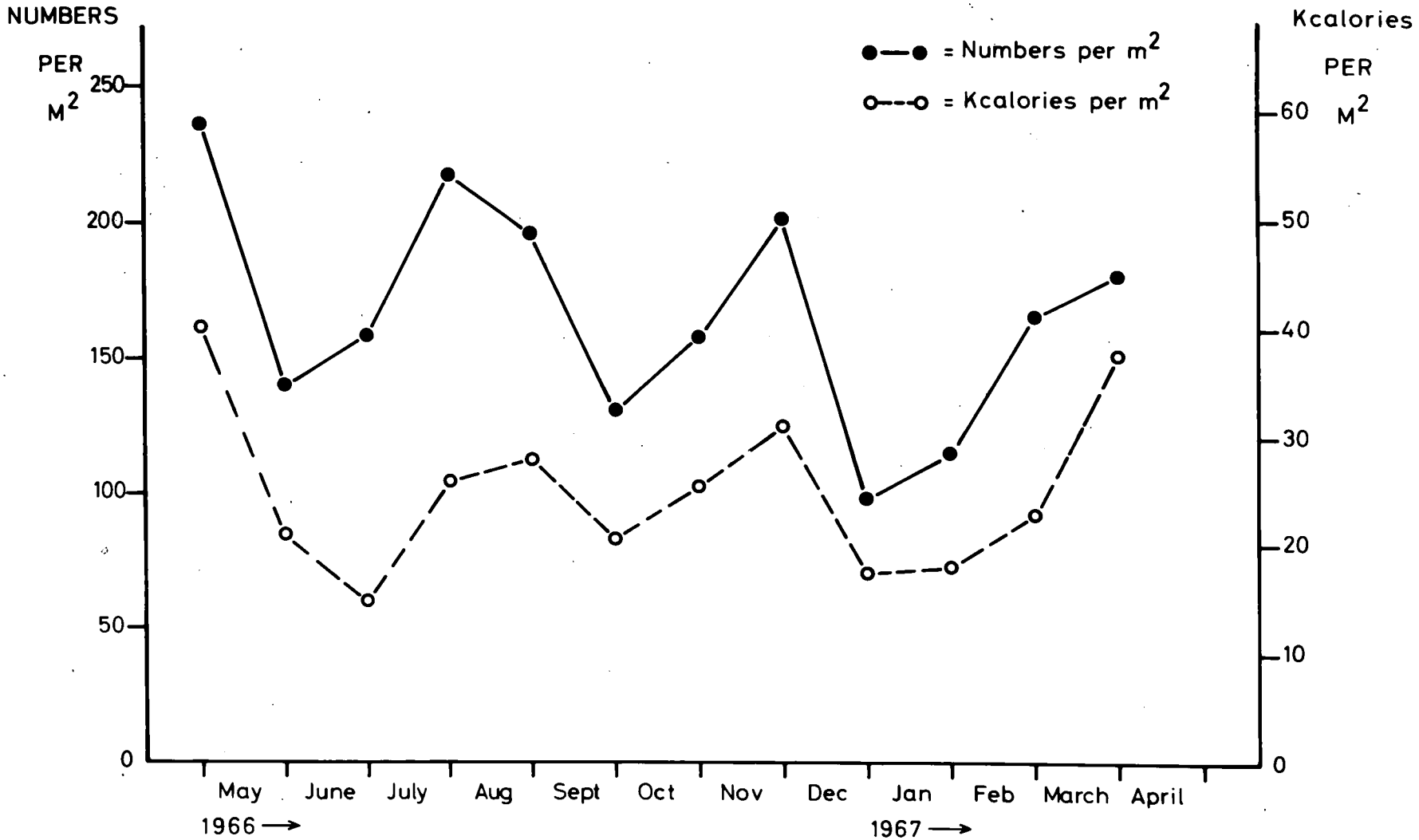


Fig. 12. Seasonal variation in density and biomass of the total lumbricid worm population occupying the top 30 cm. of soil in the grid area at Wynyard.

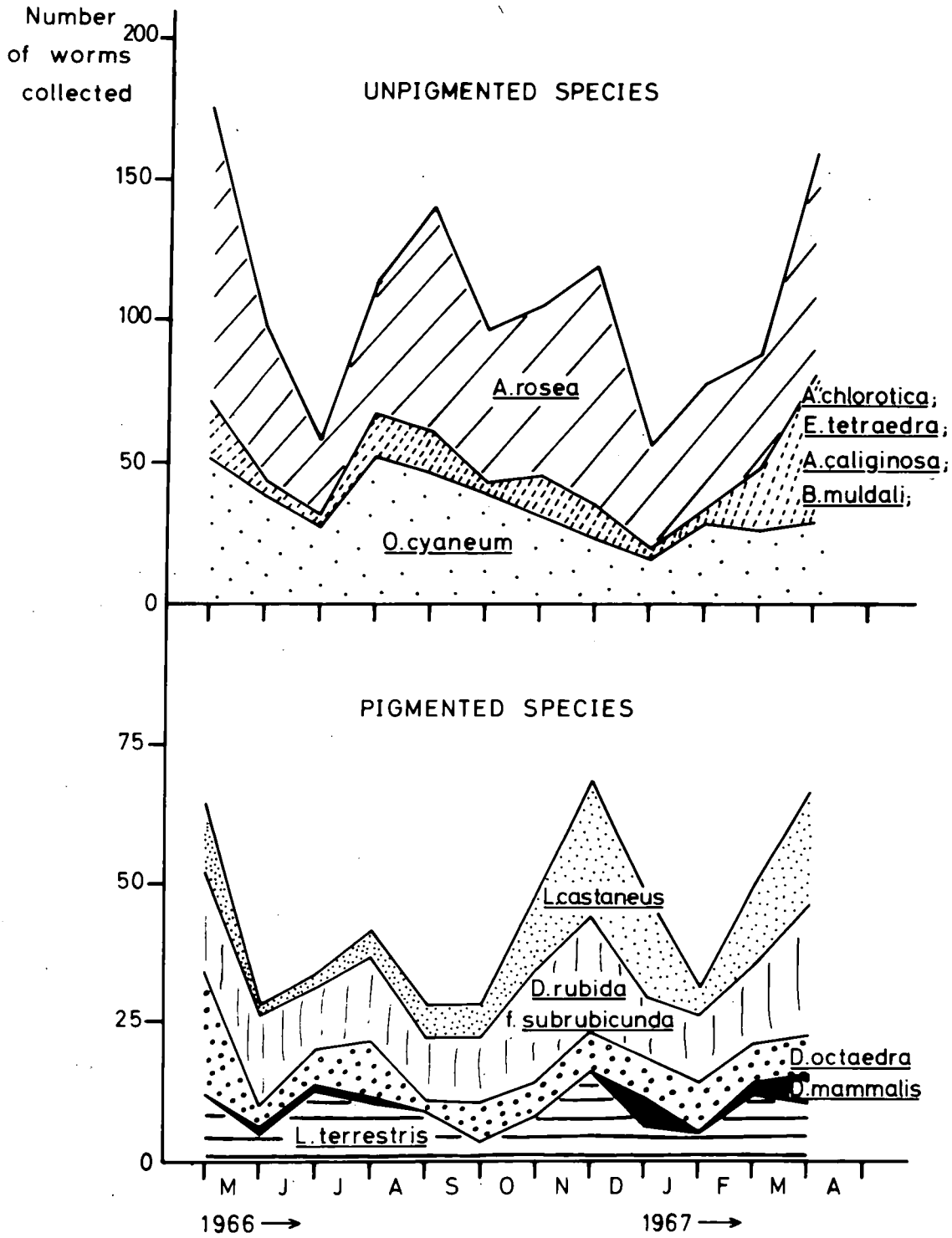


Fig. 13. Accumulative representations of the species components comprising the total lumbricid population in the grid area through the year. (See text).

groups, which show basic differences in mode of life and habitat relations (see Part I, Section II, sub-section 3). Both pigmented and unpigmented species showed the three peaks in density found for the total lumbricid population. However, the importance of the individual peaks varied between the two groups. In pigmented species the August peak was of little importance; however, the December peak was very pronounced, being of comparable size to the greatly increased population numbers in mid- spring. For most unpigmented species the situation regarding the late summer and early December peaks was reversed: the August/September peak was of sizeable proportions in all species, whilst the December peak was much less in evidence in all species except A. rosea (Sav.) Unpigmented species also showed the mid-spring period of abundance.

D. octaedra (Sav.) was the only pigmented species which did not conform to the overall analysis for the group. This species was collected more frequently in May, 1966, and August, 1966, but was present in approximately constant numbers at other times. After the August period of peak numbers, O. cyaneum showed a steady decline in numbers through late summer, autumn and early winter. There was an increase in numbers collected in mid-winter but the April, 1967, peak was not shown in this species.

The size class distribution for A. rosea was described above. Variation in numbers of O.cyaneum was primarily due to variation in immature population density, since

adults were present in a near constant level of low numbers throughout the year. E. tetraedra (Sav.) and B. muldali Omodeo showed higher numbers of immatures in mid-spring with sporadic occurrence of both adults and immatures at other times. A. caliginosa (Sav.) immatures were collected more frequently in August/September, November and mid-spring with adults occurring mainly in the summer months of July, August and September. A. chlorotica (Sav.) immatures and adults occurred very occasionally in mid-spring, with one immature recorded in December, 1966. L. terrestris immatures showed peak numbers in late summer, early December and early spring; adults occurred more frequently in early spring, mid-summer and early December. Adults of L. castaneus (Sav.) showed somewhat higher numbers in early January, being present in low numbers at other times. Immatures of this species were abundant in early December and mid-spring. Adults and immatures of D. rubida (Sav.) f. subrubicunda (Eisen) were present in approximately equal numbers throughout the year, though immatures tended to be more frequent in mid-spring and early November whilst adults showed higher densities in early December and in the early spring of 1967. D. octaedra population numbers were mainly composed of immatures throughout the year, though adults were more frequent in the late spring and early summer of 1966. D. mammalis (Sav.) adults occurred in mid-summer, 1966, and both adults and immatures were found in the mid-winter and early spring of 1967.

Though the number of species in each of the pigmented and unpigmented groups was similar - five and six, respectively - the individual species populations in the pigmented group were of more similar size than those in the unpigmented group, which was dominated by A. rosea and O. cyaneum. The mean total population density of the unpigmented species was approximately 2.5 times the similar figure for pigmented species.

The aggregation index, λ, was calculated monthly for the total lumbricid population density per metre square; the variation in λ through the year is shown in Fig. 9. The highest values were recorded in mid-spring, with minimal aggregation in mid-summer and mid-winter. For the rest of the year the population showed approximately constant aggregation at a high value (λ 2.0 to 2.3).

Table 3 shows the mean annual standing crop, in terms of dry weight per metre square, for the various species occurring in the grid area. The group totals for deep-burrowing species, small unpigmented topsoil species and surface active pigmented species are shown. The total dry weight biomass of unpigmented and pigmented species were 8.49 g/m^2 and 4.286 g/m^2, respectively; i.e. these values were in the approximate ratio of 2 : 1. The total lumbricid population was found equivalent to 51.71 Kg. dry weight per acre. Seasonal and annual mean biomass figures in Kcalories per metre square are shown in Table 4. For the deep-burrowing species O. cyaneum the spring and summer

Species	Mean Biomass ($gms.\ dry\ wt/m^2$)		
O. cyaneum	7.008	10.872 (≡ 44.00 Kg/acre)	: Deep-burrowing species
L. terrestris	3.864		
A. rosea	1.114	1.483 (≡ 6.00 Kg/acre)	: Small Unpigmented topsoil species
A. caliginosa	0.328		
E. tetraedra	0.025		
A. chlorotica	0.012		
B. muldali	0.004		
L. castaneus	0.200	0.422 (≡ 1.71 Kg/acre)	: Surface active pigmented species
D. rubida f. subrubicunda	0.086		
D. octaedra	0.123		
D. mammalis	0.013		

Table 3. Lumbricid mean annual biomass in the grid area in grams dry weight per metre square, calculated from monthly measurements over a one year period.

Season	A. rosea				Deep-burrowing species		Small unpigm. species (incl. A.rosea)	Small pigm. species	Total lumbricid population
	SI	LI	A	Total	O.cyaneum	L.terrestris			
SPRING (mid-March to mid-June)	0.751	1.364	1.838	3.953	22.337	16.223	5.616	2.241	46.417
SUMMER (mid-June to Sept/Oct)	0.419	1.621	1.271	3.311	23.923	15.932	4.438	1.143	45.436
AUTUMN (Sept/Oct to Nov/Dec)	0.401	2.844	1.373	4.618	17.733	15.073	5.710	2.049	40.565
WINTER (Nov/Dec to mid-March)	0.439	1.516	0.837	2.792	13.454	15.201	3.578	2.145	34.378
WHOLE YEAR	0.507	1.733	1.308	3.548	19.441	15.648	4.694	1.861	41.644

Table 4 'Seasonal' and Annual Mean Lumbricid Biomass in Kcalories per metre square, calculated from measurements made on or about the 1st of each month over a one year period. (SI - Small immatures; LI - Large immatures; A - Adults)

values were similar, but the autumn figure was lower with minimal standing crop during the winter months. L. terrestris, however, showed a constant biomass throughout the year. Small unpigmented topsoil species, greatly influenced by the A. rosea population, showed similar biomass in spring and autumn with lower values in summer and, especially, in winter. The standing crop of surface active pigmented species was constant for most of the year, but was reduced by approximately 50% in summer. The total lumbricid population maintained a more or less constant biomass in spring, summer and autumn but the figure was lower in winter. The deep-burrowing species (O. cyaneum and L. terrestris) contributed 35 Kcalories/m^2 to the mean annual lumbricid biomass, whilst the topsoil and surface-living species amounted to only about 6.6 Kcalories/m^2. The total unpigmented and pigmented species biomass estimates for the year, 24 Kcalories and 17.5 Kcalories/m^2, respectively, were in the approximate ratio 4 : 3.

The mean annual lumbricid biomass of 41.644 Kcalories per metre square was accumulatively constructed by species and species groups in Fig. 14 to demonstrate the importance of A. rosea relative to other topsoil and surface-living species, and of deep-burrowing species relative to the total population, in terms of standing crop. The ratio of A. rosea mean standing crop in Kcalories to that of rare unpigmented topsoil species and surface active pigmented species was approximately 6 : 2 : 3. Deep burrowing species accounted for 84% of the mean annual standing crop.

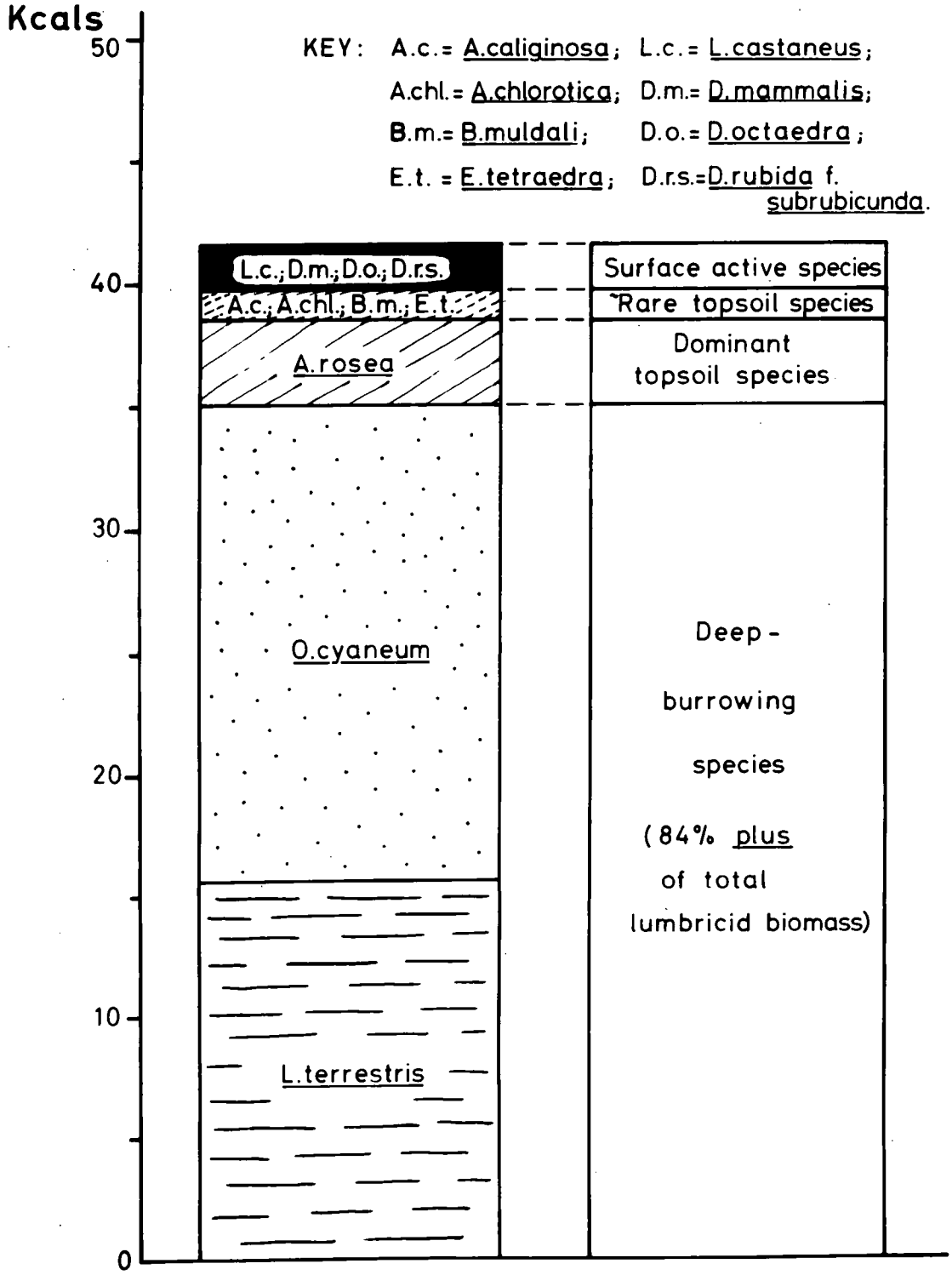


Fig. 14. Lumbricid species and species group contributions to the total mean annual lumbricid biomass on the grid area at Wynyard (1966/67).

Numbers of cocoons collected each month were totalled for the pigmented species L. terrestris, L. castaneus, D. rubida f. subrubicunda and D. octaedra, and for the unpigmented species A. rosea and O. cyaneum. These two groups were dominated by the higher recovery of L. terrestris and O. cyaneum cocoons, respectively, but the groupings were considered valid since similar trends to those of the dominant species were shown by the species with fewer recoveries. Fig. 15 shows the annual variation in collected numbers of cocoons of these two groups. The data are presented in the form of monthly means, calculated from measurements made on or about the 1st of each month. The two histograms are markedly different in form. The cocoons of pigmented species showed peak numbers in August, 1966, followed by a sharp fall in September before the major peak in November, 1966. From November, 1966, to April, 1967, cocoons of pigmented species showed a steady decline which appeared to continue until June/July, judging from data obtained for May and June, 1966. More constant numbers of cocoons of unpigmented species were collected through the year. There appeared to be two extended periods of rising numbers - from June to August and from October to April - the first being followed by a fall in numbers in September, and the second, after an interrupting fall in February, being terminated by the fall to lower numbers in May. Cocoons of both unpigmented and pigmented species were present in the field throughout the year but the unpigmented group

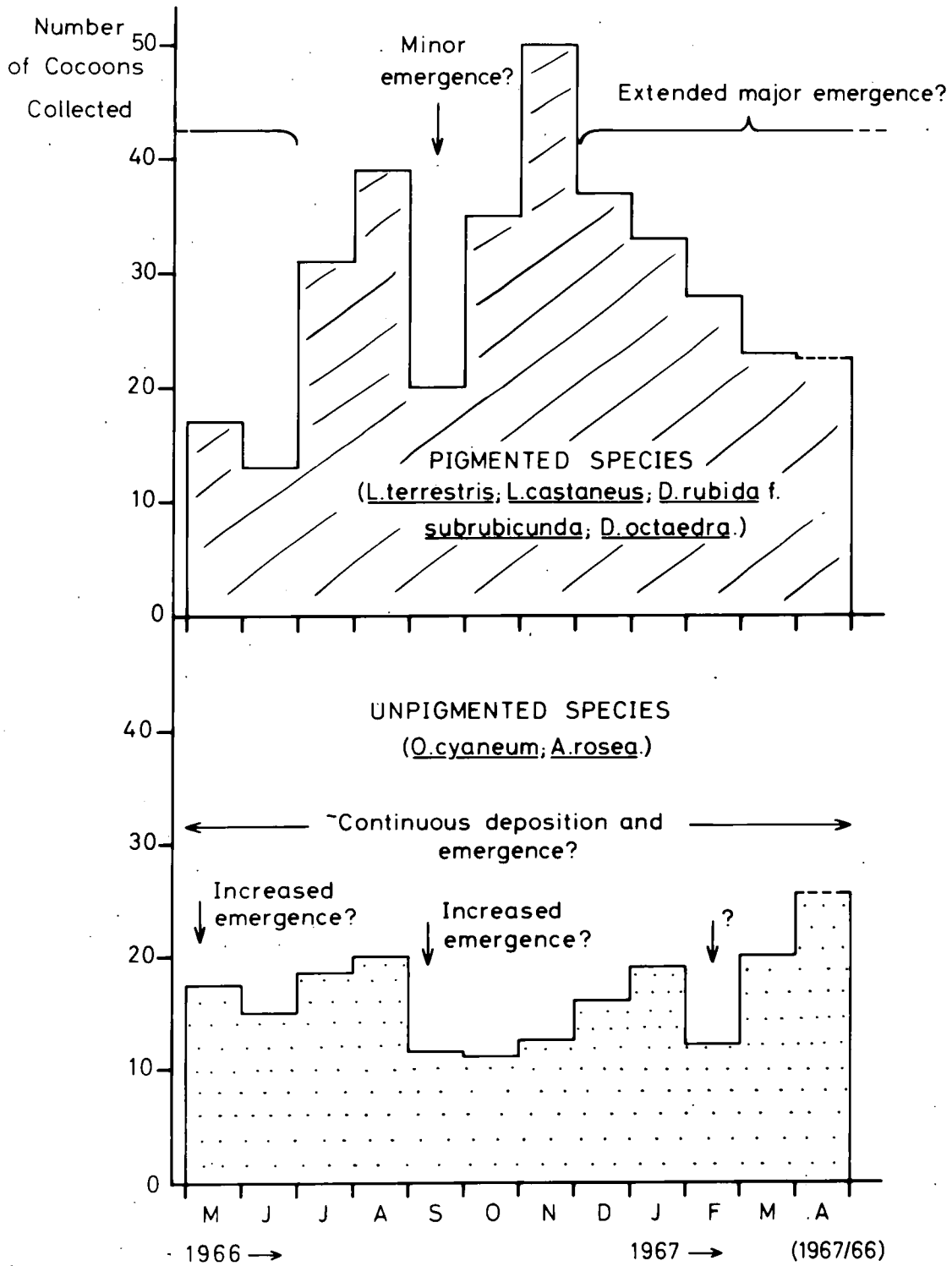


Fig. 15. Numbers of cocoons of pigmented and unpigmented lumbricid species collected during the population survey at Wynyard.

showed less synchronised variation in numbers.

Since the recovery of O. cyaneum and L. terrestris cocoons was reasonable good, yielding mean annual densities of 8.67 and 12.50 cocoons per metre square, respectively, the cocoon biomass of these two deep-burrowing species was calculated. Biomass was estimated from the mean cocoon dry weights (given for a number of species in Appendix 4) and cocoon calorific values (see Part III, Section I). The mean annual biomass of O. cyaneum cocoons was 0.081 gms. dry weight or 0.422 Kcalories per metre square; equivalent figures for L. terrestris cocoons were 0.224 gms. dry weight and 1.171 Kcalories per metre square. The total biomass of cocoons of deep-burrowing species - 1.593 Kcalories/m^2 - was equivalent to 4.5% of the deep-burrowing worms' biomass, 3.8% of the total lumbricid biomass and 24.3% of the total topsoil and surface active worms' biomass in Kcalories.

Discussion

Total lumbricid biomass in Kcalories fluctuated to a slightly greater degree, proportionally, than population numbers. However, in view of the considerably greater influence of the two deep-burrowing species on population biomass than on population numbers (see Fig. 13, Table 4 and Fig. 14), the synchronisation between these parameters was of a remarkably high order. The mid-spring and late summer peaks corresponded to peak immature numbers in most species, periods of increasing overall aggregation, and periods of maximum decrease in numbers of cocoons of both

pigmented and unpigmented species. It can therefore be assumed that these peaks were due to the emergence of both pigmented and unpigmented worms in places where conditions were locally favourable.

Early December was a time of peak numbers of immature worms of pigmented species; it was also a period of peak A. rosea large immature numbers and biomass. The very slightly increased numbers of immature A. caliginosa in November were of negligible proportions. The early December peak in total lumbricid numbers and biomass was thus assumed due primarily to increases in the pigmented worm population, supplemented by an increase in the large immature population of the dominant unpigmented topsoil species. Since the aggregation index for both the total lumbricid population and the total A. rosea population did not change significantly over this period, the population of pigmented worms - including newly-hatched worms - was assumed to have remained in the same aggregation localities but to have maintained the original density within aggregates by a degree of local dispersion (c.f. increase in A. rosea aggregation on increased emergence of young worms). This emergence phenomenon may have a similar basis to the fact that total pigmented species population density was found to be less than half that for unpigmented worms - despite the higher reproductive rates in pigmented species (Evans and Guild, 1948b.). In addition to their restriction to favourable localities, characteristic of

both lumbricid groups, the pigmented species are limited to an essentially two dimensional habitat. The latter limitation is most obvious for the small, surface active species but applies equally well to L. terrestris, which relies largely upon food obtained at the soil surface. Whereas the density-increasing effects of emergence are countered by the volume of nutritious medium available to unpigmented soil-dwelling species, for pigmented species these effects must be countered by dispersion of individuals to the limits of the favourable locality and/or regulation of population size. Population density of the pigmented species will be kept at a low level by high mortality rates: the surface active species are vulnerable to both predation and fluctuation in physical conditions; L. terrestris is eagerly sought by moles (see Appendix 6). The high mortality factors probably account for the high reproductive rates of pigmented species, but when these factors fail to regulate numbers adequately - possibly as in early December at Wynyard, following the optimum temperatures and food availability of autumn - then density may be lowered by two dimensional dispersion. Such dispersion will obviously have less effect on the aggregation index, based on area measurements, than the three dimensional and predominantly vertical dispersion typical of soil-dwelling species.

The periods of rapidly decreasing total lumbricid population numbers, following each of the peak periods described above, were attributed to an increased mortality

of young worms in late spring and summer - due to high soil temperatures and a fall in surface soil moisture content - and to high mortality of both large and small individuals in autumn and early winter as a result of predation by moles and carnivorous invertebrates.

The mean aggregation index value of 2.0 to 2.3, for the total lumbricid population, was comparable to the value of approximately 2.6 measured for oribatid mites in oak litter (Debauche, 1962). Though the lumbricid figure was, on average, somewhat lower than the value for mites, it is interesting to note that in May, 1966, and April, 1967, the lumbricid population reached degrees of aggregation described by λ indices of 2.7 and 3.2, respectively.

Satchell (1958) listed seven British authors, and twenty-seven authors from other countries, who have estimated lumbricid population densities in the range 1.25 to 3 millions per acre. Satchell considers this range to be a reliable indication of 'acceptable' population estimates. It is relevant to note that the large majority of these studies were carried out in grassland, pasture-land or arable soils where soil conditions are usually most favourable to the majority of lumbricid species. Very little information is available on lumbricid population size in 'poor quality' soils. Minimal estimates of approximately 73,000 and 50,000 lumbricids per acre were recorded by Bornebusch (1930) in spruce raw humus and by Guild (1948) in peaty acid soil, respectively. In Egyptian

heavy clay soils Bishara and El-Kifl (1954) recorded maximum densities of about 670,000 lumbricids per acre; Habib and Issa (1958) found numbers ranging from 33,000 to 165,000 per acre and of the fourteen localities studied by El-Duweini and Ghabbour (1965b) seven had lumbricid population densities of less than 500,000 per acre, four were in the range 0.5 to 1.25 millions per acre and only three were within the 'acceptable' range quoted by Satchell (1958). Van Rhee and Nathans (1961) found only 101,000 to 608,000 lumbricids per acre in 'green manure' plots compared with 1.22 to 2.03 millions per acre in grass plots. The acid clay soil with high water content found in the grid area at Wynyard was similar to that found in a nearby low moor area where waterlogged soils were overgrown by Juncus spp. A. rosea was common in both areas and it is interesting to note, in relation to the mean figure of 101.69 lumbricid worms per metre square (412,000 per acre) in the grid area, that Pickford (1926) hand-sorted the soil amongst Juncus spp. roots and found a lumbricid population density of 96.9 worms per metre square (392,000 per acre). Satchell's population density range of 1.25 to 3 millions per acre is undoubtedly correct for climax grassland or cultivated soil situations, but the lower limit of 1.25 millions per acre (about 300 worms per metre square) is to be regarded as a maximum figure for fringe habitats with soils of poor structure and conditions adverse to the growth and development of lumbricid populations.

Apart from the major controlling factors of mortality and emergence from cocoons (Evans and Guild, 1948a), the insulation effects of organic soil cover in relation to soil freezing (Hopp, 1947), 'flushes' of dead root and herbage debris (Waters, 1956) and dispersion (Murchie, 1958) have been cited as factors affecting seasonal variation in lumbricid population densities. Though winter air temperatures were frequently below $0^{o}C$, the soil in the grid area did not freeze at any time and this was attributed to the insulation effects of dense vegetational cover, supporting Hopp's observations in relation to cultivated soils. The abundance of raw organic debris in autumn and early winter was thought to affect the population size of pigmented species but to be of little importance to soil-dwelling species. A discussion of the possible effects of dispersion, considered in a three dimensional was well as the two dimensional sense, was given above.

The influence of the deep-burrowing species O. cyaneum and L. terrestris on lumbricid population biomass at Wynyard has been shown to be considerable. Examination of the seasonal mean estimates of biomass showed certain differences between the two species involved: O. cyaneum biomass was constant in spring and summer but with values decreasing in autumn and winter, whereas L. terrestris biomass remained approximately constant throughout the year. Variation in O. cyaneum numbers was shown to be due mainly to an August, 1966, peak in immature numbers, followed by a

steady decline until January, 1967, and a second period of peak immature numbers in early and mid-spring. Adults numbers were low and constant throughout the year. Immature and adult L. terrestris numbers showed similar variations throughout the year. It thus seems possible that whilst the whole L. terrestris population and adults of O. cyaneum were underestimated to a constant degree, immatures of O. cyaneum tended to be active nearer the surface of the soil in spring and mid-summer and were therefore sampled more adequately. It might be possible to calculate a correction factor on this basis but the present data were considered inadequate for this purpose.

The 2 : 1 ratio of unpigmented to pigmented worm biomass in terms of dry weight was shown to give a false impression of dominance when compared with the ratio of 4 : 3 for the same parameter in Kcalories. This was due to the high weight of inorganic material in the gut contents of soil-dwelling, unpigmented species. In terms of Kcalories per metre square, A. rosea was shown to provide 54% of the total topsoil and surface active lumbricid population biomass; absolute numbers and biomass of A. rosea were considered sufficiently large and consistent for their utilisation in constructing a population energy budget for this species. Using information from various authors, in addition to that gained in the present study, the population metabolism (as an indicator of energy flow) of each species or species group was estimated below for comparison

with the proportions of population biomass shown in Fig. 14 (see Table 22 and p.282).

The cocoons of pigmented species would seem to be deposited mainly in the summer and autumn months, deposition occurring, but at a much reduced rate, at other times of the year. Cocoons deposited in late spring and early summer probably accounted for the minor hatch indicated by the fall in cocoon numbers in September, 1966, though little information is available on cocoon incubation periods and the effects of soil temperature (Evans and Guild, 1948b). An extended period of emergence of pigmented worms, from early winter until late spring or early summer, was indicated by a steady decline in cocoon numbers. Satchell (1967) showed a well-defined spring peak in the emergence of L. terrestris in a woodland situation. At Wynyard, where L. terrestris provided the bulk of the collected cocoons of pigmented species, the spring emergence was not so synchronised.

The collected numbers of O. cyaneum and A. rosea cocoons indicated continuous deposition and emergence of cocoons, with increased emergence in early spring, late summer and possibly over a short period in mid-winter. The first two peak emergence periods were supported by the occurrence of increased immature numbers of both O. cyaneum and A. rosea. The mid-winter fall in cocoons numbers coincided with an increase in numbers of O. cyaneum immatures. Observations on cocoon deposition and emergence in A. rosea (see p. 138) indicated that cocoon deposition

in this species occurred mainly in the spring and summer months, worms emerging in September having had an incubation period of less than three months. Worms emerging at other times were thought to have had a much longer incubation period. Cocoons of O. cyaneum were kept in Wynyard soil in a constant temperature room at 10°C for more than six months without any sign of development. This suggests that long-term experimentation, involving observations at both field and laboratory temperatures, will be necessary for a better understanding of lumbricid cocoon incubation periods.

The figures given in the present study for numbers and biomass of O. cyaneum and L. terrestris cocoons have shown that the density and biomass of cocoons of deep-burrowing species can be readily estimated by even a hand-sorting method. With a more suitable technique these quantities could be measured to a much higher degree of accuracy. Experiments could be carried out under field conditions to determine the rate of cocoon deposition by adults of deep-burrowing species, and to determine the variation in incubation periods. It might then be possible to use the density and/or biomass of cocoons - deposited in the topsoil - to determine indirectly the adult population size of these species for which there is no adequate direct sampling technique.

II Secondary Production of Allolobophora rosea (Sav.) f. typica

1. Growth Studies in the Field

(i) Growth of Individual Animals

Introduction

The purpose of studying individual growth rates of A. rosea was twofold: first, to obtain a growth curve for use in ageing the field population in order to estimate field mortality rates; second, to measure rates of tissue production of the various life stages, under field conditions, throughout the year. Measurements of growth rates could have been made under laboratory conditions, but the elaborate correction techniques necessary for extrapolation from the laboratory to the field situation were avoided in the present work by performing these studies entirely under field conditions. During the course of these studies note was taken of the sexual condition of the individual animals to determine the rate of attainment of sexual maturity and the degree of adult regression.

Methods

Red-clay porous plant pots, measuring 15 cm. internal depth by 16 cm. upper internal diameter, were filled to a depth of 12 cm. with mull-type soil from a 'bramble' site adjacent to the grid area. Soil layers were placed in the pots in the same relative positions as they occurred in the field. The pots were spaced on a grid square system, allowing a minimum of 50 cm. between pot positions, and sunk into the soil of the growth studies site so that the

internal and external soil surfaces for each pot were at the same level. Ground vegetation and soil structure of the site were carefully preserved throughout the study.

Twenty to twenty-five A.rosea individuals of each of the major size classes - small immatures, large immatures and adults - were dug from the site and fresh-weighed. Parameters of size class distinction and the mode of fresh-weighing were as described for A. rosea in the population survey (p.71). Two, and occasionally three, worms of markedly unequal size were placed in each pot. To prevent escape, each pot was provided with fine mesh nylon gauze at the base and muslin sheet, secured by elastic bands, over the upper surface.

At monthly intervals over a one-year period the pots were transported to the laboratory and stored overnight at 10°C. On the following day the soil from each pot was carefully removed by layers and the worms extracted by hand-sorting. The soil layers were replaced in the pot in their original arrangement and lightly compacted to field texture and firmness. The worms were fresh-weighed, assigned to categories of sexual development and returned to the pot media for transport back to the field. All soil media were exchanged for 'fresh' soil from the same site at three-monthly intervals. Any worms which died, became damaged or parasitised, or escaped from the pots were replaced by individuals of similar size taken from the same area. Growth data were recorded separately for

individual animals; any animals progressively losing weight prior to paralysis - and eventually death - through parasitisation were thus easily detected and discounted throughout their period of measurement. In the final data analysis for growth curve construction the animals were assigned to arbitrary weight classes and mean annual growth increment rates were calculated from all individual increments measured for animals in each weight class throughout the year. The weight classes were chosen such that the mean annual increment rate for each class was calculated from data on growth rates of about fifty, or more, individuals. Calculations of mean growth increment per month by the three major size classes at different times of the year were made on a two-monthly basis to ensure an adequate sample size for each period.

A mean annual growth curve for the species was constructed as follows:

A mean emergence weight for the species was determined by the fresh-weighing of 28 newly-emerged individuals - after cocoon incubation at $10^{o}C$ in 5 cm. x 0.5 cm. glass tubes containing mull-type 'bramble' soil and a dampened cotton wool plug. The cocoons were checked daily so that the young worms were fresh-weighed within one day of emergence. The mean emergence weight was used as the starting point for the time-based growth curve. The mean annual weight increment per month for the lowest weight class was added to the emergence weight and the total

weight plotted at time, or age, one month. For greater accuracy the actual figures used were a multiple of the mean increment rate, e.g. ten times the monthly mean increment, added to the emergence weight and plotted at time, or age, ten months. The straight line lying between the emergence weight and the summated weight, located as above, was drawn from the emergence weight to the upper limit of the lowest weight class. If the summated weight was greater than the upper limit of the weight class the line was stopped short at the upper limit, and if the summated weight was within the weight class range then the line was continued through the summated weight to the upper limit of the class. The terminal weight and time, or age, thus determined for the lowest weight class were then treated as the 'emergence' weight and starting point in time for the construction of the growth curve section passing through the 2nd. lowest weight class. This procedure was continued until the growth curve was complete.

Results

The mean emergence fresh weight for A. rosea worms was found to be 6.32 ± 0.02 mg. (St. Error). This initial weight is seen as the starting point of the fresh weight growth curve for an 'average' individual shown in Fig. 16. The curve is imperfectly sigmoidal in form, with an inflexion after approximately two years (see Bertalanffy, 1949). The horizontal lines adjoining the curve demarcate the weight ranges used for purposes of calculation. The growth

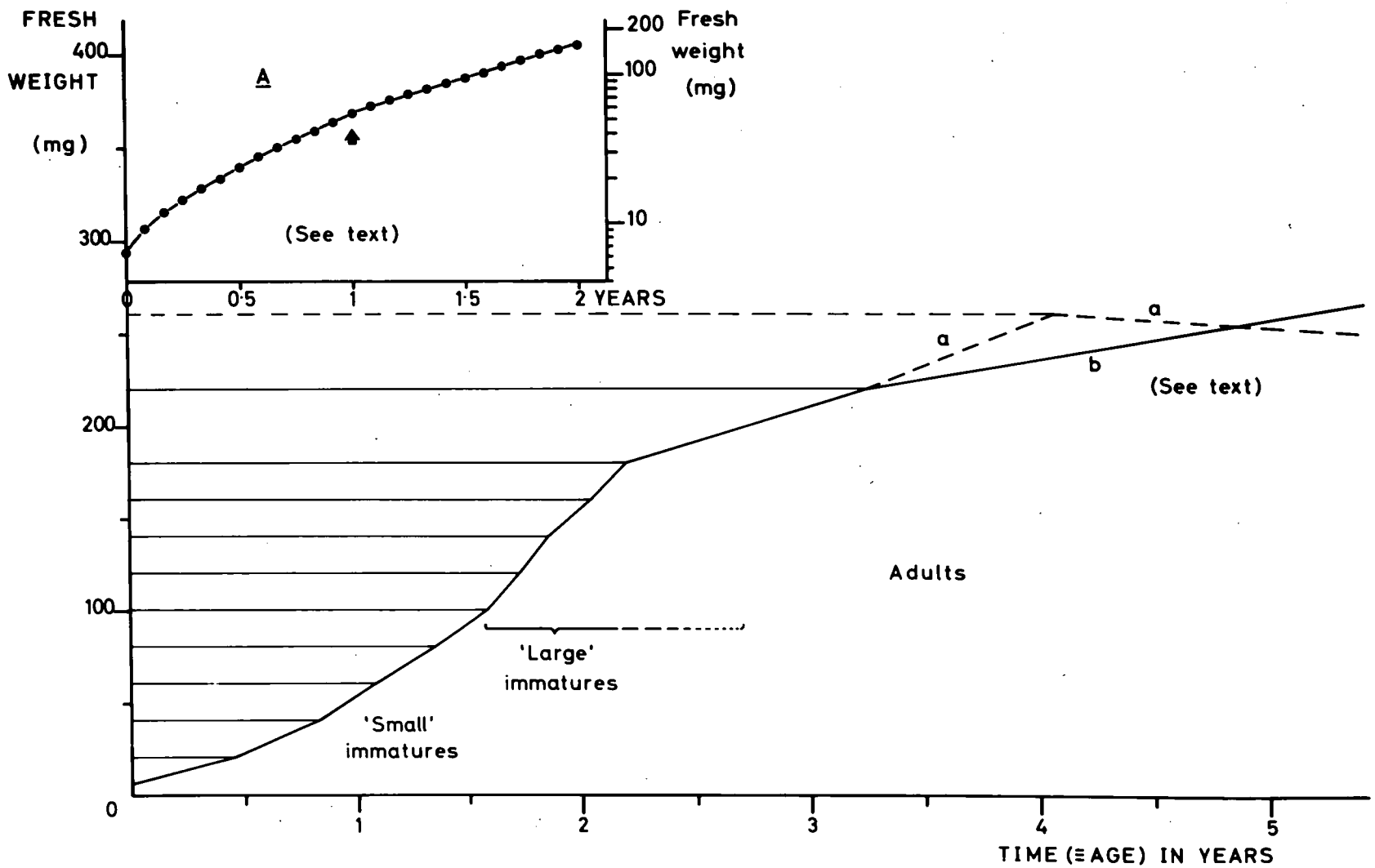


Fig. 16. The fresh weight growth curve for an 'average' A.rosea individual.

increments for worms of weight in excess of 221 mg. were initially calculated in two weight ranges - 221 mg. to 260.9 mg. and 261 mg. plus - producing the growth line 'a' in Fig. 16. Since A. rosea individuals of fresh weight greater than 261 mg. occurred in the field the broken line 'a' was considered unrealistic and unsuitable for ageing purposes. The growth increment data for all animals of weight in excess of 221 mg. were therefore pooled and a grand mean increment rate was calculated. This procedure allowed the construction of line 'b' in Fig. 16 and this line was used for ageing purposes. The growth curve has been apportioned into 'small' immature, 'large' immature and adult categories according to the parameters used in the population survey.

The inset A in Fig. 16 shows the course of fresh weight increase over the first two years, weight being plotted on a logarithmic basis. The arrow marks the point, after one year, when the rate of weight increase per unit time appears to change: during the first year the rate gradually diminished but over the second year the rate of increase was approximately constant. After two years the rate of weight increase per unit time was found to fall off rapidly. Fig. 17 shows the percentage frequency, in the sample of A. rosea individuals used for growth studies, of the various stages of sexual development, plotted against worm body weight. Stages of maturation occurred over a wide weight range - 60 mg. to 260 mg. - but no evidence of

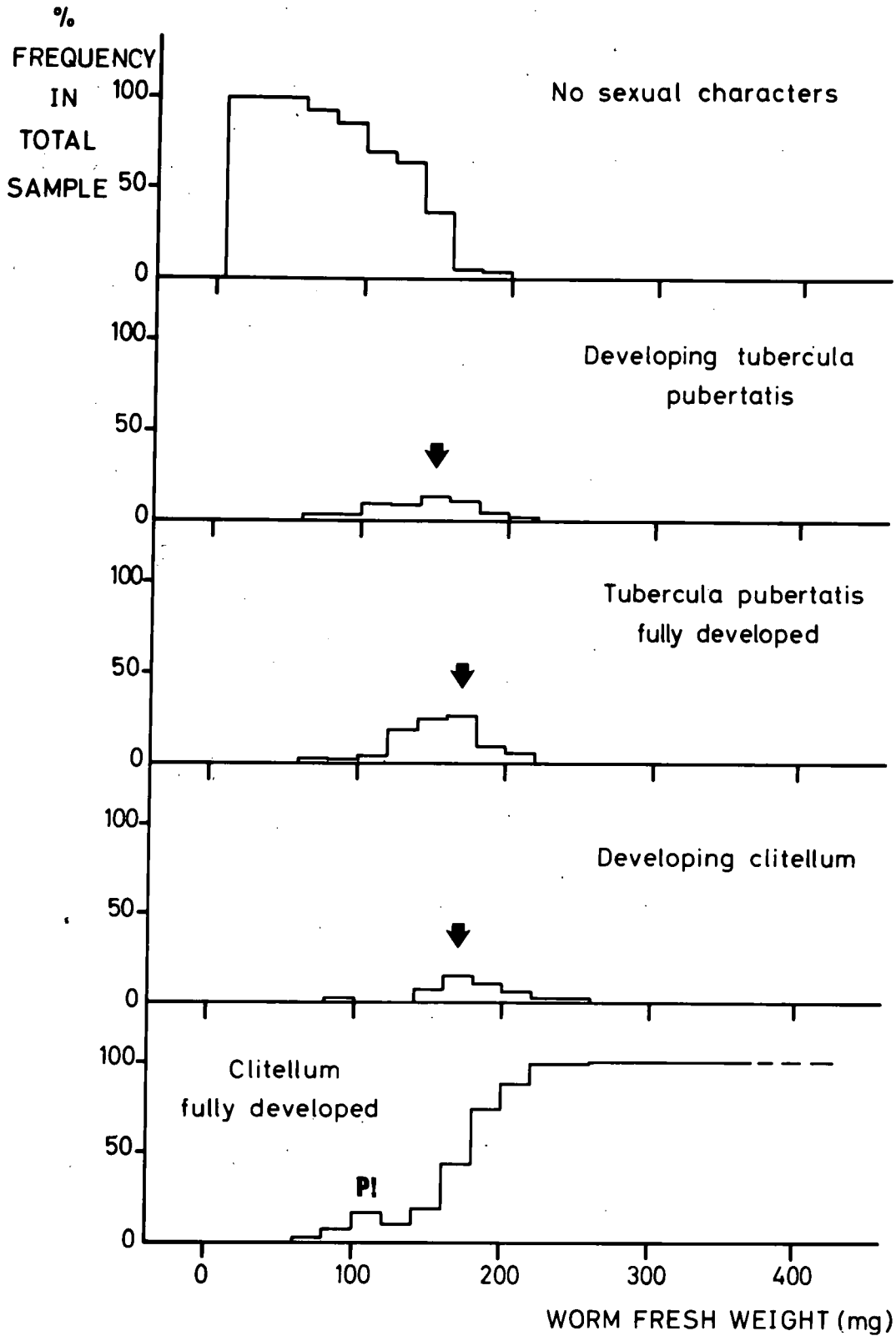


Fig. 17. Percentage frequency of A.rosea maturation stages in the sample of worms used during field growth studies.

regression was recorded during these field measurements. The arrows in Fig. 17 mark the peak percentage frequencies of the three intermediate maturation stages. These peaks, which must signify maximum maturation development, were found to occur over a quite narrow weight range and to approximately coincide with the 50% frequency of mature individuals at fresh weight 175mg. The overall percentage frequencies of the intermediate maturation stages suggest that the tubercula pubertatis develop rapidly, persisting for some time prior to the rapid development of the clitellum.

'P!' in Fig. 17 marks the minor peak of early maturing individuals, hereafter termed 'pygmy adults'. The pygmy adults were largely responsible for the width of the maturation weight range. They occurred in the field population in similarly small proportions to their frequency in the sample of worms used for growth studies.

In Fig. 18 the mean monthly growth increments - calculated from data collected over a full year - for 'average' individuals in each of the weight ranges are shown in the form of a histogram of growth production. Body tissue production, in absolute terms, showed a broad peak over the weight range 101 mg. to 181 mg. and was usually negligible in animals weighing more than 261 mg. Considerable variation occurred in the growth increment data but mean values obtained over the peak range indicated above were significantly different from the mean monthly increments of smaller or larger worms at the 95% level of

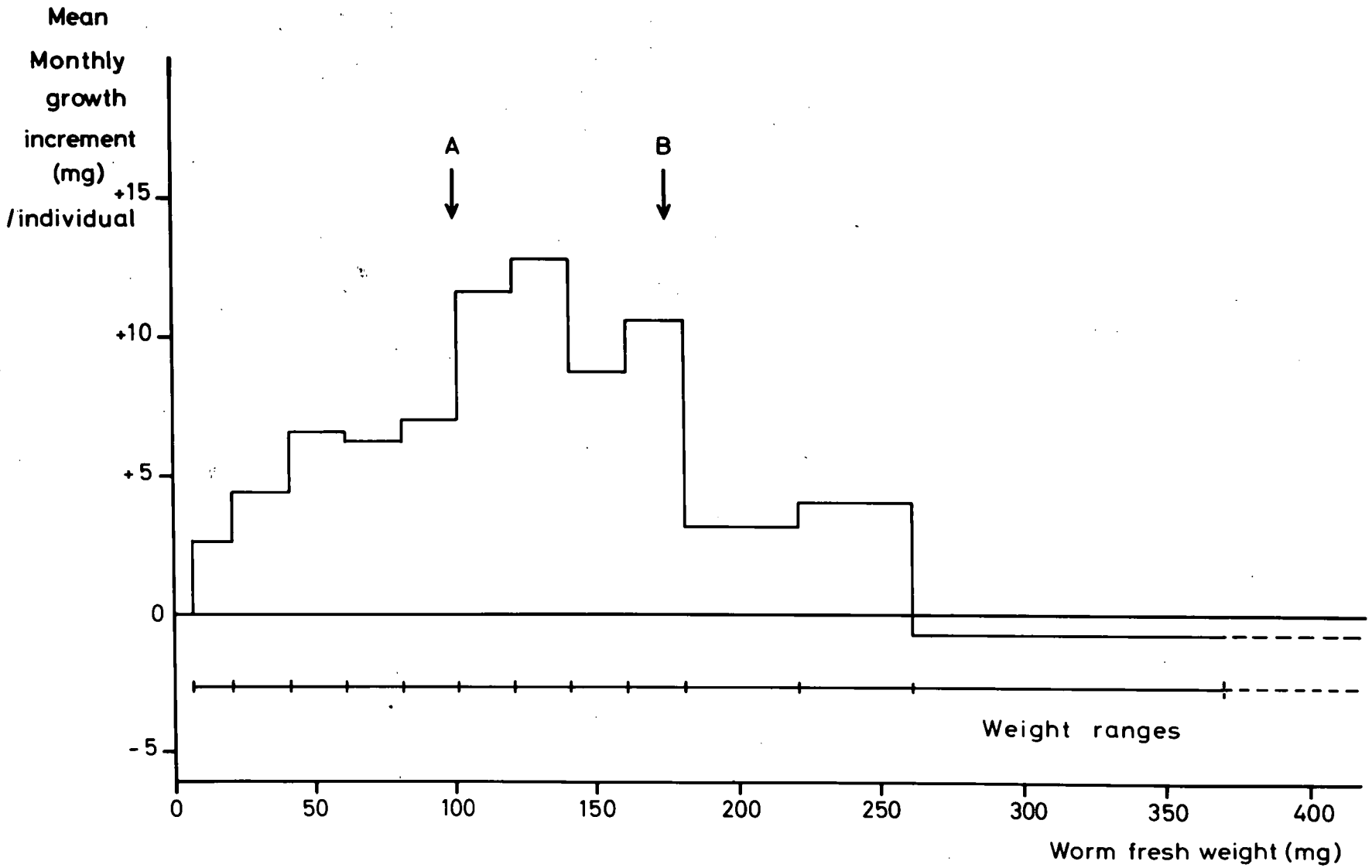


Fig. 18. Mean monthly growth increments by A.rosea individuals of various sizes.

confidence. The arrows A and B in Fig. 18 show the transition weights between 'small immatures' and 'large immatures' (as defined in the population survey) and between 'large immatures' and 'adults' (as defined by the 50% frequency of mature animals given above), respectively.

Seasonal variations in the mean monthly fresh weight growth increment for the three A. rosea size classes used in the population survey are shown in Table 5. Net body tissue production was negligible in all size classes over the late autumn and early winter period from mid-November to mid-January. Adult growth was slow in mid-summer and early autumn, showing a peak value in late winter - though adult body tissue production was low, relative to immature production, and variable throughout the year. Apart from the significantly low values in late autumn and early winter, the growth production of worms in each of the immature size classes was fairly constant throughout the year.

Discussion

Evans and Guild (1948b) observed the emergence from cocoons of various lumbricid species. They kept cocoons, for ease of observation, in beakers containing clean sand and water. During the population survey, 'empty' cocoon cases of L. terrestris L. were frequently found to contain fine textured soil resembling faecal material - and indeed this material could only have been egesta of the emerging

Period	SEASON	Small Immatures		Large Immatures		Adults	
		Mean incr. /individual /month (mg)	S.E.	Mean incr. /individual /month (mg)	S.E.	Mean incr. /individual /month (mg)	S.E.
mid-March to mid-May, 1966	SPRING	5.85	0.97 (65)	8.84	2.25 (39)	3.31	2.22 (57)
mid-May to mid-July		8.10	1.25 (53)	16.98	3.61 (39)	3.87	2.62 (46)
mid-July to mid-September	SUMMER	7.64	1.31 (56)	11.48	5.09 (29)	2.33	2.42 (41)
mid-September to mid-November	AUTUMN	6.92	1.07 (54)	13.23	2.65 (30)	0.79	2.19 (52)
mid-November to mid-January, 1967		0.40	0.95 (52)	minus 1.94	4.65 (28)	minus 0.03	1.66 (51)
mid-January to mid-March	WINTER	6.17	0.77 (61)	14.16	2.18 (34)	8.79	1.66 (50)

Table 5. Mean monthly fresh weight increments for individuals of three A. rosea (Sav.) f. typica size classes through the year. (S.E. - Standard Error; Numbers weighed in parentheses)

individual since the exit hole of the cocoon case would not allow penetration of surrounding soil. Cocoon cases of A. rosea and O. cyaneum (Sav.) also showed this phenomenon on occasion, and it seems certain from the amounts of faecal material present that young worms of soil-dwelling species can ingest considerable quantities of mineral soil prior to their complete emergence. This suggests that the emerging individual is in immediate need of food material; animals emerging into an inorganic medium such as sand were therefore considered abnormal and unsuitable for weighing purposes. Apart from the considerations outlined above, for the purpose of growth curve construction it was considered important that the mean emergence weight should be directly comparable with the weights of worms used in the growth studies, i.e. weights including gut contents. It was therefore imperative that cocoons should be kept in a soil medium, so that newly-emerged worms could ingest food material prior to their collection for weighing.

The age structure of the A. rosea field population was of a similar pattern throughout the year due to continuous emergence from cocoons and size class recruitment which produced a complex overlapping of generations. The overlapping was such that sequential cohort measurements, such as are commonly used in growth studies of insect populations, were impossible for A. rosea at Wynyard. Satchell (1963) has used the cohort method for measurements of the growth of young L. terrestris in a population with a highly synchronised

spring emergence, but even in this study considerable emphasis was laid on simultaneous measurements of the growth of isolated individuals.

The simplest method for constructing a lumbricid growth curve is the confinement of a number of newly-emerged individuals - at the time of peak emergence - within porous containers which can be sunk into the soil or litter. The body weight of these individuals can then be monitored continuously throughout their life cycle. Such a method is suitable for studies on a species with synchronised emergence and a life span covering only one or two years. However, for the construction of a growth curve for a species with a life span covering several years this method is tedious and time-consuming, involving the initial confinement of a very large number of worms if the full life cycle is to be covered, despite natural mortality. The method is also inaccurate if emergence occurs over an extended period, as is often the case in long-lived species, since environmental conditions encountered in the field will depend on the season of emergence. Satchell (1963) used this method for early stages of L. terrestris and was obliged to use initial growth rates in calculations of total population growth production.

The method used for growth curve construction in the present study was designed to provide data on growth rates of all life stages of A. rosea at all times of the year, whilst allowing the study to be completed in one year of

measurement. Van Rhee (1965) found that A. caliginosa (Sav.) preferred cages (cuvette structures, originally described by Evans, 1947) to pot conditions, but his pots were of plastic construction and packed with clay soil which had been air-dried, pounded, sieved through a 1 mm. mesh and mixed with ground leaves and fertilizer. It has since been found that this species behaves normally under pot conditions providing the soil is not packed down or 'puddled' (Van Rhee, pers. comm.) In porous plant pots containing loosely-packed soil, A. rosea showed no signs of abnormal behaviour and, with the exception of a number of parasitised individuals, the worms remained in perfect health throughout the study. All growth data from eventually parasitised individuals were discarded for two reasons: first, the parasite was found to cause weight loss in the later stages and it was not possible to determine exactly the time of initial attack, and second, parasitised worms did not occur in the grid area population. A description of the parasite, and its effects on infected A. rosea individuals, is given in Appendix 5. The soil medium in the pots was replaced by 'fresh' soil at intervals since there was a gradual degeneration of soil crumb structure with repeated hand-sorting. A renewed supply of unworked soil was also desirable in pots containing larger individuals. Various authors, including Hassan et al (1956), Barley (1959) and Miles (1963b), have studied lumbricid growth under varying soil media conditions; in the present study

the growth of A. rosea in a field soil medium was considered the most relevant parameter for computation of the A. rosea field population growth production. The mull-type soil from beneath 'bramble' vegetation, used in the A. rosea growth studies, was known to be the optimal soil type in the grid area but its use was considered justified by the close association of A. rosea with this soil - as revealed during the population survey (p. 77).

Individual variation in the weight of gut contents was assumed to account for a major proportion of the variability in the growth increment data. The hypothesis that this variation tended to cancel out when considering a large number of animals was borne out by the smoothness of the growth curve, constructed from mean values.

Worms weighing between 100 mg. and 180 mg. were found to produce the highest monthly growth increments, in absolute terms; comparison with parameters used in the population survey (based on the body fresh weight/dry weight relation) and data on maturation weights showed this weight range included the bulk of the 'large immatures'. The enhanced growth production in the large immature phase was thought to account for the temporarily halted decline in growth rate over the second year of development. Hassan et al (1956) recorded high growth rates prior to clitellum formation in A. caliginosa (Sav.) f. trapezoides (Duges), followed by greatly decreased growth production on the assumption of maturity. This situation is identical to

that found for A. rosea in the present study.

The 'small immature' phase of A. rosea was found to include the first 1.5 years of development, with maturation occurring, on average, approximately 6 months later at age 2 years. Evans and Guild (1948b) recorded an average period of approximately one year for development to maturity in this species. Their growth measurements, under conditions of optimum water and artificial food supply in a subterranean cellar, are obviously not comparable with field growth rates, even when the latter are measured in mull-type field soils with a high water content.

Pygmy adults of A. rosea were included with immature animals of similar weight for purposes of growth curve construction and ageing. Exact ageing of these individuals was impossible and their inclusion with immatures for growth curve construction could produce little error due to the small numbers involved. In all size class calculations they were included with clitellate worms of normal size. Kollmannsperger (1934) showed that A. rosea attained a larger size in loamy soils than in sandy soils. The same author (Kollmannsperger, 1956) showed 'dwarfism' in A. caliginosa living in fields around the Oasis Tazerouk mountainous region in the central Sahara. The dwarf A. caliginosa were only 50 to 65% of the normal European length. The maturation weights of pygmy A. rosea at Wynyard were found to be a similar proportion of normal maturation weights. Kollmansperger attributed the dwarfism of

A. caliginosa to 'limited space' in the Oasis soil. At Wynyard the pygmy A. rosea individuals existed in the same soil habitat as the normally developing worms so that the phenomenon can not be directly related to soil conditions. Prior to maturation, the pygmy individuals showed similar growth rates to those of normal small immature worms, suggesting that the pygmyism was induced through a factor or factors affecting only the onset of maturation. The slower growth rates observed in the pygmy adult phase - equivalent to normal adult rates - could be governed by adult growth hormones of the normal type. This hypothesis - and the use of the term 'pygmy' - presupposes a genetical basis for the phenomenon; this seems probable, despite the parthenogenetic nature of the species (Muldal, 1952a), but data on the course of development of offspring from pygmy adults would be needed for a full analysis of the situation.

Growth of A. rosea individuals was shown to be negligible during the early winter of 1966/67. However, during the late winter months from mid-January until mid-March growth production was similar to that in the spring, summer and autumn months - and even at a maximum in adult worms. Winter soil temperatures were lowest in mid-January, with similar average temperatures in the two-month periods before and after this date (see Fig. 6). It follows, therefore, that growth production in A. rosea is limited not by absolute low temperatures but by falling temperatures in a

low range. This limiting effect was observed in each of the three major size classes of A. rosea.

(ii) Population Tissue Growth

Introduction

In applying data on the growth production of individual animals to the total A. rosea population the assumption was made that the field population individual was growing under the same conditions, and therefore at the same rate - subject to individual variation, as its counterpart in the growth study 'population'.

Methods

Using the known mean population individual fresh and dry weights for each size class throughout the year, the measured mean fresh weight increments per individual were converted to their dry weight equivalent as follows:

The mean fresh weight increment for a particular period and size class was added to the mean individual fresh weight for the same period and size class. This total individual fresh weight was then converted to its dry weight equivalent, using the known fresh/dry weight relation for A. rosea. Finally, the mean population individual dry weight was subtracted from the total dry weight, obtained as above, yielding the growth increment as a dry weight.

To obtain a measure of total population growth production over the year, the mean numbers of individuals in each size class in each two-month period through the year were multiplied by twice the monthly dry weight increment per

individual of the same size class over each period. All calculations were made on a metre square basis. Total annual figures were obtained by summing the six two-monthly estimates for each size class.

Results

Table 6 shows the growth production of the A. rosea population through the year. The pattern of results is basically similar to that found for individual increments in different seasons, though the higher numbers of small immatures in early spring and late summer, and of large immatures in early autumn, enhance total growth production in these periods. There was negligible growth of small immatures and adults during the early winter and the large immatures showed a net fall in body weight. The total figures in Kcalories for annual growth production of each of the three A. rosea size classes, shown in Table 6, were calculated from the two-monthly values using seasonal estimates of calorific content (see Part III, Section I).

Discussion

Smalley (1960) and Saito (1965) calculated the population growth production of a grasshopper and an isopod, respectively, by use of the formula:

$$\left(N_t \times \frac{\Delta W}{\Delta t}\right) + \left(\frac{\Delta N}{\Delta t} \times \frac{1}{2} \times \frac{\Delta W}{\Delta t}\right) \quad \ldots\ldots\ldots 1$$

where N_t is the number of surviving individuals at time t,

Δt is the time interval t_o to t_1,

ΔN is the change in population numbers $(N_o - N_t)$ in Δt,

Period	SEASON	Small Immatures			Large Immatures			Adults		
		Mean Number /m^2	Dry wt. incr.per ind.per month (mg)	Total 2 month Pop. incr. (mg)	Mean Number /m^2	Dry wt. incr.per ind.per month (mg)	Total 2 month Pop. incr. (mg)	Mean Number /m^2	Dry wt. incr.per ind.per month (mg)	Total 2 month Pop. incr. (mg)
mid-March to mid-May, 1966	SPRING	31.51	1.291	81.358	11.66	2.566	59.840	13.81	0.964	26.626
mid-May to mid July		16.30	1.780	58.028	7.45	4.929	73.442	5.64	1.119	12.622
mid-July to mid-September	SUMMER	17.14	1.685	57.762	15.04	3.328	100.106	8.40	0.678	11.390
mid-September to mid-November	AUT	15.72	1.520	47.788	16.65	3.835	127.706	7.77	0.228	3.544
mid-November to mid-January, 1967	WINTER	16.18	0.089	2.880	17.66	minus 0.560	minus 19.780	4.70	minus 0.006	minus 0.056
mid-January to mid-March		15.23	1.357	41.334	8.50	4.104	69.768	5.52	2.545	28.096
Total annual production: (mg. dry weight)				289.150			411.082			82.222
				0.886 Kcals./m^2			1.320 Kcals./m^2			0.264 Kcals./m^2

Table 6. Growth production of the A. rosea population through the year.

and Δ W is the weight change of an individual ($W_o - W_t$) in Δt. Thus the growth production of the survivors at time t, over the period Δt ($N_t \times \frac{\Delta W}{\Delta t}$, in equation 1) is added to half the growth production of animals which were lost to the population over the same period, assuming they were all lost half way through the interval Δ t, i.e. that the mortality rate was constant over the period.

Formula 1 reduces to the form:

$$(N_t + \frac{\Delta N}{2\Delta t}) \cdot (\frac{\Delta W}{\Delta t}) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 2$$

Both Smalley and Saito were dealing with population cohorts in which numbers were constantly declining so that, despite a degree of overlapping of generations, the principles of cohort analysis could still be utilised. In a population with continuous emergence, recruitment between age classes and mortality throughout the year the situation is more complex. The growth production of newborn and recruited animals must be taken into account, in addition to that of the surviving and dying animals of the original population structure. The final population density N_t may be greater than N_o over certain periods of increased emergence or recruitment, so that Δ N becomes a negative quantity. The A. rosea population at Wynyard was of this complex type, but the calculation of growth production was found to be no more difficult than methods involving cohort measurements:

If the mortality rate over the period Δ t is M

Then total growth production P_g is determined as follows:

P_g = (Survivors' growth over Δ t) + ($\frac{1}{2}$ x dying animals' growth over Δt) + ($\frac{1}{2}$ x recruits' growth over Δt)

(assuming both mortality and recruitment (and/or emergence) rates are constant over the period Δt)

Thus:

$$P_g = \left[(1-M).N_o \times \frac{\Delta W}{\Delta t}\right] + \left[\frac{1}{2} \times M. N_o \times \frac{\Delta W}{\Delta t}\right] + \left[\frac{1}{2}\times(M.N_o+\frac{\Delta N'}{\Delta t})\times\frac{\Delta W}{\Delta t}\right]$$

where $\Delta N'$ can be positive or negative according to the balance between mortality and recruitment, and ΔW is assumed a constant for survivors, dying animals and recruits over the period Δt.

This formula reduces as follows:

$$P_g = \left[(1-M).\ N_o + (\tfrac{1}{2} \times M.N_o) + (\tfrac{1}{2} \times M.N_o) + \frac{\Delta N'}{2\Delta t}\right] \times \frac{\Delta W}{\Delta t}$$

$$= (N_o + \frac{\Delta N'}{2\Delta t}) \times \frac{\Delta W}{\Delta t} \qquad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad 3$$

Formula 3, for a population incorporating recruits, is thus similar to formula 2 above, for a declining cohort, with the substitution of N_o for N_t and the new interpretation of ΔN.

Knowing the value $\frac{\Delta W}{\Delta t}$ and the quantities N_o and N_t, it is thus possible to determine total growth production for each of the A. rosea size class populations over each of the two-monthly periods for which ΔW was calculated. However, the production was calculated even more simply by calculating the mean population density over each period Δt ($\bar{N}$) and multiplying this figure by $\frac{\Delta W}{\Delta t}$, since:

$$\bar{N} \times \frac{\Delta W}{\Delta t} = \frac{(N_o + N_t)}{2} \times \frac{\Delta W}{\Delta t}$$

$$= \tfrac{1}{2} \times (2N_o + \frac{\Delta N'}{\Delta t}) \times \frac{\Delta W}{\Delta t}$$

$$= (N_o + \frac{\Delta N'}{2\Delta t}) \times \frac{\Delta W}{\Delta t} \text{ ; i.e. formula 3 above.}$$

This method is equally suitable for a declining cohort situation; in this case:

$$\bar{N} \times \frac{\Delta W}{\Delta t} = \tfrac{1}{2} (2N_t + \frac{\Delta N}{\Delta t}) \times \frac{\Delta W}{\Delta t}$$

since ΔN is always positive, thus:

$$= (N_t + \frac{\Delta N}{2\Delta t}) \times \frac{\Delta W}{\Delta t} \text{ ; i.e. formula 2 above.}$$

Despite its relatively short age span, the large immature size class showed the highest capacity for body tissue production. This was considered due to high growth increment rates, in absolute terms, and high population numbers as a result of the unusually low mortality rate in the small immature phase (see p. 147). On entering the large immature size category, A. rosea individuals were known to experience a much higher mortality rate, so that worms surviving into the adult phase were low in number. The low adult population density, coupled with greatly reduced individual increment rates, resulted in the low figure obtained for adult A. rosea annual growth production.

2. Reproduction Studies in the Field

(i) Rate of Cocoon Production by Individual Animals

Introduction

The only quantitative measurements of lumbricid cocoon production known to the author are those by Evans and Guild (1948b). These workers kept adults of various lumbricid species - including A. rosea (Sav.) - under conditions of optimum artificial food supply and soil water content in the modified temperature regime of an underground cellar. These methods have already been shown to produce abnormally high growth rates in immature worms (see p. 119) and for the purpose of the present study the determination of field rates of cocoon production in A. rosea was considered necessary. Seasonal measurements were desirable for the calculation of a realistic mean annual figure for reproductive rate. Since calorific conversions were to be effected on a dry weight basis, it was necessary to determine the relation between fresh weight and dry weight for cocoons of A. rosea.

Methods

The site used for studies of cocoon production was in the same area as that used for growth studies. In a preliminary study attempts were made to clear soil of A. rosea cocoons by washing through a series of sieves, later reconstituting the soil - minus cocoons - by mixing and partially air-drying the components (organic debris, clay, sand and silt) with the addition of a quantity of coarse sand. In this medium, adults of A. rosea progressively lost weight and produced no cocoons. The method

described below was therefore adopted, since it allowed the clearance of viable cocoons from the soil without affecting the soil structure or constitution.

A considerable volume of mull-type soil from beneath 'bramble' was cleared of A. rosea worms by hand-sorting. The soil was then placed in a finely perforated polythene sack which was sunk into the ground so that the internal and external soil surfaces were at the same level. Soil layers within the sack were kept in their original relative positions as far as possible. The upper edges of the sack were held by stakes at a height of approximately 30 cm. above the ground surface, to prevent the entry of A. rosea adults from the surrounding topsoil. After a period of three months, the first sample of soil from the sack was used for experimental studies. The soil sample was sub-divided and lightly packed by layers into polypropylene tubes measuring 5 cm. internal diameter by 30 cm. in length. One end of each tube was corked and the other, upper end was covered by muslin sheet, held in place by elastic bands. Soil filled each tube to within approximately 5 cm. of the upper edge. Fourteen such tubes were sunk into the ground so that the internal and external soil surfaces were at the same horizontal level. Twenty-two fresh-weighed A. rosea adults, collected from the same area, were grouped in twos and threes according to similarity of fresh body weight. The worms, chosen so as to cover the full range of adult body size regardless of clitellar size, were not aged since adult

age was taken as proportional to body fresh weight in growth curve construction (see p. 111). Two A. rosea adults were placed in each of five tubes, three adults were placed in each of four tubes and the five remaining tubes were used as controls. After three months in the field, the tubes were returned to the laboratory for examination. The adults from the soil in each tube were removed by hand-sorting, washed in dechlorinated tap water, examined and fresh-weighed. A. rosea cocoons were extracted from the soil by washing through a 1 mm. mesh gauze, using a fairly strong jet of tap water. The cocoons were individually fresh-weighed and noted as to their tube of origin. Each cocoon was placed in a 2 cm. long section of suitably labelled glass tubing, containing mull-type soil and 'stoppered' at both ends with a small cotton-wool plug. After examination, each tube was refilled with a fresh portion of soil from the field sack, the adults were replaced and all tubes were kept at 10°C, being transported back to the field on the following day. As in the growth study, any animals which were damaged, missing or became parasitised were replaced by other worms of similar fresh-weight collected from the same area.

The procedure outlined above was repeated at three-monthly intervals over a one-year period. All cocoons collected were kept, within their glass tubes, in porous containers which were buried in the topsoil of the field site. These cocoons were returned to the laboratory at monthly intervals for examination and fresh-weighing.

To determine the fresh weight: dry weight relation for cocoons of A. rosea, cocoons were used from both the field and adult laboratory cultures in 'bramble' soil at 10°C. Forty-six cocoons were individually washed in dechlorinated tap water, briefly rolled on Whatman's No. 1 filter paper and fresh-weighed. They were dried in a vacuum oven at 60°C and cooled in a desiccator prior to the measurement of individual dry weights.

Results

The summarised results of the cocoon production study are shown in Table 7. The mean number of cocoons produced per adult per annum was found to be 3.13. Cocoon production in spring and summer was shown to account for 85% of the annual total. Cocoon production in winter was negligible. At no time during this study were A. rosea cocoons found in soil from control tubes. No significant differences were observed between the cocoon production rates of adults grouped in twos and in threes. One worm was missing at each examination and a small number were found to be parasitised. Total adult weight was approximately constant during autumn and spring, rising during winter and falling during the summer months. Growth rates of individual animals were similar to those found for adults during growth studies described above.

Fig. 19 shows the relation between dry weight and fresh weight for A. rosea cocoons. The positive correlation coefficient of 0.99, calculated for this linear relationship,

Period	SEASON	No. of Adults Parasitised	No. of Adults Missing	Net % change in total wt. of Adults	Total No. of health Adults * present throughout	Total No. * of cocoons produced	Mean No. of Cocoons per Adult per 3 months	Mean No. of Cocoons /Adult as a % of annual total
15th Sept, 1966 to 15th Dec	AUT	0	1	-3.20%	20	8	0.40	12.8%
15th Dec to 15th March, 1967	WINTER	2	1	+14.63%	17	1	0.06	1.9%
15th March to 15th June	SPRING	2	1	+2.96%	14	15	1.07	34.2%
15th June to 15th Sept	SUMMER	2	1	-20.61%	15	24	1.60	51.1%

* Excluding those present in tubes containing parasitised or missing adults on examination.

Table 7. Seasonal variation in field cocoon production of A. rosea (Sav.)

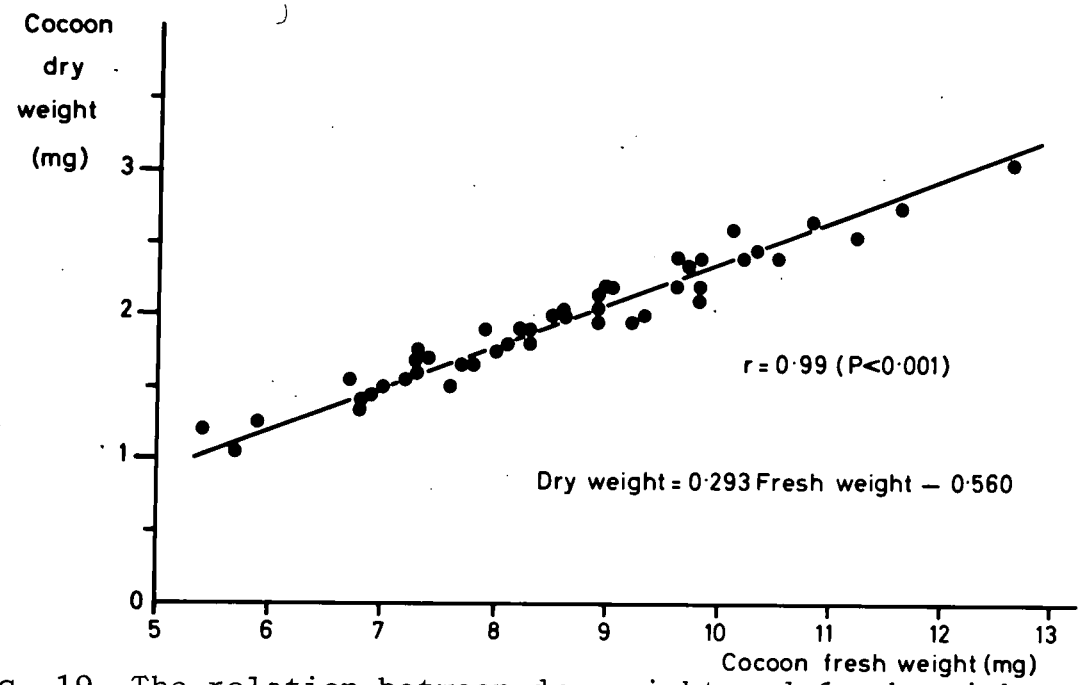


Fig. 19. The relation between dry weight and fresh weight of A.rosea cocoons.

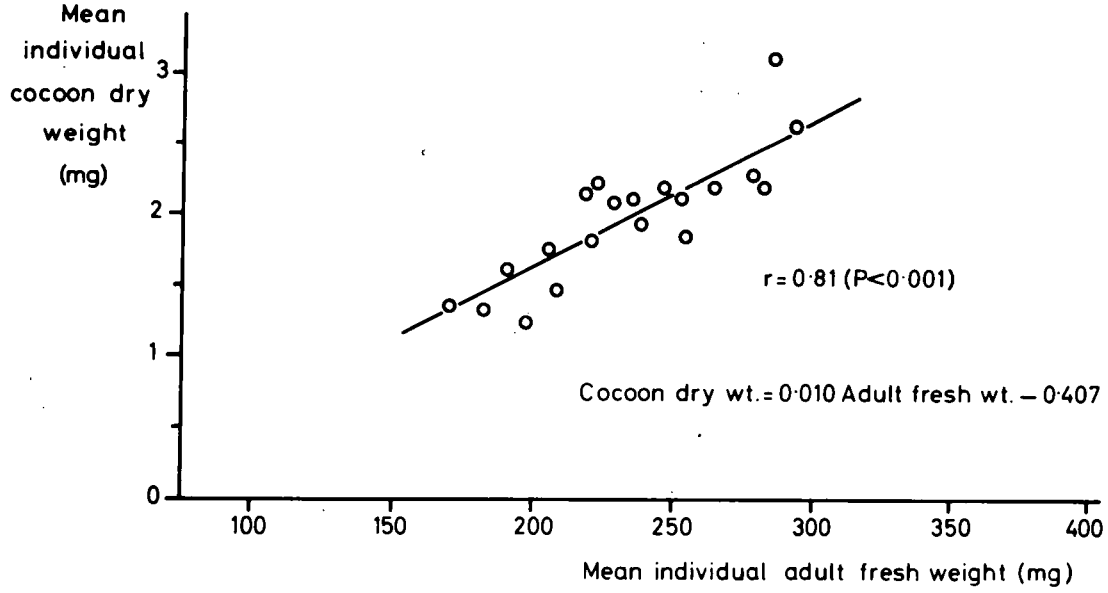


Fig. 20. Relation between mean cocoon dry weight and mean A.rosea adult fresh weight from the same cocoon production tube.

indicated an almost complete lack of deviation from the mean trend. Fig. 20 shows the relation between the mean individual dry weights of cocoons from particular tubes and the mean individual fresh weights of the adults present in the same tubes over the period of deposition. A significant degree of positive correlation was achieved.

The results of cocoon fresh weight measurements in successive months after collection were inconclusive due to the death of almost all tubed cocoons in September, 1967. At this time the glass tubes were found to be polluted with stagnant water after a period of rainfall which produced temporary waterlogging of the area in which the cocoons were kept. The incubation period for A. rosea cocoons was found to be remarkably variable. Cocoons deposited during the autumn of 1966 showed little change in fresh weight but continued viable until September, 1967, when all died; one cocoon was found to have been in the pre-emergence state prior to death. The two cocoons deposited during the winter of 1966/67 also maintained a constant fresh weight until September, 1967, when both died without signs of emergence. Cocoons deposited during the spring of 1967 - eighteen in all - maintained their weight until September, 1967, when all but three died. The three survivors, and four of the deceased, were in the pre-emergence state at that time. The thirty-two cocoons deposited during the summer of 1967, on collection in September 1967, were found to be already in an emerging or pre-emergence state -

with the exception of nine cocoons which were placed in 'bramble' soil at 10°C in the laboratory. The worms from these nine cocoons all emerged within a period of 1.5 months from the date of collection (15th September).

Discussion

The final method used for clearing soil of A. rosea cocoons was based on the fact that soil media used had been isolated from A. rosea adults for a period of up to 15 months and not less than 6 months before examination, and upon the assumption that all viable cocoons present in the soil initially would have produced young worms or deceased in periods of this duration. The latter assumption was based on the incubation period for A. rosea cocoons of 17.5 weeks, as determined by Evans and Guild (1948b). In view of the observations on viable cocoon incubation during the present study, it seems that this assumption was not entirely justified; however, since twenty control tubes failed to produce a single viable A. rosea cocoon it is safe to consider the method effective. It may have been fortuitous that the soil sample was collected during the summer months when cocoon development appears to be accelerated.

The discarding of data from parasitised worms was justified, as in the growth studies, by the observation that the parasite infection did not occur in the A. rosea population of the grid area. Although the absolute numbers of adults parasitised and missing on each examination occasion were small, the resulting reduction in available data was

considerable. Without knowledge of the exact numbers and weights of adults producing cocoons the data from complete tubes had to be discarded where any adults were parasitised or missing on examination.

The washing method used in the present study for extraction of A. rosea cocoons was similar to that used by Evans and Guild (1948b) and suggested by Raw (1960).

The mean annual production of approximately three cocoons per adult, determined for A. rosea during the present field study, was considerably less than the figure of eight cocoons per adult per annum proposed by Evans and Guild (1948b). In addition to the optimum environmental conditions used by these authors, only adults with robust clitella were used during their studies of reproductive rates. Evans and Guild (1947b) showed that A. rosea adults could produce viable cocoons less than one month after maturity, cocoon production becoming more common with increasing age. The use of well-developed adults in their later experiments must therefore have introduced an additional bias towards higher cocoon production figures. In the present study it was necessary to determine the field rate of cocoon production by the total A. rosea adult population, regardless of age. Worms representing the full adult age structure, in the approximate proportions found in the field population, were therefore used in determinations of cocoon production rates. The studies of Evans and Guild (1948b), on reproductive rates in a number of lumbricid species, were

useful in that they illustrated the fact that soil-dwelling species have much lower rates of cocoon production than small, surface-active pigmented species. However, the absolute rates determined for at least the soil-dwelling species were considerably higher than might be expected in the field situation due to the conditions of measurement used by these authors.

Evans and Guild (1948b) showed that cocoon production in a number of species, including A. rosea, increased linearly with increasing temperature but decreased curvilinearly at a high rate with decreasing temperature. They suggested a 'fatigue' of the reproductive system, producing a rapid decline in cocoon production, was responsible for the apparent 'recovery' phase following a period of high production at high temperatures. They suggested that these experimental results might be manifested in an A. rosea field population where soils were sufficiently moist throughout the year to prevent dormancy in the warmer months. Murchie (1958) considered that if moisture conditions had remained suitable on the Michigan site a large proportion of the adult A. rosea population would have remained clitellate and cocoon production would have continued throughout the summer. The findings and postulates of both these authors were supported by the seasonal rates of cocoon production observed at Wynyard, where the soil moisture requirements were amply satisfied. The rapid decrease in cocoon production after the summer peak

supported the steep curvilinear relation with decreasing temperature found by Evans and Guild, but the 'fatigue' theory was viewed with some suspicion since growth rates of A. rosea worms of all sizes had been shown to be more drastically affected by falling temperatures in a low range than by increasing temperatures in the same temperature range (see p. 120). It seems possible that declining soil temperatures have a different effect on A. rosea production in general, than increasing temperatures over a similar range.

Muldal (1952a) showed that A. rosea is parthenogenetic, the condition being obligatory and of an eccentric apomictic type. Lumbricids are adapted to infrequent pairing by the use of a storage mechanism for the seminal fluid which according to Roots (1956a) can be used for viable cocoon production for at least a year after copulation. The dispersion into, and colonisation of, fresh habitats will obviously be even further facilitated by the adoption of parthenogenetic reproduction. According to Muldal, parthenogenesis occurs in a considerable number of lumbricid species, but only five of the species studied, four of which were polyploids, showed obligatory, non-sexual parthenogenesis. Evans and Guild (1947b) demonstrated the production of viable cocoons by single individuals of most of the species they studied. However, they considered mating to be a powerful stimulus to cocoon formation in all but a few exceptional species - one of which was A. rosea. In the present study it was found that the grouping of A. rosea adults in threes, instead of twos, in containers of similar

size did not significantly increase individual cocoon production rates. This finding is in accordance with the theory of completely independent reproduction by single adult worms of A. rosea.

The high degree of correlation achieved for the relation between fresh weight and dry weight of A. rosea cocoons might be expected from the compact and uncontaminated nature of the material.

Since A. rosea adults were approximately matched for weight in each of the experimental tubes, the relation shown in Fig. 20 is equivalent to that between the dry weight of a cocoon and the fresh weight of the adult which produced it. Evans and Guild (1948b) showed a similar correlation between mean cocoon and adult weights of different lumbricid species.

Despite the inconclusive nature of most data on incubation periods in A. rosea cocoons, the information collected during the present study does have some practical significance. The glass tube confinement of cocoons was thought in itself to produce an unnaturally anaerobic situation and is not recommended for use in this type of study. Murchie (1958) showed that A. rosea cocoon emergence could be delayed by adverse external conditions and in the present study the pollution occasionally observed in soil from confinement tubes was thought to account at least in part for the extended incubation period of cocoons deposited during the autumn of 1966. However, the result for these

cocoons does indicate a considerable capacity for prolonged incubation in a viable condition. Evans and Guild (1948b) showed three modes of fresh weight progression in the course of incubation of cocoons from different species: E. foetida (Sav.) cocoons gained weight initially, then progressively lost weight at a high rate until worm emergence; cocoons of L. rubellus Hoffmeister and A. chlorotica (Sav.) maintained a constant weight throughout the incubation period; L. castaneus (Sav.) and D. rubida (Sav.) f. subrubicunda (Eisen) cocoons progressively lost weight at a low rate until worm emergence. Even allowing for the possibility of adverse external conditions, A. rosea cocoons appear to conform to the second type of progression in which a constant weight was maintained.

Data collected during the population survey showed that newly-emerged A. rosea worms occur at all times of the year, but the highest numbers occurred in early spring (April/May) and in September. Cocoons deposited during the summer months in the cocoon production study were seen to produce young worms in September/October of the same year, presumably due to an acceleration of development by the prevailing high soil temperature. A number of the spring-deposited cocoons were in the pre-emergence state in September of the same year, but the majority showed little sign of development at this time. The winter-deposited cocoons were too few to be of importance, and the prolonged incubation of autumn-deposited cocoons was difficult to interpret due to the possibility

of adverse conditions within the confinement tubes. Thus the September emergence peak seemed due to emergence from summer-deposited cocoons, and to some extent from spring-deposited cocoons. It seemed possible that a major section of the spring-deposited cocoons did not develop until after the September period, possibly overwintering to produce young worms when soil temperatures rise in the following spring. Evans and Guild (1948b) showed an overwintering phenomenon in sections of cocoons deposited by L. castaneus and A. caliginosa (Sv.). Autumn-deposited cocoons of A. rosea could have contributed to the spring peak emergence, had experimental soil conditions been favourable, but this is pure supposition. It must be concluded that, as for most lumbricid species, more information is urgently required on the process of cocoon incubation in A. rosea.

(ii) Cocoon Standing Crop

An attempt was made to estimate the standing crop of A. rosea cocoons in soil of the grid area. By a stratified random sampling technique, similar to the one used for estimates of worm standing crop, forty-eight soil cores were taken in January, 1967. Each core, measuring 5 cm. in diameter by 15 cm. in depth, was washed through a 1 mm. mesh gauze to extract lumbricid cocoons. Only two A. rosea cocoons were collected from the whole sample and the technique was considered hopelessly inadequate. Although cocoon deposition in winter was later found to be negligible, the spring peak in emergence suggested that overwintering

cocoons should have been present in the soil. The aggregation of A. rosea small immatures (see p. 84) suggested that cocoons were probably also aggregated. The mean number of adults per metre square in the grid area and their extremely low reproductive rate (as estimated in the present study) lead to the conclusion that only very small numbers of cocoons were produced per metre square in a year. When the small size, moderately high mortality rate (see p. 150) and low respiratory rate (see p.239) of A. rosea cocoons were taken into account, the mean monthly standing crop and its effect on population metabolism were taken, for all practical purposes, as negligible.

The determination of cocoon standing crop, to any degree of accuracy, would have involved an extremely laborious and time-consuming sampling survey involving literally hundreds of small samples. The figure could not be calculated from data on adult standing crop, cocoon production, cocoon mortality and cocoon emergence rates without detailed knowledge of the process of cocoon incubation. Due to the small biomass and metabolism of A. rosea cocoons, on a metre square basis, neither an extensive sampling programme nor detailed studies of incubation were considered practically justified for the present work. Estimates of cocoon production by the A. rosea population through the year are shown in Table 9 below (see also p.151); these estimates were made during investigation of standing crop stability for the total A. rosea population.

3. Excretion and Exudation

(i) Excretory Products

Lacerack (1963), in his comprehensive review of nitrogenous excretion in lumbricids, stated that the by-products of metabolism - passed out in the urine - have been shown to contain ammonia and urea. The presence or absence of uric acid and/or allantoin has not yet been satisfactorily established. Most authors agree that ammonia forms a major proportion of the nitrogenous waste products in lumbricid urine (Cohen and Lewis, 1949a, b; Needham, 1957; Barley and Jennings, 1959; Haggag and El-Duweini, 1959). Quantitative estimates have indicated that the absolute amounts involved are small. Needham's figures for L. terrestris L. excretion were similar to those of Barley and Jennings for A. caliginosa (Sav.) - less than 100 µg/gm.worm/day. Needham's estimates for A. caliginosa were much less than those for L. terrestris. Cohen and Lewis's figures for L. terrestris excretion were only about $^{1}/_{12}$th of Needham's estimates but Satchell (1962 - in discussion) attributes this to the omission of Cohen and Lewis to record ammonium adsorbed on to the faeces. Needham found that faecal material contained no measureable insoluble nitrogenous products. Laverack (1963) suggested that the amount of water in the environment was probably of great importance in determining the type of excretory products produced by a particular lumbricid species - those living in more aqueous habitats producing a greater proportion of ammonia.

We may therefore postulate that A. rosea at Wynyard would produce small quantities of urine which were mainly composed of ammonia.

Krueger et al (1968) discussed the calorific losses involved in nitrogenous excretion. They showed that if ammonia is the end product of protein combustion the urine energy loss amounts to 14.2% of the original protein energy content. Using certain assumptions made by Winberg (1956) during similar considerations, it can be postulated that approximately 50% of the protein assimilated by a growing animal will be combusted, the remainder being used for body tissue growth. The ammonia urine energy is thus reduced to 7.1% of the total assimilated protein energy. Since normal mixed diets include only about 50% protein, it can also be postulated - on the assumption of proportionate assimilation - that ammonia urine will amount to only 3.55% of the total assimilated energy. Winberg (1956) arrived at a similar percentage for the average urine energy loss from total assimilated energy. However, he made the unjustifiable assumption that ammonia has a negligible calorific value. The calorific content of ammonia is in fact 4.137 Kcalories per gram (Krueger et al, 1968). Winberg's calculation was based on a value of 28% for non-utilisable energy loss from combusted protein. Krueger et al (1968) showed this percentage to be dependent on the type of excretory product and equal to 14.2% for ammonia, 15.6% for urea and 23.3% for uric acid production. The values used in Winberg's

calculations are therefore completely erroneous and the similarity between his average of 3% for urine energy loss from assimilated energy and the above-derived value of 3.55% ammonia urine energy loss is entirely due to chance. If urea was the sole nitrogenous excretory product, urine energy loss would be 3.9% of total assimilated energy, and the equivalent value for an animal excreting only uric acid would be 5.8%.

The percentage urine energy loss from total assimilated energy will be reduced by a factor equal to the assimilation efficiency for considerations of excretion in terms of ingested energy. For A. rosea, with an omnivorous diet (see Part II, Section III) and urine containing a high proportion of ammonia, the excretory loss of ingested or assimilated energy must be of negligible proportions and no attempt was made to measure the amounts involved at Wynyard.

(ii) Mucus Production

Muco-proteins probably have a high calorific value. Teal (1957) estimated the mucus production of two planarian species by subtraction of the energy of respiration, growth and unassimilated material from the energy ingested. He obtained figures which suggested that each month these flatworms secrete an amount of mucus almost equal in energy content to that of their standing crop. However, it is known that flatworms lay down mucus continuously for purposes of traction and prey entanglement. Lumbricids are equipped with chaetae for locomotory purposes and mucus production

is more limited under favourable environmental conditions. Lumbricid mucus acts as a surface for respiratory exchange, whilst keeping the body surface clean, cementing the soil particles of the burrow walls and lubricating the passage of the worm through the soil. The amounts of mucus required for these 'normal' functions will not be great. In the present studies of burrowing activities under cage conditions (see Section III, sub-section 1), A. rosea (Sav.) was observed to initially cement the walls of new burrows with a small amount of mucus but after this, whilst the animals were feeding normally over periods of several weeks, there was no build-up of mucus in the burrow systems. If the soil medium dried out, A. rosea worms excavated cells within the medium, sealing the walls with copious mucus secretions prior to dormancy. Worms exposed to an unsaturated atmosphere also produced mucus in appreciable quantities and this behaviour was obviously used as a general protection against desiccation. Copious mucus secretion occurred initially when A. rosea worms were placed in water, and continually when they were subjected to irritating stimuli of any kind. Laverack (1963) pointed out that mucus secretion is used by lumbricids as a buffer against the external environment.

Needham (1957) reported that muco-proteins accounted for up to 50% of the total nitrogen loss in both L. terrestris L. and A. caliginosa (Sav.). However, the worms were kept in conical flasks containing water - a totally unnatural situation, seen in the present study to produce excessive

mucus secretion by A. rosea individuals. No method has yet been devised whereby the rate of mucus secretion by soil-dwelling lumbricid worms can be measured under natural conditions. Until mucus from burrowing and feeding individuals can be collected, without the introduction of media or conditions involving any degree of irritation to the worms involved, the mucus production of lumbricid worms under field conditions must remain an unknown quantity. The use of data from experiments which did not satisfy the stated requirements would be totally unjustified and probably misleading. For the purposes of the present study it was postulated, according to the cage observations outlined above, that irritants were likely to occur only rarely in Wynyard soils, so that the mucus production of A. rosea was minimal and would produce little error by its exclusion from estimates of energy flow.

4. Survivorship, Mortality and Population Change

(i) Population Survivorship and Mortality

Introduction

Population survivorship curves and/or life tables have been used in production studies to estimate the biomass mortality of animal cohorts throughout their life or age span (Kitazawa, 1959; Smalley, 1960; Wiegert, 1964, 1965a; Saito, 1965, 1967). In the present study, similar estimations were attempted by use of the mean annual population age structure for A. rosea (Sav.) f. typica in the grid area at Wynyard.

Methods

The total sample of A. rosea worms, extracted during the one year population survey, was divided and summated according to year classes. The worms were aged from their individual weights using the growth curve measured for this species during the present study. Numbers present in each year class were converted to the equivalent mean number per metre square and Δ N - the apparent number dying between successive year classes - was calculated. On the assumption of constant year class mortality through the year - for the present purposes - the mean individual dry weight between year classes, $\bar{W}$, was taken from the growth curve and multiplied by Δ N to obtain the apparent biomass mortality in each year class transition. The apparent biomass mortality losses in Kcalories were calculated from the dry weight values, using the appropriate calorific values for the

A. rosea size classes (see Part III, Section I).

An approximate percentage mortality for A. rosea cocoons was derived from data on mean numbers of adults and small immatures present in the grid area during the year of study, using information already collected on cocoon production rates and small immature age span in this species. Cocoon mortality was calculated as follows:

Mean number of adults/m^2(N_A) will give rise to mean number small immatures /m^2(N_{SI}) in a period equal to the small immature age span (T_{SI}) in years. (assuming small immature mortality is negligible - see below). Thus adults will give rise to ($\frac{N_{SI}}{T_{SI}}$) small immatures per annum; Knowing the adult cocoon production rate per annum (N_C/adult/annum), Cocoon percentage mortality (Mc) can be estimated as:

$$M_c = \frac{(N_A \cdot N_C) - (N_{SI} / T_{SI})}{(N_A \cdot N_C)} \times 100\% \text{ per annum}$$

(Assuming that each cocoon can potentially give rise to only one worm - on the evidence of Evans and Guild (1948b) and according to the hatching data collected during the present study).

Results

Table 8 shows the apparent A. rosea population biomass mortality losses during one year, on the basis of the population size and age structure determined during the year of study. The apparent mortality of small immature worms was remarkably low. Analysis of the data according to six-

Year Class	Mean No. per metre^2	Δ N	$\bar{W}$ (mg. dry wt.)	Dry wt. Loss per yr. ($\Delta N \cdot \bar{W}$)	Mortality Loss in calories/m^2
0 - 1	12.389	0.667	11.38	7.590	23.51
1 - 2	11.722	4.833	33.86	163.645	521.37
2 - 3	6.889	4.611	53.16	245.121	786.84
3 - 4	2.278	1.000	60.51	60.510	194.24
4 - 5	1.278	0.611	66.44	40.595	130.31
5 - 6	0.667	0.056	72.40	4.054	13.01
6 - 7	0.611	0.278	78.36	21.784	69.93
7 - 8	0.333	0.111	84.32	9.360	30.05
8 - 9	0.222	0.166	90.25	14.982	48.09
9 - 10	0.056	minus 0.111	96.21	minus 10.679	minus 34.28
10 - 11	0.167	0.111	102.15	11.339	36.40
11 - 12	0.056	0.056	108.11	6.054	19.43

Table 8. Apparent annual mortality loss per metre square from the A. rosea population present at Wynyard over the period 1966/67.

monthly age classes produced fluctuation in the numbers recovered, but no consistent tendency for recovery numbers to decrease with decreasing worm size. Maximum apparent mortality losses occurred in the second and third year classes, i.e. mainly the large immature and first year adult worms. The apparent total population mortality loss during one year was found to be 574.4 mg. dry weight, or 1.839 Kcalories, per metre square.

The maximum life span for A. rosea appeared to be in the region of eleven to twelve years. The highest individual adult fresh weight recorded during the studies at Wynyard was 439.4 mg. This animal, assuming its growth rate was normal, was about fourteen years old. However, this was an isolated case, being the only worm of recorded weight equivalent to an age greater than twelve years.

The approximate percentage mortality of A. rosea cocoons was found to be 48% per annum.

Discussion

The apparent mortality losses here calculated were only approximate, incorporating many broad assumptions. However, the figures were considered sufficiently reliable to be used for the investigation of standing crop stability which follows. The variation in apparent mortality rate over the year class structure will be presented and discussed during the standing crop investigation.

Korschelt (1914) kept individuals of E. foetida (Sav.), L. terrestris L. and A. longa Ude under laboratory conditions

for up to $4\frac{1}{2}$ years, 6 years and $10\frac{1}{2}$ years, respectively. The maximum life span for A. rosea - determined as approximately 12 years during the present study - was greater than any of Korschelt's figures, but the average life expectancy of A. rosea (about 3 years) is of a more similar order. Detailed comparisons between laboratory and field longevity data are extremely difficult due to the relative complexity of the factors controlling field survivorship.

The approximate estimate of annual cocoon mortality was seen to be near 50%. The considerations of standing crop stability below indicate that this value should be reduced to allow for changing population numbers. However, after allowance for these factors, cocoon mortality must still be considerable - probably in the range 25 to 35% per annum.

(ii) Change in Population Standing Crop

Introduction

In order to investigate the stability of the A. rosea standing crop in successive years, population tissue productionwas compared with the apparent biomass mortality loss to predators and decomposer organisms.

Methods

Figures for the total annual growth production of the A. rosea size class populations - i.e. growth of survivors plus half the potential growth of animals recruited and dying - were taken from Table 6. The size class estimates were summated to give total population growth production

per annum.

The calorie equivalent of the annual cocoon production was calculated from data on the population densities and mean individual weights of adults through the year, using estimates of cocoon production rate and the relation between cocoon dry weight and adult fresh weight, all determined during the present study.

The apparent biomass mortality loss (Pm) from the worm population during one year, estimated above, was added to the approximate cocoon mortality loss (Cm) and the total subtracted from the total population production - growth production (Pg) plus cocoon production (Pc) - to assess the change in A. rosea population standing crop per annum at the approximate time of these studies (1966/67). In view of the obvious trend in the results, the population age structure was examined figuratively for comparative purposes. The numbers of worms collected from those year classes which showed appreciable mortality (large immatures and adults) were plotted logarithmically against age in years.

Population turnover time was estimated from the relation between population production and the mean standing crop over the period of study.

Results

Table 9 shows cocoon production by the A. rosea population through the year. The adult population per metre square during the year of study was estimated to produce approximately 27 cocoons per annum which had a total dry weight of 46.81 mg and a calorific content of 0.247

Period	Mean No. of Adults per m^2	Mean individual adult fresh wt. (mg.)	Corresponding individual cocoon dry wt. (mg.)	Mean No. of cocoons produced per adult	No. of cocoons produced per m^2	Dry wt. cocoon production (mg.) per m^2	Cocoon production in calories per m^2
15th Sept to 15th Dec.	6.87	226.88	1.91	0.40	2.748	5.25	27.74
15th Dec. to 15th March	5.13	197.20	1.61	0.06	0.308	0.50	2.64
15th March to 15th June	11.59	205.43	1.69	1.07	12.401	20.96	110.73
15th June to 15th Sept	6.98	216.17	1.80	1.60	11.168	20.10	106.19
			Total annual estimates:		26.625	46.81	247.30

Table 9. A. rosea population cocoon production at the time of the present studies (1966/67).

Kcalories. Total population growth production was 2.470 Kcalories, so:

$$\text{Change in Standing Crop} = (Pg + Pc) - (Pm + Cm)$$
$$= (2.470 + 0.247) - (1.839 + 0.119)$$
$$= +0.759 \text{ Kcalories.}$$

This positive increase in standing crop was seen to be equivalent to 21.39% of the mean population standing crop during the period of study (3.548 Kcalories per metre square). The monthly estimates of A. rosea population biomass, made during the population survey, were calculated as a regression of biomass on time. The correlation coefficient was positive, but not significant with only twelve points and a high degree of variation through the year. It may be relevant to note that the A. rosea biomass in April 1967 was 21.5% higher than that in May 1966.

Fig. 21 shows the numbers of worms collected in each of the year classes, from the 1 to 2 year-olds onwards, plotted logarithmically against age. There appeared to be two distinct trend lines with markedly different slopes. The apparent mortality rate over the first four or five year classes (approximately 53% per annum) was considerably higher than that for adults of age greater than about five years (approximately 36% per annum).

Population turnover time was estimated as follows:

$$\text{Turnover time in years} = \frac{\text{Mean standing crop}}{\text{Total annual production}}$$
$$= \frac{3.548}{(2.470 + 0.247)}, \text{ i.e. } 1.31 \text{ years.}$$

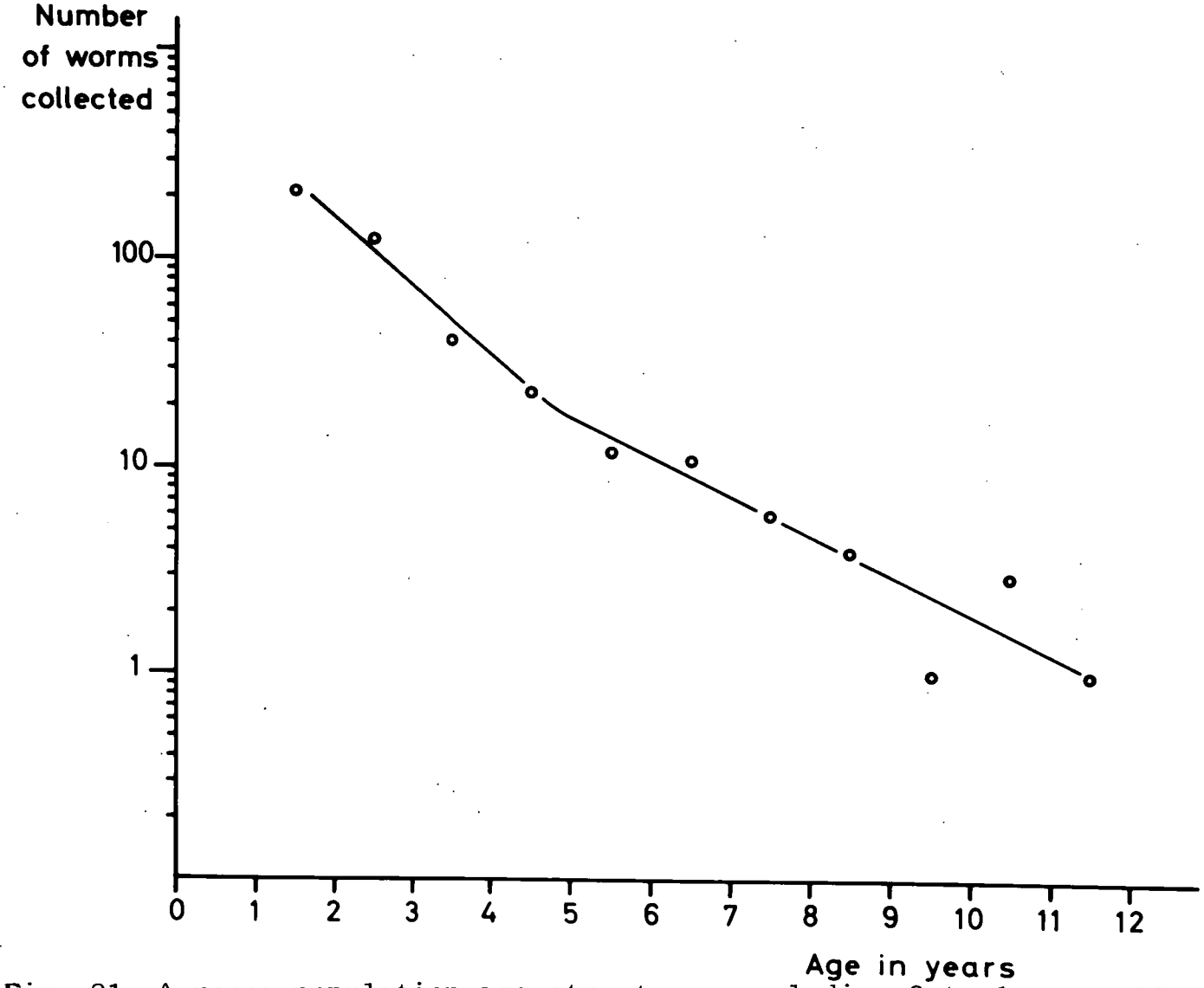


Fig. 21. A.rosea population age structure, excluding 0 to 1 year-olds, plotted in the form of an apparent log. survivorship slope.

Discussion

From the magnitude of the observed discrepancy between A. rosea population production and the apparent mortality loss, it was concluded that the A. rosea population was increasing at the time of study. The exact rate of increase could not be estimated from the data available, since these findings established that the 'apparent' mortality losses were an overestimate. The increase in population biomass must have reflected an increase in population numbers of a similar order. Increase in population numbers was indicated by the high cocoon mortality approximation and probably explains the change in slope on the graph of decreasing numbers, plotted logarithmically, with age. If the population began to increase at a constantrate four to five years prior to the present studies, then the apparently higher slope of the 'mortality line' would be produced by the increased emergence each year. There was no apparent reason why worms of age greater than about five years should have experienced a lower mortality than younger adults. It was therefore assumed that the change in the slope of the apparent survivorship line was due to a mean population increase which had progressed at a higher rate over the last five years than previously.

The turnover time of 1.31 years was thus regarded as a transient estimate, though the rate at which this would decrease with increasing annual production would be slight and diminishing.

Evans and Guild (1948a) showed that in grassland the A. rosea worm population was dormant during the summer months of high soil temperature and low soil moisture. If the production by the A. rosea population at Wynyard during the period of maximum soil temperatures (June/July to mid September) is subtracted from the measured total annual A. rosea production, the calculated change in standing crop becomes negligible. It is therefore possible that the A. rosea population increase was due to sustained production during the summer months, resulting from continued activity throughout the year. The lack of a dormant phase in the Wynyard population was attributed to the high soil moisture content on the site, even during the warmest months. It is possible that the dormant phase was eliminated approximately five years previous to this study by an increase in the moisture level of the soil due to a closing of the vegetational canopy and/or decreased drainage.

Whatever the explanation of the observed A. rosea increase, the present bioenergetic study is to be regarded as a measure o f the energy relations of a lumbricid population actively undergoing expansion. Satchell (1963) assumed 'steady state' when considering the nitrogen turnover by a woodland population of L. terrestris. He thus took population tissue production as equal to the rate of biomass yield to the 'soil'. In the absence of a detailed analysis of the relation between production and biomass mortality, as here described for A. rosea at Wynyard, such assumptions

are obviously not justified and could lead to errors of a high order.

A turnover rate of approximately three times per year was calculated by Satchell for the L. terrestris population. This extremely high rate for such a large, long-lived species was undoubtedly due to the use of immature growth rates in ageing procedures and in the calculation of total population growth production. It is therefore not comparable with the turnover time of approximately $1\frac{1}{3}$ years determined for A. rosea in the present study.

III Studies in the Feeding Biology and Faecal Output of Allolobophora rosea (Sav.) f. typica in relation to other Lumbricidae.

1. Burrow Formation and Feeding

Introduction

Soil-dwelling lumbricid species which ingest soil are often thought, by the uninformed observer, to 'literally eat their way through the soil.' For a worm placed on the surface of a soil with poor crumb structure, and thereby forced to actively burrow into the medium, the burrow may be to a large extent excavated by soil ingestion. The more normal mode of burrow formation includes the utilisation of soil spaces and material is usually pushed aside in preference to ingestion (Parle, 1963a, Arthur, 1965). Burrowing, however, does involve a higher soil intake than feeding. The two activity patterns, burrowing and feeding are not synonymous but are often difficult to distinguish. The studies described below were designed to elucidate the relation between burrowing and feeding, both by general observation and by quantitative studies under experimental conditions in the laboratory.

Methods

As a preliminary investigation into the burrowing and feeding activities of large immature and adult specimens of A. rosea, animals were placed in darkened cages containing mull-type 'bramble' soil. Each cage was composed of a perspex back-plate, measuring 10 cm. in width by 12 cm.

in length; narrow perspex strips were cemented to the outer edges of one face with two screw-tapped holes in each of the longer strips - the holes passing through the back-plate. Slits were filed into one of the shorter strips, which was defined as the upper edge. A top-plate, with appropriately drilled holes, was then screwed into the strips and back-plate so that the two perspex plates were separated by the thickness of the cemented strips (2.5 mm). During actual experiments, each cage was filled with soil to within approximately 1.5 cm of the upper edge. An artificial 'burrow' was created in the soil layer and a single worm was placed in each cage, which was kept in a vertical plane. All experiments were performed in a constant temperature room at 10°C. The mode of burrowing and feeding of a considerable number of A. rosea individuals was observed by this method and sketches and notes were made in each case.

Quantitative estimates of burrow formation were carried out in conjunction with measurements of faecal output (see below). The chambers used in these studies were of the cage type but of a more simple and inexpensive construction. Each 'cage' consisted of a glass petri-dish, a strip of plasticine and a small piece of cotton wool. To construct the experimental unit a layer was carefully sliced from a soil block of suitable moisture content (see Part I, Section III, sub-section 1). The soil layer was cut, using a sharp broad-bladed knife, to a thickness appropriate to the A.rosea size class involved. The layer was placed on the internal surface of the petri-dish top-plate and cut, using a

sharpened metal cylinder, to a perfectly circular shape which covered $\frac{1}{2}$ to $\frac{2}{3}$ of the petri-dish area. The outer edges of the soil disc were smoothed and all loose soil particles were carefully brushed out of the chamber. The smaller petri-dish plate was then inverted and lightly pressed into place on top of the soil layer. The strip of plasticine was pressed into the space between the petri-dish plate edges, leaving a half inch gap as an air-vent on the side thereby defined as the 'upper edge'. The dampened cotton wool plug was placed in this gap - more to maintain the soil moisture level than to prevent worm escape, which was rarely attempted. The petri-dish 'cages' were kept in a vertical plane, with the moistened plugs uppermost, in darkened containers under constant temperature conditions. Measurements of burrow formation were made for twenty individuals of each of the three major A. rosea size classes (small immatures, large immatures and adults) at each of three constant temperatures.

These measurements were made during experiments designed to estimate the rate of faecal output by A. rosea (see sub-section 3 below). The mean temperature and the degree of temperature variation in each constant temperature room were estimated by the use of simultaneous thermograph recordings. One worm was placed in each 'cage' by temporary removal of the cotton wool plug and worm introduction through the air-vent, aided by a small droplet of de-chlorinated tap water. The 'cages' were removed daily, the length of burrow

constructed by each worm being traced on the glass plate, using a wax pencil, and measured using a short length of fine cord. The cord was placed along the traced length of fresh burrow and subsequently straightened for measurement along a millimetre rule. The trace marks provided a basis for subsequent measurements.

In view of the burrow formation results obtained from animals used for measurements of faecal output, the burrow formation of a set of twenty adult control specimens, whose faeces were not collected, was measured at eachof the temperatures 10.0°C and 14.8°C.

Results

Fig. 22 shows the typical course of burrow formation by a large immature or adult A. rosea individual within the rectangular perspex cage. The worms initially burrowed to the bottom of the cage, followed the bottom edge to one side and subsequently burrowed up the vertical side to the surface. This procedure took approximately $2\frac{1}{2}$ to 3 days for completion. The worms then constructed one or more additional channels to the surface - but at a much decreased rate of burrowing. At this stage worms were more frequently found in a feeding position - prostomium extended over a non-terminal area of the burrow wall. It is possible that feeding could occur at the terminal wall of the burrow, but this was the position adopted by actively burrowing worms and was therefore discounted in observations of feeding behaviour.

After the construction of major channels to the soil

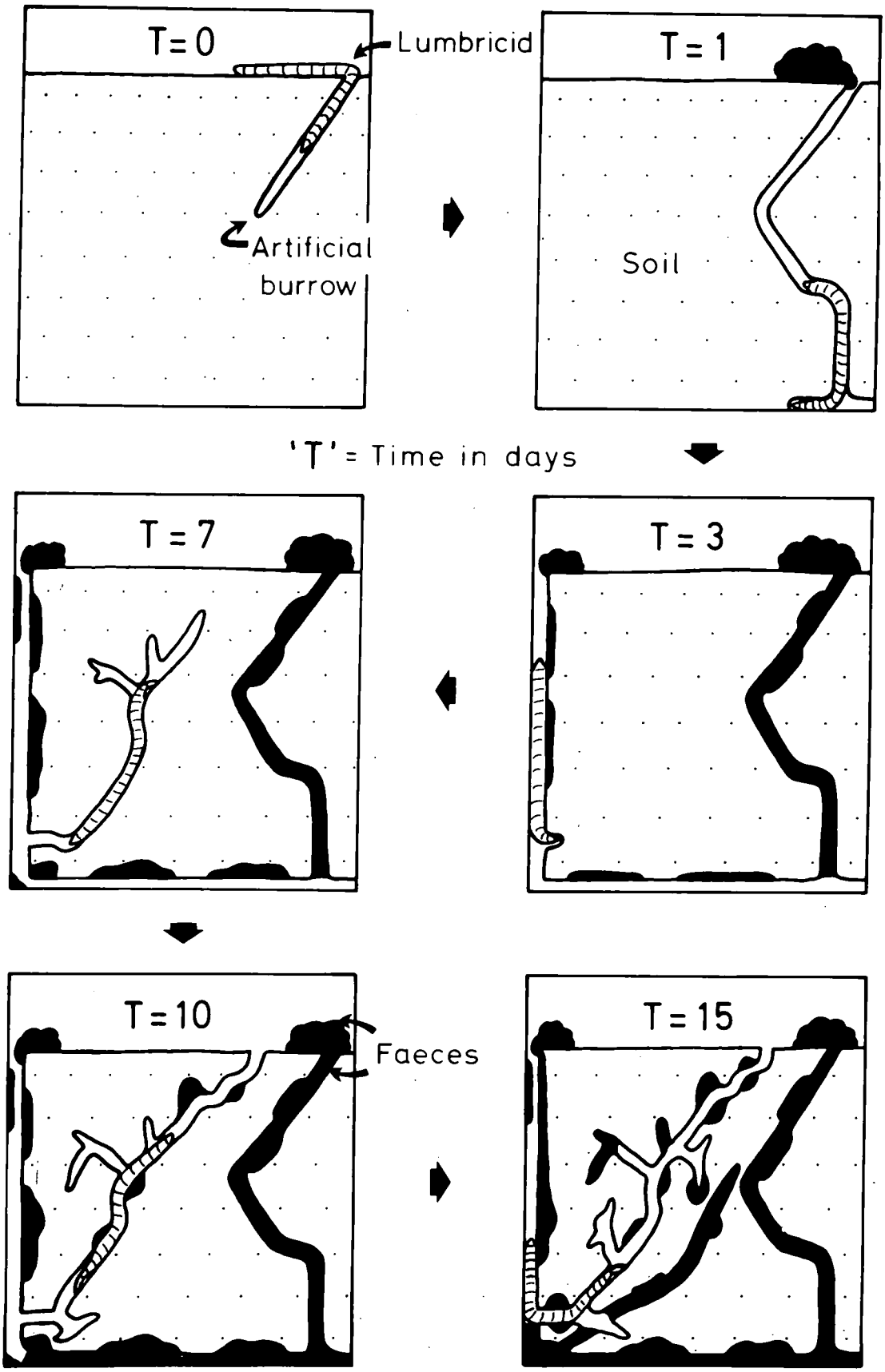


Fig. 22. The typical course of A.rosea burrow formation within a small perspex cage (see text for dimensions).

surface, the worms proceeded to produce - over a period of several days - short, inter-connecting side-burrows which gradually ramified the soil structure. The rate of burrow formation was found to be approximately constant after the third day.

Faecal material was deposited on the soil surface during the first day - the worm periodically retreating from the terminal wall of the burrow until its posterior end reached the soil surface where a faeces 'pile' was deposited. These small 'piles' accumulated until the faecal mass almost covered the entrance hole - though the hole was never seen to be completely blocked at this stage. Once the second link with the surface had been made, the entrance hole was usually blocked and the initial burrow length 'back-filled' with faecal material. Subsequent faecal material was either pressed into spaces adjacent to the burrow wall or used to back-fill disused sections of the burrow system.

Fig. 23 shows the mean daily length of burrow constructed per individual by each A. rosea size class at each of the temperatures used in petri-dish 'cage' experiments. The histograms show the equivalent mean daily length per individual for the sixty worms of all size classes involved in faecal output experiments. The arrows mark the points of initial faeces collection from the faecal output chambers (three days after worm confinement). The general course of burrow formation was shown to be constant, regardless of ambient temperature. Burrow construction during the first day after

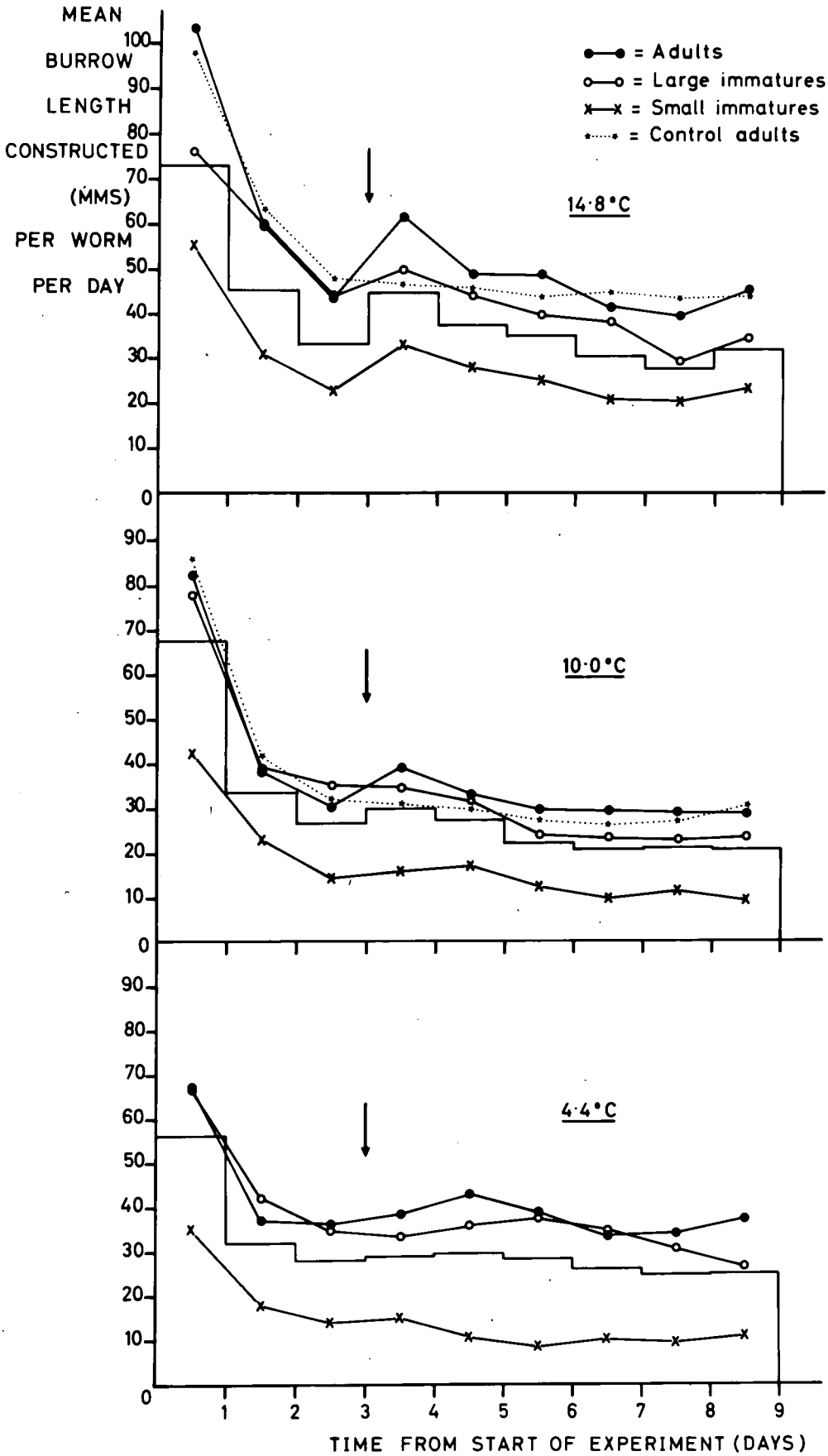


Fig. 23. Mean daily burrow length constructed by A.rosea worms confined in petri-dish cages (see text) at three temperatures.

worm confinement was seen to be maximal. At 4.4°C and 10.0°C, in all size classes, burrow construction on the second day was only 50% to 60% of the values for the first day, and close to the approximately constant level achieved later. At 4.4°C the adults showed somewhat increased burrow construction on the fifth day. At 10.0°C, burrow construction by the adults was increased on the fourth day - coinciding with a temporary halt in the decreasing daily burrow construction by large immatures. Small immature burrow construction at 10.0°C was slightly increased on the fifth day. The subsequent constant levels of burrow construction, in all size classes, were maintained until the ninth day, when the experiments were terminated. The average rate of burrow construction after the third day was approximately the same at both 4.4°C and 10.0°C.

At 14.8°C the general pattern was the same, though the decrease in burrow construction from the first to the second day was not so marked in the large immatures. Both large immatures and adults showed a greater decrease, in relative terms, between the second and the third days than was shown at the two lower temperatures. All three size classes showed an increase in burrow construction on the fourth day. These temporary increases were more marked at 14.8°C than similar effects at 4.4°C and 10.0°C. Results from adult control animals at 10.0°C and 14.8°C did not show temporary increases in burrow construction. The 'constant' level of burrow construction showed a gradual decline at 14.8°C, though

values on the ninth day showed an increase - suggesting that the consistent level had in fact been reached. This suggestion was supported by the constant level achieved by adult control animals at this temperature. The average rate of burrow construction per individual after the third day was noticeably higher for all size classes at 14.8°C than the equivalent levels at 4.4°C and 10.0°C. Table 10 shows the mean length of burrow constructed, per gram lumbricid fresh weight per day, by each of the A.rosea size classes used for faecal output estimates, at each of the experimental temperatures. These mean rates of burrow construction were calculated only from data for the 'feeding' phase - the fourth to the ninth day, inclusive. The mean rate of burrow construction, per gram fresh weight, was shown to decrease with worm size. The highest rates in all size classes occurred at the highest temperature. The rate of construction by small immatures at 10.0°C was intermediate between the values at 4.4°C and 14.8°C. The large immatures and the adults each showed similar rates of construction at 4.4°C and 10.0°C.

Despite the circular form of the soil layer in the petri-dish 'cages', the worms were seen to follow a similar pattern of burrow formation to that shown in perspex cages. One day after worm confinement the prostomia of almost all animals were near the terminal wall of the burrow, and after one or two days a 'U'-shaped burrow had been constructed - providing two outlets to the soil surface. After the second

Size Class	4.4°C	10.0°C	14.8°C
Small immatures	293.04	374.91	515.71
Large immatures	182.29	175.54	310.25
Adults	161.20	133.00	221.96

Table 10. The mean length of burrow constructed (mms.), per gram lumbricid fresh weight per day, by each A. rosea size class at three experimental temperatures. (Measurements made under normal feeding conditions - see text).

or the third day, all animals were feeding - widening existing burrows both laterally and terminally, and making only gradual extensions of the ramifying burrow system. Feeding consisted of a 'grazing' procedure in which soil materials were removed from the burrow wall and immediately ingested. The burrow expansions thus produced were non-linear, often 'fan'-like, in form; true burrow system extensions involved burrowing behaviour and were linear.

Discussion

Evans (1947) first described the cage or cuvette method for studying the burrowing activities of earthworms. This method has since become a standard technique for studies involving lumbricid confinement in the laboratory (Svendsen, 1955a; Grant, 1956; Van Rhee 1965; etc.). The perspex cage described in the present study was a miniature version of the orthodox cage structure. The petri-dish structures were eccentric in that the soil layer was in the form of a disc, to facilitate burrow tracing and faeces collection, but the lumbricids were seen to behave in a similar manner to that shown in normal cage structures. Evans (1947) described the formation of 'U'-shaped burrows, on the first day after confinement, by each of the species L. terrestris L., L. rubellus Hoffmeister and A. caliginosa (Sav.). Similar 'U'-shaped burrows were formed by A. rosea in both perspex and petri-dish cages - but they were formed in one or two days only in the smaller petri-dish units, usually appearing after two or three days in the perspex cages.

The initial entrance hole was only blocked by faeces after the second surface link had been constructed. Since this species does not forage on the soil surface for food or other materials, the formation of surface outlets would seem associated with burrow aeration.

Both in perspex cages by observation and in petri-dish units by quantitative measurement, burrow construction was seen to be extensive over the first two to three days of lumbricid confinement. During the first day of confinement burrow construction was maximal and the lumbricids were thought to derive nourishment solely from burrowing activity. The linearity and position of the burrows formed during the first two or three days suggested a prolonged 'escape reaction' to confinement, supplemented by a stimulus to create aeration channels to the soil surface. This behaviour during the first three days of confinement was considered typical of lumbricid activity in the laboratory, on introduction to a fresh medium, but could occur only rarely in the field. The faeces produced during the first three days were not included in estimates of normal faecal output (see sub-section 3 below).

After three days confinement, in both types of cage unit, the lumbricids reverted to an almost sessile state in which burrows were apparently only constructed to expose a fresh internal soil surface for feeding. This type of behaviour was considered typical of activities in the field and suitable for estimates of faecal output under natural

conditions. There was an exception to the described sequence of events, in that various A. rosea size classes at various temperatures showed somewhat increased burrowing activity on the fourth or fifth days after confinement in the petri-dish units. By measurements on adult control specimens whose faeces were not collected, the temporary increases in burrowing activity were shown due to the disturbance caused by faeces collection three days after initial lumbricid confinement. Adults were used in the control experiments - performed after the faecal output studies - since temporary increases in burrow formation had been shown to be greatest in this size class.

Absolute rates of burrow formation were shown to be dependent on worm size and ambient temperature. The dependence was of the general poikilotherm type in each case - though burrow construction rates must be influenced by variable qualities of the soil medium, in addition to metabolic considerations.

2. Food Material

(i) General Introduction

Feeding biology is perhaps the most poorly understood aspect of lumbricid ecology. Work has been concentrated on litter selection and ingestion by L. terrestris L. - a species with somewhat eccentric feeding habits which are particularly suitable for experimental analysis. Selection or rejection of litter by L. terrestris, according to the various physical and chemical properties of the material, has been extensively studied (Darwin, 1881; Mangold, 1951; Wittich, 1953; Zicsi, 1954; Satchell and Lowe, 1967). The surface active pigmented species, probably feeding on semi-decayed litter and/or humus, would seem suitable for experimental study but little information is available. Waters (1955) suggested that L. rubellus Hoffmeister fed mainly on herbage debris in New Zealand pastureland - though the suggestion was more by deduction than by experimental analysis. The unpigmented soil-dwelling species present the greatest problems for studies in feeding biology. Since it was hoped to elucidate the energy relations of A. rosea (Sav.) at Wynyard, this species was selected for studies of lumbricid feeding biology in the present work. Problems of defining the nutrient sources for soil-ingesting species such as A. rosea have yet to be solved. Lindquist (1941) found that neither A. rosea nor A. caliginosa (Sav.) would feed on leaf litter, though dung was ingested by these species. Such laboratory observations are of only limited use for inter-

pretation of food ingestion by soil-dwelling species in the field. Waters (1955) suggested that root debris was a major food source of A. caliginosa in New Zealand pastureland, though Müller (1950) regarded A. caliginosa as feeding mainly on soil fungus mycelium. Barley (1959) suggested that micro-organisms formed a major source of nutrition for A. caliginosa. Gerard (1963) regarded small unpigmented species as predominantly root-feeders - feeding on living or dead roots according to species. A considerable degree of speculation was involved in most of these estimates. Miles (1963b) demonstrated the importance of soil protozoa in maintaining normal growth of E. foetida (Sav.), but most experimental evidence for soil-dwelling lumbricid diet has involved the microbiological analysis of 'fresh' soil, lumbricid gut contents and worm 'cast' material, i.e. faeces. The evidence accumulated has been confusing and often contradictory, the variation in results probably reflecting the gradual refinement of micro-techniques in more modern times. Bassalik (1913) found that the bacteria and fungi in 'worm' casts were of similar type and numbers to those occurring in the adjacent soil. Aichberger (1914) looked at the gut contents of 'earthworms' and stated that diatoms, blue-green algae, desmids, yeasts and rhizopods were killed during their passage through the lumbricid digestive tract. Stöckli (1928) could only confirm Bassalik's findings, but Dawson (1947) suggested that bacteria were also present in smaller numbers in casts than in adjacent soil. Both Dawson and Day (1950)

agreed that fungi were little affected by passage through the lumbricid gut. Dawson's theory of bacteria destruction was supported by Day, who showed two bacterium species to be affected by passage through the gut of L. terrestris (one species was killed, the other reduced.). Ruschmann (1953, 1954) disagreed with all the above authors by stating that many micro-organisms - especially actinomycetes - actually increased within the lumbricid gut and were present in higher numbers in lumbricid faeces than in the adjacent soil. Ruschmann's findings were supported and expanded by those of Parle (1963b) who has performed the latest and probably most accurate work in this field. Parle studied A. caliginosa, L. terrestris and A. longa Ude, showing that actinomycetes and most soil bacteria multiply rapidly in the gut of these species. He showed that numbers of fungi in gut content and faecal material were dependent on the fungal concentration in the surrounding soil- and independent of lumbricid gut conditions. He did show, however, that germinating fungal spores were more frequent in casts than in the adjacent soil. Thus, for those species which Parle studied, fungi, actinomycetes and bacteria were probably not an important food source - if digested at all, being more abundant after their emergence from the lumbricid gut.

In many studies of lumbricid diet the digestive ability of the various lumbricid species has been assumed a constant factor. Tracey (1951) showed that this approach is in fact justified. He investigated the digestive enzymes present in

a large number of lumbricid species: in addition to proteases, amylases and lipases, he detected cellulase in seventeen species and chitinase in twelve of these. He suggested that both cellulase and chitinase were produced by the lumbricids themselves - not by symbiotic organisms - since the enzymes were present in extracts from washed portions of the gut wall. It is doubtful whether even minute fragments of insect cuticle could be digested by lumbricid chitinase, but the presence of the enzyme may indicate that fungal chitin could be digested.

The available evidence for the nutrition sources of a soil-dwelling, soil-ingesting species such as A. rosea is thus little more than a collection of ideas, supported by the scantiest of evidence. Tracey has shown that almost all the British Lumbricidae have the same digestive ability, so that the diet of a particular species must depend upon the availability, palatability and 'ingestibility' of different organic materials in the habitat. The investigation of palatability, by food preference experiments under natural feeding conditions, will yield the most valuable information. Ironically, it is this aspect which presents the most problems for studies involving soil-ingesting species; no method has yet been devised whereby soil organic constituents such as diatoms, algae, bacteria, protozoa, fungi or dissolved and fine particulate organic matter can be suitable isolated and presented with larger organic materials in a food selection experiment. Barley (1959a) performed food selection

experiments with A. caliginosa by placing bulky organic materials on the soil surface. This type of experimentation, originally devised for L. terrestris, is totally unsuitable for studies on the field diet of A. caliginosa or any other soil ingesting lumbricid. Rearing worms on pure diets in the laboratory provides evidence of the worm's ability to ingest the material but little or no information on feeding habits in the field. Experiments involving deprivation of particular soil organic constituents (e.g. those of Miles, 1963b) are possibly simpler to devise than selection experiments, but interpretation of results - using some worm metabolic parameter - will again be difficult in terms of field mixed diets.

In the present work, studies of A. rosea diet were restricted to investigation of the availability of food - by a soil preference experiment, the 'ingestibility' of food - by observations of ingesta particle size and worm gut contents, the possible effects of deprivation, and the possibility of food selection - by examination of soil and faeces organic content.

(ii) Soil Preference of Allolobophora rosea (Sav.)f. typica

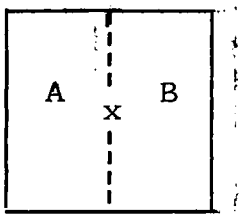
Introduction

It was assumed that if soil physico-chemical conditions were not limiting, A. rosea worms would tend to congregate in soils containing the highest quantities of food available to this species. An experiment was therefore devised whereby the soil preferences of A. rosea could be investigated both

for normal field soils, and the same soils where physico-chemical limiting factors were minimised.

Methods

Four topsoil types were used in this study; they were classified according to vegetational cover, as 'bramble', 'grass', 'bracken' and 'Juncus' soil. Glass dishes, measuring 10 cm. by 10 cm. in area by 6 cm. in depth, were filled with soils to a depth of 4 cm. in the spatial form:



where A and B were two different soil types, Worms were placed in these chambers at the point x. The combinations of soil types used were: Juncus/bramble, Juncus/grass, Juncus/bracken, bramble/grass, bramble/bracken and grass/bracken. Two such sets were prepared: topsoils from the field were cut into blocks to compose both sets, but one set was thoroughly saturated with dechlorinated tap water, and equilibrated overnight, before the introduction of lumbricids. Samples of the original soils were weighed and dried in a vacuum oven at 60°C to determine soil moisture content; the pH values of the original soils, and the waterlogged soils after equilibration, were noted.

Four A. rosea worms - two adults, one large immature and one small immature - were placed in each of the chambers, which were then covered with loose-fitting glass lids and placed in a constant temperature room at 10°c. After four

days the chambers were individually removed, the soil portions were separated and the worms removed. The number of worms found in each soil type in each chamber was noted.

Results

The soil moisture contents and pH values of the original soils were as shown (in order of decreasing magnitude for both factors) in Table 11.

Soil type	% H_2O by weight	pH
Juncus	43%	6.7
Bramble	38%	6.1
Grass	27%	5.7
Bracken	24%	4.9

Table 11. Soil moisture contents and pH values of original soils used in the soil preference experiment.

The pH values of soil combinations which had been saturated and equilibrated were variable within the range pH5 to 7, with no significant pH boundaries detectable between the soil types in any particular chamber.

The recoveries of worms from each soil type in each combination are shown in Table 12. Juncus soil was preferred to bracken soil in normal soils, and to grass or bracken soils in saturated soils; bramble soil was preferred to grass and bracken soils in both normal and saturated soils - though one adult was recovered from saturated grass soil in combination with bramble soil; grass soil was preferred to bracken soil in both normal and saturated soils. No preference was shown between Juncus and bramble soils in

Soil Type	Normal Soil	Saturated Soil
Juncus	A, LI	LI
Bramble	A, sI	A, A, sI
Juncus	A, sI	A, A, LI, sI
Grass	A, LI	NONE
Juncus	A, A, LI, sI	A, A, LI, sI
Bracken	NONE	NONE
Bramble	A, A, LI, sI	A, LI, sI
Grass	NONE	A
Bramble	A, A, LI, sI	A, A, LI, sI
Bracken	NONE	NONE
Grass	A, A, LI, sI	A, A, LI, sI
Bracken	NONE	NONE

Table 12. Recovery of A. rosea worms from normal and saturated soil combinations. (A - adult worm; LI - Large immature worm; sI - small immature worm).

either normal or saturated soils.

Of the twenty-four worms placed in chambers containing normal soils, eight were recovered from Juncus soil, ten from bramble soil, six from grass soil and none from bracken soil. Of the same number of worms placed in chambers containing saturated soils, nine were recovered from Juncus soil, ten from bramble soil, five from grass soil and none from bracken soil.

Discussion

Of the four soil types used in this experiment, three occurred in the grid area at Wynyard and the fourth - Juncus soil - was associated with a high density of A. rosea in a low moor area at Wynyard. The A. rosea population in the grid area at Wynyard was shown to be at its highest density in 'bramble' areas and negatively associated with areas of 'grasses' and bracken (see Table 2 and Fig. 11 above). The soil preference results using normal field soils could be interpreted as a positive taxis towards soils of higher moisture content and/or lower acidity. However, when both these variables were minimised, and probably removed, by water saturation, a separate group of worms showed exactly the same soil preferences. There was one exception in that the preference for Juncus soil to grass soil was more pronounced under saturated conditions. In this case it was probably the preference of one A. rosea adult and one large immature for normal grass soil which was exceptional.

The observed preferences of A. rosea for bramble and

Juncus soils, and the complete avoidance of bracken soil, thus appeared due to factors unrelated to the physico-chemical properties of the soils involved. The mull-type bramble soil was observed to have a much higher organic content than the light-coloured clay soils beneath grass, and the Juncus soil contained considerable quantities of root exodermal debris. The A. rosea soil preferences shown may therefore have been related to the availability of organic food materials in the different soil types. In the Juncus soil a major food material may have been in the form of root debris.

The soil preference experiment described in the present study was of a simple form, but it seemed evident from the results that experiments of this type could be a valuable source of information on food availability to soil-dwelling lumbricid species. The cage, or cuvette, method used by Roots (1965b) for studies of lumbricid soil preferences in relation to soil moisture levels could be adapted for use with different soil types. Such a technique would allow continuous observation of lumbricid activities in apposed soils. The water saturation technique forminimisation of soil physico-chemical variables, as used in the present study, is only recommended for use with a species such as A. rosea which is unaffected by soil saturation and frequently occurs in waterlogged soils in the field.

(iii) Ingesta Particle Size and Composition of Gut Contents

Introduction

In the course of respirometry studies involving the use of a coarse sand medium, it became obvious that only some lumbricid species could ingest the sand grains. The maximum particle size which can be ingested will set obvious limitations on the potential sources of lumbricid food supply. In view of the sand ingestion results, observations were made of mineral particle size in the gut contents of three unpigmented, soil-dwelling lumbricid species. Mineral particle size was assumed to indicate particle size 'ingestibility' in general. A. rosea gut contents were also examined for evidence of food sources in this species.

Methods

The detailed techniques used in respirometry will be described below (Part II, Section IV). For the present purposes it will suffice to note that lumbricid worms were weighed before and after a period of two days spent in a dampened coarse sand medium which was buffered to a pH of 6.5. The three major size classes of A. rosea were studied separately, but the groups of O. cyaneum, D. rubida f. subrubicunda and L. castaneus each included animals covering the full size range for the species.

Ten similarly-sized (approximately 4 cm. in length), immature individuals of each of the soil-dwelling, soil-ingesting species A. rosea, A. caliginosa and O. cyaneum were used for the investigation of ingesta mineral particle size. The worms were kept for three days in the same glass

jar containing a homogenised (see p.217) and dampened mull-type 'bramble' soil. Smears of gut contents were then taken from immediately behind the eventual clitellar region of each worm. The smears were examined using a petrological microscope with crossed Nicol prisms. Photomicrographs were prepared for typical gut smears from each species.

In addition to the above specimens, ten recently-emerged A. rosea individuals were kept in the glass jar medium. Gut content smears from these worms were taken and examined according to the procedure described for the larger individuals.

In a separate experiment, five large immature and five adult worms of A. rosea were taken from a mull-type 'bramble' soil in the field. Smears of their gut contents were taken from the gizzard, the gut midway between the gizzard and the anus, and the gut in the anal region. The smears were examined using a light microscope, with below-stage illumination, and photomicrographs were prepared for smears taken from a typical individual.

Results

Table 13 shows the percentage change in the total fresh weight of animals used in respirometry over the two-day period of confinement in a coarsesand medium. Estimates were made for each species or species size class in each month of measurement and the variation was seen to be erratic through the year. The individual values obtained are presented solely to indicate the degree of variation. The

Month	A. rosea			O.cyan.	D.rub. f. subr.	L.cast.
	sI	LI	Adults			
Sept. 1966	-7%	0%	0%	N.M.	N.M.	N.M.
Oct.	-2%	+3%	+3%	N.M.	N.M.	N.M.
Nov.	+2%	+1%	+3%	+23%	-1%	+15%
Dec.	-1%	-1%	+1%	N.M.	N.M.	N.M.
Jan. 1967	-7%	-5%	-1%	+31%	+5%	+14%
Feb.	-5%	+2%	+1%	N.M.	N.M.	N.M.
March	-3%	-2%	-1%	+18%	-1%	+24%
April	+2%	-5%	-1%	N.M.	N.M.	N.M.
May	-3%	+1%	+6%	+26%	+3%	+24%
June	-5%	+1%	+3%	N.M.	N.M.	N.M.
July	-9%	+3%	-5%	+8%	+3%	+24%
Aug.	-7%	-8%	-5%	N.M.	N.M.	N.M.
Sept.	N.M.	N.M.	N.M.	+18%	+12%	+30%

Table 13. Percentage changes in the total fresh weight of lumbricid groups during 48 hour measures of respiratory rate, performed at monthly or two-monthly intervals.

(sI - Small immatures, LI - Large immatures, A - Adults, N.M. - Not measured, O.cyan. - O.cyaneum, D.rub. f. subr. - D. rubida f. subrubicunda, L.cast. - L. castaneus)

overall pattern of weight change in each of the various groups was shown by the mean value over the year of measurement. The mean values obtained were -4% for small immatures, -1% for large immatures and 0% for adults of A.rosea,+21% for O. cyaneum, +2% for D. rubida f.subrubicunda and +21% for L. castaneus.

Plates 11, 12 and 13 - all to the same scale - show typical gut smears from the eventual post-clitellar regions of similarly-sized A. rosea, A. caliginosa and O. cyaneum immatures, respectively, viewed through crossed Nicol prisms. The size distribution of mineral particles (bright through crossed Nicol prisms) was seen to vary markedly between these three species. Fine particles were present in all smears but the maximum particle size from A. rosea worms was only 80 to 100μ whereas the similar maxima for A. caliginosa and O. cyaneum were approximately 200μ and 500μ, respectively. Plate 14 - to the same scale as those above - shows a typical gut smear from the eventual post-clitellar region of a recently-emerged A. rosea individual, viewed through crossed Nicol prisms. The mineral particle size distribution was seen to be similar to that shown for large immature A. rosea worms, considered above.

Plates 15, 16 and 17 show gut content smears from the gizzard, the gut midway between the gizzard and the anus, and the gut in the anal region of a large immature A. rosea individual. The smears, viewed through a light microscope with below-stage illumination, are all to the same scale but

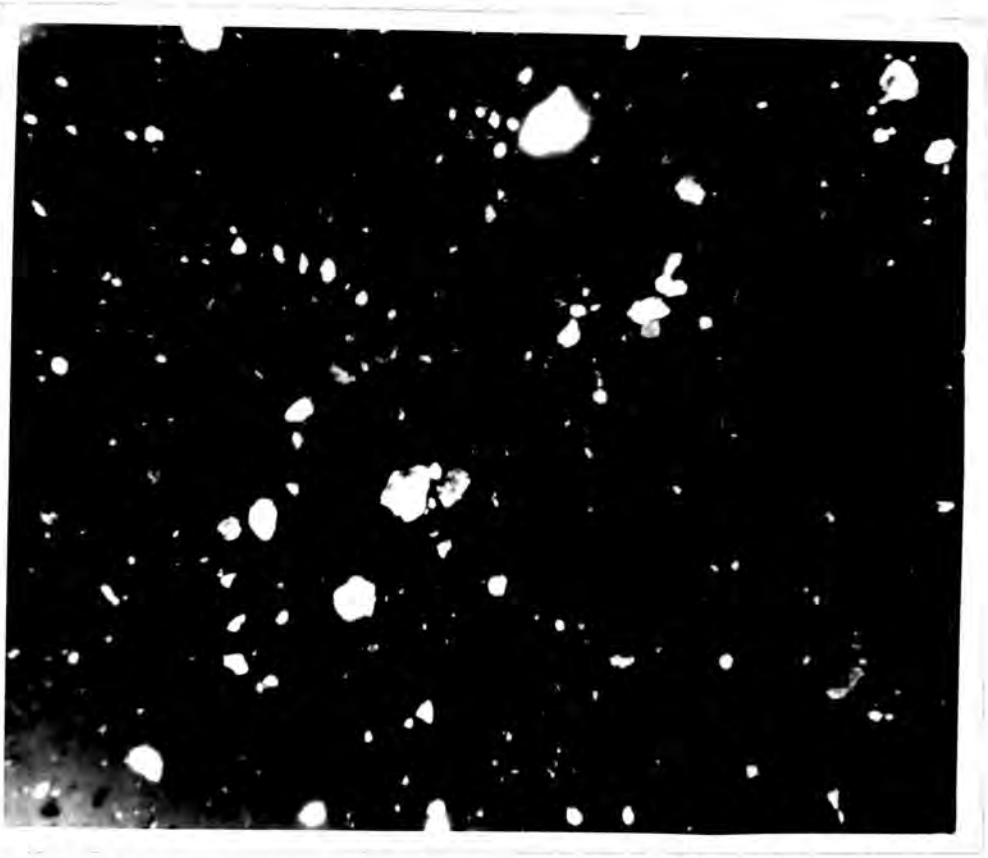


Plate 11. A.rosea: large immature gut contents (viewed through crossed Nicol prisms)

500 μ

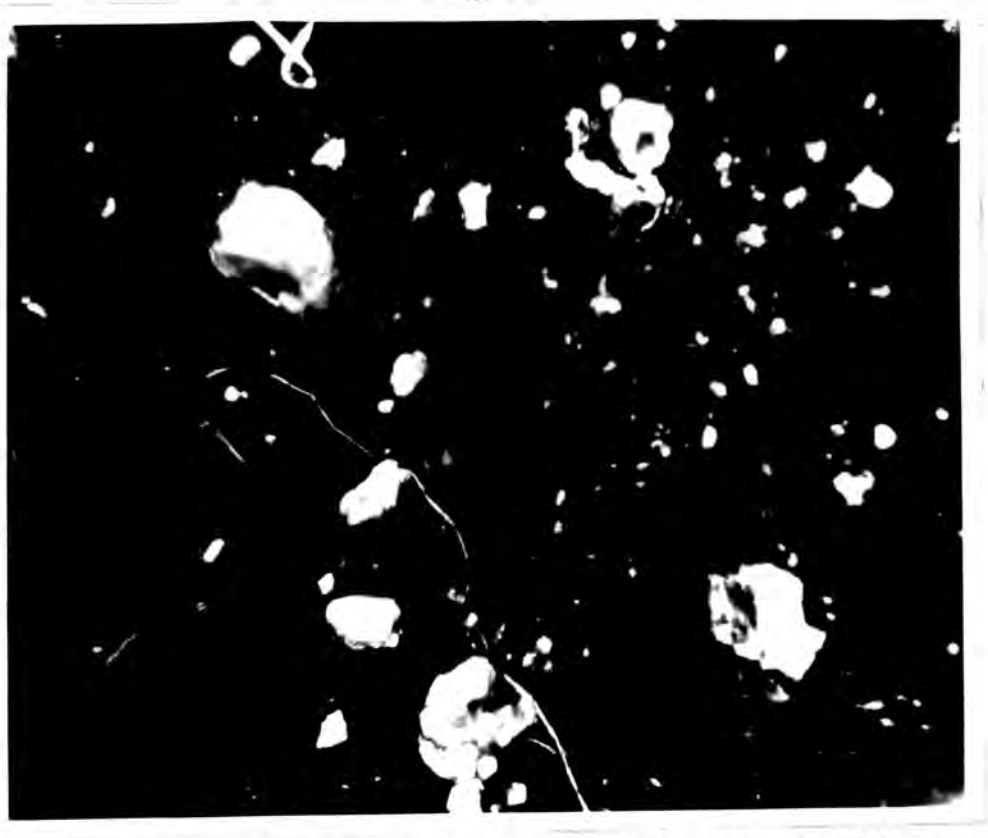


Plate 12. A.caliginosa: large immature gut contents (viewed through crossed Nicol prisms)

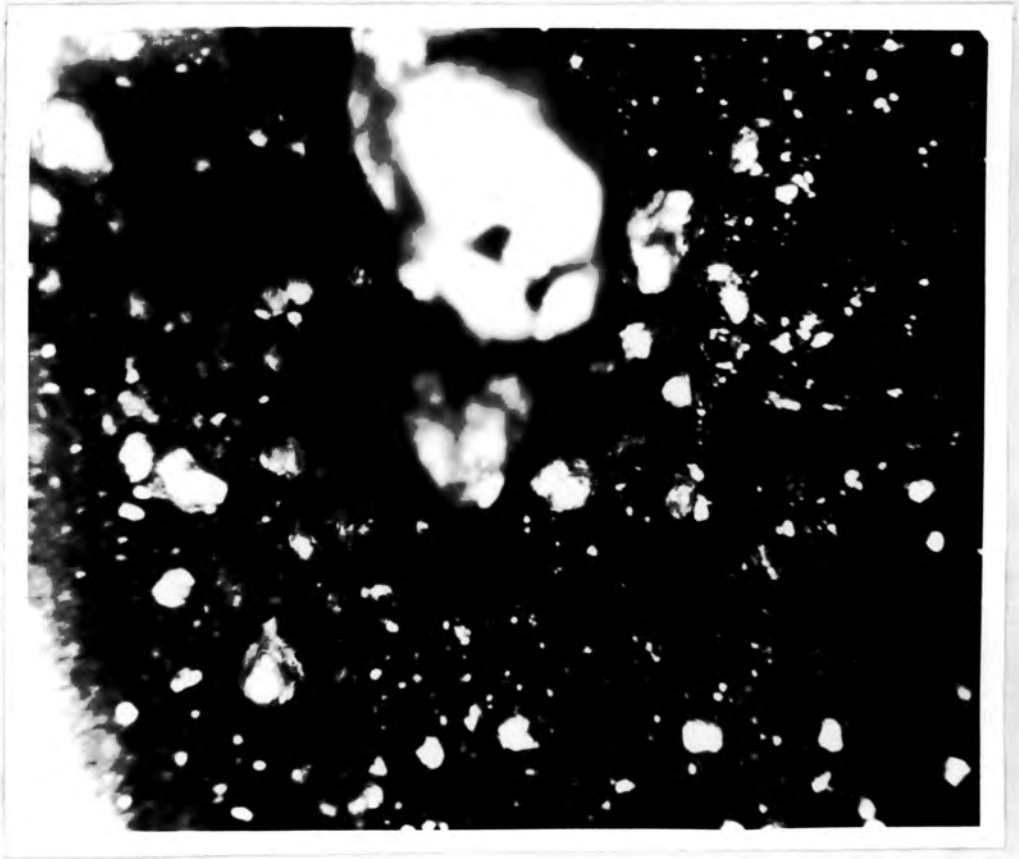


Plate 13. O.cyaneum: small immature gut contents (viewed through crossed Nicol prisms)

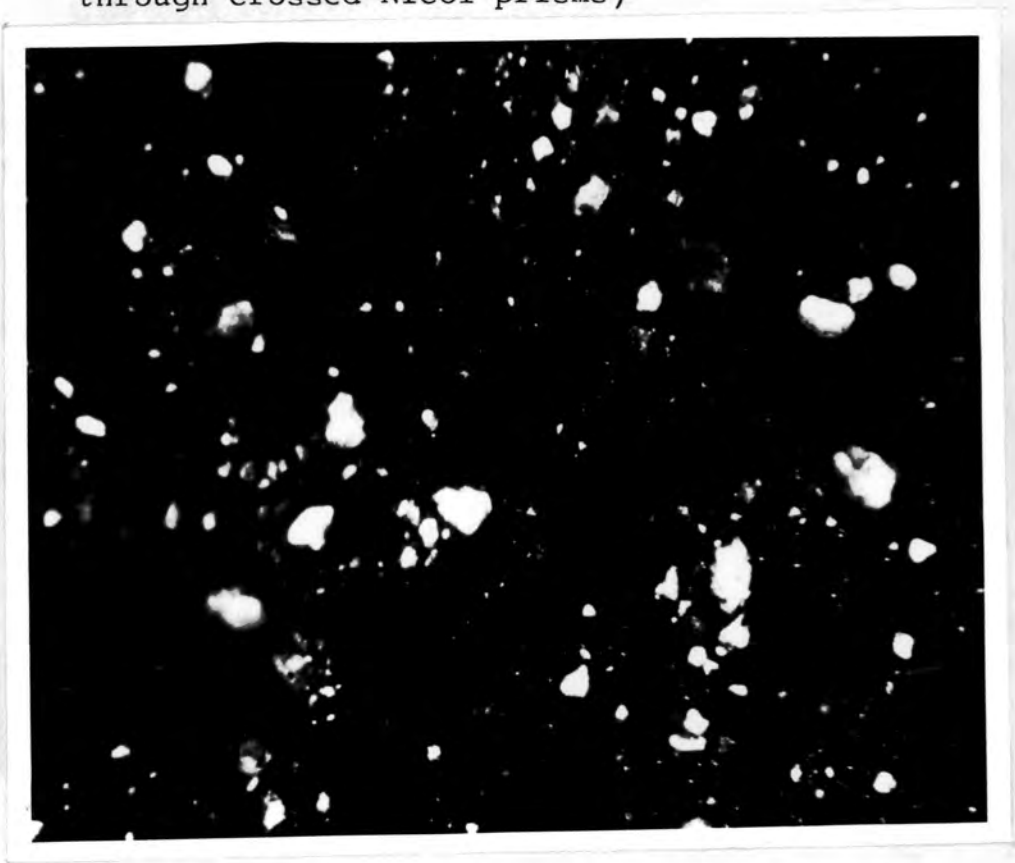


Plate 14. A.rosea: small immature gut contents (viewed through crossed Nicol prisms)

Plate 15. A.rosea gut contents - Gizzard

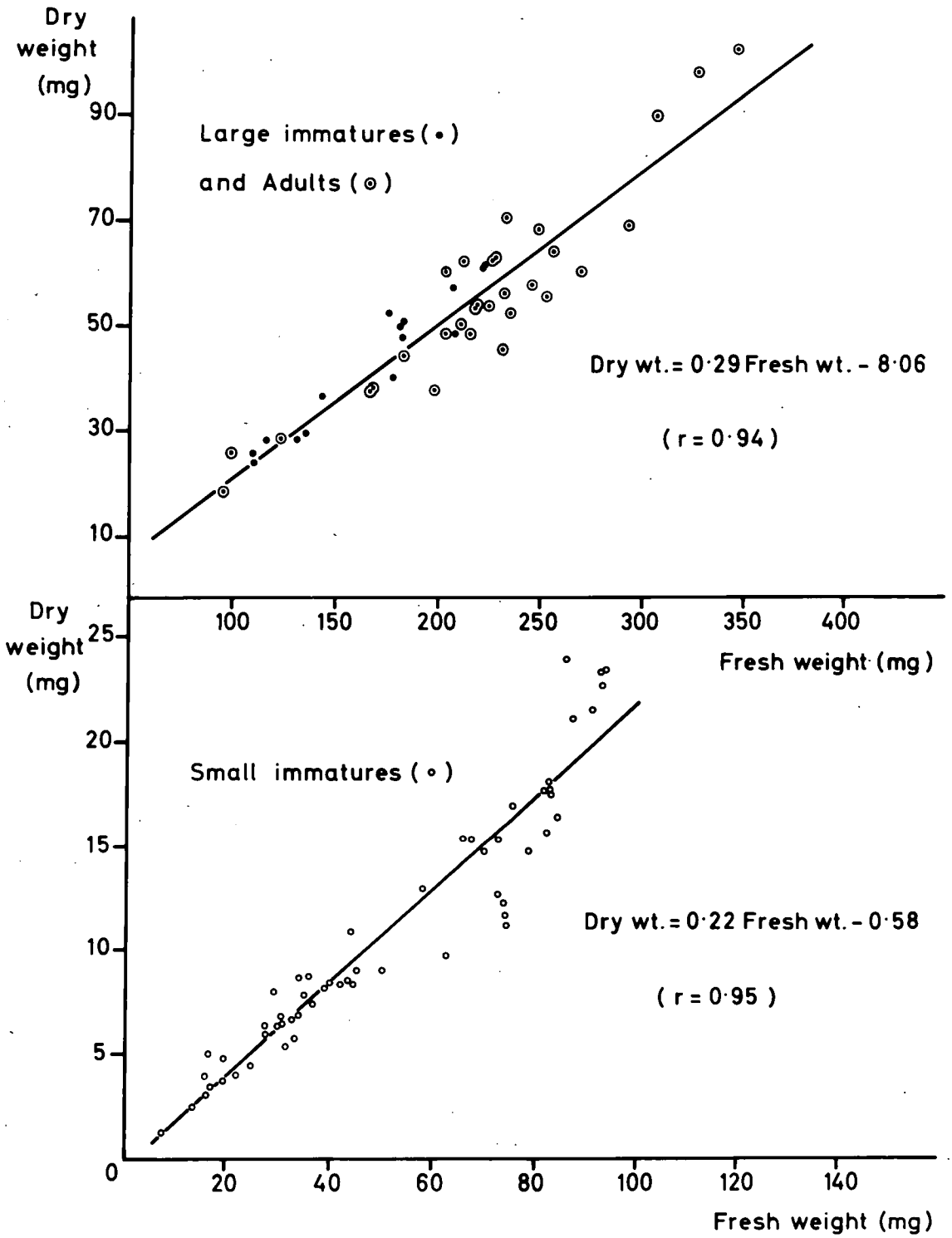


Fig. 7. The relation between total body dry weight and total body fresh weight for A.rosea worms collected at Wynyard.

Plate 16. A.rosea gut contents - Mid-intestinal region

Plate 17. A.rosea gut contents - Anal region

the actual scale involved was unfortunately not recorded. The sequence of gut contents shown was typical of both the large immature and the adult A. rosea worms examined. The gizzard contained large pieces of organic material (majority of dark particles in Plate 15) - mainly plant debris, in addition to mineral particles and small amounts of minute and finely divided organic matter. The mid-intestine did not contain large pieces of organic material, though distinct organic particles of appreciable size were still present, in addition to mineral particles and increased amounts of minute and finely divided organic matter. The prospective faeces, from the gut in the anal region, consisted of small, amorphous organic particles, mineral particles and considerable quantities of minute and finely divided organic matter.

Discussion

The coarse sand used in respirometry was not sieved and therefore contained fine material in addition to the coarse-grained material. The worms kept in the sand medium for two days might be expected to void a high proportion of their gut contents in this time. Since gut content weight formed a high proportion of total body weight in the unpigmented species A. rosea and O. cyaneum (see sub-section 3, (ii), below), a high percentage decrease in worm total fresh weight during respirometry might be expected for these species. Such decreases did not occur, suggesting that sand was ingested by both species. However, A. rosea merely maintained its body weight whilst O. cyaneum considerably increased

its body weight. Assuming that sand has a higher density than the normal gut content of these species, this suggested that A. rosea was ingesting only small quantities of the medium whilst O. cyaneum was ingesting sand at a rate similar to, or even greater than, its field soil ingestion rate. O. cyaneum individuals were observed to defaecate almost pure coarse sand on removal from the respirometer.

The pigmented species D. rubida f. subrubicunda and L. castaneus showed similar differences in fresh weight variation during respirometry. D. rubida f. subrubicunda maintained its weight whilst L. castaneus increased its weight considerably. It can therefore be suggested that L. castaneus has a much greater capacity for coarse sand ingestion.

Svendsen (1955a) took post-clitellar region gut smears from a number of pigmented and unpigmented lumbricid species found in moorland soils. He examined the smears using both a light microscope and a petrological microscope with crossed Nicol prisms. He recognised three types of material: large organic particles, fine organic particles and mineral particles. He stated that the pigmented species contained only the first two of the above types, whilst the unpigmented species contained the last two types and, rarely, the first type. From the present microscopic examinations of lumbricid gut contents, it can be stated that mineral particle size in the post-clitellar gut of unpigmented species differs according to the species involved. Since comparisons were

made between similarly-sized animals which had been feeding from the same homogeneous medium, variables of absolute body size and food medium were removed. It appeared that A. caliginosa had a greater capacity for large particle ingestion than A. rosea, and O.cyaneum had an ingestion capacity which surpassed both these species. The conclusions regarding A. rosea and O. cyaneum sand ingestion during respirometry can therefore be modified by suggesting that A. rosea can only ingest the less common, fine particles in coarse sand, whilst O. cyaneum is able to ingest the bulk of the medium. It may also be postulated that L. castaneus can ingest larger particles than D. rubida f. subrubicunda. These results appeared to indicate that lumbricid species are ecologically separated in their feeding habits by, amongst other things, their differing capacities for particle size ingestion. The similarity between gut particle size distributions for recently-emerged and large immature worms of A. rosea suggested that ingestion capacity varies little with body size in this species.

The maximum particle size of ingested organic material was shown to be drastically reduced by the action of the gizzard in A. rosea. Organic particle size was further reduced, probably through the action of digestive enzymes, by passage through the intestine of this species. It seems likely that A. rosea derives some nourishment from the digestion of large particulate organic materials - mainly plant debris.

(iv) Deprivation Effects on A. rosea Adults

Introduction

During preliminary studies on the cocoon production of A. rosea adults, animals were kept for three months in a wet-sieved and reconstituted soil medium. The use of this medium had drastic effects on the animals involved. Since lack of nourishment could have been a factor involved, the situation was examined for possible sources of food deprivation.

Methods

The sieving and reconstitution of mull-type 'bramble' soil was described above (p.127). The reconstituted medium was sub-divided and placed in eight, red-clay porous plant pots. Three fresh-weighed A. rosea adults were placed in each of the pots, which were subsequently sunk into the field soil so that the internal and external soil surfaces were at the same horizontal level. After three months the pots were returned to the laboratory; the adults were removed and fresh-weighed and the media were examined for cocoons. In view of the situation found, the bacterial and fungal status of the reconstituted soil, after three months in the field, was compared with that for field soil. A weak soil solution was prepared by mixing a quantity of 'bramble' soil with a large volume of water and filtering off the solid material. This solution was used in the preparation of agar plates for soil bacteria and fungi culture. A separate agar plate was centrally inoculated from each of the reconstituted soil pots and from each of ten field soil samples. The agar plates

were incubated at 28°C for five days, after which the radii of bacterial and fungal growth were measured, to the nearest millimetre, in each agar medium. The radii of 'coarse and/or dense' and 'fine and sparse' fungal mycelia were measured separately.

Results

Of the twenty-four adults originally confined in the reconstituted medium, seven were missing after three months and the remaining seventeen worms had all decreased in body weight - between 20% and 40% in most cases. No cocoons were produced.

Table 14 shows the radii, in millimetres, of bacterial and fungal growth in agar plates centrally inoculated from normal and reconstituted 'bramble' soils. The data were not analysed statistically due to the small number of samples involved and the irregular distribution of results. However, the mean radii shown in Table 14 suggested that the reconstituted soil medium contained less 'coarse and/or dense' fungal mycelium than the natural field soil. The 'fine and sparse' fungal growth appeared to be similar in both soils - though the results were difficult to interpret since the abundance of 'coarse and/or dense' fungal growth in most of the natural soil plates could have obscured finer fungal hyphae. Bacterial growth was low in plates from both soil types.

Discussion

It was realised that the wet-sieving procedure must have drastically altered the physical conditions within the soil media subjected to this treatment. However, A. rosea can

Sample	Natural Soil			Reconstituted Soil		
		Fungi			Fungi	
	Bact.	Coarse and/or Dense	Fine and Sparse	Bact.	Coarse and/or Dense	Fine and Sparse
i	2	25	0	2	0	35
ii	3	35	0	1	25	40
iii	2	40	0	3	20	0
iv	3	13	40	10	0	35
v	7	5	40	4	0	0
vi	0	9	40	3	4	40
vii	12	11	40	3	0	0
viii	1	40	0	5	6	0
ix	0	53	0	-	-	-
x	5	25	0	-	-	-
Mean Radius:	3.5	25.6	16.0	3.9	6.9	18.8

Table 14. Radii (mms.) of bacterial and fungal growth in agar media inoculated from natural field soils and reconstituted soils. (Bact. - bacteria)

live in a variety of habitats under a wide range of physical conditions - it occurs in soils of pH values ranging from 4.6 to 7.0 (Satchell, 1955a) and is often found in water-logged soils. Thus, the changed physical conditions within the reconstituted medium were not thought by the author to be directly responsible for the A. rosea losses in body weight. It appeared from the agar culture data that the reconstituted soil was deficient in 'coarse and/or dense' fungal mycelium growth - possibly due to the changed physical conditions in the medium. There is therefore a possibility that fungal mycelium forms a major part of the diet of A. rosea adult worms. It should be noted, however, that the wet-sieving method employed in the soil reconstitution would have involved the loss of at least a portion of the dissolved and fine particulate soil organic matter. It was shown in the present studies (see p.184) that particulate organic materials are broken down during passage through the gut of A. rosea; a deficiency of such materials in the reconstituted medium may therefore have deprived the A. rosea adults of a nutritional source.

The results of this deprivation investigation can only be regarded as marginally suggestive of A. rosea diet deficiencies. However, it is conceivable that refined studies of this type could yield positive information on lumbricid nutritional sources.

(v) Selection of Organic Matter

Introduction

The differences between burrowing and feeding in A. rosea have already been outlined (see sub-section 1, above). It was pointed out that, whilst feeding, the worms do not 'eat their way through the soil.' Material is grazed from the burrow walls and extensions in a regular manner. Lindquist (1941) stated that the amount of material passing through the gut of a soil-ingesting lumbricid is inversely proportional to the organic content of the soil. With the regulated intake shown by A. rosea, it is possible that the more organic constituents of the soil may be selectively ingested, a situation which could detract from Lindquist's suggested relation. Satchell (1960) stated that lumbricid faecal casts were usually found to be richer in nutrients than the surrounding soil, due to selective feeding by worms on the parts of the soil rich in organic matter, although he did not give the data or source from which his conclusions were drawn. In the present study, the possibility of selection of soil organic matter by A. rosea was investigated by examination of worm faeces in relation to the unworked soil medium.

Methods

Approximately 1.5 litres of mull-type 'bramble' soil were hand-sorted to remove all large organic constituents (roots, moss, plant debris, large invertebrates, etc.) and any stones or other large inorganic materials. The soil was crumbled

to pieces of about 1 cc., or less, in volume and thoroughly mixed. Twenty soil samples - each weighing approximately 2 to 3 g. after drying - were dried in a vacuum oven at 60°C, cooled in a desiccator, and weighed. They were then ashed in a muffle furnace at 500°C, again cooled in a desiccator and re-weighed - in weighed and suitably labelled 5 x 2.5 cm. glass tubes throughout. The remaining soil was divided into three equal portions. Each portion was placed in a glass container, covered with perforated polythene sheet to prevent escape. Ten small immature A. rosea worms were placed in one container, ten large immatures in the second container, and ten adults in the remaining soil medium. The worms had been previously acclimatised at 10°C for a period of four to six weeks. All containers were kept at 10°C throughout the experiment. After one week of confinement the worms were removed from the soil and rinsed briefly in dechlorinated tap water. The prospective faeces of each worm were removed on to a clean glass slide by gently pressing the worm tail region using a rounded glass rod. The faeces were carefully removed into weighed glass tubes using a surgical scalpel and a fine stream of distilled water. The faeces from all ten worms of each size class were grouped into the same glass tube. The worms were replaced in the same soil media at 10°C and, after a further period of one week, prospective faeces were again collected by the same procedure. The second quota of faeces from worms of each size class was placed in the same glass tube as the first

quota. The total amounts of grouped faeces were treated exactly as described for the soil samples above. The weight loss on ignition, for both soil and faeces samples, was calculated as a percentage of the original dry weight.

Results

The mean percentage weight loss on ignition of unworked 'bramble' soil was 11.25%, with a standard deviation of ± 1.00%. The percentage weight losses on ignition of small immature, large immature and adult A. rosea faeces were 14.17%, 15.25% and 15.40%, respectively. Analysis by Students' t test, as outlined by Bailey (1966), showed that the mean percentage weight loss of the 'bramble' soil was significantly lower than the values obtained for faeces from the three major size classes of A. rosea ($P = 0.001$ in each case).

Discussion

Hensen (1882) showed that the 'excrement burrow linings' of lumbricids lost 3.3 to 5.0% of their weight on ignition, whereas unworked soil lost only 2.3% of its weight on ignition. Salisbury (1924) found that lumbricid casts showed a higher ignition weight loss than soil in six cases out of eight. Lunt and Jacobson (1944), in a detailed study of lumbricid (probably A. caliginosa) casts in a forest soil, showed considerably higher percentage weight losses on ignition of cast material than those shown for various soil layers. Gawronski (1960) showed A. caliginosa casts to have a slightly lower ash content than the surrounding Polish soil.

In all the above studies, the results were difficult to interpret since it was not known how much of the weight loss on ignition was due to combustion of organic matter. Many authors have referred to the weight losses on ignition of soils or casts as equal to the organic contents of these materials. Of course, such assumptions are not justified, since at temperatures between 500°C and 800 to 900°C - temperatures often attained over short periods in certain types of ignition apparatus - various inorganic salts and minerals break down and are lost. Calcium carbonate is present in considerable quantities in some soils, so that its loss on ignition would be significant.

In the present study it was shown that neither 'bramble' soil nor A. rosea faeces contain calcium carbonate, or significant quantities of any other inorganic salt or mineral decomposable at temperatures below about 900°C (see Part III, Section I, sub-section 3). It can therefore be stated with certainty that the percentage weight losses on ignition of 'bramble' soil and A. rosea faeces, recorded in this study, represented the percentages of organic matter in these materials.

Since A. rosea faeces contained a higher percentage of organic matter than the soil from which the worms were feeding, these normal, healthy individuals - obviously showing a net gain in energy from the environment - must have been actively selecting for ingestion the more organic fraction of the soil. Semenova (1966) has shown that surface active, litter - and

humus - feeding lumbricids have bead-shaped intestines with a poorly developed typhlosole in the midgut. However, species (such as A. rosea) which occupy and ingest the mineral soil layers were shown to have a cylindrical mid- and hind-gut with a strongly developed and deeply cut typhlosole. A. rosea is thus adapted to the ingestion and rapid turnover of high quantities of soil medium. However, it can be stated that the carrying capacity of the A. rosea gut, as an adaptation for increased absolute assimilation rate, is supplemented by the selection of the more organic fraction of the soil during ingestion. It is not suggested that the A. rosea selection is more than remotely connected with the highly specialised food selection shown by L. terrestris (Satchell and Lowe, 1967).

(vi) The Diet of A. rosea - General Conclusions

It has been shown in the present study that A. rosea worms feed by grazing soil material from the burrow walls and extensions, selecting the more organic fractions of the medium. It has been shown that the animal prefers soil media of higher organic content and ingests materials of small particle size. Particles of plant debris were seen to be broken down during passage through the gut of A. rosea, and adult worms lost weight when deprived of a portion of the coarse fungal mycelium and/or dissolved and fine particulate organic material in the medium.

In view of the digestive ability shown by Tracey (1951) for A. rosea and the dependence of E. foetida on soil

protozoa as a nutrition source (Miles, 1963b), it was assumed that micro-organisms will be digested during passage through the A. rosea gut. It was therefore concluded that A. rosea probably has a mixed diet consisting of fine particulate herbage and/or root plant debris, coarse fungal mycelium, micro-organisms and, possibly, dissolved organic matter. Since living material was not observed during examinations of A. rosea gut contents, the ingested plant debris was thought to be predominantly dead material. Bacteria did not occur in high densities in the Wynyard soil, though they may be a more important constituent of the A. rosea diet in other soil types. Small immatures of A. rosea were shown in the present study to feed from soil in a manner similar to that shown by larger worms of this species. However, the small immatures live in the more organic surface layer of the field soil and may therefore have an increased selection efficiency for the more organic fraction of the medium as a whole.

Since the field diet of A. rosea was so intimately associated with mineral soils of variable organic content, it was not possible to estimate field ingestion rates directly. Annual field ingestion was therefore calculated as equal to the sum of annual respiration, production and egestion (see Part III, Section II).

3. Faecal Output

(i) General Introduction

Since Darwin (1881) estimated lumbricid surface cast production on two old grassland sites as 7.5 and 16.1 tons per acre per annum, the collection and weighing of surface casts has become commonplace amongst students of lumbricid ecology. Stöckli (1928) in Zürich and Dreidax (1931) in Breslau obtained figures corresponding to cast production of 30 to 40 tons per acre per annum, whilst Kollmannsperger (1934) measured only 2 to 3 tons per acre per annum in a low rainfall area of Germany. Evans and Guild (1947a), at Rothamsted, estimated values similar to those given by Darwin.

As Satchell (1958) has pointed out, surface casts in the field are only produced in quantity by three lumbricid species: Allolobophora longa Ude, Allolobophora nocturna Evans and Lumbricus terrestris L. L. terrestris produces only small surface casts (Gerard, 1960), so that the bulk of the surface cast material is produced by only two large, deeper-burrowing species which are most abundant in 'better' soils (Svendsen, 1955a; Gerard, 1964) and inactive or casting below ground for part of the year (Evans, 1948b). Evans (1948b) attempted to estimate the total faecal output of the lumbricid populations in eight fields at Rothamsted by assuming that, weight for weight, the non-casting species void the same quantity of faeces below the ground surface as the two main casting species eject above it. In view of

the widely differing feeding habits of the different lumbricid species, such a generalisation was thought by the present author to be unjustified.

Roy (1957) used data from collections of surface casts to compare annual cycles of lumbricid activity in two different ecological habitats.

There are two methods by which the faecal output of a soil-dwelling, soil-ingesting and sub-surface-defaecating lumbricid species - such as A. rosea (Sav.) - can be estimated. The mean weight of material in the worm gut can be measured, and faecal output estimated by investigation of the gut turnover time. The rate of faeces egestion can also be directly measured by collection of faecal material from the soil. Estimates were attempted by use of both these methods in the present study.

(ii) Weight of Gut Contents

Introduction

In addition to the possibility of estimating A. rosea faecal output by the gut turnover method, the mean dry weight of material carried in the gut of A. rosea was required for calculations of worm calorific content (see Part III, Section I). The relation between mean fresh gut content weight and total body fresh weight for O. cyaneum (Sav.) was required for calculation of meaningful weight-based respiratory rates for this species. The O. cyaneum study, whilst unconnected with faecal output estimates, was most appropriately included with these studies of A. rosea gut

content weight.

Methods

At times approximately half way through each of the 'seasons' delineated in Fig. 6, twenty to twenty-five individuals of each of the major A. rosea size classes were collected in the field and transported to the laboratory in soil from the collection site. Gut contents were immediately removed from each animal in the following manner: each worm was rinsed in dechlorinated tap water, briefly rolled on Whatman's No. 1 filter paper and fresh weighed in a stoppered bottle containing a small quantity of water. The worm was then placed on a clean glass plate and the gut contents removed by gripping the anterior end of the worm with the forefinger and thumb whilst gently, but firmly, stroking the body of the animal with a rounded glass rod. The stroking movements, towards the posterior end of the worm, were at first concentrated on the pre-anal region - being gradually extended to commence more anteriorly as gut contents were emptied from the more posterior regions. When the whole gut posterior to the gizzard had been emptied, the material on the glass plate was regarded as the total gut content, i.e. that the remaining material in the oesophagus and gizzard was negligible. The gut content material from each worm was placed in a separate, small, weighed glass tube using a surgical scalpel and a fine stream of distilled water. The gut contents were dried in a vacuum oven at 60°C, cooled in a desiccator and weighed.

The gut content weight proportion for O. cyaneum was measured only once - in the 'spring' of 1968. Thirty O. cyaneum individuals, representing the whole size range for the species, were collected from the field and transported to the laboratory. They were kept, in soil from the collection site, for a period of one week in a constant temperature room at 10°C. Each worm was then rinsed in dechlorinated tap water, rolled briefly on Whatman's No. 1 filter paper and fresh weighed. The gut contents were quickly removed by the method described for A. rosea above, and the worms re-weighed. The fresh weight of the gut contents of each worm was calculated by difference.

In an independent study in the course of this work, thirty O. cyaneum individuals were fresh- and dry-weighed to determine the relation between these quantities.

Results

Fig. 24 shows the relation between gut content dry weight and total body fresh weight for individual A. rosea worms in each season. No significant differences between the relations for different size classes were observed in any season - though there was a possibility of a shallower slope for small immature worms in 'autumn', 1967. For this season the data for small immatures and larger worms were initially calculated separately. The correlation coefficients obtained were 0.93 and 0.89 for small immatures and larger worms, respectively. The correlation coefficient of 0.95, obtained from the total data, thus accounted for the highest

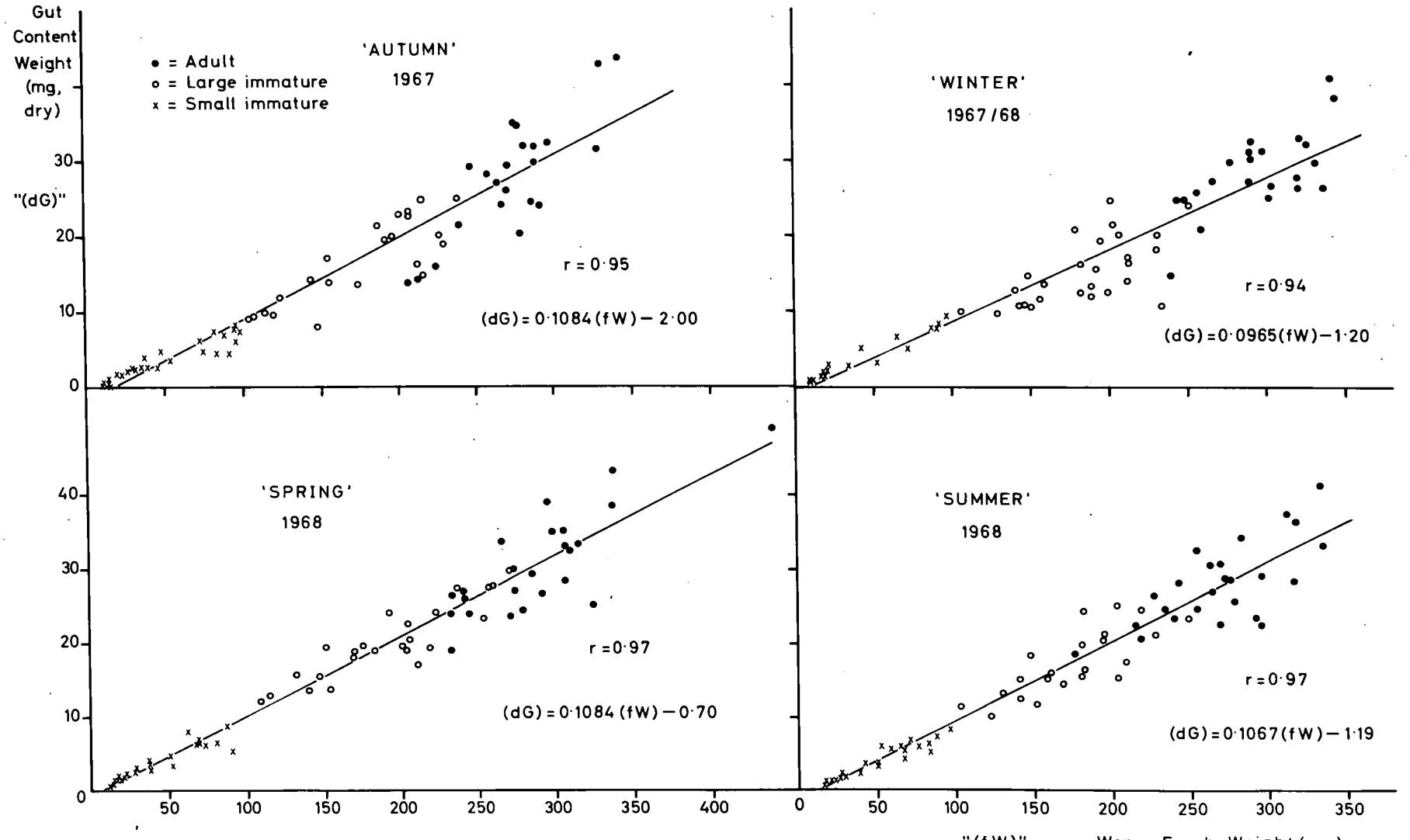


Fig. 24. The relation between A.rosea worm gut content weight and total body weight, measured in different seasons.

proportion of the variability in the results. The relation between gut content dry weight and worm total body fresh weight was found to be very similar, and highly significant, in all the 'seasons'. A. rosea gut content dry weight was found to be, on average, slightly less than 10% of the worm total body fresh weight.

Fig. 25 shows the relation between fresh gut content weight and total body fresh weight for individuals of O. cyaneum. A remarkably high degree of correlation was achieved and gut contents were seen to account for approximately 50% of the total body fresh weight of an O. cyaneum individual.

The total dry weight of an O. cyaneum individual was found to equal 0.309 times the total fresh body weight for worms of this species at Wynyard.

Discussion

Three methods have been used by previous authors to remove lumbricid gut contents. Many workers, including Avel (1929) and Barley (1959a), have allowed the worms to defaecate gut material by keeping them on damp filter paper - allowing the ingested filter paper to completely clear the gut. This procedure can take several days to complete and the loss in worm body tissue weight can be considerable - introducing errors into the calculation of gut content weight by difference. Doeksen et al (1962) used a variation of this method in which damp powdered cellulose was employed as a substrate. Bouché (1966) used an ingenious method

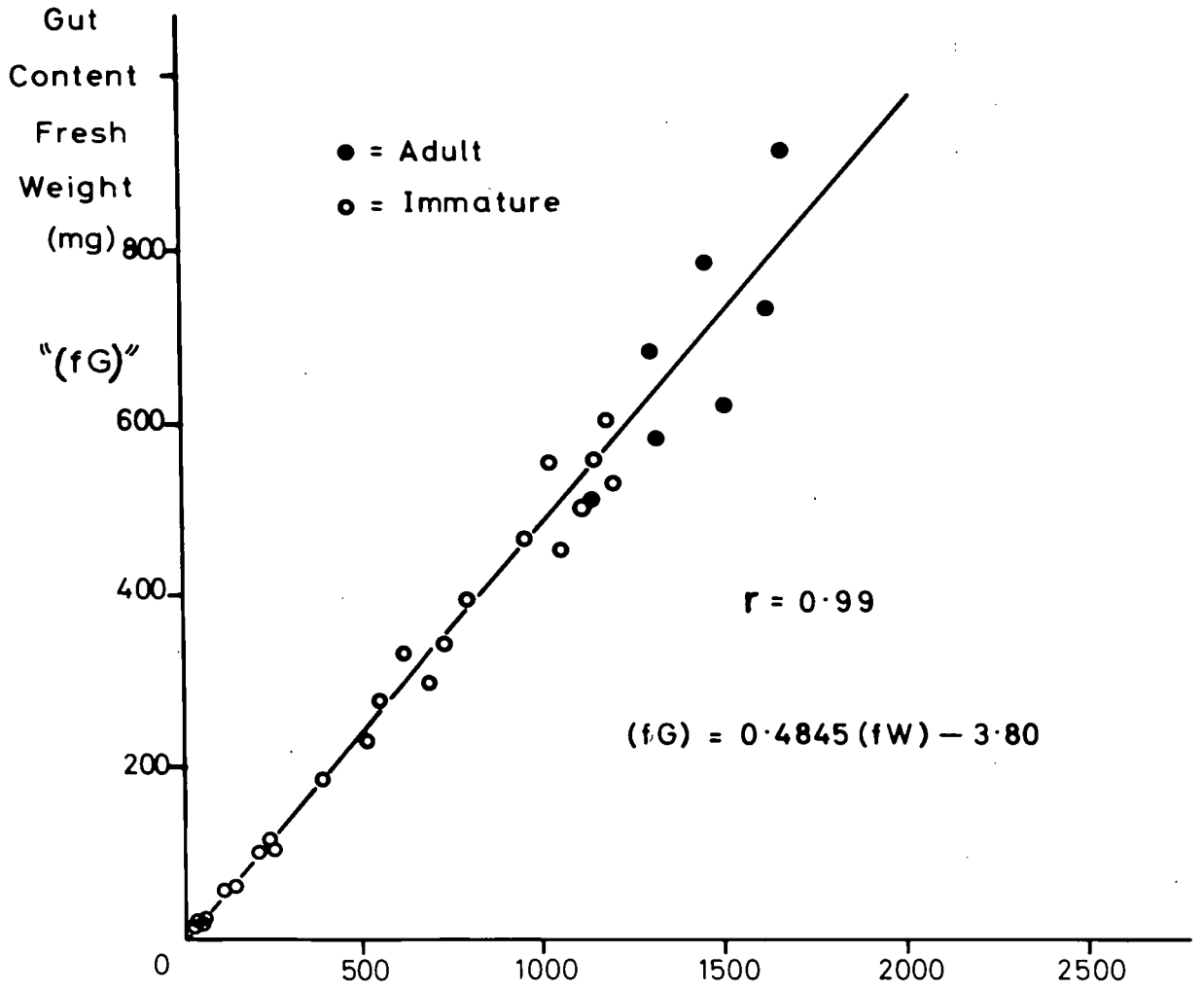


Fig. 25. The relation between gut content fresh weight and total body fresh weight for O.cyaneum individuals.

employing a fine catheter and syringe for the mechanical expulsion of lumbricid gut contents. However, as Bouché pointed out, this method is only suitable for use with large species such as L. terrestris and A. longa. Since this method requires much practice and a high degree of manual dexterity (Bouché, pers. comm.), it was not attempted for even the larger specimens in the short-term O. cyaneum study.

The method used in the present study was a refinement of that of Barley (1959a) and Barley and Jennings (1959) who manually 'expressed soil from the gut' of A. caliginosa. Whilst this method may appear crude, the results of the present study show that, if employed with care, it can be highly effective.

Barley and Jennings (1959) found that the dry gut content weight of A. caliginosa worms in Australian pasture was approximately 16% of worm fresh weight. Byzova (1965) suggested that the fresh weight of gut contents represented approximately 10% of total body fresh weight in both A. rosea and A. caliginosa. As long ago as 1877, Hensen pointed out that body weights of lumbricids from different soils may be difficult to compare, due to variation in gut content weight. Byzova's estimates were based on data from worms collected in Moscow garden soils of high organic content (Byzova, pers. comm.) and comparison of these with data from worms in Australian and Wynyard soils is obviously impossible to within an order of magnitude.

The gut contents of O. cyaneum were easily removed and the relation between gut content and total body weight was near perfect. These facts suggest that O. cyaneum may be highly adapted to the rapid passage of mineral soil through a gut with few restrictions or convolutions, with the exception of the typhlosole. The constant in the equation shown in Fig. 25 was negligible, compared with the range of body weight in this species. Body tissue fresh weight was therefore taken as 0.5155 times the total fresh weight of O. cyaneum individuals in conversions associated with measurements of respiratory metabolism. In view of the A. rosea results, and the fact that O. cyaneum measurements were made at 10°C (i.e. near the annual mean soil temperature at Wynyard), the measured O.cyaneum gut content proportion was taken as applicable in all seasons.

The mean dry weight/fresh weight proportion of 0.309, calculated for O. cyaneum individuals at Wynyard, was similar to Bouché's estimate of 0.293 (Bouché, 1967) for the same species.

(iii) Faeces Collection and Population Egestion

Introduction

Barley (1959b) studied a permanent pasture near Adelaide, where A. rosea (Sav.) and A. caliginosa (Sav.) were the dominant lumbricid species. He found that surface casts did occur, but he though they constituted less than 15% of the total faeces produced. The only quantitative estimates of faeces production by purely soil-ingesting lumbricids have

been those by Barley (1959a) and Barley and Jennings (1959). A. caliginosa was studied in both cases. These workers kept worms in pots containing moist field soil; they collected faeces, after a known period, by breaking the medium into fragments of decreasing size - extracting the egesta by use of a fine spatula. Efforts to employ this technique in the collection of A. rosea faeces from Wynyard soil were unsuccessful in the present study. The dark, but fine-textured, A. rosea faeces could be distinguished from the dark 'bramble' soil only with difficulty and on close examination. Fragmentation of the soil rendered the 'back-filled' burrow systems of A. rosea inaccessible for quantitative collection of faeces. A method was therefore devised whereby the burrow system was displayed for faeces collection.

Methods

The two-dimensional petri-dish cage structure was described above (see sub-section 1). The numbers, sizes and environmental conditions of animals used for faeces collection experiments were identical to those given for worms used in studies of burrow formation (see p.159). The faecal material egested by each worm in each cage during the first three days after confinement was carefully removed, using a fine spatula, and discarded. After a further six days the faecal material in each cage was carefully removed, placed in a weighed, labelled tube and dried in a vacuum oven at 60°C. In all faeces collections, the egested material

was located with the aid of the wax pencil tracings of the cage burrow systems (see p.160). After drying, the faeces deposited from the fourth to the ninth day, inclusive, was cooled in a desiccator and weighed by difference. The rate of faeces production, over the experimental period, was calculated for each individual worm of known size. The constant temperature room thermograph traces were examined to determine the mean ambient temperature, and the degree of variation, over each experimental period.

Results

Fig. 26 shows the relation between the logarithms of the rate of faeces production, per fresh gram of lumbricid worm per day, and worm individual fresh weight at three temperatures. The temperature variation encountered, due to thermostat lag, was plus or minus 0.6°C at the mean temperatues 4.4°C and 14.8°C, and plus or minus 0.5°C at 10.0°C. The log. rate of faeces production per gram worm was found to decrease rapidly with increasing worm size (log. fresh weight) at 14.8°C, considerably less so at 10.0°C, and hardly - if at all - at 4.4°C. This changing slope of the relation was superimposed on a general decrease in faeces production rate with decreasing ambient temperature. The slopes of the trend lines at 10.0°C and 14.8°C were significant at the 99% and the 99.9% levels of confidence, respectively. The 4.4°C slope was not significantly different from the horizontal (broken line in Fig. 26). Assuming that the slope changes represented a continuous series, the 4.4°C slope was thought

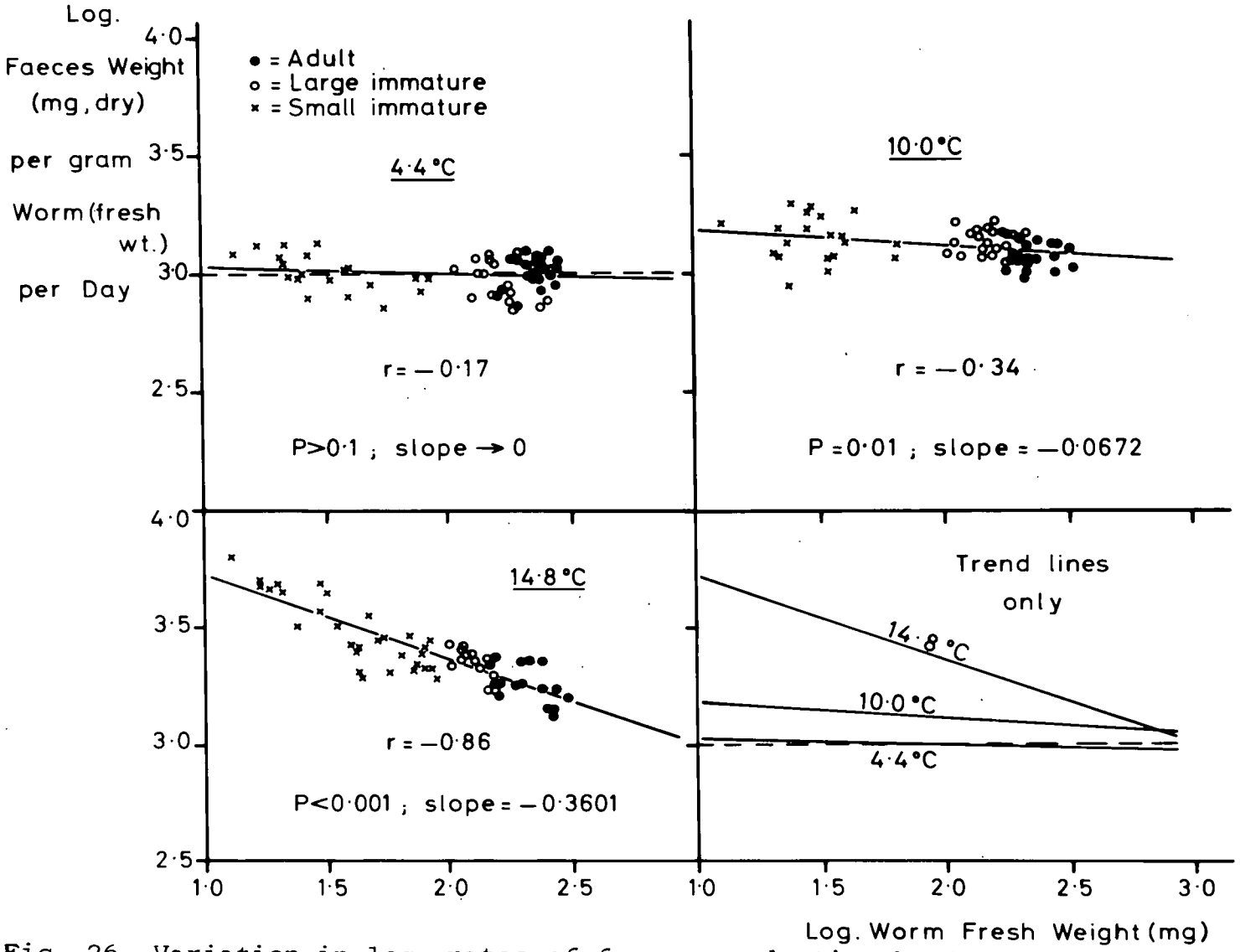


Fig. 26. Variation in log. rates of faeces production by A.rosea individuals according to worm size, measured at three temperatures.

to have a real value near to zero - though it would take a much greater number of measures to prove it. From the relation between the 10.0°C and 14.8°C slope values, and the approximate value - near zero - of the 4.4°C slope, the slope of the log. faeces production rate/worm size relation was assumed to conform to an exponential rate of increase with ambient temperature. The 4.4°C slope was thus estimated as -0.0095.

Since the logarithm of the rate of faeces production per worm unit weight varied independently with worm size and with ambient temperature, the lines relating log. faeces production rate and ambient temperature for worms of different sizes were neither parallel nor even straight in almost all cases. Difficulty was therefore encountered in attempting to relate the laboratory results to the field population. This was finally achieved as follows:

Knowing the slopes of the log. faeces production rate/worm size relation at the three experimental temperatures, the single worm fresh weight was calculated, at which the line relating log. faeces production rate to ambient temperature conformed to a positive straight line trend. This fresh weight was found to be 394.1 mg. - approximately equal to the maximum individual weight for this species at Wynyard. Knowing the mean field soil temperature and the mean worm individual weight (for each size class) for a particular period, the size class population egestion was estimated by determining the log. faeces production rate for a worm

weighing 394.1 mg. at the field soil temperature. The exponential relation between log. faeces production rate/ worm size slope and ambient temperature was used to estimate the relevant slope at the field temperature. From this slope, and the known factors of log. faeces production rate and log. worm fresh weight for an individual weighing 394.1 mg. the faeces production rate at the mean individual worm weight in the field could be calculated. Multiplication by a factor equal to the field biomass then yielded an estimate of total population egestion.

Total annual figures for A. rosea population egestion per metre square, estimated by the method described above, are given in Table 15. Egestion was calculated in terms of both grams dry weight and Kcalories - from the organic calorific content of faecal material (see Part III, Section I). It was estimated that 2.148 Kgrams dry weight of soil material were passed through the guts of the A. rosea worm population each year. The dry-weighing of cut and measured soil cubes showed that the A. rosea annual turnover represented approximately 2% of the total dry weight of a square metre of 'bramble' soil to a depth of 10 cm.

Discussion

The increased slope of the log. faeces production rate/ worm size relation at higher temperatures suggested that gut turnover rate was influenced more by ambient temperature in smaller worms (c.f. contrast between adult and immature Q_{10} values for respiratory metabolism in this species: Fig 27

Size class	Faeces Grams Dry Weight /m^2	Faeces Kcalories /m^2
Small Immatures	406	344 .022
Large Immatures	1003	919 .590
Adults	739	601 .696
Total A. rosea Population	2148	1865 .308

Table 15. Estimates of total annual faeces production by the A. rosea population at Wynyard.

and p.242).

The estimates of population egestion were considerably higher than expected from the general activity of the animal. Also, the calculated gut turnover rates from these data (see p.214) were extremely high, compared with previous estimates for a similar species. These estimates of population egestion were made under feeding conditions - i.e. after the initial 2 to 3 days of high burrowing activity, though it was acknowledged that the cage structures did not perfectly simulate field soil conditions. The soil layers within the chambers were compressed during construction of the cages, so that few soil spaces existed. The feeding levels of burrow formation during these experiments may therefore have been somewhat higher than those in the field. However, this factor was not thought by the author to constitute the major source of error. The major difficulty in this study was the accurate delimitation of faecal material - deposited in soil crevices, lining wider burrows and filling disused burrows - from the surrounding soil. Despite the care taken, it was thought that a proportionate amount of unworked soil was recovered during A. rosea faeces collection. It was difficult to estimate even the approximate extent of these errors, but in the author's opinion they could not have involved more than a doubling of the faeces production estimate.

It was therefore concluded that the A. rosea population at Wynyard was capable of egesting 'bramble' soil faeces in

the ranges of 1.08 to 2.15 Kgrams, dry weight, and 933 to 1865 Kcalories per metre square per annum. The possible effects of different soil types on the mean population rates of egestion at Wynyard were unknown - but were thought to be only slight due to the marked preference of A. rosea for 'bramble' soil (see p.77). The only comparable estimates of faeces production are those in the approximate range of 200 to 420 mg. dry weight, per fresh gram worm per day, for A. caliginosa (Barley, 1959a, 1961; Barley and Jennings, 1959). These A. caliginosa measures were made at 15°C, so that - allowing for the possible maximal doubling factor outlined above - the A. rosea output was approximately five to ten times that of A. caliginosa. Clearly, A. rosea is highly adapted to the rapid turnover of large quantities of soil material.

(iv) Rate of Gut Clearance

Introduction

The rate of gut clearance by lumbricids is a highly variable quantity. If individuals of soil-ingesting species are separated from a particulate solid medium, material is retained in the gut for periods of several days after separation - sometimes even until death by starvation. Pigmented species feeding on a more organic diet do not show this behaviour to the same extent. Burrowing rates of gut clearance are known to be greater than feeding rates of clearance (Parle, 1963b). A. rosea has been shown in the present study (see p. 192) to select food material, and this must provide

an additional degree of variability in the mean rate of gut clearance.

In spite of these difficulties, attempts were made to determine the gut turnover rate of A. rosea individuals to obtain an independent measure of faecal output - knowing the mean weight of worm gut contents. The rate of gut turnover was finally estimated from the known gut content weight and data on field population egestion derived from faeces collection experiments.

Methods

Twenty large immature A. rosea worms were fresh-weighed and placed in petri-dish cages - as used for the faeces collection experiments described above. After three days in the 'bramble' soil layer medium, the worms were temporarily removed from their burrow systems by carefully removing the inner petri-dish plate. Each burrow system was dusted with carmine powder (Michrome No. 73, Gurrs); the powder was also sprinkled on the unworked soil surface. The worms and plates were replaced, and each chamber was examined at 30 min. intervals for signs of red-stained faecal material.

In the final estimation of field gut turnover rate, the estimated field dry faeces production rate per fresh gram worm was converted to the rate per individual for each size class, knowing the mean individual fresh weights in the field population throughout the year. The mean dry weights of individual worm gut contents were also calculated from the mean individual fresh weights in the field. Faecal output

divided by gut content weight for the 'average' size class population individual thus gave a measure of the mean rate of gut clearance.

Results

Of the twenty worms confined in their cage burrow systems after carmine sprinkling, fourteen produced visible quantities of red-stained faeces after periods varying from 4h. to 16h. Of the remaining six individuals, three produced fresh burrow systems in carmine-free areas and three showed signs of stress (feeding ceased; gut partially emptied; body flaccid). On removal of the inner petri-dish plates after 20h., detailed examination revealed traces of red-stained faeces in the three chambers containing worms which had resumed extensive burrowing away from the carmine powder. Red-stained faeces already recorded were found to be much more extensive than could be seen by simple observation with the chambers and excess carmine powder intact.

Using field soil temperature data and mean population individual fresh weights obtained during 1966 and 1967 at Wynyard, the gut clearance rates (times per day) and times (hours) were estimated monthly for each of the three major A. rosea size classes. The estimated ranges and mean monthly values are shown in Table 16. The mean gut clearance rates and times for large immatures and adults were not significantly different but the small immature values were significantly different from those for larger worms (P less than 0.002, greater than 0.001). The gut clearance time for A. rosea

Size Class	Gut Clearance Rate (times per day)			Equivalent Gut Clearance Times (hours)		
	Maximum (Month)	Minimum (Month)	Mean (± St.Dev.)	Minimum	Maximum	Mean (± St.Dev.)
Small Immatures	28.33 (July)	13.08 (April)	19.09 (± 5.30)	0.85	1.83	1.35 (± 0.36)
Large Immatures	16.90 (July)	11.11 (April)	12.96 (± 1.92)	1.42	2.16	1.89 (± 0.25)
Adults	15.80 (July)	10.95 (April)	12.49 (± 1.57)	1.52	2.19	1.95 (± 0.22)

Table 16. Mean estimates, and ranges encountered, for A. rosea individual gut clearance rates and times at Wynyard (1966/67).

worms appeared to be generally in the range 1 to 2 h.

Discussion

Parle (1963b) used P^{32} in H_3PO_4 to mark the food - pellets of a dung and peat soil mixture - of L. terrestris L. By use of a Geiger microprobe he then recorded the passage of material through the worm gut by examination of separate individuals, removed at intervals from the medium. He found the gut clearance time to be approximately 20 h. Van der Drift (1959) estimated the similar value for L. rubellus Hoffmeister to be in the range 16 to 48 h. From information on gut content weight and the rate of faeces production, the gut clearance time for A. caliginosa during feeding has been estimated as in the range 10 to 20 h. (Barley, 1959a; Barley and Jennings, 1959) - though, in the author's opinion, the block collection methods employed by these workers may have involved the loss of faeces used to backfill disused burrow systems. The turnover times obtained during the preliminary experiments using carmine stain were of a more similar order to those given by previous workers for other lumbricid species. However, these measures were thought completely unreliable since the red-stained faeces was not readily visible in the presence of moist carmine powder, and the worms showed obvious signs of irritation by the carmine particles. Various organic dyes have been used to mark animal foods and faeces (New, 1959; Kindel, 1960; Brown, 1961) but, as with the carmine stain, problems arise regarding the uniform introduction of dyes into lumbricid soil food medium after the cessation of

burrowing activity, and without then disturbing the formed burrow system. Any major disturbance at this stage would involve the recommencement of extensive burrowing activity. The use of a fluorescent dye such as rhodamine-B in aqueous solution - as outlined by Gast, 1963 - may be possible, since the dye could be sprayed on to the soil layer. Such a dye treatment would not irritate the worms, but there would be problems regarding the degree of dye penetration. Also - though Gast did not point this out - the expensive ultra-violet emitter, used to detect fluorescence, must provide energy of a precise wavelength dependent upon the dye-type employed.

Arthur, 1965, used a medium consisting of a mixture of equal quantities of barium sulphate and soil to X-ray the gut contents of L. terrestris. He subsequently found that soil alone was sufficiently opaque for this purpose (Arthur, pers. comm.). It is possible that some translucent medium, with subsequent introduction of an opaque dye in aqueous solution, could be used to advantage in this type of study - where the necessary X-ray equipment was available. Isotopes undoubtedly offer the most likely source of accurate measurement. Parle's method would be extremely difficult with worms of a small lumbricid species, but small samples of prospective faeces could be easily removed from successive samples of worms and examined under a standard Gieger probe of small size. The main difficulty, as with dye materials, is in uniformly permeating and labelling the soil food medium, whilst leaving

the soil layer or block intact and worm burrow systems undisturbed.

Even if a maximum error - the additional collection of soil equal in weight to the faecal material - was sustained during the faeces collection experiments, the average time for A. rosea gut clearance would only be 2 to 4 h. These estimates are markedly higher than those previously found for other lumbricid species. Large pigmented species, such as those studied by Van der Drift (1959) and Parle (1963b), are thought to accumulate faecal material in the lower intestine, defaecating only once or twice daily. This is probably made possible by the high organic content and low overall weight and volume of the food material of these worms. For species ingesting soil material of low organic content and high overall weight and volume, the situation is more complex. Despite a degree of organic selection, the postulate of Lindquist (1941) - that the amount of soil ingested will be inversely proportional to its organic content - must be generally true. Lumbricids such as A. rosea, ingesting mineral soil of low nutritive value (see p. 193), must therefore pass a bulky medium through the digestive tract at a high rate, if sufficient food is to be assimilated. Daily, or near daily, defaecations have often been assumed for soil-ingesting topsoil species, simply by analogy with species such as L. terrestris. This is obviously not possible if the above arguments are correct, and in the present study the hypothesis was confirmed for A. rosea by cage observations.

Worms of this species defaecated at frequent intervals, depositing small units of faeces either in one location or along the walls of the wider burrows. Each faeces unit was similar to an inverted egg-cup in shape, since the tapering terminal segments of the worm were pushed into the faecal 'pellet' during egestion. Each unit was considerably less than the full gut content of the animal - approximately four or five units probably comprised a complete gut clearance. Accurate delineation and counting of faecal units was difficult since the material was semi-fluid and the units tended to merge when accumulated. Using the 'dimple' marks as unit indicators, forty to fifty faecal units were counted in the faeces produced by one large immature worm in one day, under cage conditions.

A gut clearance time of 1 to 4 h., depending on worm size and environmental conditions, was thought to be of the correct order for A. rosea worms feeding on 'bramble' soil at Wynyard. Such rapid gut clearance rates were thought by the author to be characteristic of worms with a similar mode of life to A. rosea. The values obtained by Barley (1959a) and Barley and Jennings (1959) for A. caliginosa may indicate a more organic diet in this species, which is more common in 'better' soils. However, the estimates of gut clearance time made by these authors may be an overestimate due to the use of a faeces collection method less efficient than that described in the present studies.

4. Assimilation of Soil Organic Matter by Allolobophora rosea (Sav.) f. typica

Lindquist (1941), Franz and Leitenberger (1948) and Guild (1955) measured the rate of weight loss of litter and dung samples in the presence of lumbricids. There were difficulties regarding the material combusted during the respiration of micro-organisms, but it was thought possible that an approximate measure of lumbricid assimilation could be obtained by 'before and after' measurements of soil organic content. A prerequisite for the success of this study was the near-absolute homogenisation of a 'bramble' mull-type soil.

A soil sample of selected uniformity was fragmented by hand; large soil components of 'pure' composition (stones, twigs, large roots, mosses, clay lumps, lumbricid cocoons and large invertebrates) were removed. The soil was passed through a 6 mm. mesh gauze, thoroughly mixed and again sorted to remove the above constituents. By this technique, three soil samples were homogenised so that their percentage contents of organic matter were 10.53 ± 0.14% (St. Dev.), 10.31 ± 0.33% and 10.39 ± 0.22%. Even with this degree of uniformity - thought maximal for this soil type - it was not found possible to obtain significant changes in soil composition due to the activity of large immature A. rosea worms at any of the ambient temperatures 5°C, 10°C, or 15°C. These tests were performed with a minimal quantity of soil medium, thought sufficient to provide unworked soil for one individual for

approximately two to three weeks. With the remaining variation in soil organic content and the observed slow rate of assimilation of organic material, it was subsequently estimated that it would take a period of some months before a reliable estimate of assimilation could be achieved with even small amounts of medium. Micro-organism combustion of organic materials would influence the results over such a time span and the worms would be ingesting their own faeces - or more probably assuming dormancy - after the first few weeks. Such experiments were therefore regarded as unfeasible for this species.

Englemann (1961), Crossley (1966) and various other workers have employed radioisotopes in the estimation of food intake and assimilation rates for invertebrate animals. Golley (1968) and Odum (1961, 1968) regard techniques involving the monitoring of isotope losses as valuable tools for the elucidation of the energy relationships of invertebrate species. These methods depend on the uniform labelling of food materials and consumer body tissues to a constant equilibrium level. With the present knowledge of A. rosea nutritive sources, it was not thought possible to achieve sufficient labelling efficiency - though the use of these techniques in studies of lumbricid energetics may be possible in the future.

Teal (1957) estimated field assimilation rates for the aquatic oligochaete Limnodrilus sp. by summating field estimates of respiratory metabolism, population mortality and the change in population standing crop. A similar

technique was adopted in the present work: A. rosea field assimilation was estimated by summating respiratory metabolism and secondary production (tissue growth and cocoon production)- see p. 311.

IV Studies in the Respiratory Metabolism of Lumbricidae found in the Study Area at Wynyard

1. Comparative Studies using the Warburg Apparatus

(i) Respiratory Quotient

General Introduction

Konopacki (1907) studied the respiratory metabolism of L. terrestris L. at ambient temperatures ranging from 2.5°C to 24°C. He measured R.Q. values for this species ranging from 0.6 to 1.0, the majority being in the range 0.7 to 0.8. Apart from this work there is, to the author's knowledge, no information on the relation between oxygen and carbon dioxide gaseous exchange in lumbricids. In the present studies attempts were made to elucidate the pattern of gaseous exchange by each of the species A. rosea (Sav.), O. cyaneum (Sav.), L. castaneus (Sav.) and D. rubida (Sav.) f. subrubicunda (Eisen). The main work was concerned with determining the CO_2/O_2 ratio of gaseous exchange for animals taken from the field at various times of the year. These measurements were necessary to allow the conversion of CO_2 output rates, determined by continuous respirometry (see below), to their equivalent O_2 consumption rates. Rates of O_2 uptake were considered more suitable for comparison with previous measures of lumbricid respiration, and more reliable for use in the calculations of respiratory heat loss which were performed for A. rosea.

In addition to these measurements of seasonal R.Q.'s, laboratory investigations of 'abnormal' R.Q. values (below

0.7), and the effects of starvation and variation in ambient temperature on the R.Q. of A. rosea, were performed. The latter investigations, under laboratory conditions, will be considered separately.

(a) Seasonal Measurements of Respiratory Quotient

Methods

At times approximately mid-way through the arbitrary 'seasons' shown in Fig. 6, worms were collected from sites adjacent to the grid area at Wynyard. The worms of each species were placed in jars containing soil from the species collection site and transported to the laboratory. The jars were covered with perforated polythene sheet to prevent escape and stored in a constant temperature room at 10°C. After two days the worms were removed from the soil media, rinsed in dechlorinated tap water, rolled briefly on Whatman's No. 1 filter paper and fresh-weighed - either individually or in small groups as the occasion demanded. A. rosea adults and large immatures were measured in groups of two or three, and small immatures in groups of five or six, to a Warburg flask; O. cyaneum adults and large immatures were measured singly and small immatures in groups of two or three; L. castaneus and D. rubida f. subrubicunda (adults and immatures separate) were measured in groups of three or four worms to a flask. The worms were placed in 20 ml. Warburg flasks containing 1 ml. of phosphate buffer solution at pH 6.5. The net pressure effects of gaseous exchange were then measured over one hour at 10°C. All Warburg studies were performed in total darkness

at a shaking speed of approximately 25 complete strokes per minute - except during the short periods of actual measurement. After the first hour of measurement, the flasks were removed from the manometers and 0.2 ml. of 15% KOH solution, plus paper 'fan', was placed in each centre well. The flasks were replaced on the manometers and pressure changes monitored during a further hour of measurement. The procedure was standard for all lumbricid species, though for L. castaneus it was necessary to insert a very small piece of cotton wool in the neck of each manometer arm to prevent the worms from entering the manometer capillaries.

Results

Table 17 shows the mean R.Q. values determined for four lumbricid species, and their size class components, at different times of the year. The numbers of worms used in these determinations are indicated in parentheses. The years of measurement are also shown. The R.Q. values measured for A. rosea were almost exclusively in the 'normal' range (0.7 to 1.0), and fairly consistent. In spring, autumn and winter A. rosea R.Q. values were mainly in the range 0.8 to 0.9. However, in the summer of 1966 values of about 0.7 were measured for all size classes. A repeat of the summer measures in 1967 yielded very similar results to those of 1966. O. cyaneum - the other unpigmented species - showed highly variable, and usually 'abnormal', R.Q. values. Individual measurements ranged from near zero to 0.8 or 0.9. The lowest R.Q. values were recorded for adults and large immatures of

Season	Year	A. rosea				O. cyaneum				L. castaneus			D. rubida f. subrubicunda		
		A	LI	sI	All	A	LI	sI	All	A	I	All	A	I	All
SPRING (April/May)	1967	0.81 (24)	0.81 (22)	0.92 (24)	0.85 (70)	-	-	-	-	-	-	-	0.55 (17)	0.74 (32)	0.65 (49)
	1968	-	-	-	-	0.47 (2)	0.72 (2)	0.66 (10)	0.62 (14)	0.52 (16)	0.62 (11)	0.57 (27)	-	-	-
SUMMER (Aug/Sept)	1966	0.70 (24)	0.74 (24)	0.66 (24)	0.72 (141)	-	-	-	-	-	-	-	-	-	-
	1967	0.72 (24)	0.76 (21)	0.75 (24)		0.40 (7)	0.62 (12)	0.56 (17)	0.53 (36)	0.53 (15)	0.77 (12)	0.65 (27)	0.66 (16)	0.76 (12)	0.71 (28)
AUTUMN (Oct/Nov)	1967	0.74 (24)	0.86 (24)	0.85 (24)	0.82 (72)	0.58 (5)	0.60 (5)	0.59 (12)	0.59 (22)	0.58 (16)	0.82 (16)	0.70 (32)	0.68 (20)	0.93 (12)	0.81 (32)
WINTER (Jan/Feb)	1967	0.89 (24)	0.85 (24)	0.88 (23)	0.87 (71)	-	-	-	-	-	-	-	0.99 (16)	0.95 (16)	0.97 (32)
	1968	-	-	-	-	0.29 (4)	0.14 (2)	0.85 (3)	0.43 (9)	0.70 (11)	0.67 (15)	0.68 (26)	-	-	-

Table 17. Seasonal values for the Respiratory Quotient of four lumbricid species. (A - Adults; I - Immatures; LI - Large immatures; sI - Small immatures; All - Species means; Numbers of worms used in measurements shown in parentheses).

this species. Adults of L. castaneus also showed abnormally low R.Q. values, variability occurring in both adults and immatures. The more normal values for L. castaneus were in the range 0.7 to 0.8. R.Q. values measured for D. rubida f. subrubicunda were consistently near 1.0 in the winter of 1967; autumn values were somewhat lower, and in spring and summer abnormally low values were measured - particularly for adult worms.

Discussion

The results for A. rosea, showing little variability and no signs of abnormality, were considered reliable and suitable for use in conversions of CO_2 output data to rates of oxygen consumption. The A. rosea R.Q. values for most of the year were indicative of a mixed diet, or one containing a high protein proportion - though the former was more likely (see p. 193). In the other three species, a disturbing factor, or factors, was thought to have influenced the measured R.Q. values so that these deviated from the normally accepted range (0.7 to 1.0). These factors were thought minimal for D. rubida f. subrubicunda during the winter months, when the gaseous exchange ration was near the value for pure carbohydrate metabolism. The phenomenon of abnormally low R.Q. values was subjected to the detailed investigations described below.

(b) Abnormally Low R.Q. values

Introduction

Attempts were made to determine the factor(s) causing the observed R.Q.'s of abnormally low value in some lumbricid

species. Laboratory investigations under controlled conditions were performed with worms of the species D. rubida f. subrubicunda and O. cyaneum.

Methods

Two series of experiments were performed to test the effects of medium water content on D. rubida f. subrubicunda R.Q. In the first series, twelve adults and sixteen immatures were placed in 4 cm. diameter glass tubes containing a mixture of 'bramble' topsoil, semi-decayed plant debris and glass beads (1 mm. diameter). The artificial medium was wetted with phosphate buffer, pH 6.5. Adult worms were grouped three to a tube and immatures were grouped four to a tube. After one week at 10°C R.Q. values for the worms from each tube were measured according to the Warburg technique used in the seasonal measurements (see above). During the R.Q. measurement period, the tube media were emptied into a large dish, thoroughly mixed and sub-sampled for estimates of water content. Each sub-sample was weighed immediately, dried in a vacuum oven at 60°C and re-weighed. Meanwhile, the bulk of the medium was partially dried, re-mixed and distributed between the glass tubes. The worm groups were replaced in these tube media, suitably labelled, after R.Q. measurements. The tubes were again kept at 10°C - the temperature used throughout the series. After three days the R.Q. values of the worms were again measured; the water content of the medium was determined as before and the medium was further dried before re-introducing the same worm groups. After a

further three days, worm R.Q. values were measured, medium water content was determined and the medium was thoroughly wetted with dechlorinated tap water. The worms were kept in the wet medium for a final three day period, after which the R.Q. values of the worms and the moisture content of the medium were determined. The procedure during the second series of experiments was similar to that in the first series except that fresh medium was prepared, and a new set of twenty adult worms (grouped in sets of four) was used for R.Q. measurements. Two sets of measurements were made: the first at a low medium water content and the second at a high moisture level.

For investigations of O. cyaneum R.Q. values, worms were kept in natural clay media taken from the collection sites. Twelve worms, including three adults, three large immatures and six small immatures, were taken from the Wynyard site and a similar set of twelve worms was taken from a wet glassland site near to the Durham laboratories. The R.Q. values of each set (adults and large immatures singly, small immatures in pairs) were determined under conditions of low and high soil medium water content, at each of the laboratory temperatures 10°C and 5°C, and in Warburg flask buffer solutions at pH 6.5 and 7.0.

Results

D. rubida f. subrubicunda R.Q. values at various levels of medium water content are shown in Table 18. Considerable R.Q. variation occurred at most moisture levels, though the

Series	Expt.	Medium % Water Content (by weight)	Mean R.Q.	Minimum R.Q. Value	Maximum R.Q. Value
1	1	7.30%	0.76	0.55	0.97
	2	5.27%	0.58	0.39	0.80
	3	2.45%	0.52	0.42	0.58
	4	10.25%	0.72	0.63	0.85
2	1	5.51%	0.56	0.41	0.66
	2	10.18%	0.76	0.68	0.87

Table 18. Recorded R.Q. values for D. rubida f. subrubicunda worms, kept at 10°C in laboratory media of different water contents.

values recorded at the highest and the lowest levels of medium water content were more consistent. At medium moisture levels higher than about 7% water, by weight, the R.Q. values were usually in the normally acceptable range of 0.7 to 1.0. At medium moisture levels of about 5% water, and below, recorded R.Q. values were almost invariably abnormally low, i.e. below 0.7. These findings were seen to apply to situations of both decreasing and increasing medium water content.

O. cyaneum R.Q. values for different individuals ranged, apparently at random, from near zero to about 0.8. This situation invariably occurred, regardless of the soil type from which the worms were collected, the water content of the laboratory medium, laboratory acclimatisation temperature or Warburg flask buffer solution pH. During some of the Warburg measures of O. cyaneum R.Q. values 'air' bubbles, within mucus films, were seen to form around the worms and in the buffer solutions. However, these were not consistently associated with low R.Q. values.

Discussion

Apart from the remote possibility of the utilisation by certain lumbricids of highly eccentric metabolic pathways, the abnormally low R.Q. values recorded for O. cyaneum, D. rubida f. subrubicunda and L. castaneus could only be due to either experimental error or high activity of the calciferous glands.

The calciferous glands occur along the oesophageal tract

of most lumbricid species, though their size varies according to the species habitat, e.g. the glands are especially large in species occurring in highly calcareous soils. Laverack (1963), in his excellent review, quotes evidence by Dotterweich (1933), Voigt (1933), Robertson (1936) and Clark (1957) before concluding that the glands are effective in helping to maintain the salt and water balance of the body fluid by removing excess calcium ions. He also acknowledges that the glands may have a role in the complex pH buffering systems of lumbricid body fluids. Carbon dioxide - as bicarbonate ions - is linked with calcium and removed in the solid form of calcium carbonate which is voided with the faecal material. Wieler (1914) thought that lumbricids were restricted to calcareous soils to ensure replacement of calcium ions lost via the calciferous glands; Russell (1950) thought that a calcium supply was necessary to 'satisfy' the calciferous gland requirements. Neither of these views - though often quoted - seems worthy of serious consideration. However, calcite crystals are voided by lumbricid calciferous glands when excess calcium occurs in the ingested soil or plant materials (Robertson, 1936; Puh, 1941; Ponomareva, 1948; Baltzer, 1955) and it seems likely that the glands enable lumbricids to occupy nutritious calcareous soils without suffering the effects of excess calcium levels in the body fluid. In soils deficient or, as at Wynyard (see Part III, Section I), almost or completely lacking in calcium the calciferous glands of lumbricids present could not be performing this function.

Stephenson (1930) pointed out that calciferous glands were relatively larger in species living in dry habitats, and in species of large body size - regardless of habitat. He postulated that carbon dioxide diffusion would be restricted under dry conditions. This could lead to high acidity in the lumbricid body mucus layer and/or increased levels of CO_2 in the burrow atmosphere. Stephenson noted that CO_2 diffusion was also restricted in larger worms, due to the lower body surface area to volume ratio. M'Dowall (1926) first postulated CO_2 excretion as an important function of lumbricid calciferous glands and his theories have recently been supported by Crang et al (1968) who have shown the glandular epithelial mitochondria to be intimately involved in lumbricid calcite production.

The experiments here described were only of a preliminary nature, but the results have suggested that the occurrence of low R.Q. values in D. rubida f. subrubicunda may be associated with low moisture levels in the environment. Stephenson's postulates, regarding inter-species variation in calciferous gland activity according to the dryness of the habitat, may be relevant intra-specifically for D. rubida f. subrubicunda in seasons of varying habitat moisture levels. This surface active, pigmented species may have experienced dry conditions during the summer months, even at Wynyard. Worms taken from the field during the warmest and driest months were shown in the present studies to have the lowest R.Q. values. L. castaneus was not studied under experimental laboratory

conditions, but the seasonal R.Q. measures for this species were lowest in the spring and summer. It may be that in this species too the dryness of the soil surface environment - with the consequential reduction in CO_2 diffusion efficiency - necessitates the removal of metabolic CO_2 in solid form, via the calciferous glands. It may be argued that these surface active species are in open contact with the atmosphere, which contains only 0.03% CO_2. Such an argument would, however, be fallacious since it was observed during the present studies that these 'surface active' species were in fact usually associated with the top 1 to 1.5 cm. of the soil, which was overlain by a variable thickness of partially decayed organic debris.

It was not possible to determine any positive causal factor for the low R.Q. values shown on some occasions by some O. cyaneum individuals. It is possible that calciferous gland activity was involved in this large species - but if so, it was certainly not obligatory or directly due to the environmental conditions simulated in this investigation. O. cyaneum occurred in dense clay soil, often at considerable depth. In these confined conditions a mechanism for removal of CO_2 in a non-toxic form might be advantageous, though the moisture content of the surroundings may have been sufficiently high to obviate any necessity for such a system. The bubbles observed in some flasks during O. cyaneum measures may have contained CO_2 which was prevented from reaching the absorbent during the second phase of R.Q. measurement. However, large

bubbles were occasionally observed in flasks containing worms which showed perfectly normal - even high - R.Q. values. No bubbles were observed in flasks containing other species. It may be that calciferous gland activity in O. cyaneum is stimulated by any form of irritation. The conditions within a glass Warburg flask, in constant motion, must result in a degree of irritation to any lumbricid species. O. cyaneum is a large species living under conditions of maximum soil stability; it is known to be highly sensitive to flooding - evacuating such burrows completely - and may be hypersensitive to stress. The apparently random variation in R.Q. values may therefore have been due to variation in stress response between individuals and/or to the particular set of irritating conditions impinging on any individual during the period of R.Q. measurement.

A. rosea occurred in the topsoil at Wynyard, a habitat which was moist throughout the year and contained negligible amounts of calcium. It was not therefore suprising that no evidence of calciferous gland activity was seen during the seasonal studies of R.Q. in this species. There may have been some excretion of CO_2 via the glands, slightly lowering the R.Q. value - e.g. in summer, but this was of little consequence in the studies of respiratory heat loss since all rates of CO_2 output were converted to corresponding O_2 consumption rates for calculations of calorific equivalents. When a species such as A. rosea enters dormancy within a watertight soil cell, the removal of CO_2 in a non-toxic form

must be vital for survival. This is probably an important function of calciferous glands in such species - a function very similar to that suggested above for the surface active species under dry soil conditions. At Wynyard, A. rosea did not undergo dormancy, so that the calciferous glands may have been almost entirely functionless in this situation.

(c) The Effects of Starvation and Variation in Ambient Temperature on the Respiratory Quotient of Allolobophora rosea f. typica

Introduction

Since there was a possibility that a degree of starvation occurred during the monthly measurements of CO_2 output rates for **A. rosea** (See sub-sections 2 and 3), an experiment was performed to assess the effects of starvation on the A. rosea respiratory quotient. During CO_2 respirometry, animals were collected from the field over a wide range of field soil temperatures. Since seasonal R.Q. measures involved collection of animals at only some of these field soil temperatures, it was considered desirable to test the effects of ambient temperature on the A. rosea R.Q. in the isolation of laboratory conditions.

Methods

Twenty A. rosea adults were taken from laboratory culture, in 'bramble' mull-type soil from the Wynyard site, after acclimatisation for several weeks at $10^{o}C$. The animals were grouped in pairs and the R.Q. of each pair was measured, at $10^{o}C$, by the Warburg technique described in the seasonal R.Q. investigation above. The animals were then starved in a

sterilised coarse sand medium, moistened with phosphate buffer at pH 6.5, for one week at 10°C. The R.Q. values of the original worm pairs were then measured again at 10°C by the same technique.

The worms were then replaced in the original 'bramble' soil medium and kept at a constant acclimatisation temperature of 15°C for three weeks. The R.Q. values of the original worm pairs were then measured once more, by the same Warburg technique - though this time at 15°C.

Results

The mean R.Q. value for fed worms at 10°C was 0.84; the mean R.Q. value for starved worms at 10°C was 0.85. The mean R.Q. value for these same worms, after feeding at 15°C for three weeks, was 0.87. The significance of the differences between the R.Q. mean values for fed and starved worms at 10°C, and for fed worms at 10°C and 15°C, were tested according to the standard techniques for small samples (Bailey, 1966). The differences were not significant ($P > 0.10$) in either case.

Discussion

It was concluded that the respiratory quotient of **A. rosea** adult worms is unaffected by either starvation or increased soil temperature, under the laboratory conditions of experimentation used in these studies. The sand medium used for prolonged starvation was similar to that used for shorter periods during respirometry. The temperature range covered in these experiments was similar to that measured in

the field over most of the year. The A. rosea R.Q. values measured seasonally were therefore considered reliable for use in conversions involving monthly measurements of A. rosea CO_2 output in the respirometer.

(ii) Effect of Ambient Temperature on the Rate of Oxygen Consumption by Allolobophora rosea (Sav.) f. typica

Introduction

In order to calculate field rates of oxygen consumption by A. rosea worms, from data collected at 10°C, it was necessary to determine the Q_{10} relation between rates of oxygen consumption and the ambient temperature. For this comparative work, the Warburg apparatus was considered suitable - though the absolute measures obtained were expected to be somewhat higher than normal field rates at equivalent temperatures (see p.265). During these experiments, possible effects of thermal acclimatisation on the worm Q_{10} relation were investigated. The relation of A. rosea cocoon respiration to ambient temperature was studied in the course of this work.

Methods

Sixty adult worms, sixty large immatures and sixty small immatures were collected from a site adjacent to the grid area at Wynyard. The animals were kept in the laboratory in large glass vessels (46 cm. in diameter by 23 cm. in depth), filled to a depth of 15 cm. with 'bramble' mull-type soil from Wynyard. Thirty animals of each size class were kept at each of the constant temperatures 5°C and 10°C for a period of eight weeks. The rates of oxygen consumption by these animals were then measured at each of the ambient temperatures 6°C, 10°C and 15°C. Rates of worm oxygen uptake were measured in 20 ml. Warburg flasks, each containing 1 ml. of phosphate buffer (pH 6.5) in the base and 0.2 mls. of 15% KOH solution

in the centre well. Adult and large immature worms were measured individually but small immatures were measured in groups of five worms per 20 ml. flask, to obtain reliable data for these small individuals. Between measurements, each worm or worm group was kept for two days in a suitably labelled glass tube (4 cm. diameter), filled with 'bramble' mull-type soil, at the original acclimatisation temperature.

Eighteen A. rosea cocoons were collected from the field and eighteen from laboratory cultures. Two plastic containers (5 cm. in diameter by 5 cm. in depth) were half-filled with wetted 'bramble' soil, overlain by a moist disc of Whatman's No. 1 filter paper. Each group of eighteen cocoons was allocated to one of these containers which was darkened and placed in a constant temperature room at 10°C. After one week the total rate of oxygen consumption by each cocoon group was measured at 10°C in the Warburg apparatus, using 10 ml. flasks. After measurement, the cocoon groups were replaced in the original containers and kept at 15°C for the following week. Oxygen consumption rates were then measured at an ambient temperature of 15°C. Finally, the cocoons were kept for one week at a constant acclimatisation temperature of 5°C, and the oxygen consumption rate of the whole group of cocoons was measured at an ambient temperature of 5°C.

Results

The mean rates of oxygen consumption by A. rosea worms of the three major size classes at three ambient temperatures are shown in Table 19; the relevant acclimatisation

Acclimatisation temperature:	5°C			10°C			5°C and 10°C		
Ambient temperature:	6°C	10°C	15°C	6°C	10°C	15°C	6°C	10°C	15°C
Adults	59.38 (6.52)	63.08 (4.82)	93.96 (7.06)	75.67 (8.22)	79.64 (9.36)	88.72 (9.02)	67.53 (5.43)	71.36 (5.48)	91.34 (5.61)
Large immatures	84.95 (9.79)	106.69 (8.85)	173.29 (12.59)	46.04 (1.83)	136.55 (9.55)	140.31 (12.48)	66.52 (6.85)	122.41 (7.27)	156.80 (9.42)
Small immatures	75.80	120.87	147.39	64.62	104.77	135.25	70.21	112.82	141.32
Adults and Large immatures	72.17 (6.43)	83.74 (7.00)	133.63 (11.49)	61.64 (5.53)	108.09 (9.23)	114.52 (9.55)	67.04 (4.23)	96.23 (6.00)	124.07 (7.44)
Adults, Large immatures and Small immatures	-	-	-	-	-	-	68.09	102.20	129.82

Table 19. Mean oxygen consumption rates (mm^3O_2/fresh gm. worm/h.) for worms of three A. rosea size classes, acclimatised at two temperatures and measured at three ambient temperatures. Standard errors are shown in parentheses.

temperatures are shown. At the 95% level of confidence, the temperature of acclimatisation produced no significant differences in the relation between oxygen consumption rate and ambient temperature for either adult or large immature worms. The only anomaly was a significantly higher respiratory rate at 6°C for 5°C -acclimatised large immatures than for 10°C-acclimatised worms of this size class. The small immature worms showed a consistent translation of the relation between oxygen consumption and ambient temperature. This was not thought statistically significant since there was some overlap in the data from worms of the two acclimatisation groups. Statistical examination of the small immature data was not possible, due to the grouping of these worms for respiratory measurements.

The results for adult and large immature worms were temporarily grouped, at one stage, to allow examination of possible acclimatisation effects with a larger worm sample size. No significant differences were observed in the Q_{10} relations for worms acclimatised at the temperatures 5°C and 10°C.

In view of these findings, the data for all worms in each size class were pooled; these results, together with the mean relation for all A. rosea worms measured, are shown in Fig. 27. The rates of oxygen consumption by large immature worms at 10°C and 15°C were found to be significantly different from those measured for adult worms at these ambient temperatures. The mean oxygen consumption rates measured for small

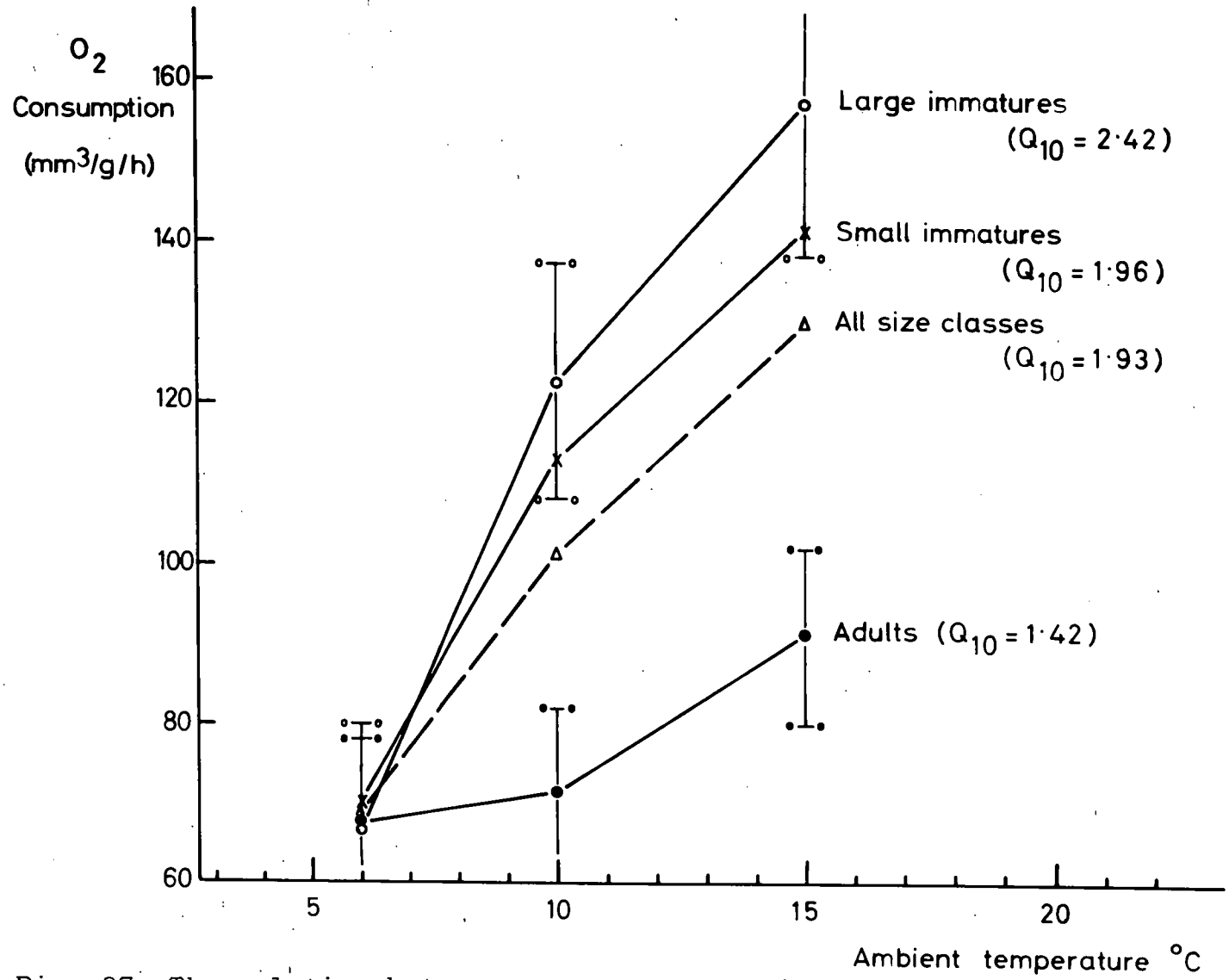


Fig. 27. The relation between oxygen consumption rate and ambient temperature for A.rosea individuals of various size categories.

immatures at these temperatures were nearer to those found for large immature worms than to those for adults. Mean rates of oxygen consumption for worms of all size classes were almost identical at an ambient temperature of 6°C. Since the linearity of the respiration/ambient temperature relations could not be improved by logarithmic transformation of the respiratory data, Q_{10} values were calculated for regression analyses of the arithmetic measurements. The Q_{10} values, between 6°C and 15°C, for adult, large immature and small immature worms were found to be 1.42, 2.42 and 1.96, respectively. An average Q_{10} value for the species was calculated and found to be 1.93.

Fig. 28 shows the relation between the rate of oxygen consumption, plotted logarithmically, and ambient temperature for cocoons of A. rosea. The results for the two cocoon groups at 5°C and 10°C were very similar and the log. mean values for all cocoons at 5°C, 10°C and 15°C showed an almost perfectly linear relation with ambient temperature. The mean trend line, shown in Fig. 28, was drawn by eye since any consequent errors appeared negligible. The cocoon Q_{10} value between 5°C and 15°C was found to be 1.63. The mean rates of oxygen consumption by A. rosea cocoons at 5°C, 10°C and 15°C were 21.47, 27.44 and 35.02 mm^3 O^2/fresh g. cocoon/h., respectively.

Discussion

Pomerat and Zarrow (1936) stated that Vernon, in 1897, had suggested 'the earthworm' to be exceptional amongst

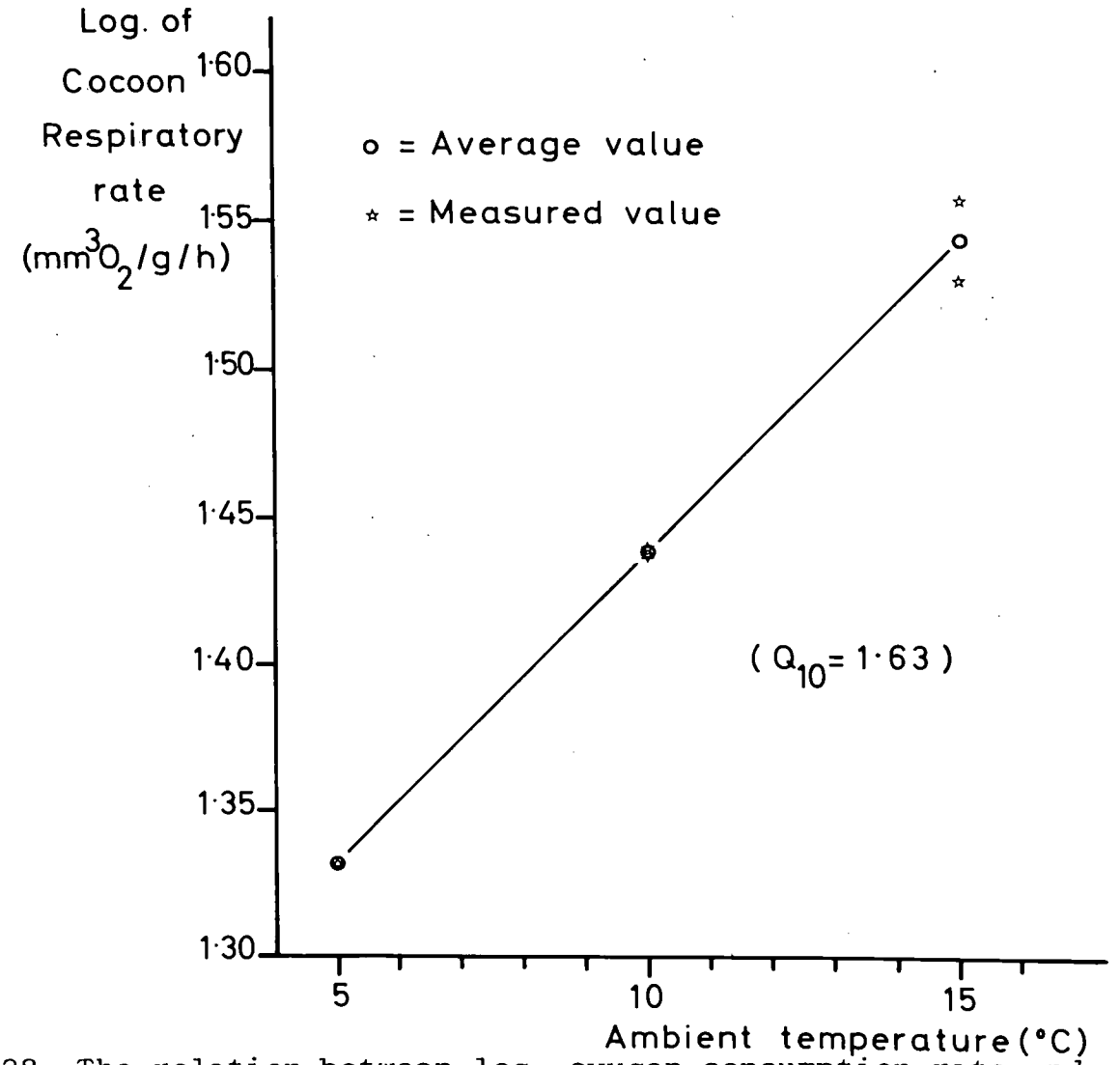


Fig. 28. The relation between log. oxygen consumption rate and ambient temperature for A.rosea cocoons.

poikilotherms in not showing increased respiratory activity with rising temperature. Konopacki (1907) demonstrated Q_{10} values in the range 2.0 to 2.5, at temperatures in the range 25°C to 30°C, for the lumbricid species L. terrestris L., L. rubellus Hoffmeister and L. communis. Alsterburg (1922) demonstrated a Q_{10} value of 2.1 for the frequency of respiratory oscillation in tubificid worms. Pomerat and Zarrow (1936) measured respiratory rates for L. terrestris over the ambient temperature range 9°C to 27°C, using the Warburg apparatus, and showed a positive relation between respiratory activity and rising temperature. Kirberger (1953) studied E. foetida (Sav.) and the aquatic oligochaete Lumbriculus variegatus Müll. She measured Q_{10} values, over the temperature range 15°C to 25°C, similar to those found by Konopacki (see above). Positive relations between respiratory rate and ambient temperature have also been shown by Knoz (1957), for E. foetida and E. tetraedra (Sav.), by Saroja (1959, 1964), for the megascolecid Megascolex mauritii and the tropical worm Octochaetona serrata, and by Mendes and Almeida (1962) for the tropical oligochaete Pheretima hawayana. It may be concluded that the normal poikilotherm relation between respiratory rate and ambient temperature has been established for the Oligochaeta in general and the Lumbricidae in particular. The results of the present studies on A. rosea support these general conclusions.

The Q_{10} value is not a particularly meaningful expression,

varying according to the range of temperature used, but it is useful for comparative purposes since it has been extensively used in the past. Krogh (1914, 1916, 1941) postulated a general Q_{10} value of approximately 2 for a wide range of animal species. Amongst the many authors who have used this generalisation, Bornebusch (1930) used it for various extrapolations from laboratory to field conditions and Satchell (1963) used it in conversions of Needham's figures for the rate of nitrogenous excretion by L. terrestris (Needham, 1957). MacFadyen (1963a) has pointed out that the curve is probably more useful as a generalisation than as a practical tool for relation to particular species. Q_{10} relations for poikilotherm species are almost invariably displaced from the Krogh curve, and are often affected by the temperature acclimatisation regime of the animals, prior to respiratory measures. Prosser and Brown (1961) have reviewed the available information on temperature acclimatisation, and its possible translatory and/or rotatory effects on the Q_{10} relation. Kirberger (1953) showed an upward translation of the Q_{10} relation for cold-acclimatised individuals of the aquatic oligochaete species Lumbriculus variegatus. However, she found no statistically significant differences between the Q_{10} relations for E. foetida worms acclimatised at 15°C and 25°C, measured over the ambient temperature range 15°C to 25°C. These temperatures may be regarded as abnormally high for most British lumbricid species, but E. foetida is exceptional in normally occurring under

conditions of high habitat temperature.

The results of the present studies on 5°C- and 10°C-acclimatised worms of A. rosea suggested that the respiratory metabolism of A. rosea, like that of E. foetida, was not significantly affected by temperature acclimatisation. In view of the high variability in weight-based respiratory rates shown by A. rosea, it was possible that variation in worm gut content ballast weight - superimposed on a low rate of tissue respiration - masked any acclimatisation effects. The removal of worm gut contents, prior to respiratory measures, was unjustifiable for either monthly measures of CO_2 output or the present investigations of R.Q. and temperature effects on oxygen consumption. Gut content removal would have involved considerable disturbance to the animals and respiratory measures would have borne little relation to the field situation. Variation due to worm ballast weight variation was thus inevitable, though the mean estimates of respiratory rate, from large samples of worms, were thought reliable.

Immature A. rosea worms were shown in these studies to have a significantly greater slope of the Q_{10} relation than that found for adult worms. In the present studies of A. rosea faecal output rates (see Section III, sub-section 3) a similar depression of Q_{10} slope was found in worms of larger size. However, faecal output Q_{10} decreased continuously with increasing worm size, being maximal in the small immatures. The small immature respiratory Q_{10} slope was

less than that found for large immatures. All three respiratory rate Q_{10} slopes were highly significant ($P < 0.01$ for adults and $P < 0.001$ for small immatures and large immatures). The phenomena of low Q_{10} relation slopes, for rates of respiration and faecal output, in adult A. rosea worms were thus basically dissimilar in form. A threshold factor - possibly the assumption of maturity - may be involved in determining the respiratory rate Q_{10} slope for A. rosea worms.

Byzova (1965) showed that there was no significant relation between respiratory rate and body weight in adult A. rosea worms. Her findings were so conclusive that monthly estimates of A. rosea respiratory rate were performed on animal groups during the present work, though groupings were according to the major worm size classes. The present faecal output results, the Q_{10} investigations, and the results of monthly respiratory measures (see below) have indicated that body weight does influence A. rosea respiratory rate. However, a great deal of individual variability occurred so that, whether real or apparent, the differences in respiratory rate recorded for worms of different sizes were, for practical purposes, of a threshold type. The calculation of respiratory mean values for the three major A. rosea size classes was therefore justifiable as the most practicable procedure for a species which, by its form and metabolism, imposed natural limitations on the accuracy of measurement.

The linearity of the relation between log. respiratory

rate and ambient temperature for A. rosea cocoons was of the typical poikilotherm type. The slopes of the calculated regression lines for the respiratory rate Q_{10} relations of the A. rosea worm size classes were used in extrapolation from monthly respiratory measures at 10°C to respiratory rates in the field. The slope of the cocoon respiratory rate Q_{10} relation was intended for similar usage. However, the absolute rates of cocoon respiration and the estimated size of the cocoon standing crop (see pp. 239 , 140) were both found to be extremely low. The field respiratory metabolism of A. rosea cocoons could therefore be regarded as negligible in calculations of field respiratory metabolism for the total A. rosea population.

2. The Respirometer used in the present study for Absolute Measures of Carbon Dioxide Output by Lumbricids

Introduction

For the purpose of estimating the A. rosea (Sav.) field population respiratory metabolism, it was necessary to estimate worm respiratory rates under conditions which were similar to those in the field. Two approaches were possible: the respiratory rates could be measured in standard apparatus (incorporating various factors likely to influence lumbricid behaviour and/or physiological stability) or a new device, in which the major disturbing factors were eliminated, could be constructed for measurements of lumbricid respiration under simulated field conditions. The former approach would involve detailed studies of the effects of disturbing factors such as light, temperature, humidity, pH, lack of burrowing medium, limited movement, high CO_2 or low O_2 tensions in the atmosphere, or irritants of a more specialised nature. Correction factors would then be necessary for the interpretation of respiratory measures in terms of the field situation. Such an approach was considered totally unsatisfactory and a new type of respirometer was devised.

Continuous gas flow analysis was chosen since this technique allowed maximum freedom in choosing the size of animal chambers, whilst maintaining a near constant atmospheric composition within the apparatus. Sterilised soil could not be used as a medium since it would not be possible to accurately standardise the physical conditions impinging on

the worms during respiratory measures. Infra-red gas analysis was used in these studies since this was the most sensitive technique available.

The Respirometer

Fig. 29 shows, in diagrammatic form, the infra-red gas analyser with its associated components in the complete apparatus. The various components were connected by copper tubing, which prevented leakage of CO_2 from the atmosphere. A Watson-Marlow peristaltic pump, capable of generating a maximum air flow of 9 litres per minute, provided the gas flow into the apparatus. A T-junction flow-controlling device allowed more than 98% of the flow to escape into the atmosphere, thereby minimising flow pulsation due to the pump and eliminating changes in flow rate caused by tube deterioration. The residual air flow from the pump passed through tubes containing 'carbosorb' - a self-indicating soda lime (a mixture of calcium oxide with 5 to 20% sodium hydroxide and containing 6 to 18% water) - which removed all CO_2 from the air stream. This CO_2 - free air could then be directed along either of two possible routes: one route led to the experimental chambers, whilst the alternative route led directly to the gas analyser. The latter route, for use when purging the analyser during zero and calibration checks, was provided with separate tubes for the removal of water vapour and solid particles.

During actual experiments, the gas flow to the chambers passed through CO_2 - free distilled water and a water trap

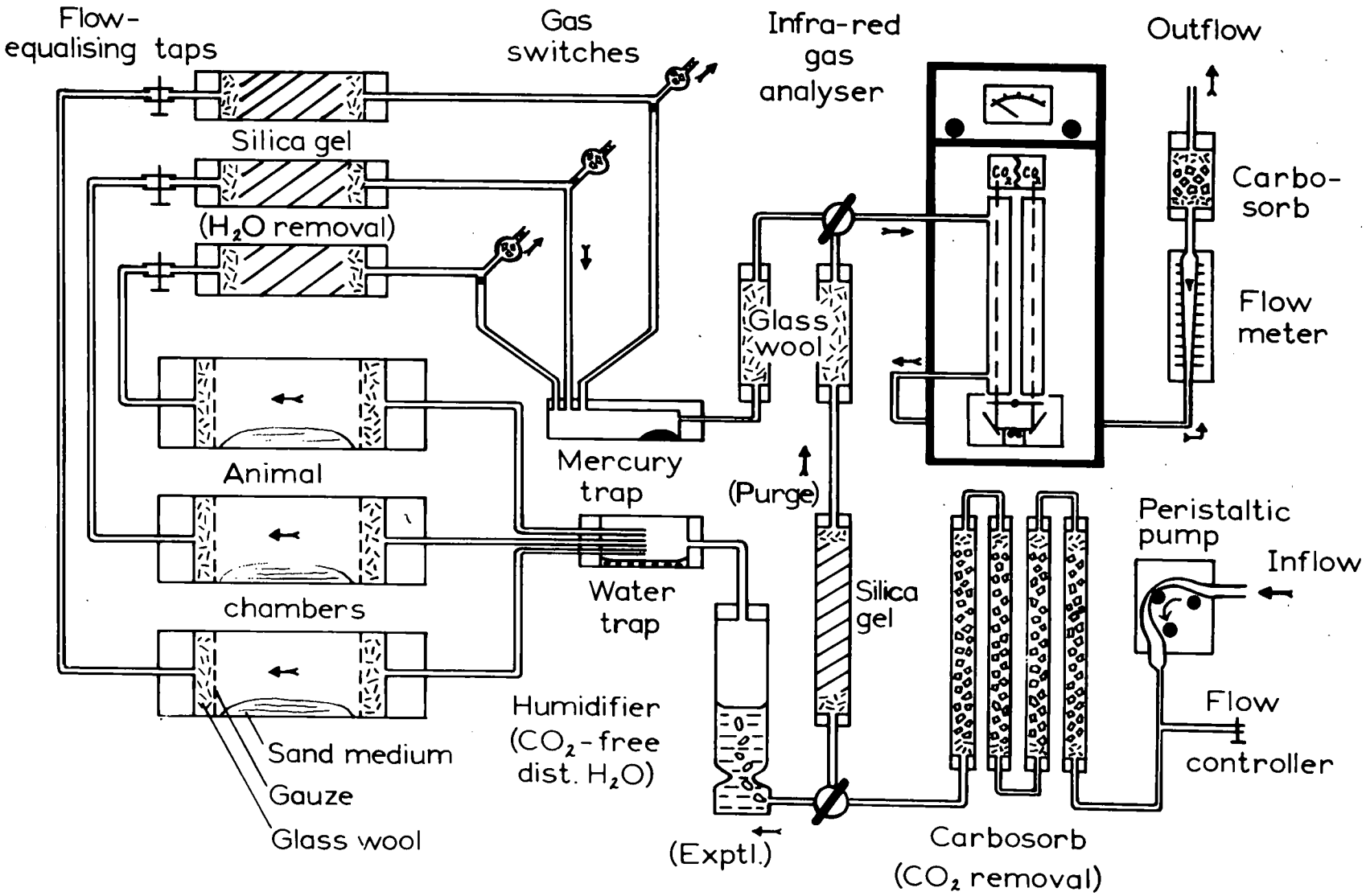


Fig. 29. The infra-red gas analysis apparatus (see text).

before entering the three channels, each of which led to an experimental chamber. Each chamber consisted of a wide-bore glass tube (4.8 cm. in diameter by 26 cm. in length) fitted with a bored rubber-bung inlet and similar outlet; gauze and glass wool barriers prevented worm escape. A sand medium, previously sterilised and buffered at pH 6.5, was used during experiments, and all chambers were confined within a darkened box. The gas mixture leaving each chamber was dried by passing through a tube filled with self-indicating silica gel, before entering its appropriate mercury gas switch. Each switch permitted a continuous flow of gas which could either be released into the surrounding air or passed, eventually, through the infra-red gas analyser. Fig. 30 and Plate 18 show a mercury gas switch in detail. It consisted of a glass tubing cross-piece with a single T-shaped inlet and two possible outlets: a 'non-analysis' outlet to the atmosphere and an 'analysis' outlet which eventually led to the gas analyser. The inlet and 'analysis' outlet were ground glass joints, of which the female sections were fixed rigidly to the supporting framework. The male sections then formed rotating pivots for the cross-piece. The base of each female section was in two parts, with a rubber tube connection which gave flexibility to the joint and facilitated removal of the central portion of the switch. The cross-piece, at the lowest level on the switch, was half-filled with clean mercury and attached to it by metal clips was an aluminium strip, the ends of which were bolted to plunger solenoids. Operation

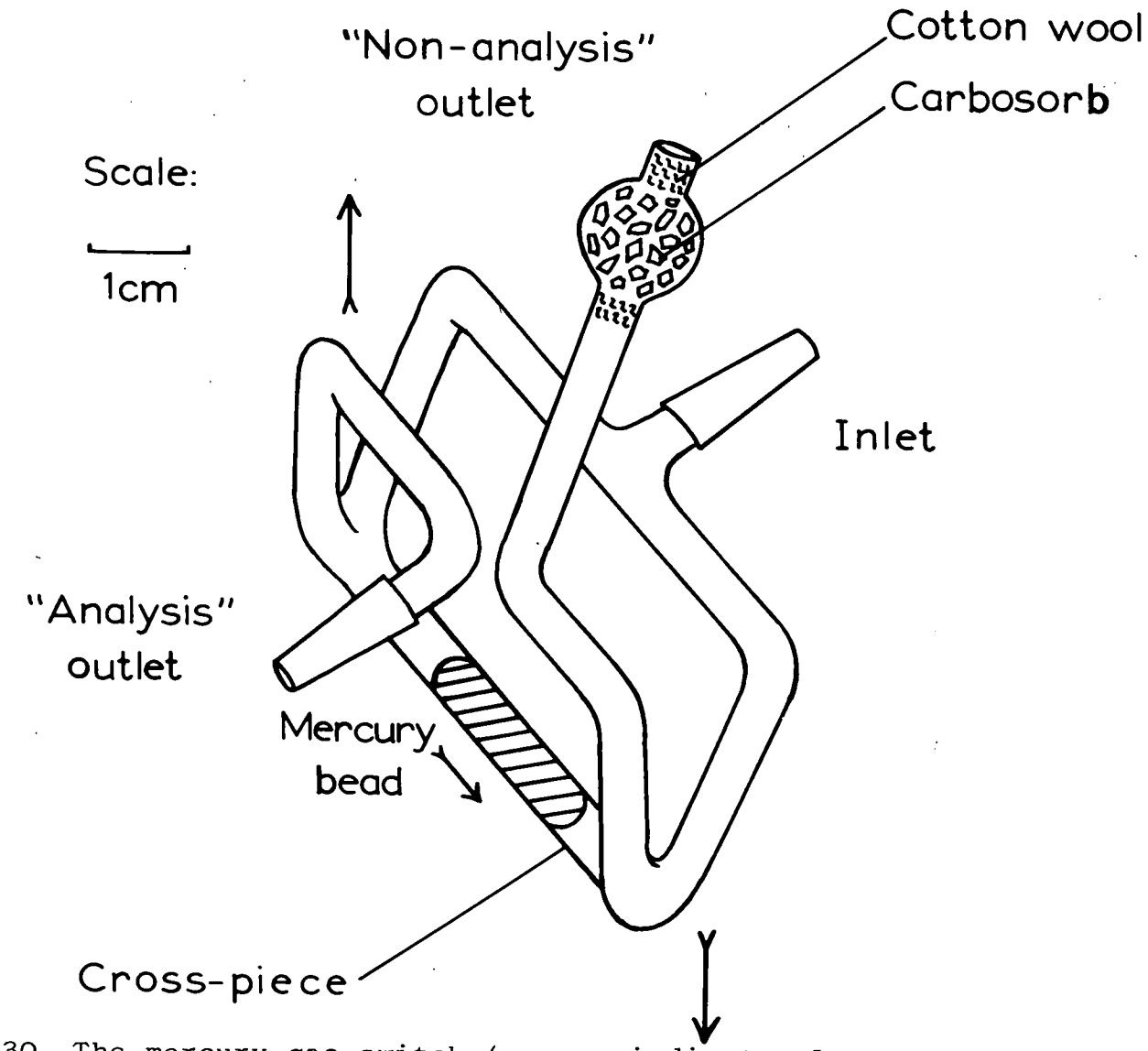


Fig. 30. The mercury gas switch (arrows indicate plane of rotation and movement of the mercury bead - see text).

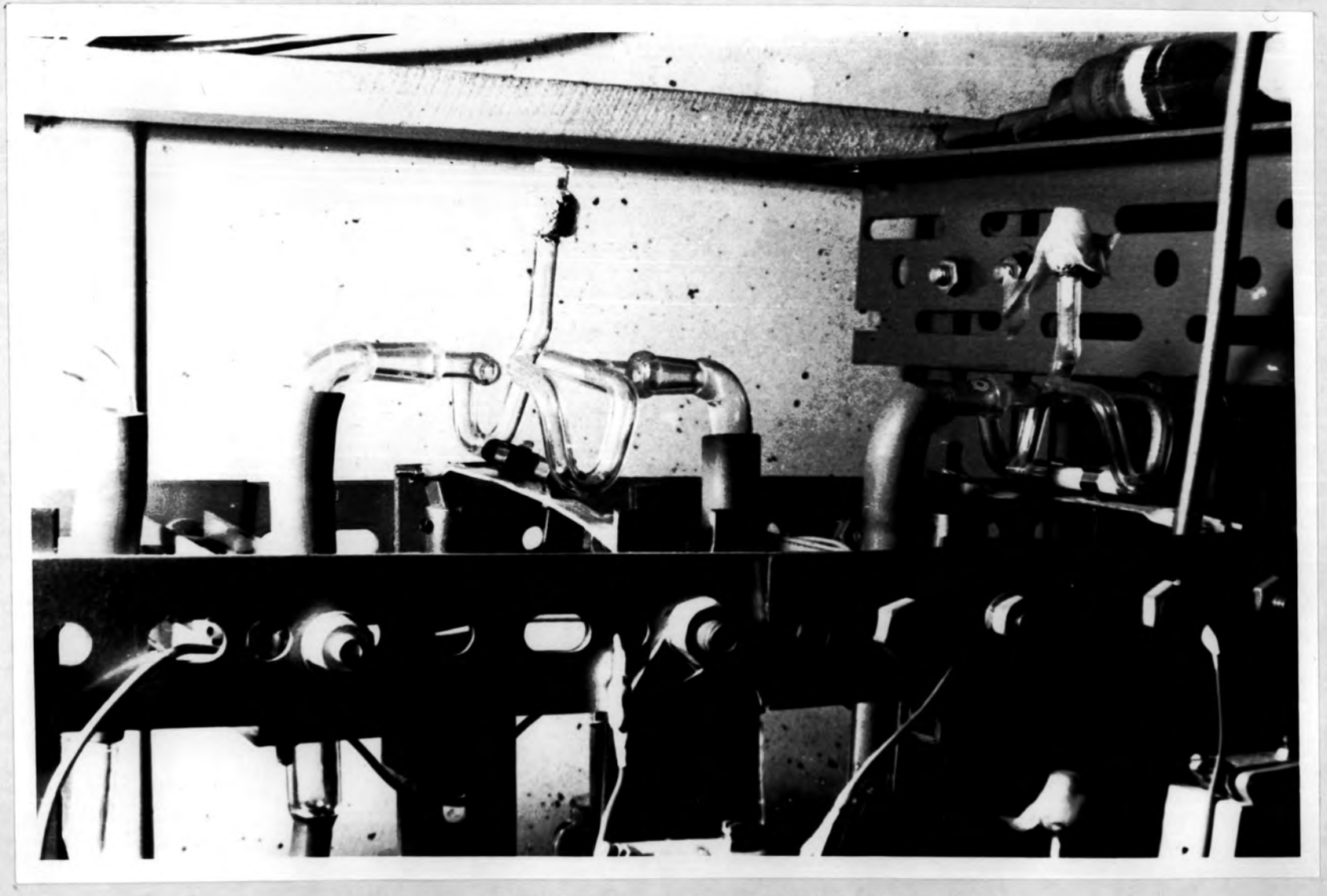
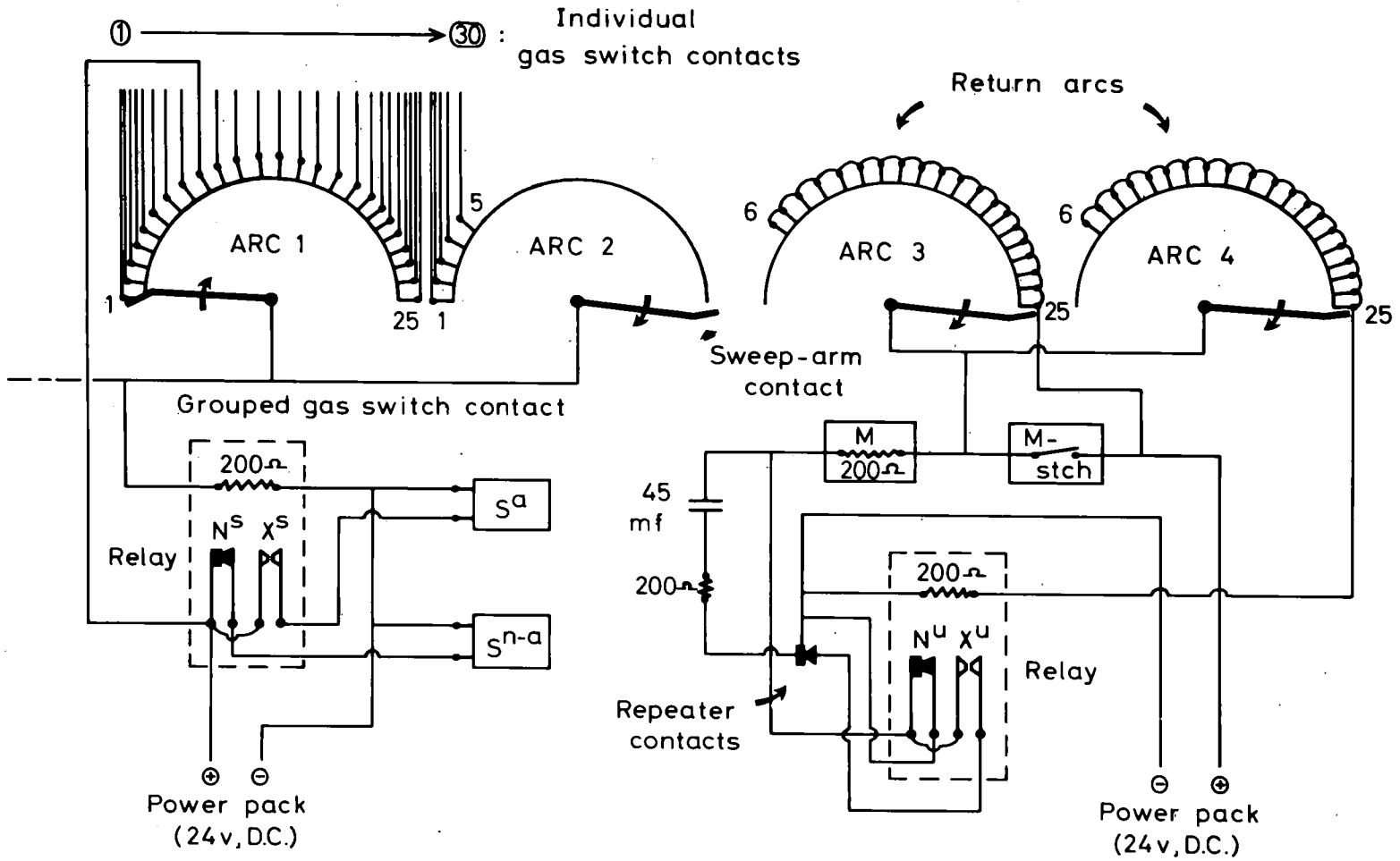


Plate 18. The mercury gas switch 'in situ'.

of the solenoid on one side resulted in a tilting of the cross-piece sufficient to cause the blocking of one outlet by accumulation of the mercury on that side. The ground glass joints were lubricated with silicone grease for efficient operation at the working temperature. A 'carbosorb' section on the 'non-analysis' outlet prevented CO_2 leakage from the atmosphere and a cotton wool plug, suitably compressed, ensured a 'non-analysis' flow rate similar to that in the 'analysis' period. Any difference between 'analysis' and 'non-analysis' flow rates was shown on the chart recording as a change in gas flow CO_2 concentration at the beginning of the 'analysis' period. A small tube and clip attachment on the 'non-analysis' outlet would have facilitated the necessary flow adjustment.

A timing device automatically synchronised the gas switches so that the gas stream from each switch in turn was passed through the analyser for 20 minutes in every hour. The apparatus originally incorporated thirty experimental chambers, with gas switches on 'analysis' for two minutes in every hour. A 'hollow disc' gas flow distributor, with a centrally inserted inlet (1.3 cm. bore) and thirty outlets (0.6 cm. bore) on the perimeter, allocated equal flow rates to the animal chambers. A length of stainless steel tube (0.6 cm. bore) with angled inlets (0.6 cm. bore) served as a gas flow collecting device. A standard Post Office uniselector was used in the time switch mechanism: Fig. 31 shows the devised wiring circuit for this arrangement. Only



KEY : M = Uniselector electromagnet; M-stch = Cam-operated micro-switch; 1,5,6,25 = Arc contacts; N^S, X^S, N^U, X^U = Relay contacts; S^a = 'Analysis' solenoid; S^{n-a} = 'Non-analysis' solenoid

Fig. 31. Original wiring circuit for thirty gas switch/uniselector system.

the arc contacts actually utilised are shown in Fig. 31. One 'activating' lead from each gas switch system was connected to one of the utilised contacts on arc 1 or arc 2. All arc 1 contacts, and five of the arc 2 contacts, were used as individual connectors for the thirty gas switch systems in the original apparatus. Contacts 6 to 25 on each of the arcs 3 and 4 were linked together for the operation of the uniselector return mechanism. The arc sweep-arms were orientated as shown in Fig. 31 so that the normal switching operation, with arcs 3 and 4 non-functional, proceeded as follows: the arc 1 sweep-arm rested on one of the switch contacts (e.g. arc 1, contact 1 - as in Fig. 31), activating the 'analysis' solenoid of this switch system. Closure of the micro-switch contacts, operational - through a rotating cam - at two minute intervals, activated the uniselector magnet which advanced all sweep-arms to their next contact position. The magnet movement automatically separated the repeater contacts. Opening of the micro-switch contacts de-activated the uniselector magnet, completing the changeover from one gas switch contact to the next. This system was operative from the first switch contact of arc 1 to the fifth switch contact of arc 2 - representing the individual 'activating' contacts of all thirty gas switch systems.

As the sweep-arms of arcs 2, 3 and 4 moved on to their respective sixth contacts, the uniselector return mechanism became operative. Arcs 1 and 2 became momentarily non-functional. The arc 3 circuit by-passed the micro-switch so

that the uniselector relay was activated through arc 4. The uniselector magnet, normally operative through the relay contacts N^u (see Fig. 31), was then activated through relay contacts X^u. This temporary magnet circuit included the repeater contacts. Since the latter were automatically separated by positive movement of the magnet, the magnet circuit was automatically broken and the magnet released, leaving arc 2, 3 and 4 sweep-arms on their seventh contacts. Magnet release closed the repeater contacts, reactivating the magnet - and so on, until the arc 2, 3 and 4 sweep-arms passed their twenty fifth contacts. Arcs 3 and 4 again became non-functional so that the uniselector relay was de-activated, the micro-switch resumed control of the uniselector electromagnet and the arc 1 sweep-arm rested on its first switch contact. The whole return procedure was completed in a fraction of a second, so that the net effect was a rapid movement from the 5th switch contact on arc 2 (30th gas switch contact) to the 1st contact on arc 1.

The wiring circuit for one gas switch system is also shown in Fig. 31. The system is shown in the 'non-analysis' phase, with no circuit through the 'activating' uniselector arc contact and the 'non-analysis' solenoid operative through the relay contacts N^s. When the sweep-arm of arc 1 came to rest on the contact for the switch system shown, the gas switch relay would be activated, opening contacts N^s, and closing contacts X^s. The 'non-analysis' solenoid would thus be de-activated, whilst the 'analysis' solenoid would become

operational.

The original gas flow distribution and uniselector timing and switching devices, described above, were found to function efficiently in the thirty pathway system. However, it was not found possible to achieve sufficient sample delineation using this time scale, at the low flow rates necessary for optimum detection efficiency. This was undoubtedly due to the 'common pathway' gas flow collection device. If separate pathways had been provided from each gas switch to the analyser, the necessary delineation would, in the author's opinion, have been achieved. However, each return pathway would have required separate absorption tubes and the whole apparatus would have become too bulky for the limited space available. In view of information available on the extent of individual variation in A. rosea respiratory rates (Byzova, 1965), the complex arrangement was abandoned in favour of a simpler apparatus incorporating only three experimental chambers and gas switches for respiratory measures on groups of lumbricid worms. The timing device was easily adapted to the simpler system: contacts 1 to 10 on arc 1, 11 to 20 on arc 1, and 21 to 25, arc 1, plus 1 to 5, arc 2, were linked together in three groups. Each group formed an individual gas switch contact, so that each gas switch was on 'analysis' for ten 2 min. periods (i.e. 20 mins) in every hour.

During an 'analysis' period the selected gas stream passed through a mercury trap, where the three channels were

re-united along a common path to the infra-red gas analyser. This common path was of minimal length, preventing excessive mixing of the gas streams when changing channels for 'analysis'. A tube containing glass wool removed any solid particles from the stream before passage through the analyser which registered CO_2 concentration in parts per million by volume. The gas flow rate was measured by a Platon 'Gapmeter' which operated on the weighted float principle. A 'carbosorb' tube, on the flow channel outlet, prevented back-leakage of CO_2 from the atmosphere, This tube was removed for conversion of the flowmeter reading to its equivalent at atmospheric pressure. A flow rate of 180 ccs. per minute was found to give suitable readings on the gas analyser without stressing the gas switch system. The Grubb-Parsons infra-red gas analyser, Model SB2, was linked to a Honeywell 'Electronik' recorder with a chart speed of 9 ins. (23cm.) per hour. Plate 19 shows the type of chart recording obtained for animals of the three major size classes of A. rosea.

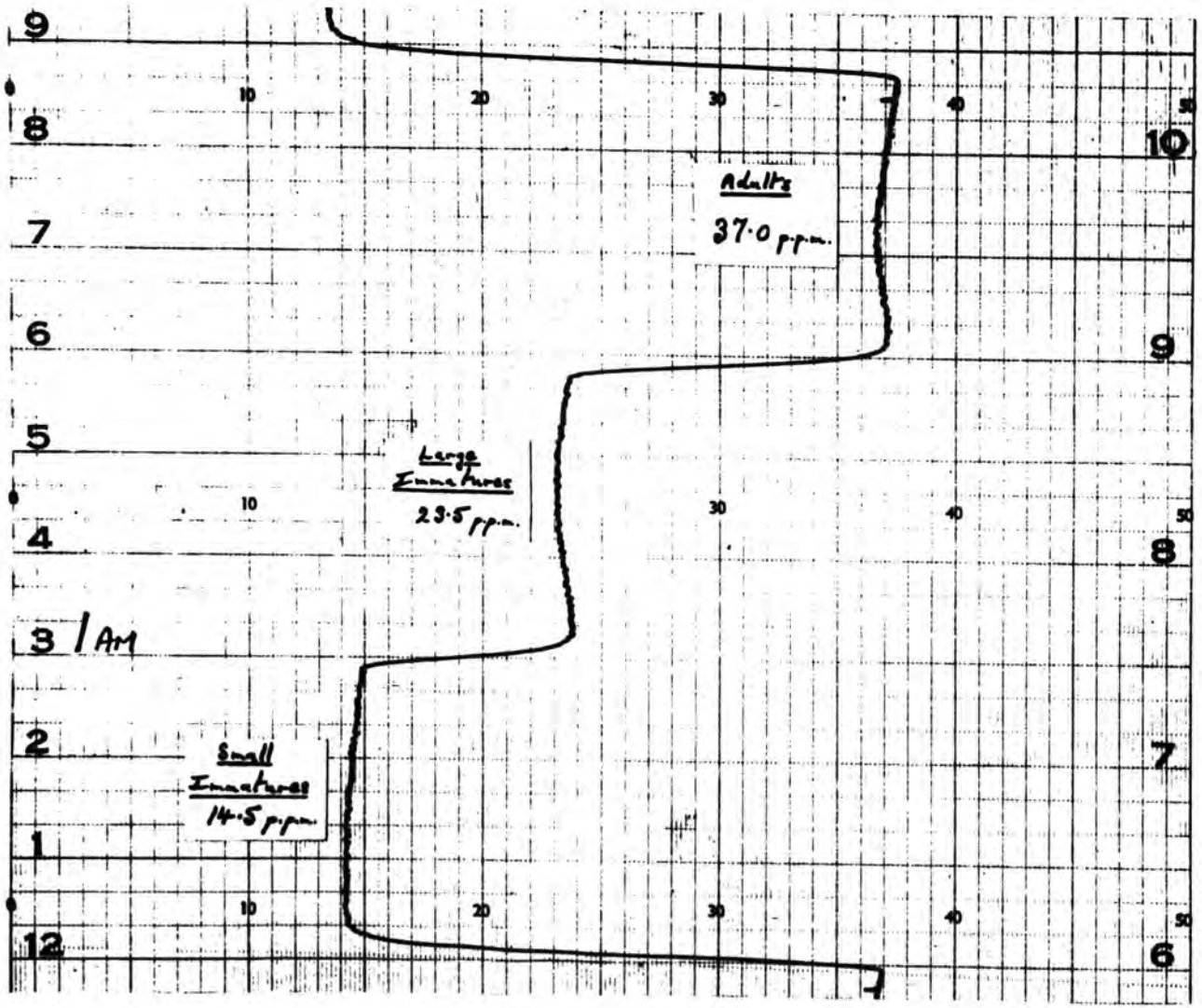


Plate 19. Sample of chart recording from the infra-red gas analyser.

3. Seasonal Variation in Lumbricid Respiratory Metabolism

(i) Respiratory Measures at 10°C

Introduction

In order to evaluate a major component of the field population energy budget for Allolobophora rosea (Sav.) f. typica, it was necessary to obtain estimates of field respiration for this species throughout the year. Since field measures were impracticable, a laboratory apparatus - capable of respiratory measurement under simulated field conditions - was constructed (see preceding sub-section). For O. cyaneum (Sav.), D. rubida (Sav.) f. subrubicunda (Eisen) and L. castaneus (Sav.), unlike A. rosea, quantitative population data was not available. However, seasonal respiratory measures were made for these species, using the gas analysis apparatus, for comparisons with data obtained by previous workers using conventional apparatus.

Methods

The infra-red gas analysis apparatus has been described in considerable detail in the preceding sub-section.

In any one experiment individuals of a given species were collected from a field site adjacent to the grid area at Wynyard; they were kept for two days at 10°C in jars containing soil and litter media taken from the collection site at the time of sampling. The jars were covered with perforated polythene sheet to prevent worm escape. The gas analyser was subjected to zero and calibration checks and the darkened animal chambers were charged with a small amount of

sand medium, previously sterilised and buffered at pH 6.5. The apparatus was then operated until the CO_2 level recorded from each chamber was constant (usually 4 to 5 p.p.m. after 24 hours). These 'background' values were noted and the previously weighed animals were placed in the experimental chambers. The apparatus was operated for a further 48 hours, after which time the animals were removed and re-weighed; finally the analyser was checked for zero drift. The initial weights of the animal groups were used in calculations of respiratory rates per gram worm fresh body weight. Any weight changes over the experimental period were related only to variation in the weight of gut contents (see p.180 above). Respiratory rates for O. cyaneum were calculated both from the total body fresh weight and from body weight excluding gut contents (from relation given above - p.201). The hourly readings (p.p.m. CO_2) for each chamber, over the 48 hour period, were plotted graphically to determine the course of respiration throughout the experiment.

In the case of A. rosea the respiratory rate over a 24 hour period, for twenty individuals of each size class, was measured monthly over a one year period. The mean daily respiratory rates of D. rubida f. subrubicunda and L. castaneus were measured at two-monthly intervals for the first twenty individuals collected in the field, regardless of size or reproductive condition. The estimation of similar rates for O. cyaneum involved measures for only about ten individuals on each occasion, due to high absolute rates of CO_2 output

and lack of time for further duplications of experiments.

The 24 hour period immediately after consistent readings were obtained was used in calculations of mean respiratory rate.

Results

Fig. 32 shows the typical course of respiration ($mm^3 CO_2$ per gram worm total fresh weight per hour) for lumbricid groups of the four species studied. Hourly measurements are shown over the 48 hour period after confinement in the respirometer; the examples were drawn from actual measures. L. castaneus showed consistent levels of CO_2 output from the first hour onwards, though the rate tended to fall after about 30 hours. The other pigmented species, D. rubida f. subrubicunda, and the unpigmented species A. rosea and O. cyaneum showed declines in rates of CO_2 output over the first few hours. These declines lasted 3 to 4 hours for D. rubida f. subrubicunda and A. rosea, and up to 6 or 8 hours for O. cyaneum. D. rubida f. subrubicunda showed considerable fluctuation in respiratory activity, with a decline to a lower level after about 30 hours. Respiratory rates of A. rosea and O. cyaneum were more consistent after the initial decline. Preliminary studies showed that A. rosea respiratory rate fell rapidly to a low, constant level after 50 to 60 hours in the respirometer. In these same preliminary studies, O. cyaneum individuals were kept in the respirometer for up to 72 hours with no sign of major decrease in respiratory rate.

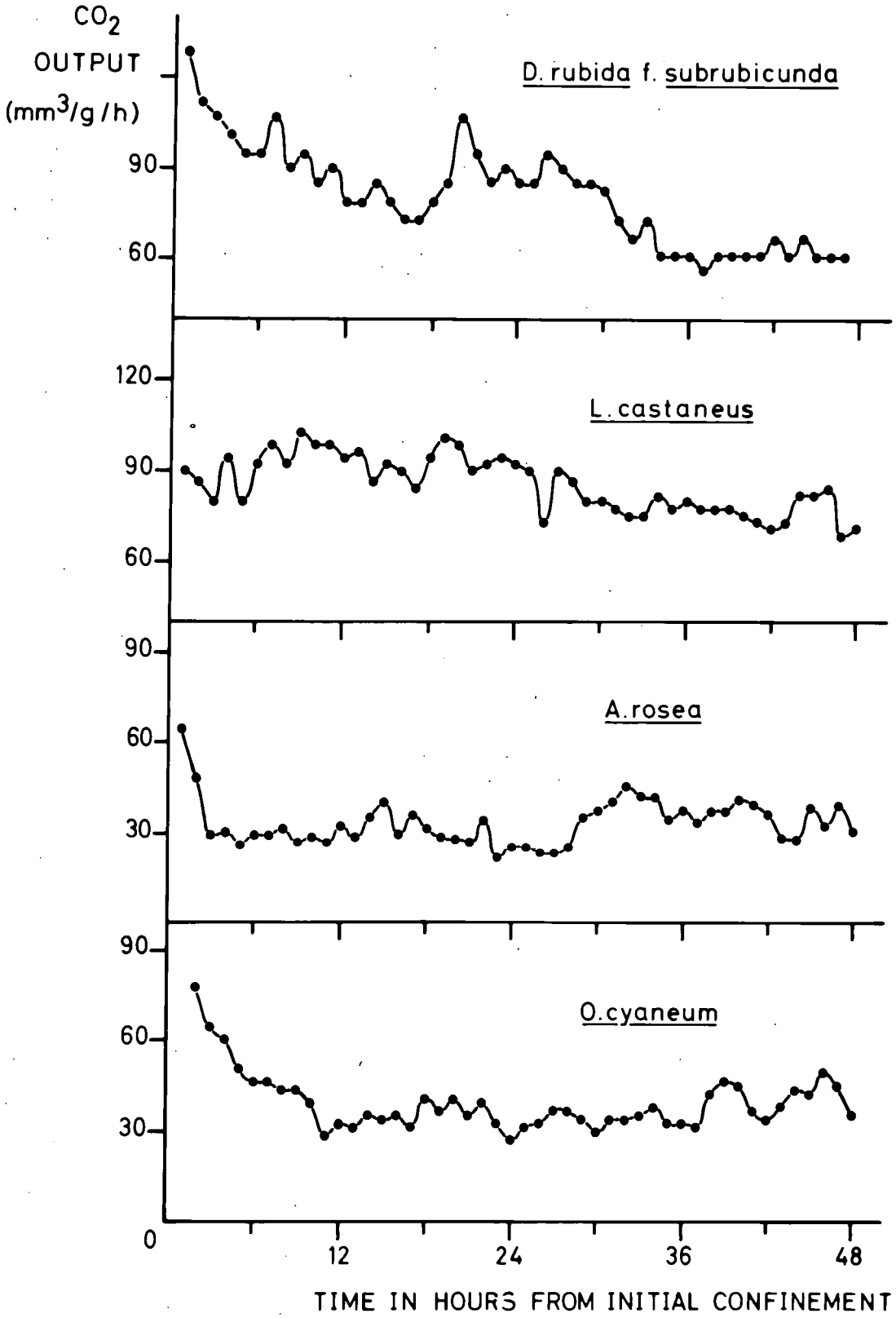


Fig. 32. Typical course of respiratory activity for each of four lumbricid species confined in the infra-red gas analysis apparatus.

Fig. 33 shows the rates of CO_2 output ($mm^3CO_2/g/h$ at 10^oC, volumes corrected to N.T.P.) for the three major size classes of A. rosea, measured at approximately monthly intervals over one year. The field soil temperature curve (5 cm. depth beneath 'bramble') is shown in Fig. 33 for comparative purposes. Using the seasonal R.Q. values, determined for A. rosea in the present study, the CO_2 output rates were converted to rates of oxygen consumption at 10^oC. Both CO_2 output rates and O_2 consumption rates were plotted against field soil temperatures at the times of worm collection. Highly significant correlation coefficients of 0.70 and 0.79 were obtained for CO_2 output and O_2 consumption rates, respectively ($P < 0.001$ in both cases).

Rates of CO_2 output at 10^oC for O. cyaneum (including and excluding gut content weight from total body weight), D. rubida f. subrubicunda and L. castaneus are shown in Table 20. The rate for L. castaneus fluctuated slightly at a high but consistent level throughout the year. D. rubida f. subrubicunda showed a fall in CO_2 output rate during the spring and probably early summer. O. cyaneum rates of CO_2 output were very similar throughout the year, though a slightly higher rate was recorded in May/June, 1967. Accurate conversions of CO_2 output rates to rates of O_2 consumption were not possible for these species, since all showed variable R.Q. values of uncertain reliability. However, approximate estimates of O_2 consumption at 10^oC would be in the ranges 45 to 60 (or 90 to 115), 100 to 130, and 135 to 155

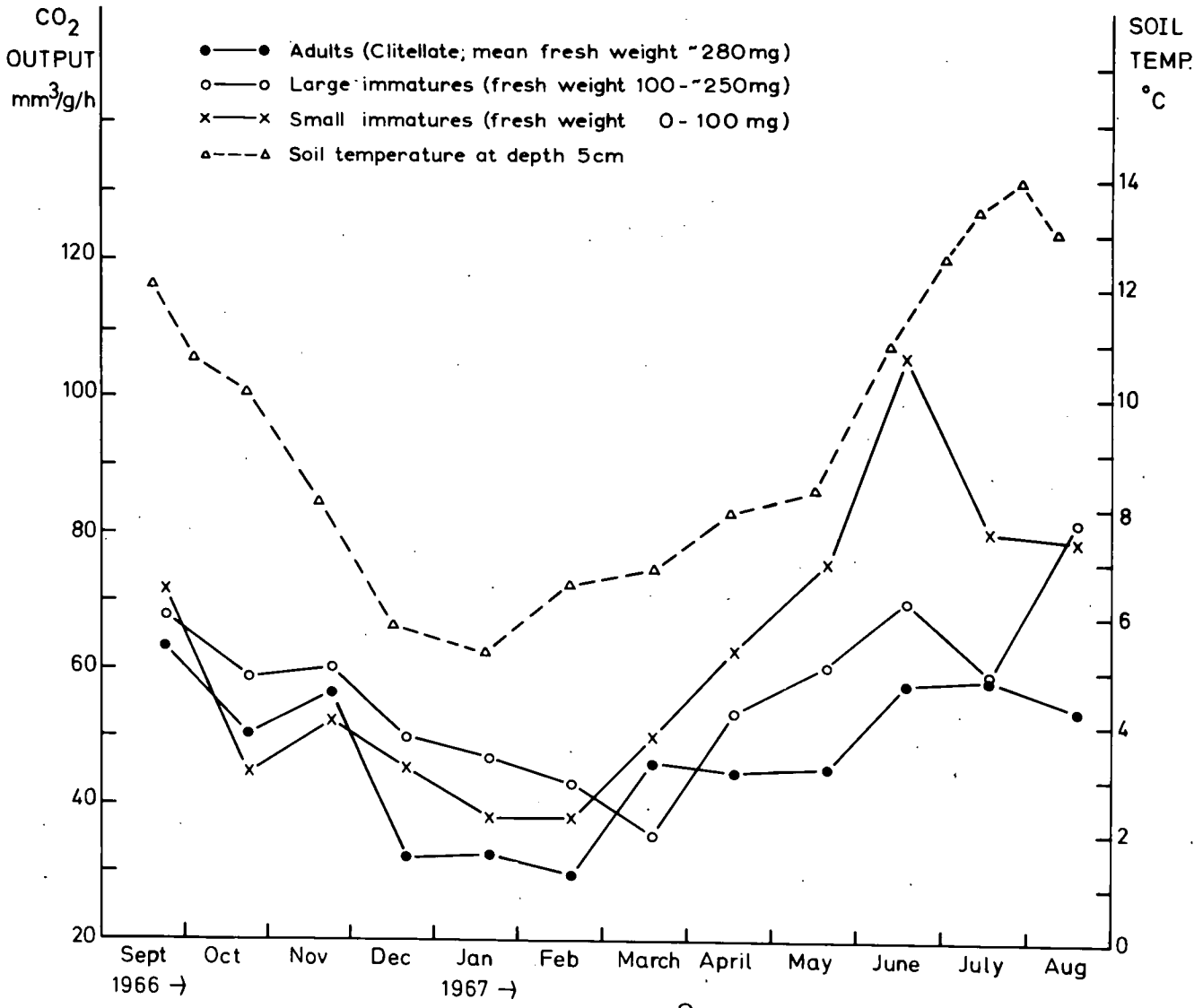


Fig. 33. Rates of carbon dioxide output at 10°C by each of the three major A.rosea size classes according to season. Field soil temperature shown for comparison.

Time of Year	D.rubida f. subrubicunda	L.castaneus	O. cyaneum	
			From total body weight	From body weight minus gut contents
Nov/Dec 1966	95.56	107.76	36.73	71.25
Jan/Feb 1967	90.47	111.15	35.63	69.13
March/April	80.05	94.39	32.86	63.74
May/June	73.56	107.57	42.16	81.77
July/Aug	97.55	99.90	37.69	73.11
Sept/Oct	96.58	105.91	35.89	69.63

Table 20. Rates of CO_2 output at 10°C ($mm^3 CO_2$/g. worm/hour) for three lumbricid species collected at Wynyard. Rates measured at two-monthly intervals over one year; all volumes corrected to N.T.P.

$mm^3O_2/g/h$ at 10^oC for O. cyaneum with (or without) gut contents, D. rubida f. subrubicunda and L. castaneus, respectively.

Discussion

Open system infra-red gas analysis has been used, amongst other things, for field measurements of photosynthesis and respiration in plants (Billings et al, 1960; Golley, 1965; Lemon 1960; Mooney and Billings, 1961; Scott and Billings, 1964; Hadley and Bliss, 1964) and the CO_2 output of soils (Loissant and Rapp, 1968). It has also been used in laboratory studies of carbon dioxide discharge by individual insects (Hamilton, 1959, 1961, 1964; Matsumoto, unpubl. - Saito, pers. comm.). In all studies known to the author, only a single experimental chamber was employed for infra-red gas analysis; the apparatus employed in the present studies incorporated an automatic gas switch system which allowed the sequential analysis of gas from three or more chambers, whilst maintaining continuous gas flows through all chambers throughout the experiment.

Although direct measures of oxygen consumption are more common in respiratory studies, the measurement of CO_2 output has been utilised quite extensively. In the apparatus used by King (1921), CO_2 output was measured as the weight increase of absorption columns containing soda lime. Rabinowitch and Bazin (1926) used the 'katharometer' - a device which utilised the comparative properties of CO_2 and air for heat conductivity,

applied to a Wheatstone bridge circuit. Closed system measures have been used by Gromadska (1962), Ito (1964) and other workers, but long-term respiratory measures in atmospheres of increasing CO_2 pollution were not thought reliable by the present author. CO_2 can depress respiratory activity in many organisms, and enhance it in others. Atmospheres containing little or no CO_2, such as were used in the present studies, have not been shown to affect respiration in any way.

Considerable attention has been devoted to the analysis of the soil atmosphere and its possible effects on the respiratory activity of soil-dwelling organisms. Raffy (1930) stated that the respiration of 'earthworms' was depressed in atmospheres of decreasing O_2 tension. Johnson (1942) studied the respiration of L. terrestris L. - the species probably studied by Raffy - at 10°C, in atmospheres of decreasing O_2 partial pressure. At partial pressures higher than about 80 mm. Hg the respiratory rate was constant. Below 80 mm. Hg, L. terrestris respiration decreased rapidly in proportion to the O_2 deficiency. Mendes and Almeida (1962) studied the tropical worm Pheretima hawayana and found a similar fall in respiration in atmospheres containing less than about 15% O_2. Byzova (1966) investigated the relation between percentage O_2 in the atmosphere and respiratory activity for four lumbricid species with differing habits. The respiration of D. octaedra (Sav.) - a surface active species - decreased uniformly with falling O_2% in the

atmosphere. E. foetida (Sav.) respiration showed a similar decline below about 15% O_2. In contrast, the soil-dwelling species A. caliginosa (Sav.) and A. rosea showed maintained levels of respiration in atmospheres reduced to only 10% O_2. Haughton et al (1958) showed different O_2 disocciation curves for haemoglobin from L. terrestris and A. longa Ude, the latter species requiring less O_2 for saturation, and related this to the different habits of the worms. Manwell (1959) found that O_2 disocciation curves for lumbricid haemoglobin were affected by pH, outlining the importance of the complex buffering systems employed by lumbricids for maintenance of pH stability in the body fluid.

Though Kupka and Schaerffenberg (1947) showed that E. foetida and L. terrestris could completely recover after 6 hours in an atmosphere of pure CO_2, it is known that high concentrations of CO_2 have a depressing effect on lumbricid respiration. However, Arthur (1965) has pointed out that lumbricids are more sensitive to O_2 lack than to increases in CO_2 in the surrounding atmosphere.

The commonly supposed situation of low O_2 and high CO_2 concentrations in the soil atmosphere has been shown fallacious in a variety of soil types. Russell (1961) gave a value of only 0.25% for CO_2 in the soil atmosphere at 15 cm. depth. Both Boynton and Compton (1944), in silty clay orchard soils, and Martin and Pigott (1965), in woodland soils, found that maximum CO_2 levels were only about 5% by volume. Boynton and Compton also showed that even in silty

clay soils the concentration of O_2 in the soil atmosphere was always greater than 10% by volume, and usually in the range 15 to 20%.

It may therefore be concluded that though the surface active lumbricid species may be sensitive to even small changes in atmospheric composition, the unpigmented, soil-dwelling species maintain constant respiratory levels regardless of minor variations which may occur in the soil atmosphere. Davis and Slater (1928) showed that any oxygen debts incurred by L. terrestris, during experimental periods of anaerobiosis, were compensated by the intake of equal amounts of O_2 on return to an aerated atmosphere. They concluded that the oxidation process was merely deferred during anaerobiosis. By the 'law of constant heat sums', respiration measurements in aerated atmospheres will therefore be equivalent to field rates for even the pigmented species, despite minor and temporary variations which may occur in the field soil atmosphere.

The gas pressure within the experimental chambers during the present respiratory estimates was not measured. It was assumed that this pressure would be slightly in excess of atmospheric pressure; Nielsen (1961) pointed out that pressure did not affect respiration in enchytraeids and nematodes and there is no reason to suppose that the situation differs for lumbricids.

The two day period of temperature acclimatisation was used in these studies for several reasons. Initially it had

not been established that the A. rosea respiratory Q_{10} relation was unaffected by temperature acclimatisation. Only two days were allowed to avoid masking field activity levels and to avoid the possible effects on absolute respiratory rates of long periods of acclimatisation (Agrell, 1947). The two day acclimatisation period was maintained throughout the study, despite the Q_{10} findings, since it eliminated any disturbance effects due to field collection, thereby ensuring comparability of data for the whole year.

The method described above was thought to be the most reliable technique available for estimations of lumbricid respiratory rates in the field at the time of the present studies. Attempts have been made to estimate field respiratory rates in various animal species using rates of radioisotope elimination (Odum and Golley, 1963; Reichle, 1967; Edwards, 1967). Such techniques could undoubtedly be applied to lumbricids and may prove valuable in the future.

On confinement in the respirometer, three of the lumbricid species used in the present studies showed initially high respiratory rates - more comparable to rates measured in the Warburg apparatus - which rapidly declined to a lower level over the first few hours. These three species were known to be normally inactive in the field. L. castaneus, the species showing the highest levels of activity in the field, was the only species which showed a consistent level of respiration from the outset. L. castaneus was observed to show high locomotory activity within the experimental

chambers throughout the periods of measurement. In contrast, A. rosea, O. cyaneum and D. rubida f. subrubicunda were only active during the first 2 to 4 hours of confinement. All the species retained a high proportion of their gut contents until the end of the first day; A. rosea, D. rubida f. subrubicunda and probably L. castaneus retained some food material in the gut throughout the 48 hour period. It was therefore concluded that the initially high respiratory rates for A. rosea, O. cyaneum and D. rubida f. subrubicunda were associated with abnormally high levels of locomotory activity, probably induced by the necessary handling of the worms prior to experiments. As this activity diminished, the respiratory rate adopted a consistent level, assumed to be equivalent to that in the field. The active mode of life shown by L. castaneus was considered responsible for the maintained high levels of respiration measured for this species. These levels were thus considered suitable for estimates of field respiration.

The variability in respiratory rate shown by D. rubida f. subrubicunda was mainly due to the low weights of worm tissue involved - this species was, on average, smaller at Wynyard than has been reported from other localities. Even small changes in gas stream CO_2 concentration produced appreciable fluctuation when converted to weight-based respiratory rates. Measures for this species were thus regarded as being at the sensitivity limits of the apparatus. The decline in respiratory rate to a low, consistent level

after about 30 hours for D. rubida f. subrubicunda and L. castaneus, and after about 50 to 60 hours for A. rosea, was considered due to the adoption of starvation levels of respiration. O. cyaneum was presumably more resistant to starvation, possibly an adaptation to life in deep, mineral soils.

The absolute respiratory rates measured for all A. rosea size classes during the summer months were similar to those obtained from animals measured using the Warburg apparatus, regardless of season or pre-treatment. These rates were also equivalent to those found by Byzova (1965) using the Warburg method. Respiratory activity at 10°C in this species was shown to be associated with the field soil temperature at the time of worm collection (see also Fig. 34 below). A. rosea respiration was not found to be significantly affected by temperature acclimatisation, and the animals had a similar gut content throughout the year (see Section III, sub-section 3, above). Q_{10} shift and starvation effects were therefore discounted and the observed annual cycle of A. rosea respiration was attributed to variation in field levels of locomotory activity. Locomotory activity was assumed to be directly related to field soil temperature, and to be not significantly affected by the two day acclimatisation period allowed in these studies. The animals were noted to be more generally dispersed within the 10°C experimental chambers during the warmer months. Evans and Guild (1948b) recorded a winter dormancy in A. rosea found in pastureland

soils. A. rosea at Wynyard became quiescent during the coldest months but did not cease feeding. The quiescence may have included some metabolic changes, normally associated with dormancy, which could have accentuated the decreased respiratory rates in these months. True dormancy, as shown for A. rosea in pastureland soils in the warmest and driest months (Evans and Guild, 1948a), has been shown to produce a lowering of lumbricid basal metabolic rate (Galissian, 1967). The summer dormancy was not shown by A. rosea in the permanently wet soils at Wynyard, hence the complete annual cycle of respiration rate in relation to soil temperature. Variation in poikilotherm respiration according to the level of locomotory activity has been shown by many workers, and extensively studied in relation to fish metabolism (Winberg, 1956; Edwards, 1967). Nielsen (1949) showed a positive relation between metabolic rate and activity in small nematodes, though it was not detectable in larger specimens.

This activity hypothesis suggests that lumbricid respiratory rates measured in the Warburg apparatus are abnormally high in normally inactive species, due to induced maximum levels of locomotory activity. These arguments were thought sufficiently feasible for absolute respiratory measures obtained by previous workers, using the Warburg method, to be re-assessed for inactive lumbricid species before application to the field situation.

Baldwin (1917) and Szymanski (1918) showed 24 hour

rythmns of activity in whole and bisected specimens of L. terrestris. Harker (1958) regarded the L. terrestris cycle of activity as endogenous. Ralph (1957) showed diurnal, lunar-day and lunar cycles of O_2 consumption in this species. However, the mode of life adopted by L. terrestris is somewhat eccentric - involving nocturnal habits of feeding and copulation. It was not, therefore, thought suprising that no such short-term cycles were found in any of the species used in the present studies.

Byzova (1965) showed that respiratory rate, per gram worm, was totally unrelated to worm body weight in adults of A. rosea. In the present studies, the estimation of A. rosea population metabolism was considered more reliably achieved by summation of estimates for the three major size classes, than by simple measurement of species mean values. However, Byzova's findings were thought sufficient justification for ignoring any possibility of significant respiration/worm weight dependence within these categories. Small immature worms were found to have the highest respiratory rates at $10^{o}C$ during the summer months. Large immatures showed slightly lower or intermediate rates in this season, and adult rates were minimal for the species. However, this classical situation was not maintained throughout the year. For most of the year the respiratory rates of all size classes were of a similar order. In winter, the rate of O_2 consumption (mm^3O_2/g.worm/h) by adults in the field was actually greater than that of the immature worms (see Fig. 34

below).

Zeuthen (1947, 1953, 1955) was amongst the first authors to postulate variations in metabolic rate within the life cycles of individual animals. More recently, Phillipson (1960, 1962, 1963) demonstrated seasonal variation in phalangid respiration which seemed associated with cycles of breeding activity. Studies on millipedes and isopods have also shown seasonal variations in respiratory rate associated with changes in body size, reproductive activity, moulting and the general physiological condition of the animals (Phillipson, 1966a; Phillipson and Watson, 1965). These workers have emphasised the importance of metabolic measures throughout the year for computation of mean annual figures in the formulation of energy budgets (Phillipson, 1966a, b; Phillipson and Watson, 1965). The life cycle of a very slowly reproducing lumbricid species, such as A. rosea, does not include the sharply defined changes in morphology and physiological condition shown by most arthropods. However, very marked seasonal cycles of respiratory activity at a constant temperature were shown for A. rosea in the present study. Differences in body size also produced some variation in respiratory rate per unit weight. Thus, even for a species of this type, year-round measures on individuals of different size classes were essential to obtain a realistic annual estimate of respiratory metabolism.

It was appreciated, from Byzova's results (Byzova, 1965) that the pigmented species D. rubida f. subrubicunda and

L. castaneus probably showed a significant relation between individual body weight and respiratory rate per gram worm, though this was doubtful for O. cyaneum. Group measures were considered suitable for comparison with data from other sources, but it should be pointed out that such mean rates are less suitable for estimates of population respiration where there exists a significant body weight/respiratory rate relation. Previous data on the species studied in this work are confined to those of Byzova (1965) since Gromadska's work, from the individual worm weights reported, could not be associated with L. castaneus - as claimed by this author (Gromadska, 1962). Byzova's measurements for A. rosea were of a similar order to those determined in the present studies - when the latter were converted to O_2 consumption rates, taking account of her experimental temperature (19°C), restriction to adult worms and exclusion of worm gut contents. However, there were differences in detail which have been discussed above. The mean respiratory rate of 75mm^3O_2/g. worm/hour for A. caliginosa (Sav.), measured by Barley and Jennings (1959) at an ambient temperature of 15°C, was similar to the present values obtained for adult A. rosea worms at similar temperatures.

Byzova's mean Warburg estimate of 152mm^3O_2/g. worm/hour for adults of L. castaneus was somewhat lower than the present mean species estimates in the respirometer, considering the ambient temperature used in her studies. However, she showed a highly significant inverse proportionality between

body weight and respiratory rate for adults of this species, and the extension of this relation into the immature size categories would seem probable. The higher respiratory rates measured in the present studies were thus most likely due to the inclusion of immature worms. Since locomotory activity was thought maximal for this species, regardless of environmental conditions, it seemed unlikely that measurements would be significantly affected by the use of conventional apparatus. However, L. castaneus respiratory rates measured in the Warburg apparatus during the present studies were also slightly lower than those measured in the respirometer. This could be due to the spacial limitations within a Warburg flask for a species as active as L. castaneus.

There was, to the author's knowledge, no published information on the respiratory rate of D. rubida f. subrubicunda at the time of the present studies. The present Warburg measurements for this species during the winter months were 30 to 40% higher than those measured in the respirometer, In other seasons, when the metabolism of the animal seemed more limited by environmental conditions (see R.Q. investigation above), respiratory measures were similar in both types of apparatus. Lower rates of CO_2 output by D. rubida f. subrubicunda in the spring and early summer were thought to be associated with increased activity of the calciferous glands. It was thought that in winter this species was physiologically capable of showing abnormally high levels of locomotory activity, and hence respiratory rate, in response

to the irritating stimuli within a Warburg flask. In other seasons, the species may have been under varying but continual degrees of physiological stress at Wynyard; it may thus have been incapable of significant locomotory responses to irritating stimuli.

There were no published measures of O. cyaneum respiratory rate previous to these studies, but Byzova gave figures for adult O. lacteum (Oerley) at 19°C. O. lacteum respiratory levels (mean: 75mm^3O_2/g.worm/hour) were higher than those measured for O. cyaneum at 10°C in the present study. O. lacteum is a smaller species, with habits which differ from those of O. cyaneum; there was no information available on the respiratory Q_{10} relations for either species. The generic affinities of these lumbricids may have little relevance to their rates of metabolism and critical comparisons would be meaningless on the information available. Warburg measurements of O. cyaneum respiration, performed in the present studies, were generally of a similar order to those found in the respirometer. The Warburg measures, however, were highly variable and of dubious reliability - yielding R.Q. values over an abnormally wide range. It was concluded that estimates of O. cyaneum respiratory rate will be most reliable when measured under simulated field conditions, such as were found in the respirometer used in the present studies.

(ii) Field Population Respiratory Metabolism of Allolobophora rosea (Sav.) f. typica at Wynyard

Introduction

Englemann (1966) outlined three approaches to bioenergetics studies: study of the metabolic rate of individual organisms and the changes associated with environmental stress, analysis of population maintenance energy, and the tropho-dynamic or food-chain approach. Investigations of individual metabolic rates have usually been associated with studies in comparative physiology, but when applied to field populations - as in the second energetics approach (usually attributed to Bornebusch, 1930) - they assume a new ecological importance. Obviously, the third approach (originally after Lindeman, 1942) - where all aspects of energy flow through species populations, food chains and community webs are quantitatively evaluated - will yield the most ecologically valuable information. However, an integral part of such quantifying analyses involves the estimation of population respiratory metabolism - or maintenance energy - since this aspect of energy flow has been shown to account for a high proportion of the energy assimilated by many organisms.

Estimates of population biomass and the rate of heat loss per unit weight are required for the calculation of maintenance energy. Heat loss can be measured by direct calorimetry, but the measurement of gaseous exchange rates, with subsequent use of a calorific conversion factor, is more accurate for small organisms. These calculations may

seem simple, but there are many complicating factors in the interpretation of laboratory measurements in terms of the field situation. Direct field measurements of respiratory metabolism are rarely possible, and even these estimates present problems of reliability according to the habitat and behaviour of the species concerned.

In the present study, for a woodland population of the lumbricid A. rosea, attempts were made to eliminate the artificial effects of laboratory conditions. Conditions in the field habitat were assessed throughout the year and simulated or compensated, wherever possible, in the computation of a reliable estimate of A. rosea annual maintenance energy expenditure.

Methods

The monthly measurement of A. rosea weight-based CO_2 output rates at 10°C, under simulated field conditions of substrate volume and density, light intensity, pH, moisture levels and non-limiting gaseous atmosphere, was described in detail above. These CO_2 output rates were converted to rates of O_2 consumption at 10°C, using the seasonal measures of R.Q. for this species. The slope of the respiratory Q_{10} relation for A. rosea was used to convert the monthly respiratory estimates at 10°C to values for O_2 consumption at the measured field soil temperatures. These 'spot' field estimates were then used to compute mean monthly estimates of O_2 consumption rate, per gram of worm fresh body weight. This complete procedure was carried out for each of the

major A. rosea size classes.

Using the fresh/dry/weight relation for A. rosea individuals - determined during the present studies - the monthly estimates of A. rosea numbers and dry weight biomass per metre square at Wynyard were converted to their fresh weight biomass equivalents. A simple multiplication then produced an estimate of field O_2 consumption rate per metre square for each A. rosea size class in each month throughout the year. An oxycalorific coefficient (see Part III, Section I, sub-section 4) was employed to assess the equivalent rates of respiratory heat loss per metre square. Monthly totals were calculated for each size class and these were summated for total species estimates. Seasonal and annual totals were also calculated for each size class, and for the total A. rosea population per metre square.

Results

Fig. 34 shows the mean monthly rates of O_2 consumption (mm^3O_2/g.worm/h) in the field for each of the three major A. rosea size classes. All size classes showed a pronounced annual cycle of field respiratory rate. Respiratory activity was maximal during the warm summer months, falling rapidly during autumn to the minimal winter values. Respiration increased rapidly during the spring, though at a generally slower rate of change than the corresponding autumn decline. The complete annual cycle covered a narrower range in adults, whose respiratory rates were higher in winter and lower in summer than those of immature worms.

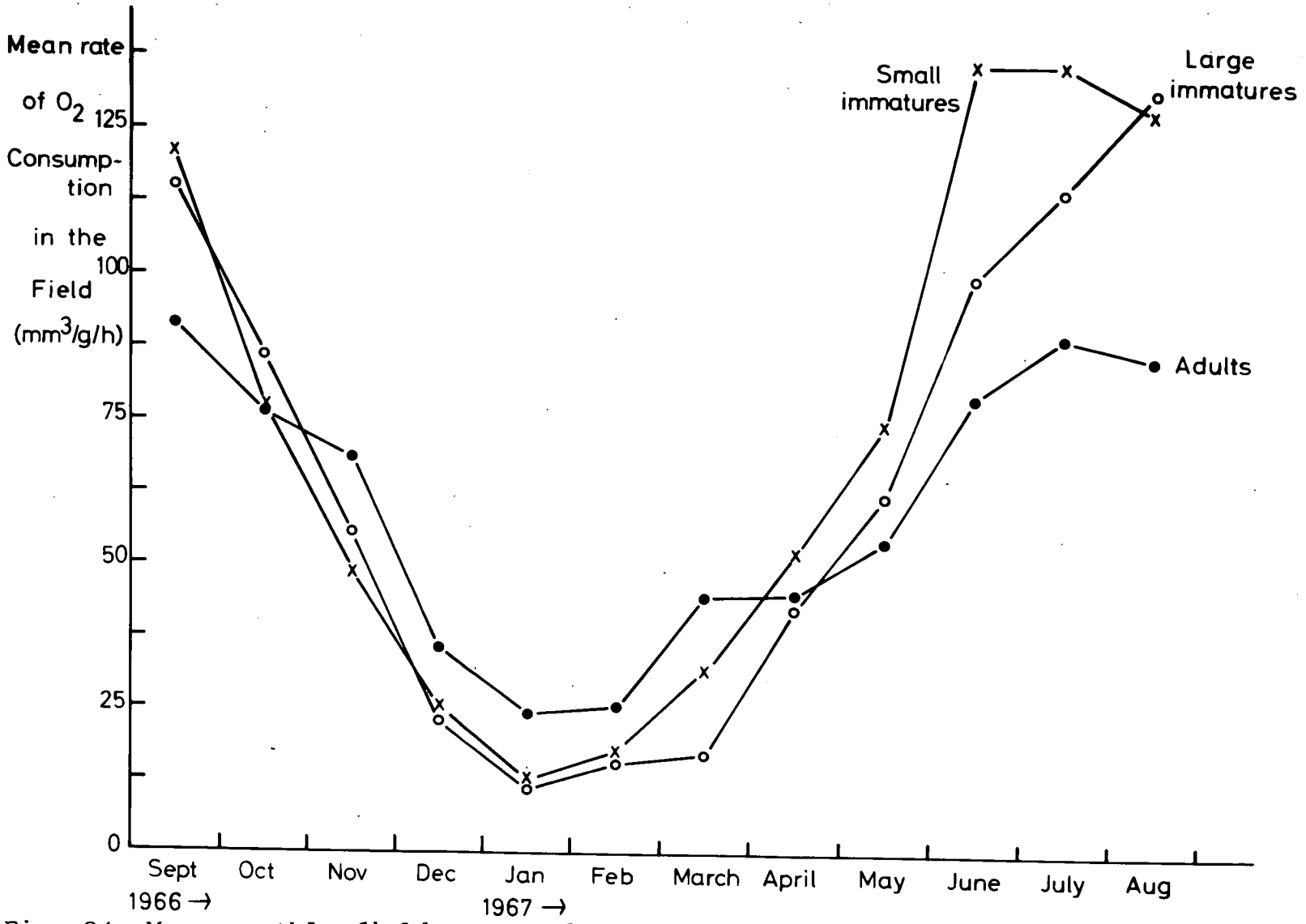


Fig. 34. Mean monthly field rates of oxygen consumption by each of the three major A.rosea size classes at Wynyard (1966/67).

Table 21 shows the monthly respiratory heat losses per metre square, and seasonal totals, for each of the A. rosea size classes. Maintenance energy expenditure was minimal during the winter months. Variation in biomass was of secondary importance to the temperature-dependent variation in respiratory rate in the determination of population heat energy losses through the year. The annual totals for maintenance energy expenditure were 2.270, 6.259 and 4.298 Kcals/m^2 for the small immatures, large immatures and adults, respectively, and 12.727 Kcals./m^2 for the total A. rosea population.

Discussion

In a poikilotherm species which shows a positive relation between respiratory rate and ambient temperature, the field respiratory metabolism of individual animals might be expected to accord with variations in the field soil temperature regime. In A. rosea this accordance appeared to be accentuated by a temperature-dependent behavioural response which bore a positive relation to the rate of respiratory metabolism. It was noted that the annual cycle of oxygen consumption rate was better defined (i.e. with less short-term fluctuation) than the equivalent cycle for CO_2 output. It seemed probable that seasonal R.Q. variations were more related to calciferous gland activity than to variation in substrate metabolism.

The narrower annual range covered by adult respiratory rates was to be expected from the lower respiratory responses to ambient and field temperature variation found in this size class.

Month	SEASON	Small Immatures		Large Immatures		Adults	
Oct.	AUTUMN	0.160	0.264	0.826	1.713	0.478	0.860
Nov.		0.104		0.887		0.382	
Dec.	WINTER	0.069	0.183	0.261	0.423	0.120	0.433
Jan.		0.032		0.040		0.079	
Feb.		0.036		0.069		0.080	
March		0.092	0.793	0.107	0.967	0.306	1.253
April	SPRING	0.272		0.298		0.534	
May		0.365		0.402		0.439	
June		0.220	1.029	0.427	3.056	0.255	1.753
July	SUMMER	0.237		0.493		0.457	
Aug.		0.338		1.247		0.647	
Sept.		0.343		1.103		0.521	

Table 21. Monthly and seasonal total estimates of population respiratory heat loss (Kcals./m^2) for the three major A. rosea size classes at Wynyard (1966/67).

Nielsen (1961) estimated the annual maintenance energy expenditure of three grassland nematode populations to be in the range 150 to 340 Kcals./m^2. He also gave estimates for grassland enchytraeid populations in the range 34 to 154 Kcals./m^2. O'Connor (1963) measured a similar figure (150 Kcals./m^2) for three enchytraeid species in coniferous forest soils. The oxygen consumption of an oribatid mite population in a deciduous forest soil was estimated by Berthet (1963, 1964) as 4.49 litres/m^2/yr. - or 21.6 Kcals/m^2/yr. Estimates for soil collembolan populations have ranged from 12 Kcals./m^2/yr. in moorland soils (Healey, 1967b) to 153 Kcals./m^2/yr. in grassland soils (Macfadyen, 1963b). These selected examples give some idea of the range of values to be expected in estimations of population respiratory metabolism for soil-dwelling organisms. There have been many other annual estimates, ranging from 30,000 Kcals./m^2 for the respiratory heat losses by primary producers in a salt marsh community (Teal, 1962) to 23 Kcals./m^2 for elephants in a savannah ecosystem (Petrides and Swank, 1965), but these are more useful for general considerations such as the comprehensive surveys undertaken by Englemann (1966, 1968).

The quoted figures for soil organisms were all for species groups and it must be noted that even numerically dominant species, considered singly, will contribute only a limited proportion of the total heat losses for a species group hierarchy in a climax or sub-climax situation. This is the essence of a community web structure. Thus Healey

(1967b) found that a common moorland collembolan species, Onychiurus procampatus Gisin, contributed only 2.6 Kcals./m^2 to the estimated annual total heat losses by the whole collembolan population (12 Kcals./m^2). The present estimate of 12.7 Kcals./m^2/yr. for A. rosea at Wynyard appeared reasonable for a large soil-dwelling invertebrate, with a normally inactive mode of life, living in a poor quality soil of low nutrient status. The maintenance energy expenditure of the total lumbricid population occupying the top 30 cm. of the soil at Wynyard has been estimated below. The secondary importance of biomass variations in determining the A. rosea population maintenance energy expenditure might be expected in such a long-lived, slowly reproducing animal. An interesting feature of the seasonal analysis was the observation that this topsoil lumbricid species, despite continued feeding and apparently normal behaviour, was metabolically dormant during the winter months (see also: suppression of tissue production during winter - Part II, Section II, sub-section 1).

(iii) An Estimate of the Total Lumbricid Population Respiratory Metabolism at Wynyard

Introduction

Despite the generally poor quality of the soils at Wynyard, an estimate of the total maintenance energy expenditure by the lumbricids occupying the top 30 cm. of the soil was considered useful for comparisons with data for other soil-dwelling groups. Rates of heat loss by the various

species and species group components of the lumbricid population at Wynyard could be compared with biomass determinations made during the present studies.

Methods

For A. rosea (Sav.), O. cyaneum (Sav.), D. rubida (Sav.) f. subrubicunda (Eisen) and L. castaneus (Sav.) the respiratory rates determined in the present studies were used in these approximate calculations of population respiratory metabolism. For A. caliginosa (Sav.), E. tetraedra (Sav.), L. terrestris L. and D. octaedra (Sav.) the respiratory rates given by Byzova (1965) for adult worms were used in these analyses. The use of these adult rates for worms of all sizes was assumed to largely compensate for any accentuation of respiration in the Warburg apparatus. B. muldali Omodeo and A. chlorotica (Sav.) were assumed to have respiratory rates approximating to those of A. rosea, since these species have a similar mode of life. For the same reason, D. mammalis respiratory rates were assumed to approximate to those found for D. rubida f. subrubicunda in the present studies.

In view of the present findings for A. rosea, and the results of previous work in this field (Konopacki, 1907; Kirberger, 1953; Gromadska, 1962), the general lumbricid relation between respiratory rate and ambient temperature was assumed to approximate to Krogh's curve (Krogh, 1914, 1916, 1941), i.e. to a Q_{10} value of 2 over the normal soil habitat temperature range (approximately $3^{o}C$ to $20^{o}C$).

The biomass figures used for these estimates were given in Section I, sub-section 2, above. Worm dry weight/fresh weight relations were derived from the present findings and those of Durchon and Lafon (1951), Grant (1955b), French et al (1957), Bouché (1967) and Satchell (1968).

Population respiratory metabolism was estimated monthly for each lumbricid species present in the grid area at Wynyard. These estimates were summated to give annual total figures for individual species and species groups.

Results

Total annual estimates for the population respiratory metabolism of lumbricid species and species groups at Wynyard are shown in Table 22. The annual maintenance energy expenditure of the total lumbricid population - occurring to a depth of 30 cm. in the soil- was estimated as 101.059 Kcals. per metre square. The deep-burrowing species, L. terrestris and O. cyaneum, contributed approximately 75% of the total heat losses. The contribution of A. rosea equalled the combined total of all other topsoil and surface active species.

Discussion

The estimates shown in Table 22, as indicators of energy flow, may be compared with the relative biomass proportions shown in Fig. 14. The estimated contribution of O. cyaneum was similar in both cases. However, L. terrestris contributed less to maintenance energy expenditure than to the total lumbricid biomass. Thus the contribution of these deep-

Lumbricid Species and Species Groups	Kcals./m^2	Major Lumbricid Groups	Kcals./m^2
L. terrestris	27.115	All topsoil and surface active species	25.451
O. cyaneum	48.493	Deep-burrowing species (O. cyaneum L. terrestris)	75.608
A. rosea	12.727	All unpigmented species	64.201
Rare unpigmented species (A.caliginosa; A. chlorotica; E. tetraedra; B. muldali.)	2.981	All pigmented species	36.858
Surface active, pigmented species (L. castaneus; D. rubida f. subrubicunda; D. octaedra; D. mammalis)	9.743	Total lumbricid population	101.059

Table 22. Total annual estimates for the population respiratory metabolism (Kcals./m^2) of various species and species groups comprising the total lumbricid population at Wynyard (1966/67).

burrowing species was reduced from 84% of the total biomass to 75% of the population heat losses. The proportions contributed by both A. rosea and the small, surface-active, pigmented species were correspondingly increased by consideration in terms of population respiratory metabolism. The minimal contributions of the rare, unpigmented topsoil species were similar in both cases. Contributions to the total lumbricid population maintenance energy expenditure by the pigmented species and the unpigmented species were in the approximate ratio of 3 : 5.

The total lumbricid heat loss at Wynyard (101.059 Kcals./m^2/yr.) was intermediate between the similar estimates for enchytraeid worms in grassland - 34 and 154 Kcals./m^2/yr. - by Nielsen (1961). The lumbricid estimate was lower than that by O'Connor (1963) for three enchytraeid species in coniferous forest soils (149.5 Kcals./m^2/yr.). Enchytraeids are known to be better adapted to acid soil conditions - such as occur in coniferous forest soils; more neutral grassland soils and acid clay woodland soils may therefore be similarly restrictive habitats for enchytraeids and lumbricids, respectively. Total lumbricid population respiratory metabolism may certainly be expected - from the density estimates reviewed by Satchell (1958) - to be considerably greater in 'better' soils than the present estimate for the Wynyard habitat.

It was noted that with the temperature regime, lumbricid

species composition and average total population density (101.69 worms/m^2) prevailing at Wynyard, the 'average' annual heat loss was almost exactly 1 Kcalorie per lumbricid worm.

PART III

I Determination of Calorific Values

1. Introduction

Studies of energy accumulation, transformation and transfer within the biosphere - in contrast to studies of nutrient cycling - evaluate biological systems in terms of a single unit of measurement. This unit - the calorie, or its multiple the kilogram-calorie (Kcalorie) - thus forms the basis for all analytical and comparative analyses of ecological energetics. This approach necessitates the evaluation of all materials relevant to an energetics study in terms of their calorific contents. These evaluations are achieved by the complete oxidation of samples of each item, with simultaneous (or subsequent) measurement (or estimation) of the energy released as heat. In other words, the oxidative metabolism practised by organisms for the breakdown and utilisation of high energy food sources is artificially simulated for the purpose of quantification.

Ivlev (1934) outlined a wet combustion method for calorific determinations. The energy released during chromic acid digestion of organic materials was estimated subsequently from the volume of oxygen consumed. The estimation was essentially a determination, by difference, of the chromic acid used in the digestion process - involving titrations with ferrous ammonium sulphate solution, using diphenylamine as an indicator (after Knop, 1924). The major difficulty with

this technique was that proteins were incompletely digested and inaccuracies arose, despite corrections by nitrogen determination. The method was also time-consuming and involved a considerable degree of expertise in the preparation and administration of solutions.

Bomb calorimetry, in which the heat released by combustion of materials in an atmosphere of pressurised oxygen is measured directly, is the method of calorific content determination in more common usage. Adiabatic instruments, incorporating a temperature-equilibrating water jacket, give maximum accuracy since heat exchanges are minimised. Such instruments were originally of bulky construction, suitable for only large samples. Since the amounts of biological material available are often limited, the arrangement was unsatisfactory in this form. However, Slobodkin and Richman (1960) - also Richman and Slobodkin (1960) - miniaturised the adiabatic system for use with samples weighing as little as 6 mg. Phillipson (1964) has since described a miniature bomb calorimeter in which the water jacket has been discarded with a negligible decrease in accuracy of determination.

Slobodkin and Richman (1961) showed that the calorific values of individuals from 17 animal species, representing 5 phyla, had a skewed normal frequency distribution with a low variance. Animal tissues may thus appear to have similar calorific contents, regardless of taxonomic type. However, as Macfadyen (1963a) pointed out, the energy values involved are quantitatively very large so that even an apparently

small degree of variation can involve substantial differences in absolute terms. Wiegert (1965b) showed that different life stages of the meadow spittlebug (Philaenus spumarius L.) have different calorific values which cover the complete range measured by Slobodkin and Richman. It is thus imperative that quantitative assessments of the calorific values of individual species, in all their life stages, are performed in studies of ecological energetics.

Cummins (1967) has accumulated data from 38 authors and compiled a list of calorific equivalents for a wide range of biological materials. As check-lists of this type are expanded in the future, workers in the field of ecological energetics may be spared the necessity of numerous calorific determinations. Unfortunately, information on lumbricid calorific equivalents is extremely limited and no values for lumbricid worms appear in the Cummins list. Calorific determinations for a variety of lumbricid species were therefore undertaken in the present work, both for the purposes of the present studies and for the benefit of future workers in this field.

Calorific values for the faeces of A. rosea (Sav.), and for the soil medium inhabited by this species, were determined in the present studies in order to evaluate certain parameters of population energy flow. These materials could not be analysed by the common methods, the technique used representing a new application for an apparatus not previously employed in studies of ecological energetics.

The only other calorific equivalent employed in the present studies was an oxycalorific coefficient for indirect calculations of maintenance energy expenditure. The oxycalorific coefficient selected for use in this work and the reasons underlying this selection are presented in sub-section 4, below.

2. Methods Employed in the Present Study for Calorific Content Determination

(i) Bomb Calorimetry

All materials used for determinations of calorific content were initially dried in a vacuum oven at 60°C and stored over desiccants (anhydrous calcium chloride and self-indicating silica gel). Worms and cocoons of the lumbricid species D. rubida (Sav.) f. subrubicunda (Eisen), D. octaedra (Sav.), L. castaneus (Sav.) and L. terrestris L. collected during the one year population survey at Wynyard were used for calorific content determinations. Worms of the species E. tetraedra (Sav.) and cocoons of O. cyaneum (Sav.) and A. rosea (Sav.) were also derived from this source; cocoons of E. tetraedra were not available for these studies. Worms of the species A. caliginosa (Sav.) and O. cyaneum were collected from Wynyard in June, 1968, and their gut contents removed before drying. Cocoons of A. caliginosa were not available for these studies. A. rosea worms were collected seasonally (November, 1967; January/February, 1968; April/May, 1968; July/August, 1968.) from Wynyard and their gut contents removed before drying.

Worms whose gut contents had been removed (A. rosea, A. caliginosa and O, cyaneum), worms of the species L. terrestris and cocoons of the species L. terrestris and O. cyaneum were ground and homogenised using a Culatti/Glen Creston mill - taking great care to recover all material from the internal surfaces of the mill after grinding. All other materials

were ground and homogenised in a small agate pestle and mortar. Adult and immature worms were treated separately in all cases; A. rosea small immature and large immature worms were also treated separately. The three major size classes of A. rosea worms were analysed separately for each season of collection. All other materials were grouped to obtain mean estimates.

Each ground material was formed into compressed pellets suitable for accurate weighing and calorific analysis. Pellets were weighed before and after ignition, using an E.M.B.-1 electromicrobalance (Research and Industrial Instruments Co., London). The bomb calorimeter used was that produced by the Gentry-Wiegert Inc. (Aiken, S. Carolina) on the design described by Phillipson (1964). Three or four determinations were performed for each sample material and the bomb was calibrated by the similar ignition of fifteen dried pellets of pure benzoic acid.

From knowledge of the relation between worm dry weight including and excluding gut contents, weight-based calorific values for whole dried worms of A. rosea, A. caliginosa and O. cyaneum were calculated. Calorific values based on ash-free dry weights were calculated for all lumbricid materials.

(ii) Differential Thermal Analysis

A group of twenty worms of each of the major A. rosea size classes was placed in each of three 3 litre glass beakers containing moist, homogenised (see p. 216 above), mull-type 'bramble' soil from Wynyard. One of the three beakers for

each size class was placed at each of the constant temperatures 4.4°C, 10.0°C and 14.8°C. The beakers were covered with perforated polythene sheet to prevent worm escape. Samples of the original homogenised soil medium were kept in 100 ml. glass beakers at 10.0°C.

After one week prospective faeces were collected from individual worms by gentle pressure on the terminal segments, using a rounded glass rod. The ejected faeces from all individuals of a particular size class living at the same temperature were grouped into a single glass tube. All faeces samples, and the original soil samples, were then dried in a vacuum oven at 60°C and stored over desiccants. Prior to analyses, all materials were ground to a fine, but not powdery, texture and homogenised using an agate pestle and mortar. Two or three approximately 4 mg. samples of each material were subjected to calorific analysis, using the Micro Differential Thermo Analyser, model M1 (Bureau de Liaison). The analyser was coupled to a Kipp and Zonen potentiometric recorder, model BD 1, and control unit.

Each sample was weighed, by difference, in a platinum crucible. Crucible and sample were placed in a thermocouple unit which functioned as a crucible-holder within the cylindrical base of the combustion chamber. An adjacent thermocouple held a crucible containing a thermally inert material of similar texture to that of the sample. Loose-fitting platinum lids were placed on both crucibles and a cylindrical cap completed the combustion chamber enclosure.

The complete combustion chamber, in the centre of which the crucibles were supported on the thermocouple wires, measured 8mm. in diameter by 10mm. in depth and was loosely jointed to facilitate internal and external gaseous exchange. Throughout these analyses a continuous stream of oxygen at low pressure was passed over the combustion chamber which was supported by a narrow 'stem' attached to the base of the instrument. The combustion chamber and 'stem' unit were covered by a thermoequilibrating jacket in the form of an inverted 'test tube'. A wide-bore, cylindrical electric furnace (uniformly coil-wound) was preheated to approximately 200°C and lowered over the thermoequilibrating jacket; the temperature controller and recorder were then activated. The temperature controller raised the furnace temperature by 13°C, ± 0.5°C, per minute and the recorder continuously registered any temperature differences between the sample and reference crucibles. Upscale recorder deflections represented exothermic reactions in the sample and downscale deflections represented endothermic reactions. Extended reactions were recorded as deflection peaks, the areas of which were proportional to the total heat exchanges in calories. Peak areas were measured using a planimeter. For maximum accuracy and comparability, the instrument was calibrated using standard materials previously analysed by use of the Gentry-Wiegert bomb calorimeter. Of the materials tested, ground cocoons of O. cyaneum were chosen for the final calibration

since this material showed maximum uniformity of calorific content, whilst producing peak areas of similar form to those produced by faecal and soil materials.

3. Calorific Values

(i) Lumbricid Worms and Cocoons

Table 23 shows average seasonal and annual calorific values for **A. rosea** (Sav.) worms of the three major size classes. No significant differences in calorific content occurred between either size classes or seasonal values determined for the same size class. Significance was assessed according to the 'acceptable' range of 3 to 4% variation between individual determinations for a single material (Golley, 1961); this range coincided with that found during the present studies. The only exception to this constancy of calorific content was the slightly higher value for large immature worms in 'summer'. This difference was not apparent in the ash-free values and was attributed to chance.

Mean annual values for adult and immature worms of **A. rosea** are shown in Table 24 with the results of determinations for worms and cocoons of a variety of lumbricid species. Calorific values based on ash-free dry weights were remarkably similar for different species, though the complete range (approximately 5.3 to 5.7 Kcals./ash-free gram dry weight) extended beyond the 4% maximum for individual determinations. Values for adult and immature worms of the same species were also similar (with the 4% range) in most cases. Immature worms of D. rubida (Sav.) f. subrubicunda (Eisen) showed a slightly higher calorific content, per ash-free gram dry weight, than that found for

Size Class:		Small immatures	Large immatures	Adults	All worms
AUTUMN	Dg	5.185	5.275	5.235	5.232
	W	3.370	3.080	3.201	3.217
	Af	5.525	5.651	5.621	5.599
WINTER	Dg	5.189	5.364	5.327	5.293
	W	2.994	3.558	3.386	3.313
	Af	5.632	5.653	5.667	5.651
SPRING	Dg	5.314	5.292	5.161	5.256
	W	2.966	3.042	3.076	3.028
	Af	5.632	5.616	5.581	5.610
SUMMER	Dg	5.005	5.621	5.257	5.294
	W	3.057	3.328	3.175	3.187
	Af	5.585	5.636	5.602	5.608
WHOLE YEAR	Dg	5.173	5.388	5.245	5.269
	W	3.097	3.252	3.210	3.186
	Af	5.594	5.639	5.618	5.617

Table 23. Average calorific values (Kcals./gram dry wt.) for worms of the species A. rosea at Wynyard. Dg - Excluding gut content weight; W - Including gut content weight; Af - Based on ash-free dry weight.

Species	Adults			Immatures			Cocoons	
	Dg	W	Af	Dg	W	Af	W	Af
A. rosea	5.245 (92)	3.210	5.618	5.281 (187)	3.175	5.617	5.283 (35)	5.383
A. caliginosa	4.798 (15)	2.927	5.312	5.123 (15)	3.125	5.517	-	-
O.cyaneum	4.576 (15)	2.791	5.369	4.518 (16)	2.756	5.356	5.205 (156)	5.255
E.tetraedra	-	4.098 (14)	5.617	-	4.087 (40)	5.560	-	-
D.rubida f. subrubicunda	-	4.209 (71)	5.410	-	4.359 (104)	5.734	5.236 (40)	5.321
D.octaedra	-	4.648 (27)	5.531	-	4.676 (62)	5.424	5.139 (49)	5.361
L.castaneus	-	4.191 (33)	5.571	-	4.416 (93)	5.568	5.154 (43)	5.279
L.terrestris	-	3.983 (19)	5.528	-	4.117 (76)	5.435	5.227 (225)	5.362

Table 24. Calorific values (Kcals./gram dry wt.) for worms and cocoons of several lumbricid species found at Wynyard. Number of individuals used in each group analysis shown in parentheses. Key as for Table 23, above.

adults of this species. Cocoon calorific equivalents were similar for all species studied, and in each case similar to, or less than, worm calorific contents for the same species. Calorific equivalents based on worm weights including gut contents were substantially lower for the unpigmented species A. rosea, A. caliginosa (Sav.) and O. cyaneum (Sav.) than those for E. tetraedra (Sav.) and the pigmented lumbricid species.

Discussion

In contrast to the findings of Wiegert (1965b) for a cercopid species, the present studies have shown a consistency of A. rosea worm calorific values regardless of the stage of development. Worms of A. rosea were also shown to have the same calorific equivalent throughout the year - though this may not be the case in situations where a summer dormancy occurs.

Ivlev (1934) reported that 'earthworm' (probably L. terrestris L.) protein constituted 72% of total worm dry weight and 76% of the ash-free dry weight. Similar protein estimates, in the range 54% to 72% of worm dry weight, were derived from the studies of Russell (1910), Lawrence and Miller (1945), French et al (1957) and Satchell (1963). Kollmansperger (1952) and Watson and Smith (1956) reported the protein content of lumbricid tissues to be 'high'. It was not suprising, therefore, that lumbricid worm calorific values were found to be very similar to that of pure mammalian muscle protein (5.65 Kcals./gram dry weight - Brody 1945). The consistency in the present results for different

species suggested a uniformity of lumbricid tissue composition, regardless of mode of life. Since muscle protein must be reduced in the less active, unpigmented species, it was concluded that other body constituents are reduced to a proportionate extent - possibly to be replaced by the extra gut volume found in these soil-ingesting species.

The only previous determinations of lumbricid worm calorific content were those by French et al (1957). They analysed 'one pint' of each of the species L. terrestris, L. rubellus Hoffmeister and a species 'tentatively classified' as A. rosea, estimating values of 4.42, 4.70 and 4.73 Kcals./ gram dry weight, respectively. They 'partially' removed gut contents from these worms before analysis, yet the L. terrestris estimate was only slightly higher than that found in the present study for intact worms of this species. Since they reported a 'partially cleared' L. terrestris ash content of 23% whilst whole dried worms in the present study contained only 24 to 28% ash, it seemed probable that these workers achieved only a slight degree of gut clearance for this species. Their value for 'A. rosea'- whether this 'very active' species was in fact A. rosea or A. caliginosa - was greater than the present whole worm estimates for Allolobophora species. Their estimate was close to the present value obtained for gut-cleared A. caliginosa, but significantly less than that for gut-cleared A. rosea worms. It remained in some doubt whether their value was for efficiently gut-cleared A. caliginosa specimens or inefficiently

cleared A. rosea worms. The practical significance of their value for L. rubellus was doubtful, though the situation regarding the similarly pigmented L. terrestris (see above) suggested that the L. rubellus figure was more applicable to studies involving worms with gut contents intact.

Paine (1964, 1966) outlined problems in calorific determinations where the inorganic constituents of sample materials include salts, such as calcium carbonate, which break down endothermically at ignition temperatures. Since the gut contents of soil-ingesting lumbricid species were entirely removed before thermal analyses in the present study, these arguments have little relevance to the present results of bomb calorimetry. However, they are discussed more fully in relation to faeces and soil calorific equivalents (see below).

By eliminating gut contents from calorific analyses for soil-ingesting species, it was assumed that the gut material had negligible calorific value in relation to that of the lumbricid tissue. Thermal analyses of A. rosea faeces and the soil medium (see below) and the present estimates of A. rosea gut content weight (see Part II, Section III, subsection 3) supported this assumption. It was also noted that the yield of A. rosea, as an 'energy unit', does not include gut content energy when passed to the mole predator, since moles usually squeeze out lumbricid gut contents prior to worm ingestion (Godfrey and Crowcroft, 1960).

The lumbricid calorific equivalents estimated in the present studies were for worms collected from clay soils on

the Wynyard site, and this should be considered in future applications of these data. The calorific values based on ash-free dry weights and on dry weights of gut-cleared worms may be regarded as reliable for any situation. However, values based on whole worm dry weights may have been influenced by the gut content weight - related to the Wynyard habitat - of the specimens used. These considerations were probably negligible for E.tetraedra and the pigmented species, but possibly significant for the soil-ingesting species A. rosea, A. caliginosa and O. cyaneum. For the latter species group it would be necessary to determine worm gut content dry weight proportions in order to calculate calorific equivalents, in terms of total dry weight, for worms from other soil types. The present estimates of cocoon calorific equivalents should be applicable in any situation. Cercopid egg calorific values, determined by Wiegert (1965), were considerably higher than those for the hatched insects. The values obtained for lumbricid 'eggs', or cocoons, were probably reduced by the influence of the cocoon capsule - thought to be of low energy content.

(ii) Soil and Faecal Materials

Calibration of the differential thermo-analyser, using O. cyaneum cocoon material, yielded highly consistant peak area conversion factors (mean : 5.181 cals. ± 0.078 cals. (St. Dev.) per peak area unit).

Homogenised, mull-type 'bramble' soil from Wynyard had an organic calorific equivalent of 0.6186 Kcals. ± 0.0137

Kcals. (St. Dev.) per gram total dry weight.

The results of organic calorific content determinations for **A. rosea** faeces are shown in Table 25. The faeces calorific values showed little variation, and were consistently higher than the soil medium calorific content (see above). Both small and large immature faeces showed maximum calorific equivalents when worms were kept at 14.8°C. However, since only a small number of samples were analysed, the differences in faeces calorific content could not be regarded as significant - hence the calculation of size class mean values, regardless of collection temperature. Faeces collected from large immature worms showed the highest mean calorific content; the significance of this difference is doubtful, being entirely due to the high value for faeces collected at 14.8°C.

Discussion

The only previous measures of 'soil' calorific equivalents have been those by Gorham and Sanger (1967). However, these workers merely estimated calorific equivalents for the highly organic soil layers of woodland humus, swamp peats and lake sediments. They were thus able to derive estimates by use of standard bomb calorimetric techniques. There have been no previous calorific content determinations for mineral soil - or its near equivalent: soil-ingesting lumbricid faeces. The present estimates were solely for the calorific equivalents of dried soil and faeces in terms of their organic constituents. Exothermic organic combustion proceeded at comparatively low

Size class	Collection Temp. °C	Sample No.	Calorific content (Kcals./g. dry wt.)	Size Class mean (± St. Dev.)
Adults	4.4	1	0.8871	0.8139 (± 0.0870)
		2	0.8690	
	10.0	1	0.6908	
		2	0.7140	
	14.8	1	0.8597	
		2	0.8627	
Large immatures	4.4	1	0.7640	0.9172 (± 0.1699)
		2	0.8287	
	10.0	1	0.8720	
		2	0.7725	
		3	0.8676	
	14.8	1	1.1631	
		2	1.1528	
Small immatures	4.4	1	0.8727	0.8469 (± 0.0595)
		2	0.8624	
	10.0	1	0.7620	
		2	0.8565	
		3	0.7650	
	14.8	1	0.9046	
		2	0.9049	

Table 25. Individual measurements and mean estimates for A. rosea faeces calorific equivalents. (Measurements performed for the three major size classes each taken from three ambient temperatures).

temperatures (300 to 400°C) and was quite separate from the endothermic breakdown of mineral salts at higher furnace temperatures. The clay mineral with the lowest breakdown temperature was kaolinite, which produced a very small endothermic peak at about 450 to 500°C. All other endothermic reactions were at temperatures in excess of 900°C. Paine (1965, 1966) pointed out that materials containing calcium carbonate would suffer weight loss and endothermy at ignition temperatures within bomb calorimeters due to the decomposition of $CaCO_3$ (at approximately 800 to 850°C). However, he has shown that these effects are negligible were $CaCO_3$ constitutes less than 25% of the total dry weight of ignition materials. It was shown, during the present differential thermal analyses, that soil and A. rosea faeces from Wynyard contained no $CaCO_3$ detectable in the sensitivity range used for organic analyses. All endothermic reactions were easily differentiated and eliminated by use of the thermal analysis technique.

Wet combustion methods were originally considered for soil and faeces calorific content analyses. Murchie (1958) estimated soil organic carbon by the wet combustion technique of Piper (1947), using a Klett-Somerson colorimeter. However, this method was not suitable for calorific content determinations. Kononova (1961) outlined the Tyurin method, a refinement of Ivlev's original chromic acid digestion technique, for the oxidation of soil organic carbon. It was, however, pointed out that this method was inaccurate when applied to soils rich in raw humus or containing any of a variety of salts

which react with chromic acid.

Quantification and calorific content estimation of soil organic constituents may be possible, but any such estimates would be time-consuming and very approximate. An advantage of such a method would be the possibility of isolating, in calorific terms, the soil organic components available to lumbricids.

Bomb calorimetry could only be performed after removal of the soil mineral fraction, e.g. by treatment with hydrofluoric acid (Bremner and Harada, 1959), or sodium hypobromite (Troell, 1931; Bourget and Tanner, 1953). The efficiency of these powerful solvents in separating off the soil organic fraction for calorific analysis was unknown and in some doubt. It was not attempted during these studies.

According to Mackenzie (1964), the principle of differential thermal analysis originated in the metallurgical studies of Roberts-Austen (1899). The first recording arrangements were cumbersome and the technique was advanced considerably by the invention of photographic recording methods. Modern potentiometric recording systems have made the detailed analysis of heat exchanges a comparatively simple process, thereby increasing the value of the technique for thermal investigations. Most of the early applications were in metallurgy, but more recently the technique has been most widely used in studies of clay mineralogy. Controlled-atmosphere apparatus allows the enhancement or suppression of exothermic combustion reactions by sample permeation with

oxygen or nitrogen, respectively. Various refinements related to uniform heating of the sample, and to the positioning of thermocouples for maximum heat exchange detection efficiency, have made this technique a valuable tool for the detailed study of both qualitative and quantitative thermal properties of a wide range of materials. The present studies represent the first quantitative assessment of the heat exchanges associated with combustion of organic materials using this technique. The method is obviously invaluable to the ecological energeticist dealing with materials incombustible by standard techniques.

Benzoic acid was unsuitable for calibration purposes due to its volatile nature when subjected to slow increases in ambient temperature. Starch and cellulose were also unsuitable since they produced complex or polymodal peak forms totally different from the broad, single peak associated with combustion of soil and faeces organic matter. Dried cocoon material produced a peak of similar form to that of the soil and faeces samples and proved eminently suitable for calibration purposes.

The time available for use of the thermal analysis apparatus was limited. Since each analysis required 1 to $1\frac{1}{2}$ hours for completion the number of sample analyses was therefore also limited - hence the restriction to only two samples of most materials. Whilst this consideration rendered the significance of certain dissimilarities in some doubt, the general consistency in the results was sufficient

for the calculation of reliable mean values. Little error might have resulted from the use of a grand mean value for A. rosea faeces calorific content. However, size class means were in fact used for estimations of field faecal output in calorific terms (Part II, Section III, sub-section 3). The observed dissimilarity between soil and faeces calorific equivalents served to accentuate the previous findings (see p. 192 above) regarding selection of soil organic matter during feeding by A. rosea worms.

4. The Oxycalorific Coefficient

Adams and Poulton (1937) regarded carbon dioxide production during respiratory metabolism as more consistent with changes in R.Q. ratio than values for oxygen consumption. The wider heat equivalent range for CO_2 output was regarded by these authors as an apparent, rather than a real, source of error in calorific conversions, due to difficulties in interpretation of carbohydrate/fat interconversions. They postulated that minimal errors would be incurred by considering CO_2 output alone and re-defining basal metabolism as the 'heat of combustion in the post-absorptive state.' These authors were concerned with medical problems relating to human metabolism. Whilst their theories have undoubted relevance in the medical field, their postulates concerning 'post-absorptive metabolism' have only a limited significance in relation to field estimates for continuously feeding species, or species with irregularities in the mode of CO_2 excretion. Brody (1945) pointed out that, in addition to the generally acknowledged wide range for normal heat conversions from CO_2 output data (Swift and French, 1954), processes such as fermentation or CO_2 storage during alkalosis further decrease the efficiency of calorific interpretations from CO_2 output data alone.

Measurement of O_2 consumption rates would thus appear to be the only reliable method for achieving estimates of respiratory heat losses using data on gaseous exchange during respiration. However, whilst the actual calorific conversions

must be achieved from O_2 consumption data, it is perfectly feasible for the relevant rates of O_2 intake to be calculated from data on CO_2 output and R.Q. values. This technique was employed in the present studies.

The choice of an oxycalorific coefficient would seem to depend on the relevant R.Q. value for the species concerned and a table of coefficients was produced by Brody (1945) on this basis. However, such refined procedures depend upon the reliability of the R.Q. estimate in terms of cellular metabolism. For lumbricids, there is always the possibility, however slight, of irregularities in CO_2 output due to calciferous gland activity. Indeed, the absolute reliability of R.Q. measures for whole organisms - in terms of cellular metabolism - has recently been questioned for variety of animal species (Bowler, pers. comm.).

The complete range of oxycalorific coefficients, for R.Q. values varying from 0.7 to 1.0 represents a deviation of only $\pm$ 3.5% from the mean value of 4.825 Kcals./litre O_2 at R.Q. 0.82. Since the R.Q. value for protein combustion is also 0.82, any protein breakdown in respiratory metabolism will tend to further decrease the $\pm$ 3.5% deviation from the mean coefficient. In addition, it was noted that A. rosea R.Q. values, measured in the present studies, were in the range 0.8 to 0.9 for most of the year. It was therefore evident that the use of the mean 'mixed diet' oxycalorific coefficient (4.825 Kcals./litre O_2), regardless of R.Q., would involve negligible error in calculations of respiratory heat losses.

Mean or 'mixed diet' oxycalorific coefficients have previously been used by many authors, including Nielsen (1961), Slobodkin (1962), O'Connor (1963), Englemann (1966) and Krueger et al (1968). Nielsen, Slobodkin, Englemann and Krueger et al used a value of 4.8 Kcals./litre O_2, whilst O'Connor used 4.775 Kcals./litre O_2 - after Heilbrunn (1947). Brody's mean estimate of 4.825 Kcals./litre O_2 corresponded exactly with that of Ivlev (1934), suggesting maximum reliability.

II Population Energy Budgets for Allolobophora rosea (Sav.) f. typica at Wynyard (1966/67)

1. Introduction

Studies in ecological energetics can be performed at the community, trophic association or population levels of biotic organisation. In contrast to the other types of study, community energetics can be studied without reference to species or individuals, an approach exemplified by the work of Odum, H.T. and Odum, E.P. (1955). Such 'whole system' studies are, however, extremely limited in application. The detailed pattern of energy utilisation by entire communities can only be elucidated by investigation and inter-relation of their species population components. Energy budgets for species groups (e.g. families or trophic associations) are only meaningful when the component species have very similar modes of life. Families rarely approach this situation and trophic associations often deviate from the apparent hierarchy of food utilisation. The present study represents an attempt to evaluate the annual population energy budget for a soil-dwelling, soil-ingesting lumbricid species. This work formed part of an extensive survey, performed by the author and his associates, of the Wynyard woodland litter production and its utilisation by a variety of decomposer organisms.

It was hoped that monthly energy budgets for 'average' individuals of the three major A. rosea size classes might be evaluated in the course of this work. Since energy budgets calculated for individual animals are little affected by

specific estimates of population density, they are immediately more reliable for use in different situations or in general considerations involving population models, Even so, individuals - like populations - can not be assumed to behave similarly in any situation. The absence of an A. rosea summer dormancy at Wynyard is an admirable example of the influence of the physical environment. The present data for A. rosea was not considered suitable for detailed presentation at the level of the individual worm, or in sub-divisions of the annual cycle. Soil-dwelling lumbricids, such as A. rosea, impose natural limitations on the accuracy of individual measurements. Population energy budgets for the three major A. rosea size classes, calculated on an annual basis, were considered reliable; they are presented in this Section with the proviso that they may only be applicable to an expanding population in a Wynyard-type habitat.

2. Calorific Components of the Population Energy Budgets

The terminology and symbols used in this analysis were basically similar to those proposed by Odum (1968). The Odum parameters, and minor modifications for the present purposes, are listed below to serve as a key for the symbols used in this study.

B - Biomass or standing crop

I - Ingested energy

A - Assimilated energy

NU - Egested or faecal energy ('Not Used')

P - Production (= Secondary production, in this case)

R - Respiratory or maintenance energy expenditure

G - Biomass growth, here subdivided into:

G' - Tissue growth

and Rp - Reproductive products (cocoons)

The Odum parameter E (excretion and exudation) was regarded as negligible in the present studies (see Part II, Section II, sub-section 3). The biomass and maintenance energy expenditure of cocoons were also regarded as negligible.

The parameters outlined above were associated by the three conventional energy budget equations:

$$I = A + NU \quad \ldots \quad 1$$

$$A = R + P \quad \ldots \quad 2$$

and $P = (G + E) = G$, in the present study,

i.e. $P = G' + Rp$ ... 3

Populations of the three major A. rosea size classes were considered separately and subsequently summated to obtain

energy budgets for the total A. rosea population. Energy flow parameters were in Kcals./m^2/yr., and the mean annual standing crops are shown in Kcals./m^2.

The derived energy budgets were as follows:

Small Immatures (B = 0.507 Kcals./m^2)

Equation 3:

P = 0.886 + 0, hence P (= 0.886)

Equation 2:

A = 2.270 + 0.886, hence A (= 3.156)

Equation 1:

I = 3.156 + 344.022, hence I (= 347.178)

Large Immatures (B = 1.733 Kcals./m^2)

Equation 3:

P = 1.320 + 0, hence P (= 1.320)

Equation 2:

A = 6.159 + 1.320, hence A (= 7.479)

Equation 1:

I = 7.479 + 919.590, hence I (= 927.069)

Adults (B = 1.308 Kcals./m^2)

Equation 3:

P = 0.264 + 0.247 hence P (= 0.511)

Equation 2:

A = 4.298 + 0.511, hence A (= 4.809)

Equation 1:

I = 4.809 + 601.696, hence I (= 606.505)

Total A.rosea population (B = 3.548 Kcals./m^2)

Equation 3:

P = 2.470 + 0.247, hence P (= 2.717)

Equation 2:

$$A = 12.727 + 2.717, \text{ hence } A\ (= 15.444)$$

Equation 1:

$$I = 15.444 + 1865.308, \text{ hence } I\ (= 1880.752)$$

3. Assimilation and Secondary Production Efficiencies

The following definitions of efficiency, from Ivlev (1945, transl. 1966), Ricker (1946, 1968) and Slobodkin (1962), were used in the present analyses.

Assimilation Efficiency

$$\text{Digestion efficiency} = \frac{A}{I}\ \%$$

Secondary Production Efficiency, sub-divided into:

Gross growth efficiency $= \frac{P}{I}\ \%$ (i.e. including Rp, where appropriate)

Net growth efficiency $= \frac{P}{A}\ \%$ (- do -)

These efficiencies were evaluated for the A. rosea population at Wynyard (1966/67) on the basis of total annual energy flow estimates (see above). The results are shown in Table 26. A. rosea assimilation and gross production efficiencies were invariably less than 1%. Even allowing for a maximum overestimate of NU (see p.207), and hence I, assimilation efficiencies could not be greater than 2% and gross production efficiencies must still be less than 1%. Net production efficiencies were in the range 10 to 30%. Assimilation and secondary production efficiencies were all maximal for small immature worms. Assimilation efficiencies were similar for large immatures and adults, but large immature secondary production efficiencies were intermediate between those for small immatures and adults. The total A.rosea population expended 82.4% of assimilated energy as respiratory heat.

Size class	Assimilation efficiency	2^{o} Production efficiencies	
		Gross growth efficiency	Net growth efficiency
Small Immatures	0.91%	0.26%	28.07%
Large Immatures	0.81%	0.14%	17.65%
Adults	0.79%	0.08%	10.63%
Total population	0.82%	0.14%	17.59%

Table 26. Efficiencies of energy utilisation, calculated from annual energy flow parameters (Kcals./m^2), for the A. rosea population at Wynyard (1966/67).

4. Discussion

Whilst monthly or seasonal energy budget analyses were unfeasible, it was noted that A. rosea energy utilisation (respiration, tissue growth and reproduction) was almost suspended during the coldest winter months. The unusual importance of summer activity - in a species prone to dormancy in this season - was also an important feature of A. rosea energy utilisation at Wynyard.

The outstanding feature of the A. rosea population energy budgets was the enormous quantity of potential energy collected and ingested by this species, only to be egested - with no apparent benefit to A. rosea. The implications of this situation will be discussed below (see p.324).

Assimilation efficiencies have been found to vary according to the trophic level of the species - whether vertebrate or invertebrate. As the amount of available energy decreases along food chains, and the decreasing spirals of community food webs, survival becomes increasingly dependent upon the efficiency of food utilisation. Thus top carnivores, whether weasels (Golley, 1960), wireworms (Dutton, pers. comm.) or damsel-fly larvae (Lawton, pers. comm.), assimilate 80 to 100% of ingested food energy. In contrast, a herbivorous insect (grasshopper) and a detritus-feeding snail were only found to have assimilation efficiencies of 35% and 43%, respectively (Odum and Smalley, 1959). A. rosea thus appeared to represent the ultimate in inefficiency of food utilisation. However, soil organic matter is a complex of

humic derivatives and dissolved material, in addition to the biotic components which are more apparent but constitute a much smaller proportion of the total organic energy. It was appreciated that not all ingested energy is available for assimilation by many animal species, e.g. cellulose in the diet of many omnivores. However, it was thought that soil-ingesting lumbricids consume an abnormally large proportion of organic materials which are not available for digestion. A. rosea assimilation efficiencies calculated on the basis of ingested energy available for digestion would undoubtedly be more comparable with similar estimates for other decomposer organisms. There was, at the time of the present studies, no method of separating the soil energy fractions available and non-available to A. rosea, but the present findings indicated that A. rosea assimilation efficiency must be of a low order, even with respect to the digestible elements of the food material.

A. rosea net efficiencies of secondary production were very similar to those found for other detritus feeders: oribatid mites (21.4%), an isopod (17.9%), a mussel (29.8%), nematodes (24.7%) and a snail (14.0%) - to quote data from the review by Golley (1968). Homoiotherms and active carnivores (both vertebrate and invertebrate) expend an even greater proportion of assimilated energy as the heat of respiratory metabolism, often having net secondary production efficiencies of only about 1 to 5%. In contrast, herbivorous invertebrates such as grasshoppers convert 30 to 40% of assimilated energy

into biomass available to predators and decomposers. The proportion of assimilated energy devoted to maintenance metabolism is probably a function of food availability, population mortality, individual longevity and environmental stress. Thus homoiotherms expend a high proportion of metabolic energy in maintaining a constant high body temperature. Carnivores usually require large energy resources simply to search out prey organisms ('work loop' of Odum, 1968). Animals with a long life span and a low mortality rate can gain little, in terms of population survival, by acquiring an enormous biomass, e.g. the extinction of Mesozoic reptile populations with small numbers of very large individuals, or the 'crashes' of small mammal populations whose large numbers of small individuals have exhausted the available food resources. A. rosea is a long-lived species, with only a moderate yield to predators and decomposers, In addition, the worms have to expend a high proportion of metabolic energy in the mechanical processing of huge quantities of food material; this could be related to the carnivore 'work loop'. Herbivorous insects usually have a short life span and suffer a high yield to predatory species, necessitating rapid replacement of individuals. Their food is usually readily available, so that maximum production efficiency can be achieved - thereby ensuring population survival.

The higher production efficiencies found in populations of younger A. rosea individuals were in accordance with the

more rapid growth rates in smaller worms, and the low adult reproductive rates characteristic of this long-lived species. Phillipson (1960) showed declining assimilation efficiency in successive instars of Mitopus morio (F.) due to variation in the mode of food ingestion. The earliest instars were unable to ingest the harder parts of prey items and were therefore restricted in diet to the easily digestible prey body fluids. Lawton (pers. comm.) also found lower assimilation efficiencies in later instars of a damsel-fly larva. The reason for the higher assimilation efficiency in small immature A. rosea worms was obscure. The difference may have been real, but it may also have been due to the use of the same mineral soil for all size-classes in faeces production experiments: assimilation was derived from estimates in the field - where small immatures tend to occupy the more organic topsoil surface layer.

A. rosea comparative logarithmic rates of secondary production and respiratory metabolism were closely related to the general relation between these parameters for invertebrate species - calculated linearly by Englemann (1966), and suggested to be more curvilinear by Golley (1968). Englemann (1966) has pointed out that the general invertebrate relation, as distinct from that for vertebrates, is probably more correctly related to poikilothermy than to skeletal structure. If the general poikilotherm relation was assumed to apply to the total lumbricid population at Wynyard (i.e. occupying the top 30 cm. of soil and litter), the present estimate of

101.059 Kcals./m^2/yr. for total lumbricid population respiratory metabolism (see p. 281) would have yielded estimates for total lumbricid secondary production as 40.74 Kcals./m^2/yr. (by the Englemann equation) or approximately 30 Kcals./m^2/yr. (by the curvilinear postulate).

Since no quantitative data was available on the A.rosea yield to predatory species, and the calculated mortality yield (including that to decomposers) was only an apparent value due to population increase, accurate estimates of ecological efficiency were not possible. Net ecological efficiencies (yield to predator(s)/prey assimilation) were expected to be slightly less than net growth efficiences - e.g. 10 to 15% for the total A. rosea population - if decomposers were included in the yield to 'predator(s)'. Exclusion of yield to decomposers would further reduce the net ecological efficiency of A. rosea by an unknown amount.

The approximate A. rosea net ecological efficiency (including yield to decomposers), for an expanding population, was of a similar order to previous general laboratory and field estimates of gross ecological efficiency for 'steady state' animal populations (Slobodkin, 1968). It thus appeared that assimilated energy was transmitted by A. rosea to successive food web populations at a rate comparable with those shown by other species populations for transmission of ingested energy. It was necessary to include A. rosea yield to decomposers with 'yield to predators' since the proportions of these two mortality sources were unknown.

The ecological efficiency of the A. rosea population was not assessed in terms of ingested energy, since meaningful comparisons with other species populations would not have been possible. A. rosea gross efficiencies of food utilisation and transfer, like food digestion efficiency, were exceptionally low due to the highly unusual mode of feeding adopted by this species.

PART IV

GENERAL DISCUSSION

In any climax or sub-climax situation there is a complexity of habitats, even within small areas. The present studies of vegetational and soil structure on the grid area at Wynyard showed that such habitat diversification was considerably advanced within the alder/birch woodland. This was thought to account for the wide range of lumbricid species which occurred in the area. However, the woodland had not reached the maximal complexity of the climax state and studies of soil moisture levels, temperature and pH revealed a degree of primitive simplicity, supported by the stabilising influence of the diverse vegetational cover. The lumbricid population of the grid area was dominated by species better adapted to 'poor quality' soils: L. terrestris L., A. rosea (Sav.) f. typica and O. cyaneum (Sav.).

Studies of lumbricid ecology have been hampered in the past by taxonomic difficulties and difficulties in adequately assessing population densities. The taxonomic difficulties - with regard to immature worms - can be overcome by practised familiarity with the individual species and the term 'earthworm' will eventually sink into the oblivion it richly deserves. Techniques of sampling lumbricid populations have been subjected to intensive experimentation. Until washing and flotation methods have been suitably refined, hand-sorting of soil - after sample removal with a tool such as that described in this study - can be regarded as the most reliable

method for sample extractions of the total lumbricid population. It should be noted, however, that refined techniques are available for the extraction of particular species or species groups from situations where hand-sorting may be less reliable. Special features of the life cycle or annual cycle of activity (Avel, 1928) should be noted for particular species when deciding the optimum method(s) for sampling throughout the year. Such considerations are vital in any studies relating to lumbricid physiology.

The lumbricid population density at Wynyard was low in comparison to densities in pastureland or cultivated soils of higher nutritional status. This was not considered abnormal for poorer quality or 'developing' soils such as those at Wynyard. Pigmented and unpigmented lumbricid species - delineated ecologically in a moorland situation by Svendsen (1955a) and physiologically by Byzova (1965, 1966) - showed distinct patterns of ecological activity at Wynyard. The continued separation of these groups in ecological and physiological studies is therefore recommended. In addition to this major division, the habitat preferences and niche relationships of individual lumbricid species are now well-defined; it is hoped that species niches will be increasingly clarified by future researches and given due consideration by all students of the lumbricid 'modus vivendi'.

The present investigations of lumbricid ecology, physiology and ecological energetics were focussed primarily on a single species: Allolobophora rosea. This was

necessitated initially by the inadequacy of population size for other lumbricid species at Wynyard. This approach was considered amply rewarding in the final analysis, since it allowed - in the time available - a more detailed enquiry into lumbricid functional relationships than might have been envisaged for studies relating to a number of species.

Although it was the dominant topsoil species, A. rosea at first appeared - from its absolute population density and rates of metabolism and tissue production - to be of little consequence to the development of soil structure and nutritional status. Studies of faecal production by this species revealed the true extent of the influence of A. rosea on habitat succession. The worms were shown to pass enormous quantities of soil material through their digestive tracts each year. Since they actively selected the more organic constituents of the soil, the energy content of the processed material represented an even greater proportion of the soil standing crop of organic matter than that suggested by the mass of material defaecated. Edwards and Heath (1963) showed the rate of leaf litter disappearance in soil to be trebled in the presence of lumbricids. Since micro-organisms wæe thought responsible for the bulk of litter energy dissipation, the influence of lumbricids was assumed to be exerted through facilitation of micro-organism activity. A. rosea did not ingest raw litter, but this species was shown to reduce particulate plant debris to a much more finely divided form by trituration and digestion. This increase in surface area

of organic matter, possibly coupled with the demonstrated multiplication of bacteria and activation of fungal spores within the lumbricid gut (Parle, 1963a, b), must result in optimum conditions for the activity of micro-decomposers. A. rosea possibly forms the second stage in a three-link decomposer chain leading from the raw-litter-feeders (e.g. millipedes, isopods or L. terrestris and surface active lumbricids such as L. castaneus (Sav.)) to the 'combustion house' of the micro-decomposers.

Hughes (pers. comm.) estimated the annual litter production at Wynyard to be approximately 2,500 Kcals./m^2/yr. If this energy was completely respired by the decomposer organisms - as it would be in a climax situation - the total lumbricid population at Wynyard would have accounted for about 4% of the total heat production. Since soil organic matter in the area was below peak standing crop, some annual accumulation of soil organic energy was likely; lumbricid respiration must therefore have accounted for a slightly higher percentage of the actual combustion. Macfadyen (1963a) estimated the proportion of organic energy released by grassland soil macrofauna to be only about 8 or 9% of the total heat loss. If the lumbricids in the top 30 cm. of a poor quality woodland soil respired 4 or 5% of the total annual energy expenditure, the total expenditure of grassland soil macrofauna would appear to have been underestimated. Macfadyen's later estimate (Macfadyen, 1963b) for the soil macrofaunal contribution as about 20% of the total energy

release would seem to be more realistic. Micro-organisms undoubtedly respire the bulk of the organic debris entering the soil, but the metabolism of the 'masticators' must not be discounted.

Although A. rosea was shown to be a food source for the mole population at Wynyard (see Appendix 6), the rate of biomass production by this lumbricid species was found to be exceeding the population mortality yield. A net increase in population size appeared to have occurred in each of the 4 or 5 years previous to the present studies. Assuming the habitat conditions during the year of production estimates were not grossly abnormal, which seemed likely from the stability of the habitat structure and the sequence of climatic conditions, the production increase was attributed to environmental change. Golley (1968) pointed out that secondary production is one of the energy budget parameters most responsive to change in environmental conditions. Saito (1967) suggested that production estimates may be afforded an extra significance if calculated per animal generation. However, he acknowledges that this poses extremely complex problems for species - such as A. rosea - with a long life span and/or overlapping generations.

O'Connor (1968b), studying the energy relations of enchytraeid worms, concentrated attention on estimates of respiratory activity - stating that other parameters were almost impossible to measure in terms of the field situation. In the present studies, even the respiratory metabolism of

certain lumbricid species was shown to pose physiological problems in relation to carbon dioxide excretion. In formulating energy budgets for the A. rosea population it was necessary to derive estimates for ingestion and assimilation from measurements of secondary production, respiration and faecal output. It was not possible to measure ingestion directly or to obtain a separate estimate of assimilation under controlled experimental conditions. Future advances in this field may facilitate such studies in the feeding biology of soil-ingesting lumbricids. The absolute values for A. rosea energy budget parameters were related to the biomass of the population at Wynyard at the time of these studies. The calculated efficiencies of energy utilisation were thought to be of more general application since Saito (1967) showed gross production, net production and gross ecological efficiencies, for final instar and adult diplopods, to be constant - regardless of population density.

Soils of the alder zone at Wynyard - in which the A. rosea population was markedly aggregated - were of a higher nutritional status than those in the birch areas. Ovington (1965) measured total nitrogen concentrations in the mineral soils of a mixed woodland. He found that soils beneath alder trees (whose roots incorporate nodules containing nitrogen-fixing bacteria) contained the highest nitrogen concentrations. Alder is usually found in wetter areas, so that the general situation at Wynyard may have been typical for a woodland of this type. Such habitat similarities will be a necessary

pre-requisite for the wider application of data relating to the population ecology and physiology of lumbricids. Apinis, in recorded discussion of the work by Satchell (1963), pointed out that rates of nitrogen turnover have shown wide variation between different forest types. Similar variation in energy flow estimates can be expected and lumbricids occurring in a variety of habitats must not be assumed to make similar quantitative contributions to energy flow in each situation.

As available information is increased, the role of lumbricids - and other soil organisms - in promoting and achieving energy flow will be evaluated for a range of soil community structures. It is hoped that the emerging pattern will indicate means whereby stability can be incorporated into the utilisation of energy resources by civilised mankind.

SUMMARY OF RESULTS

1. The grid area at Wynyard incorporated a complexity of habitat conditions, though the soil structure was occasionally primitive and generally of a low nutritional status.
2. Fourteen lumbricid species occurred in the region; eleven species were found in the grid area. D. rubida (Sav.) f. subrubicunda (Eisen) and L. castaneus (Sav.) were the commonest surface active species; A. rosea (Sav.) f. typica was the dominant topsoil species; O. cyaneum (Sav.) and L. terrestris L. occupied the subsoil layers.
3. The habitat preferences and niche relationships of lumbricid species at Wynyard were compared with previous findings in other situations. Habitat preferences at Wynyard were generally in accordance with previous records of occurrence. A. rosea and A. caliginosa (Sav.) were found to occupy the same soil stratum but were separately dominant in different soil types. D. rubida f. subrubicunda and L. castaneus utilised the 'litter wads' accumulated by L. terrestris and penetrated the surface mounds of clay produced by moles.
4. The water table in the grid area was within 38 cm., and commonly within 25 cm. of the ground surface for most of the year. There was a marked fall in water table depth during June, July, August and September, but the topsoil remained moist throughout the year.
5. A positive relation was shown between water table depth and surface soil moisture tension. This relation was used in

preparation of laboratory experimental media.

6. Soil temperatures, measured at different depths, followed a normal cycle of annual variation, but with a reduced annual range due to damping at the summer and winter extremes. Soil temperature varied only slightly with depth but showed classical inversions in early spring and autumn. Soil temperatures at similar depths beneath bramble and grass vegetation were almost identical throughout the year. The measurements of soil temperature were used in the relation of laboratory energy flow measures to the field situation.
7. The soils of the grid area were slightly acid. Topsoil pH values were similar beneath all major vegetational types; the mean topsoil pH was 6.22 ± 0.07 (St. Error). A topsoil sample taken from beneath bracken showed a minimal pH value (5.25). Topsoil pH was simulated in laboratory experiments involving lumbricids, where data obtained was intended for quantitative relation to the field.
8. Lumbricid extraction techniques were briefly reviewed.
9. A new sampling tool was constructed for use in the woodland soils at Wynyard.
10. The relation between fresh and dry individual body weights was determined for worms and cocoons of the lumbricid species A. rosea.
11. Worms of all sizes were found in the A. rosea population at Wynyard throughout the year. Seasonal fluctuations in

biomass were recorded: 'small immature' worms showed increased numbers and biomass in April/May and September; 'large immatures' showed similar peaks in September and December; adult worms showed maximum numbers and biomass in May, 1967, and April, 1966. A. rosea did not exhibit a summer dormancy in Wynyard soils.

12. Small immatures and large immatures of A. rosea were aggregated throughout the year, maximum aggregation approximately coinciding with their respective times of peak population numbers per metre square. Adult worms were randomly dispersed for most of the year.
13. The A. rosea population was shown to be less dense in soils where grasses and bracken formed the dominant ground vegetation. The population showed aggregation in soils beneath vegetation including bramble.
14. The density and biomass of the total lumbricid population occupying the top 30 cm. of the soil both showed peak values in April/May, August/September and early December. Both pigmented and unpigmented lumbricid species contributed to the three peaks in total population density; the spring and early December peaks were most important for pigmented species whilst the spring and late summer peaks were of corresponding prime importance for most unpigmented species.
15. The mean lumbricid population density and biomass over the year of study were 101.69 worms and 12.771 grams dry weight ($\equiv$ 41.644 Kcals.) per metre square,

respectively. These mean estimates were approximately equivalent to 400,000 worms and 52 Kg.dry weight per acre.

16. The total lumbricid population was shown to be highly aggregated for most of the year. Aggregation was maximal in mid-spring and minimal in mid-summer and mid-winter.

17. Cocoons of four pigmented lumbricid species (predominantly L. terrestris cocoons) appeared to have more synchronised deposition and emergence patterns than those for cocoons of two unpigmented species (predominantly O. cyaneum cocoons).

18. The total biomass of cocoons of deep-burrowing species (1.593 Kcals./m^2) was equivalent to 24.3% of the total biomass of topsoil and surface active worms.

19. The mean emergence fresh weight for A. rosea worms was 6.32 mg. $\pm$ 0.02 mg. (St. Error). This body weight was used as the starting point in the construction of a fresh weight growth curve for A. rosea.

20. On average, A. rosea worms attained maturity after approximately two years. The growth curve was used for ageing the field population and growth increment data was used in tissue production estimates.

21. There was no evidence of adult regression in the field but a small proportion of 'pygmy' adults were found to occur.

22. From mid-November to mid-January net body tissue production was negligible in all A. rosea size classes. Adult growth was slow and variable for the rest of the year, whilst immature worms showed an approximately uniform rate of

tissue production apart from the late autumn/early winter interruption. Population tissue production was enhanced by increased small immature biomass in spring and by the large immature biomass peak in autumn; seasonal variation was otherwise in parallel with that for individual growth rates.

23. A. rosea adults - of all ages - only produced, on average, 3.13 cocoons per annum. Cocoon production in spring and summer accounted for 85% of the annual total.

24. A. rosea cocoons could maintain their fresh weight and remain viable - though showing little sign of development - for more than nine months in Wynyard soil. The cocoons were shown to be sensitive to waterlogging of the soil and long periods of anoxia. Cocoons deposited during the summer months emerged in 3 to 4 months or less; cocoons deposited at other times had long incubation periods.

25. Small immature A. rosea individuals were shown to have a remarkably low apparent annual mortality during their first year. Worms aged between one and five years old suffered an apparent mortality of approximately 53% per annum, whilst numbers of older worms appeared to be reduced by approximately 36% per annum. Cocoon mortality was eventually estimated to be in the range 25 to 35% per annum.

26. The maximum life span of an A. rosea worm was approximately eleven to twelve years. The average life span was

approximately three years (i.e. two years immature, one year adult).

27. A. rosea standing crop was estimated to be increasing by more than 20% per annum at the time of these investigations. The net annual increase in biomass appeared to have been initiated four to five years previous to these studies performed during 1966/1967.

28. The A. rosea population turnover time, probably only slightly affected by overall population expansion, was estimated at 1.31 years.

29. Qualitative and quantitative distinctions were demonstrated between the burrowing and feeding activities of A. rosea worms confined in two-dimensional cage structures. Burrow formation during feeding activities was shown to be related to worm body size and the ambient temperature.

30. A. rosea worms showed a marked preference for soils taken from beneath Juncus sp. or bramble vegetation, and corresponding avoidance of soils from beneath grasses or bracken, regardless of soil moisture content or pH.

31. O. cyaneum can ingest coarse sand in bulk and was found to ingest the largest mineral particles of those found in the gut contents of three unpigmented, soil-ingesting lumbricid species feeding on Wynyard soils. A. caliginosa ingested soil mineral particles of intermediate size and A. rosea worms, regardless of body size, ingested soil mineral particles of the smallest diameter. A. rosea was thought to ingest only the finer fraction of a

coarse sand medium. Worm body weight variation in a coarse sand medium suggested that L. castaneus could ingest larger mineral particles than D. rubida f. subrubicunda.

32. Adults and large immatures of A. rosea were shown to triturate larger particles of plant debris and reduce them to a fine particulate and amorphous state by the action of the worm gizzard and intestinal juices.

33. A. rosea adults decreased in body weight, and produced no cocoons during a three month period, when confined in a medium deficient in coarse fungal mycelium and fine particulate and dissolved organic matter.

34. Measurements of percentage organic matter and calorific content for worm faeces and the surrounding soil medium showed that A. rosea worms, of all sizes, actively select for ingestion the more organic fraction of the soil.

35. A positive relation was shown between the dry weight of gut contents and individual body fresh weight for A. rosea worms of all sizes. The relation was negligibly affected by season for A. rosea worms collected at Wynyard.

The fresh gut content weight of O. cyaneum individuals was shown to equal 0.4845 times the total body fresh weight for worms collected at Wynyard. Dry body weight equalled, on average, 0.309 times the fresh body weight for O. cyaneum individuals collected at Wynyard.

36. The logarithmic rate of faeces production per gram worm for A. rosea individuals was shown to be inversely

proportional to the logarithm of worm fresh weight. The slope of this relation was shown to increase with increasing ambient temperature. Absolute log. rates of faeces production, at particular worm body weights, were also shown to be greater at higher ambient temperatures.

37. It was estimated that 2.148 Kg. dry weight (≡ 1,865 Kcals.) of faecal material were egested each year by the total A. rosea population per metre square at Wynyard. It was acknowledged that this may have been an overestimate, but maximum errors were defined and a general gut clearance time of 1 to 4 hours was estimated for A. rosea individuals. The use of powder stains was shown to be impracticable for independent estimates of A. rosea gut turnover rates. Possible staining techniques were reviewed and the difficulties defined.

38. The respiratory quotient for A. rosea worms was shown to be in the range 0.8 to 0.9 for most of the year, with values of about 0.7 during the summer months of both 1966 and 1967. A. rosea seasonal R.Q. values at 10°C were used to convert monthly measures of A. rosea CO_2 output to their equivalent rates of oxygen consumption.

R.Q. values for O. cyaneum, L. castaneus and D. rubida f. subrubicunda showed a high degree of variability, maximal in large individuals of O. cyaneum. Values frequently occurred below the normal range of 0.7 to 1.0. Values for D. rubida f. subrubicunda were near 1.0 in

winter and therefore assumed 'normal' at this time; respiratory quotient was shown to be related to the moisture content of the surrounding medium in this species.

39. The respiratory quotient of adult A. rosea worms was shown to be unaffected by worm starvation or variation in ambient temperature.

40. The rate of oxygen consumption per gram worm was proportional to ambient temperature for A. rosea individuals. The slope of this relation was shown to be greater for immature worms than for adults. The relation was unaffected by temperature acclimatisation of worms prior to measurement at three ambient temperatures.

Mean oxygen consumption rates for A. rosea worms of all sizes were almost identical at an ambient temperature of 6°C. Q_{10} values, between 6°C and 15°C, for A. rosea adults, large immatures and small immatures were found to be 1.42, 2.42 and 1.96, respectively. The average Q_{10} for the species was 1.93. The slopes of the Q_{10} relations for the different size classes were used in calculations of field respiratory metabolism.

The log. rate of oxygen consumption per unit weight for A. rosea cocoons was proportional to ambient temperature. the cocoon Q_{10}, between 5°C and 15°C, was 1.63.

41. A new respirometer apparatus, incorporating an infra-red gas analyser and a new gas switch system, was devised for the measurement of lumbricid respiratory rates under

simulated field conditions.

42. Respiratory rates of A. rosea, O. cyaneum and D. rubida f. subrubicunda were shown to be initially high - and more comparable with Warburg measures - during early confinement in the respirometer apparatus. Respiration rapidly decreased over the first few hours and assumed a more constant rate over the next 24 hours or more. After about 30 hours for L. castaneus and D. rubida f. subrubicunda, 50 to 60 hours for A. rosea, and an indefinite period for O. cyaneum, respiratory rates declined to a low constant level - assumed to be associated with worm starvation.

43. A. rosea worms of all sizes showed a pronounced annual cycle of respiratory activity at 10°C, shown to be related to field temperatures at the times of worm collection. This relation seemed to be associated with field levels of locomotory activity. CO_2 output rates for O. cyaneum, L. castaneus and D. rubida f. subrubicunda were fairly constant throughout the year, though D. rubida f. subrubicunda rates of CO_2 output declined during the spring and early summer (assumed due to calciferous gland activity). Approximate estimates of average oxygen consumption rates for O. cyaneum with (or without) gut contents, D. rubida f subrubicunda and L. castaneus were in the ranges 45 to 60 (or 90 to 115), 100 to 130, and 135 to 155 mm^3 $O_2/g/h$ at 10°C, respectively.

44. The total annual maintenance energy expenditure of the A. rosea population at Wynyard was found to be 12.727 Kcals. per metre square. Small immatures respired 2.270 Kcals./m^2, large immatures 6.259 Kcals./m^2 and adults 4.298 Kcals./m^2. Annual variations in biomass were of secondary importance to the temperature-dependent variations in A. rosea respiratory rate.

45. The total annual maintenance energy expenditure by the lumbricid population occupying the top 30 cm. of soil at Wynyard was estimated at 101.059 Kcals./m^2. 75% of this total heat loss was due to the respiration of the deep-burrowing species O. cyaneum and L. terrestris. Contributions by pigmented and unpigmented worms were approximately in the ratio 3 : 5.

46. Calorific values for worms of eight lumbricid species and cocoons of six lumbricid species were determined by bomb calorimetry. Calorific values for A. rosea worms of the three major size classes were shown to be similar in all seasons. Cocoon calorific values based on ash-free dry weights (in the range 5.26 to 5.38 Kcals./gram) were consistently lower than similar values for worms of the same lumbricid species. Worm calorific equivalents were in the range 5.3 to 5.7 Kcals./ash-free gram dry weight.

47. A thermal analysis technique, not previously used in studies of ecological energetics, was described with reference to the determination of calorific equivalents

for mineral soil and faeces of soil-ingesting lumbricids.

48. Homogenised, mull-type mineral soil, collected beneath vegetation dominated by bramble at Wynyard, had an organic calorific equivalent of 0.6186 Kcals./gram total dry weight. A. rosea faeces organic calorific equivalents were generally in the range 0.8 to 0.9 Kcals./gram total dry weight.

49. Population energy budgets were formulated for each of the three major A. rosea size classes. The total A. rosea worm population at Wynyard (mean biomass: 3.548 Kcals./m^2) ingested approximately 1,881 Kcals./m^2/yr. Of this total amount, only 15.444 Kcals./m^2 were assimilated and approximately 1,865 Kcals./m^2 were egested. 12.727 Kcals./m^2 of the assimilated energy were dissipated as the heat of maintenance metabolism, the remainder (2.717 Kcals./m^2) represented secondary production. Tissue growth accounted for 2.470 Kcals./m^2 and cocoon production amounted to 0.247 Kcals./m^2.

50. A. rosea worms assimilated less than 1% of the total ingested energy. Net efficiencies of secondary production for small immatures, large immatures and adults were 28.07%, 17.65% and 10.63%. The net efficiency of secondary production for the total A. rosea population was 17.59% - i.e. 82.41% of the energy assimilated was dissipated as the heat of maintenance metabolism.

51. Using the present estimate of population respiratory

metabolism for the total lumbricid population in the top 30 cm. of Wynyard soil, the secondary production of this total lumbricid population was estimated (from the general poikilotherm relation) as approximately in the range 30 to 40 Kcals./m^2/yr.

52. The net ecological efficiency, including yield to decomposers, was estimated for the total A. rosea population as in the range 10 to 15%.

Appendix 1

Plant Species List for Newton Hanzard Grid Area, Wynyard, Co. Durham

(mainly from data supplied by Mr. M. K. Hughes, Durham University)

Nomenclature after Clapham, Tutin and Warburg (1959) and Watson (1963)

Grid Squares : (x indicates presence)	A	C	E	B	D	F	Common Name
Trees							
Betula sp. L	-	x	x	x	x	x	Birch (hybrid)
Alnus glutinosa (L.)Gaertn.	x	x	x	-	-	-	Alder
Acer pseudoplanatus L.	x	-	x	x	-	-	Sycamore
Quercus robur L.	-	-	x	-	-	-	Oak
Sambucus nigra L.	-	-	-	x	-	-	Elder
Crataegus monogyna Jacq.	x	x	x	x	x	x	Hawthorn
Shrubs and Forbs							
Rosa canina agg.	-	-	-	x	x	x	Dog Rose
Rubus fruticosus L.	x	x	x	x	x	x	Bramble
Mercurialis perrenis L.	-	x	x	-	-	-	Dog's Mercury
Ajuga reptans L.	-	-	x	-	-	-	Bugle
Cirsium sp. Mill.	-	-	-	x	-	-	Thistle
Viola riviniana Rchb.	x	x	x	-	x	-	Common Violet
Circaea lutetiana L.	x	x	x	-	x	-	Enchanter's Nightshade
Potentilla sterilis (L.) Garcke	-	-	x	x	-	x	Barren Strawberry
Potentilla reptans L.	x	-	-	-	-	-	Creeping Cinquefoil
Geum urbanum L.	-	-	x	-	-	x	Wood Avens

Grid Squares :	A	C	E	B	D	F	Common Name
Chamaenerion angustifolium (L.) Scop.	x	x	x	x	x	x	Rose-bay Willow-herb
Ferns							
Dryopteris filix-mas agg.	x	x	x	x	x	x	Male Fern
Polystichum setiferum (Forsk.) Woynar	x	x	x	x	x	x	Soft Shield-fern
Pteridium aquilinium (L.) Kuhn	-	-	-	-	-	x	Bracken
Grasses							
Deschampsia caespitosa (L.) Beauv.	-	x	x	x	x	x	Tufted Hair-grass
Agrostis tenuis Sibth.	-	x	x	x	x	x	Common Bent-grass
Arrhenatherum elatius (L.) Beauv. ex J.&C.Presl	x	x	x	-	-	-	Oat grass
Holcus mollis L.	x	x	x	-	-	-	Creeping Soft-grass
Poa trivialis L.	x	-	-	-	-	-	Rough-stalked Meadow-grass
Mosses							
Mnium hornum Hedw.	x	-	x	-	-	-	Swan's Neck Thread Moss
Polytrichum formosum Hedw.	x	x	x	-	-	-	Bank Hair Moss
Eurhynchium striatum (Hedw.) Schp.	x	-	-	-	-	-	Feather Moss
Eurhynchium praelongum (Hedw.) Hobk.	x	-	-	-	-	-	Long Trailing Feather Moss
Thuidium tamariscinum (Gedw.) B. & S.	x	x	x	-	-	-	Tamarisk-leaved Feather Moss
Liverwort							
Pellia epiphylla (L.) Corda	x	x	-	-	-	-	Wide-nerved Liverwort

Appendix 2

An Artificial Key to Immature Lumbricids occurring commonly in the Alnus/Betula Woodland at Wynyard, Co. Durham

1. Worms with red brown or violet colouration, commonly called 'pigmented' 2

   Worms without red brown or violet colouration, commonly called 'unpigmented' 5

2. Posterior segments flattened dorso-ventrally; prostomium tanylobous 3

   Posterior segments cylindrical or octagonal; prostomium epilobous 4

3. Posterior segments broadly flattened; pigmentation almost confined to a mid-dorsal stripe; chaetae paired more widely in terminal regions ... Lumbricus terrestris Linnaeus

   Posterior segments less flattened; body red brown throughout; chaetae closely paired throughout ... ... Lumbricus castaneus (Savigny)

4. Posterior segments octagonal; anterior segments more violet or purple; chaetae distant Dendrobaena octaedra (Savigny)

   Posterior segments cylindrical, with yellow or orange pigment; body segments red or red brown throughout; 'bundling' of individuals in tap water, with adhesion of detritus due to copious mucus secretion ... ... Dendrobaena rubida (Savigny) f. subrubicunda (Eisen)

5. Posterior 2 - 3 segments containing conspicuous yellow pigment; chaetae widely paired ... Octolasion cyaneum (Savigny)

Posterior segments without conspicuous yellow pigmentation; chaetae closely paired 6

6. Posterior segments quadrangular; medium brown colouration ... ... *Eiseniella tetraedra* (Savigny) f. *typica*

Posterior segments cylindrical or somewhat flattened dorso-ventrally; pink, pale grey or white colouration 7

7. Anterior segments indistinct, pale pink, retracted to give a rounded shape when irritated; posterior segments cylindrical, with same colouration as preceding segments ... ... *Allolobophora rosea* (Savigny) f. *typica*

Anterior segments well-defined, reddish colouration, extended to give a pointed shape when irritated; posterior segments slightly flattened, paler than preceding segments ... ... *Allolobophora caliginosa* (Savigny)

Appendix 3

The registration numbers of specimens of Wynyard Lumbricidae stored at the British Museum

Allolobophora rosea (Savigny) f. *macedonia* (Rosa)	B.M.(N.H.)	Reg.	No.	1966	.25	.2.
Bimastos muldali Omodeo	"	"	"	"	"	1.
Bimastos eiseni (Levinsen)	"	"	"	"	"	3.
Dendrobaena rubida (Savigny) f. *tenuis* (Eisen)	"	"	"	"	"	7.
Octolasion cyaneum (Savigny)	"	"	"	"	"	4-6.

Appendix 4

Dry weights of Cocoons collected during the Population Survey

Species	No. of cocoons weighed	Mean cocoon dry weight (mg.)	St.Deviation	St. Error
L. terrestris	203	17.88	3.10	0.22
O. cyaneum	146	9.37	1.98	0.16
A. rosea	41	2.19	0.53	0.08
D. octaedra	48	1.04	0.20	0.03
D. rubida f. *subrubicunda* and *L. castaneus*	57	0.84	0.24	0.03

Appendix 5

The Major Parasite Infection of Allolobophora rosea (Sav.) f. typica at Wynyard, Co. Durham

Introduction

During studies involving the lumbricid species A. rosea, individuals were collected from a mull-type soil beneath bramble vegetation at a site close to the study area at Wynyard, Co. Durham. A small proportion of these individuals were infected by a parasitic micro-organism which did not occur in the A. rosea population of the study area. However, if infected individuals were confined with normal worms in small quantities of soil medium, the previously normal worms became infected within a few weeks. Since the possibility existed that the infection might arise in the study area during the course of this work, the syndrome of the infection was studied in detail and attempts were made to identify the parasitic organism. The infection did not, in fact, spread to the study population of A. rosea.

The Syndrome

The post-clitellar region of A. rosea is usually pale-grey in colour, sometimes with a trace of green. In a recently infected worm the tail segments became white or greenish-white, the loss of colouration spreading forward as the infection became established. The epidermal tissue in the infected regions lost its texture, becoming flaccid, amorphous and eventually breaking down to release copious quantities of coelomic fluid. The worm segments in the infected regions were almost or totally paralysed. The infection eventually

reached the clitellum/gizzard region and it was assumed that worm death occurred at this stage, since no worms were collected from the field with a more extensive infection and worms in laboratory confinement were invariably 'missing' or found dead after this stage had been reached. Studies on infected worms kept in pot media, sunk into the field soil, showed that the infection resulted in a gradual fall in worm body weight. The complete cycle, from visible infection in the early stages to death of worms infected throughout their length posterior to the clitellum/gizzard region, took 4 to 6 months for completion.

The Parasite

Infected worm segments were teased out in a water droplet on a glass slide and examined beneath a coverslip, using a light microscope. Cigar-shaped cysts, containing one to four nuclei - arranged linearly, were found in great profusion. The cysts were occasionally arranged in star-shaped groupings though the majority were individually suspended in the fluid. Larger spherical, and occasionally club-shaped, bodies filled with numerous small nuclei occurred amongst the cysts. Samples of exuded coelomic fluid, taken from the infected but undamaged regions of parasitised worms, were found to contain cysts in abundance. The parasite was thus shown to be at least partially coelomic in nature. The stages observed were reminiscent of those shown by coccidian parasites (Sporozoa), but Professor Garnham (pers, comm.) discounted this possibility since the organism was Gram

positive. He consulted mycologists, at the London School of Hygiene and Tropical Medicine, who agreed with his diagnosis of the parasite as an algal infection. Dr. George (pers. comm.) suggested that if the organism was an alga it should be possible to produce cultures on soil-extract agar. Attempts along these lines have so far been unsuccessful. The identity of the parasite must therefore remain in some doubt.

Appendix 6

Investigations of Mole Predation on the Lumbricidae at Wynyard

Introduction

Lumbricids have been known, in various situations, to form a prey source for birds, moles and centipedes (Chilopoda) - especially Geophilus spp., (Friend, 1924; Kühnelt, 1961). Kühnelt also cited a predacious snail (Testacella sp.) and adult and larval ground beetles (Carabidae) as species preying on lumbricid worms. Very few studies have been attempted with a view to ascertaining the quantitative effects of predation on lumbricid population size and/or species diversity. Walton (1928) reported that the United States Biological Survey found lumbricid remains in the stomach contents of forty-five species of birds. Satchell (1955a) compared measures of lumbricid abundance with corresponding population estimates of Chilopoda and Staphylinidae (known to include species predacious on earthworms) in Park Grass plots at Rothamsted. The regressions were negative and significant at the 5 per cent level for A. rosea (Sav.) on Chilopoda and for L. castaneus (Sav.) on Staphylinidae in only one out of two years in both cases. The results were therefore regarded as inconclusive.

Numbers of mole-hills were also counted in the Park Grass plots and of six regressions for lumbricid abundance on mole-hill numbers which were significant at the 10 per cent level, five were positive. Mole-hill numbers have been shown to be related to mole activity but not mole numbers (Godfrey and

Crowcroft, 1960). Satchell's conclusions could thus be rephrased as follows: the Park Grass results indicated that moles were more active in regions of lumbricid abundance, but mole predation was not responsible for lumbricid scarcity in other regions. Numerous examinations of mole stomach contents have been carried out by various authors, but quantitative estimates from these studies are very difficult since digestion rates vary according to food type (Godfrey and Crowcroft, 1960). The main categories of food in mole stomachs can be identified fairly easily, since the prey is often swallowed whole or in large fragments. The identification of lumbricid species has rarely been possible since the anterior end and clitellum of the same adult worm have been needed for this purpose.

Of the invertebrate carnivores known to prey on lumbricid worms, geophilids and staphylinids were known to occur in the grid area. From their small size it was assumed that they would only attack small immatures of A. rosea and from the exceptionally low mortality found in the small immature size class it appeared that their predacious behaviour towards this species was not a serious cause of mortality to the total A. rosea population. The mortality of larger worms was of a much higher order (see p. 153). The bird population of the area was sparse and could not have accounted for a measurable proportion of lumbricid mortality. Thus the only possible sources of appreciable mortality were mole predation, death due to variation in physical factors of the environment,

and parasitism. Parasitism was important in some regions at Wynyard, but not in the study area. The environmental factors of soil pH, water content and temperature were shown in the present study to be fairly stable and never beyond the limits of lumbricid tolerance; food supply was not thought to be limiting for this soil-dwelling species (see Part II, Section III). Unless some unknown factor was involved, mole predation was thus the only source of appreciable mortality in the A. rosea population of the grid area at Wynyard.

Quantitative counts of mole-hill distribution over the grid area were not impossible, if the ground vegetation was to be preserved. However, mole activity was seen to be highest in the alder zone where A. rosea was found to be most abundant. Moles were known to occur throughout the grid area and two fortresses were located - one in B3 and one, off the grid area, near E4. The mere presence of moles, and the logical arguments given above, did not amount to direct evidence for mole predation on A. rosea. A seasonal study of mole stomach and gut contents was therefore undertaken: to elucidate not only the degree of predation on A. rosea, but also the hitherto impossible field feeding habits of moles with respect to different lumbricid species. Data on total stomach contents - including non-lumbricid prey items - was collected in order to assess the range of food sources available to moles at Wynyard. The assessment of the importance of lumbricid prey, in relation to other food sources, was not attempted due to the errors introduced by varying digestion rates (Godfrey and Crowcroft, 1960). Variation in the mole digestion rate for

different lumbricid prey species did not affect comparisons of mole diet with respect to lumbricids, since the chaetal examination method employed in the present study excluded such errors.

Methods

As a preliminary to the survey of mole stomach and gut contents, a pictorial key to the chaetal forms of various lumbricid species was formulated. Fifteen to twenty variously sized individuals of each of the lumbricid species found in the grid area - with the exception of the rare species A. chlorotica (Sav.) and B. muldali Omodeo - were collected for examination. Each worm was placed in boiling KOH solution (15%) and the chaetae, plus gut content and other insoluble debris, were pipetted on to a glass slide. The chaetae were examined using a light microscope, with below-stage illumination, and drawings were made of the most abundant chaetal form for each individual. Genital chaetae, malformed chaetae and chaetae in early stages of development were not included in this investigation. The normal, fully-developed somatic chaetal forms, for the various lumbricid species, were carefully defined and used in the seasonal investigation of mole diet.

Moles were trapped seasonally using pincer traps. The traps were inserted into occupied runs in quarter squares A1 and F4 - areas characteristic of 'bramble' and 'grasses' vegetation respectively. The traps were laid, and checked daily over a one-week period, in the middle of each 'season'.

Trapped moles were labelled according to the date and area of capture and stored in a refrigerator at -20°C. At a later date, each mole was thawed slowly and dissected to remove the whole of the gut system, excluding the oesophagus. The sex of the mole was noted and the stomach and lower gut were placed separately in 70% alcohol.

The stomach was bisected and the contents washed into a 10 cm. diameter crystallising dish containing alcohol. All distinguishable pieces of tissue - larger than approximately 2 mm. in diameter - were rinsed and arranged in a petri dish containing alcohol. These items were examined ('macro-analysis') under a binocular light microscope, with surface illumination. Each item was identified, noted and discarded. Fragmented worms were reconstructed to determine the number present.

The remaining stomach contents and the lower gut - with contents intact - of each mole were separately subjected to detailed examination ('micro-analysis') of lumbricid chaetal forms. Each material was boiled in a small beaker containing a suitable quantity of 15% KOH solution. Two pieces of glass tubing were placed in the beaker, and the solution was stirred continuously, to prevent 'bumping'. Boiling was continued for three to five minutes. The chaetae were already loosened from the lumbricid tissues by the mole digestive juices, but it was necessary to remove KOH-soluble organic material for subsequent clarity of examination. The stirrer and glass tubing were washed in tap water and the KOH, plus undigested

material, was poured into a centrifuge tube. The material was spun at approximately 2,000 revs per minute for one minute and the bulk of the supernatant discarded. Five samples - each being a droplet just sufficient to fill the area beneath a 2.2 cm. x 2.2 cm. coverslip - were pipetted from the concentrate and examined individually using a light microscope with below-stage illumination. Chaetae of each lumbricid species or type were counted separately in each sample and, using the maximum number found in any sample unit, classified as 'abundant' (more than thirty chaetae), 'common' (less than thirty, more than ten) or 'occasional' (less than ten). A petrological microscope, with crossed Nicol prisms, was occasionally used to discern chaetae from a concentration of background material, but this was not usually necessary.

Results

Fig. a1 shows the 'average' chaetal forms for lumbricid species of the grid area. The numbers shown indicate the relative lengths, in approximate terms, of shaft portions external ('1' for all chaetae in Fig. a1) and internal to the nodulus in life. The chaetae were not drawn to the same scale since chaetal size varied according to chaetal position on the individual and to the size of the individual worm. Chaetae from adult worms of most species were found to be of similar total length (about 400μ) from tip apex to base. L. terrestris chaetae (shown larger in Fig. a1) from large immature or adult worms were approximately 550μ, or more, in length. Chaetae of D. rubida (Sav.) f. subrubicunda (Eisen) never attained a length of more than

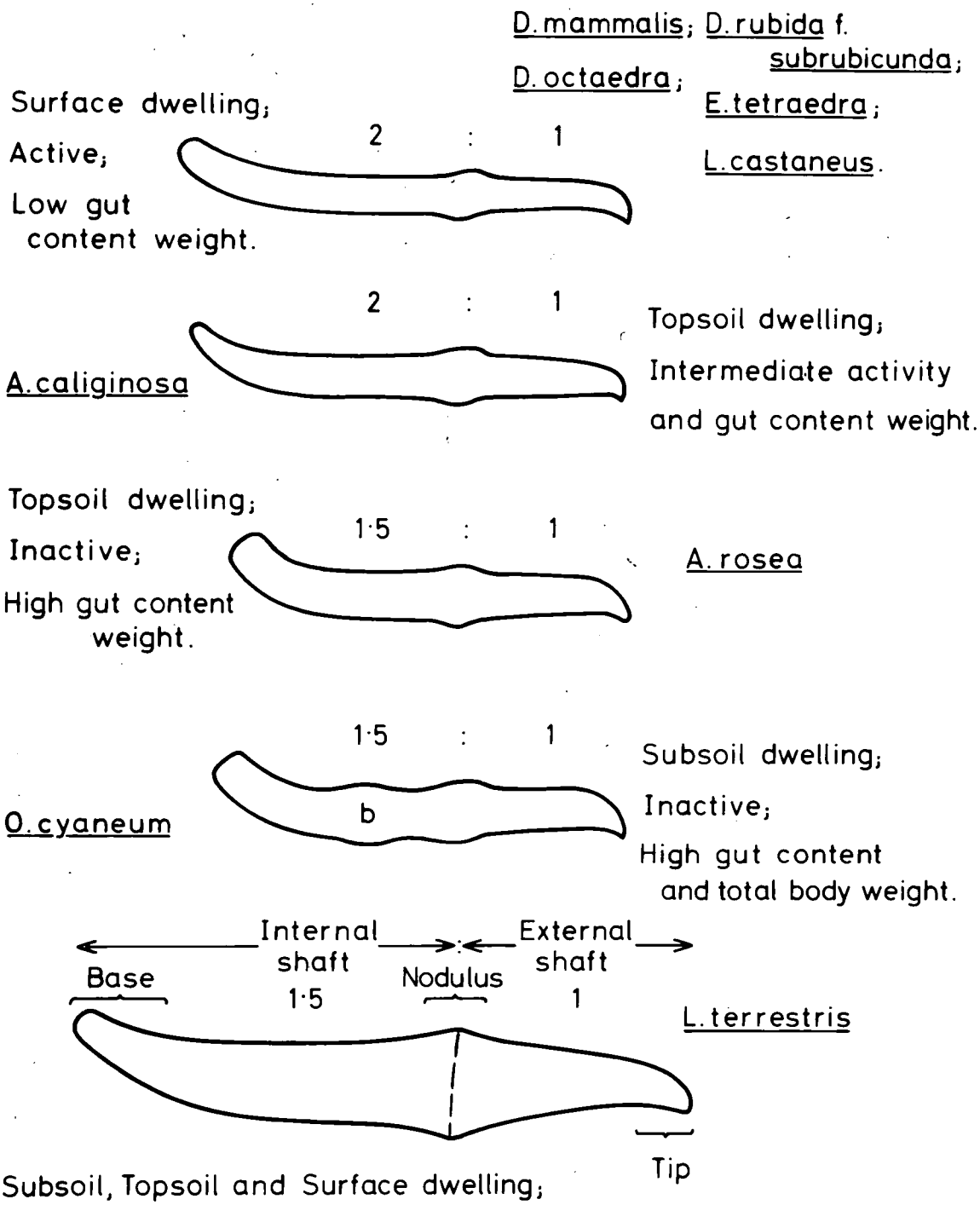


Fig. a1. Lumbricid chaetal forms; the associated ecological and morphological features of the species and species groups are shown.

about 250μ.

There was considerable variation in chaetal form between individuals of a particular species, but each individual showed remarkable constancy of chaetal shape. Chaetae of D. mammalis (Sav.) D. octaedra (Sav.), D. rubida f. subrubicunda, E. tetraedra (Sav.) and L. castaneus - collectively termed 'the surface active species' hereafter - showed a uniformly slim shaft; the base was gently but markedly curved. A. caliginosa chaetae showed a somewhat stouter shaft, varying in width; the base was curved and tapered. A. rosea chaetae were uniformly stout with a broad, blunt base showing more sharply-angled curvature; the shaft external to the nodulus was often near conical in shape. O. cyaneum chaetae were of similar general form to those of A. rosea, but with slightly less curvature of the base, which was never broader than the shaft internal to the nodulus - as sometimes occurred in A. rosea. O. cyaneum chaetae usually showed a secondary swelling ('b' in Fig. a1) on the shaft internal to the nodulus. This secondary swelling varied in size and was occasionally more internally placed than shown in Fig. a1, but its occurrence was diagnostic of the species. L. terrestris chaetae showed an external shaft of conical form with an internal shaft gently tapering towards the base. The base itself was often sharply tapered as shown in Fig. a1 but could be broader with a 'fractured' appearance. Chaetae of larger L. terrestris individuals - as were usually found in mole stomach and gut contents - were easily distinguishable by their large size.

The tips and noduli of lumbricid chaetae were generally of the form shown in Fig.a1. The chaetal nodulus of L. terrestris showed a sharp crest which often appeared as a hairline (broken line in Fig.a1). The noduli of other species were more bulbous in form. Noduli of all species were displaced from the line at right angles to the shaft, the crest being nearer to the chaetal tip on the side of concave base curvature. The curvatures of the chaetal tips for the different species were usually as shown in Fig.a1, but certain species - notably A. caliginosa and O. cyaneum - showed a degree of variability. The sharpness of the tip was probably affected by the amount of abrasion encountered - i.e. the age and mode of life of the individual - but chaetal tips in A. rosea and L. terrestris were most often in the blunted condition.

Recently ingested worms of the smaller lumbricid species were found to be whole, or merely bisected, in mole stomachs. Large L. terrestris individuals were in pieces 1 to 1.5 cm. in length. Lumbricid gizzards, and to some extent clitella, were found to be resistant to digestion by gastric juices. Gizzards, which were often contracted so that they still gripped a quantity of the lumbricid gut contents, were found in mole stomachs where other body tissue of the original lumbricid was no longer recognizable.

Tables a1, a2, a3 and a4 show the stomach and gut contents of a number of moles - as determined by macro- and micro-analysis - captured in autumn (1967), winter (1967/68), spring (1968) and summer (1968), respectively. In these tables the

Trap Region						Square A		
Mole Sex						♂	♀	♀
STOMACH	Macro-analysis	Dipterous larvae	Tipulidae			8	2	4
			Other			0	0	0
		Cockchafer larvae				1	0	2
		Slugs				0	0	0
		Lumbricidae	Identified worms & fragments	Species		L.t.	L.t. O.c. L.c. A.r.	L.t.
				No. & Age	A	1	1 1	
					LI		1 1	1
					SI			
					C		1	
			Gizzards (Diameter & Contents)	3 mm.	Veg.			
					Soil			
					Nil			
				3 2 mm.	Veg.			
					Soil			1
					Nil			1
				2 mm.	Veg.	1		
					Soil			1
					Nil			
	Micro-analysis (chaetae)			Abundant		L.t.	L.t.	L.t.
				Common		S.A.	O.c. S.A. A.r.	O.c. A.r.
				Occasional		Dr.s? A.r.		
GUT	Micro analysis (chaetae)			Abundant				
				Common			L.t.	
				Occasional		L.t. S.A. A.c. O.c.	O.c. A.r.	L.t. O.c. A.r. S.A.

Table a1. Mole stomach and gut contents in 'Autumn' (31st October to 6th November, 1967). Key in Text.

	Trap Region					Square A					Square F				
	Mole Sex					♂	♂	♀	♀	♀	♂	♂	♂	♀
STOMACH	Macro-analysis	Dipterous larvae	Tipulidae			12	1	0	22	0	1	1	1	0
			Other			1	1	1	0	0	0	0	0	0
		Cockchafer larvae				0	1	2	0	0	0	0	1	1
		Slugs				2	1	0	0	0	0	0	0	1
		Lumbricidae	Identified worms & fragments	Species		Lt.		Lt.		Lt.	Lt.		Lt.	Lt.
				No. & Age	A	2				1	1		1	
					LI	1		2		2	1		1	1
					SI									
					C	6								
			Gizzards (Diameter & Contents)	3 mm.	Veg.		1					1		
					Soil									
					Nil									
				3 2 mm.	Veg.									
					Soil		1							
					Nil									
				2 mm.	Veg.				1			1		
					Soil				2			1		
					Nil									
	Micro-analysis (chaetae)			Abundant		Lt.		Lt.			Lt.		Lt.	Lt.
				Common		Ar.			Drs.? Ar. Ac. Oc.	Lt. Ar.				Ac.
				Occasional		Ac.	Lt. SA.		Lt.	Ac. Oc.		Lt.		Ar. Drs.?
GUT	Micro-analysis (chaetae)			Abundant			Lt.	Lt.				SA.	Lt.	
				Common		Lt. Ac.	SA.		Drs.? Ar. Ac. Oc.		Lt.	Lt.		
				Occasional		Ar.	Ac.		Lt.	Lt. Ar. Oc.	SA. Ar.	Drs.? Oc.		Ac. Ar Drs.?

Table a2. Mole stomach and gut contents in 'Winter' (22nd to 29th January, 1968). Key in Text.

Trap Region							Square A							Square F		
Mole Sex							♂	♂ (P)	♂		♂ (P)			♂	♂	♀
STOMACH	Macro-analysis	Dipterous larvae	Tipulidae				4	2	8		0			0	2	1
			Other				0	0	0		1			0	0	0
		Cockchafer larvae					1	0	0		0			2	1	0
		Slugs					0	0	1		0			0	1	0
		Lumbricidae	Identified worms & fragments	Species			Lt. Ac.	Lt.	Lt. Lc. Dc.					Ar.	Lt.	Lt.
				No. & Age	A		1 1		1 1 2						1	1
					LI									2	1	
					SI				1 1							
					C			1								
			Gizzards (Diameter & Contents)	3 mm.	Veg.		1									1
					Soil				1							
					Nil									1		
				3 2 mm.	Veg.		1									
					Soil			1							2	3
					Nil											1
				2 mm.	Veg.										4	
					Soil		1		1					1	3	1
					Nil									1		2
	Micro-analysis (chaetae)			Abundant										Ar.	Lt.	Lt. Oc. Ar.
				Common			Lt. Ac.		SA. Oc.					Lt.	Ar.	SA. Drs?
				Occasional			Ar.	Lt. Ac.	Lt. Ar.					Oc. SA.	Oc SA Drs?	Ac.
GUT	Micro-analysis (chaetae)			Abundant			Lt. Ar.	Ac.						Lt.	Oc.	
				Common				Lt.	Oc.							Lt. Ar.
				Occasional			Ac. SA.	SA.	Lt. SA.		Lt. Ar.			Oc. SA. Ar. Ac. Drs?	Lt Ac. Ar.	Oc. Ac.

Table a3. Mole stomach and gut contents in 'Spring' (11th to 18th May, 1968). Key in Text.

Trap Region						Square A			Square F		
Mole Sex						♂	♂	♂	♀		♀
STOMACH	Macro-analysis	Dipterous larvae	Tipulidae			0	1	0	0		0
			Other			0	0	0	0		0
		Cockchafer larvae				0	0	0	4		0
		Slugs				0	0	2	0		0
		Lumbricidae	Identified worms & fragments	Species		L.t.	L.t.	L.t.	L.t.	A.c.	D.o.
				No. &Age	A	1					
					LI		1	1	1	1	1
					SI						
					C	1					
			Gizzards (Diameter & Contents)	3 mm.	Veg.						
					Soil						
					Nil			1			1
				3 2 mm.	Veg.						
					Soil			1			1
					Nil						1
				2 mm.	Veg.						
					Soil						
					Nil						
	Micro-analysis (chaetae)			Abundant		L.t.	L.t.	L.t.	L.t. A.c.		
				Common							L.t. O.c. S.A.
				Occasional			A.r.	O.c.	A.r.		A.r.
GUT	Micro-analysis (chaetae)			Abundant					L.t.		L.t.
				Common		L.t.	A.r.	L.t.			A.c.
				Occasional			L.t.	O.c.	A.c. A.r.		O.c. A.r.

Table a4. Mole stomach and gut contents in 'Summer' (4th to 11th August, 1968). Key in Text.

recognizable lumbricid worms or fragments found in the stomach contents of each mole were shown according to species and age group(s). Any cocoons present were also indicated according to species. Gizzards recovered from the stomach contents were recorded according to size and the material, if any, which they contained: plant debris ('Veg.') or soil. The key for abbreviations used in the analysis tables is as follows:

Lumbricid Number and Age categories:

A - Adult worms

LI - Adolescent worms ('Large Immature')

SI - Young worms of small size ('Small Immature')

C - Cocoons

Chaetal categories: Species abbreviations:

L.t. - Lumbricus terrestris L. : L.t.

O.c. - Octolasion cyaneum (Sav.) : O.c.

A.c. - Allolobophora caliginosa (Sav.) : A.c.

A.r. - Allolobophora rosea (Sav.) f. typica : A.r.

S.A. - Larger surface active species (Dendrobaena mammalis (Sav.); Dendrobaena octaedra (Sav.); : D.o.
Eiseniella tetraedra (Sav.) f. typica; Lumbricus castaneus (Sav.).) : L.c.

D.r.s.?-Chaetae of 'Surface active' form but of sufficiently small size to indicate the presence of Dendrobaena rubida (Sav.) f. subrubicunda (Eisen).

(these chaetae could, of course, be from small immature specimens of the larger surface active species).

Mode of Mole Capture:

(P) - Paw capture; indicating a period spent in the trap prior to death.

No differences were shown between the diet of male and female moles in any season. Of the seven moles trapped and examined in spring, only one was female. This was thought due to the presence of newborn young in the fortress - as suggested by Godfrey and Crowcroft (1960).

In autumn, 1967, only three moles were trapped - all from quarter-square A1. All three stomachs contained tipulid larvae, and cockchafer larvae had been eaten by two of the moles. Large specimens of L. terrestris had been recently ingested by all three moles and one stomach contained an L. terrestris cocoon in addition to an adult worm of this species. One stomach contained recently ingested specimens of O. cyaneum, L. castaneus and A. rosea, but the micro-analyses of both stomach and gut contents revealed the presence of chaetae from a range of lumbricid species, including A. rosea, in each of the moles captured.

Nine moles were trapped and examined in winter, 1967/68, five from the 'bramble' area and four from the 'grasses' area. Tipulid larvae had been ingested by six of the moles and non-tipulid dipterous larvae, cockchafer larvae and slugs were all found in the stomachs of various moles. The only

identifiable worm fragments were those of large L. terrestris specimens in six of the nine stomachs examined. L. terrestris cocoons were also found in the stomachs of two moles. The chaetal examination revealed that a range of lumbricid species had been ingested by seven of the nine moles. A. rosea had been ingested by five of the moles. O. cyaneum was present in only three of the moles, and its chaetae were only 'occasional' in two of these. L. terrestris chaetae were the most commonly occurring type. Gizzard recoveries were seen to roughly correspond with the results of the chaetal examination. No significant differences were observed in the diet of moles trapped in the two vegetational areas.

Five of the seven mole stomachs examined in spring, 1968, contained tipulid larvae, One non-tipulid dipterous larva, four cockchafer larvae and two slugs were recovered from various moles. The variety of lumbricid prey species ingested was indicated by the recognizable presence of worms of four of the smaller lumbricid species,and a number of small gizzards, in the mole stomachs, in addition to specimens of L. terrestris. However, the chaetal examination revealed the full extent of the mole diet variation. Every mole examined had ingested a range of lumbricid species. All the lumbricid species or species types were represented, occasionally within the diet of a single mole. A. rosea had been ingested by six of the seven moles and its chaetae were 'abundant' in the stomach or gut contents of three of the predators. O. cyaneum and A. caliginosa chaetae were also

found 'abundant' on occasion, and chaetae of all the smaller lumbricid species categories were occasionally 'common'. L. terrestris was still the most common prey species, worm fragments occurring in the stomachs of five moles and its chaetae in the stomach and gut contents of all. One L. terrestris cocoon was recovered. Two of the 'spring'-trapped moles had spent some time in the traps before death. The stomach of one of these moles was found to contain a L. terrestris specimen; chaetae in this stomach were only 'occasional' - though A. caliginosa chaetae were 'abundant' in the gut contents. The stomach of the other paw-trapped mole contained no lumbricid remains of any kind, and chaetae of L. terrestris and A. rosea were only 'occasional' in the gut contents. Gizzard recoveries corresponded well with the results of chaetal analyses. O. cyaneum was found to have been ingested by all three moles trapped in the 'grasses' area, but only by one of the moles from the 'bramble' area. Apart from this, there seemed little difference between mole diets in the two areas.

In summer, 1968, the five mole stomachs examined contained much less food material than might have been expected in this season. One stomach contained four small cockchafer larvae, another contained a single tipulid larva and a third contained two small slugs. The recognizable lumbricid fragments were mainly of L. terrestris, though single immatures of A. caliginosa and D. octaedra had been recently consumed by two of the moles. The chaetal

examinations of mole stomach and gut contents revealed a slightly wider range of lumbricid species in the mole diets. A. rosea had been eaten by three of the five moles, and O. cyaneum chaetae were present in the digestive tracts of two moles. The sample of moles was numerically too small for comparisons between male and female predators, or between predators captured in different vegetational areas.

Discussion

The 'average' lumbricid chaeta is an anchoring device, protracted when the longitudinal muscles are contracted and retracted when the circular muscles are operative. In the protracted position the posteriorly-pointing chaetal tip grips the substrate, whilst the contracted longitudinal muscles presumably brace the internal shaft. It would seem from the results of the present study that chaetal shape in the Lumbricidae is related to the mode of life of the various species. Variation in shape may be primarily due to the observed degree of uniformity in overall chaetal size. It may be significant that in L. terrestris, the only large species studied which showed an increase in chaetal size, the chaetal form was of the most generalised type. This species may be exceptional, therefore, in that mechanical problems may have been overcome by selection of individuals with adaptations of formative follicle size, rather than chaetal shape. D. rubida f. subrubicunda showed reduced chaetal size in addition to modified chaetal shape, and may be highly specialised in this respect.

The surface active species, with rapid locomotory activity but a low body weight: volume ratio, were found to possess slender chaetae with a relatively shorter external portion. This form will give an efficient, shallow anchoring system with maximum bracing length internally. Additional chaetal strength would give little advantage since the chaetae of these light, surface-dwelling species rarely bear a significant proportion of the weight of the individual.

Chaetae of the slow-moving, soil-dwelling species A. rosea and O. cyaneum must regularly bear a proportion of the considerable body weight of these worms. This probably accounts for the stoutness of the chaetae found in these species. The external shaft was shown to be relatively longer in these forms, presumably to provide more grip on the burrow wall - at the expense of internal bracing length. The secondary bulge on chaetae of O. cyaneum, the largest purely soil-dwelling species studied (with the highest body density), may be a bracing adaptation to compensate for the loss of internal length.

A. caliginosa is of body density and mode of life intermediate between the surface-active species and the above soil-dwelling species. It may be significant, therefore, that the chaetae of A. caliginosa were found to be of a form intermediate between the two types so far described.

L. terrestris is exceptional amongst the pigmented species examined in the present study, in that though the body density is low, the overall body weight is considerable. Despite the size of the animal, locomotory activity is high and the chaetae

must regularly bear a high proportion of the full weight of the individual in its near-vertical burrow. High substrate grip, bracing length and mechanical strength are thus all demanded by the body size and mode of life of worms of this species. The possession of large chaetae, described in the present studies, may thus have been a primitive, essential development in the evolution of L. terrestris.

The constancy of chaetal form for individual worms suggested that the variation between individuals was of genetical origin.

The micro-analysis of mole stomach and gut contents was shown to be a valuable technique for the elucidation of mole predation on lumbricids. The results for macro and micro-analysis, viewed comparatively, suggested that 'abundant' or 'common' chaetae in the stomach indicated a very recent meal, of which the identifiable remains were often present in the stomach. 'Occasional' chaetae indicated a lumbricid meal from which the body tissue, with the exception of the gizzard, had been totally digested. Gut content chaetal counts were more difficult to interpret, since chaetae were in a greater dilution and mole gut turnover rates were unknown. However, 'abundant' chaetae probably indicated a recent meal at a late stage of digestion; 'common' chaetae could have been from either a meal in an early stage of stomach digestion or a meal whose remains had been partly egested; 'occasional' chaetae in the gut could have been from either a meal still identifiable in the stomach or a meal whose remains had been

almost completely egested. Gut content 'occasional' chaetae, when of the latter type above, provided the widest scope of diet detection by this technique. Some indication of the gut turnover time in moles was gained from the data for the two paw-trapped moles in spring. From the stomach and gut contents of other moles trapped in this season, it was assumed that lumbricid prey were available to moles in plenty. One of the paw-trapped moles had almost cleared the entire stomach and gut contents of lumbricid remains. It seemed probable, therefore, that mole gut turnover time was less than the 24 hours between trap clearances, and that the present investigation was related to mole diet during a maximum of 24 hours previous to the time of capture.

The moles at Wynyard obviously enjoyed a varied lumbricid diet in all seasons. L. terrestris was the most common lumbricid species constituent of the diet; this species was more frequently eaten, proportionally, during the winter and summer months. A. rosea, with most other lumbricid species, also figured significantly in mole diet throughout the year. The more frequent detection of these small species by chaetal analysis, than by identification of whole or fragmented worm specimens, suggested a higher mole digestion rate for these worms. This could be due to the higher surface area/volume relation in smaller worms.

The lower numbers of O. cyaneum in the diet of winter-trapped moles may have resulted from deeper burrowing habits of O. cyaneum in the winter months. This soil-feeding

lumbricid species is much less dependent on surface soil and organic debris than the other deep-burrowing species, L. terrestris.

The ease with which moles were trapped, and the range of lumbricid prey species detected in their diet, during the winter months showed that the predators were actively hunting during this season. If L. terrestris was stored in winter (see Dahl, 1886, 1891; Evans, 1948a; Godfrey and Crowcroft, 1960; Skoczeń, 1961), it was certainly not the only lumbricid species eaten. A. rosea had been eaten as commonly by moles trapped in the 'grasses' area (low A. rosea population density - see population survey) as by those trapped in A1. This was probably a feature of the wide-ranging habits of the moles during foraging. Both the occurrence of a number of L. terrestris cocoons in a single mole stomach and the similar occurrence of small tipulid larvae suggested a 'digging out' procedure by a mole which had located one such food item. Both L. terrestris cocoons and tipulid larvae were inactive organisms occurring in local aggregations in Wynyard soil. Such a 'digging out' procedure would be highly effective if applied to A. rosea population aggregations. A. caliginosa figured much more prominently in the mole diet than might have been expected from the low numbers of this species over the grid area. A. caliginosa is a moderately active species and this may have been the reason for its frequent capture, with other active lumbricid species, by moles. There are various theories, but little evidence for the mode of lumbricid capture by moles (Evans,

1948a; Godfrey, 1955; Skoczeń, 1961). Godfrey and Crowcroft (1960) suggested that smell and hearing may be used by the mole in detecting prey at close quarters, the final capture being stimulated by the texture and body movements of the prey. The Pyrenean desman - a close relative of the European mole - detects the presence of trout prey by use of the tactile hairs on the snout. Though these hairs are less well-developed in the mole, it may be that the 'hearing' suggested by Godfrey and Crowcroft is in fact a tactile sense which allows the mole to detect the movement of prey within a short distance of the excavated run. The prey could then be captured during a short excavation from the run. The numerous side-branches of short length, observed in mole runs at Wynyard, were thought to be explained by mole behaviour of this type.

Larkin (1948) estimated the mole population density of a grassland area as four to eight moles per acre. The numbers of moles trapped seasonally at two locations on the grid area at Wynyard - roughly a half acre in extent - suggested that by Larkin's standard the study area was densely populated by moles. Since the predators were expected to have been most active in regions of high prey density (Satchell, 1955a), the aggregated A. rosea population - shown to have been a species common in the mole diet - was probably subjected to a continuous and high predation mortality of larger worms throughout the year.

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