

ResearchOnline@JCU

This file is part of the following reference:

Tyndale-Biscoe, Marina (1985) *An ecological study of two dung beetles (Coleoptera, Scarabaeninae) with contrasting phenologies*. PhD thesis, James Cook University of North Queensland.

Access to this file is available from:

<http://researchonline.jcu.edu.au/33795/>

If you believe that this work constitutes a copyright infringement, please contact
ResearchOnline@jcu.edu.au and quote
<http://researchonline.jcu.edu.au/33795/>

An ecological study of two dung beetles (Coleoptera, Scarabaeinae)
with contrasting phenologies.

Thesis submitted by
Marina Tyndale-Biscoe BSc (Hons) (Tas.) in March 1985

for the degree of Doctor of Philosophy in the Department of
Zoology at James Cook University of North Queensland.

Access to thesis.

I, the undersigned, the author of this thesis, understand that James Cook University of North Queensland will make it available for use within the University Library and, by microfilm or other photographic means, allow access to users in other approved libraries. All users consulting this thesis will have to sign the following statement:

"In consulting this thesis I agree not to copy or closely paraphrase it in whole or in part without the written consent of the author; and to make proper written acknowledgement for any assistance which I have obtained from it."

Beyond this, I do not wish to place any restriction on access to this thesis.

(signature)

.....28/8/85.....
(date)

Declaration

I declare that this thesis is my own work and has not been submitted by me in any form for another degree or diploma at any university or other institution of tertiary education. Information derived from the published or unpublished work of others has been acknowledged in the text, in the acknowledgements, and a list of references is given.

Acknowledgements

I wish to thank Dr. Max Whitten, Chief of Division, for permitting me to do the work for this thesis on a part-time basis while employed in the Dung Beetle Section of the Division of Entomology, CSIRO, Canberra.

I also wish to thank my supervisors, Drs. Rhondda Jones of James Cook University of North Queensland, and Lindsay Barton Browne of the Division of Entomology, CSIRO, Canberra, for all the help and advice they gave me during the course of this work.

Special thanks are due to Dr. Murray Wallace of the Division of Entomology, CSIRO, Canberra, who provided the information on the distribution of Onthophagus granulatus in south-eastern Australia, and for innumerable discussions about dung beetles over the years.

I would especially like to thank Rhondda Jones, and Neil Gilbert of this Division, for help in the statistical analysis of the incidence of resorption in both dung beetle species; as well as Jo Walker, John Feehan and Erik Holm for all the technical help they provided. Trap catches, collections of weather data and dissections of Ot. granulatus at Armidale from 1977-1979 were made by M. Porter and I am grateful for his help.

Finally, I acknowledge the help of John Green who did the photography, Carole Wilson who typed the manuscript and Chris Hunt, who prepared the illustrations for this thesis.

Abstract

The literature on age-grading techniques of adult insects, published since 1968, is reviewed. This is followed by a study of the phenologies of two dung beetle species: the native Onthophagus granulatus Boheman, and the introduced Onitis alexis Klug, using age-grading techniques together with field and laboratory experiments.

At Uriarra, NSW, Ot. granulatus is a univoltine species which emerges in the summer or early autumn. It spends the remainder of the warm season in the maturation-feeding phase, a period which generally coincides with the presence of dung produced from a senescent and dried-off pasture. A proportion of beetles may begin breeding before the onset of cold weather, but the progeny do not survive the winter. The species over-winters underground in the adult stage, and reappears in the dung pads in the spring, when dung is generally produced from actively growing pastures. Oviposition occurs in the spring and early summer, and the adults die about the time of the emergence of the new generation. Periodic drought reduces the population by causing follicle resorption in the females and larval mortality. This was confirmed in laboratory experiments which showed that dung from hayed-off pasture caused high mortality in teneral adults, reduced fecundity in sexually mature females and caused high mortality in the pre-adult population. Additionally, dry conditions during larval development caused total mortality in the laboratory.

At Armidale, NSW, the species has basically the same life cycle except that a small proportion of larvae survive the winter and emerge as adults in the following spring.

Newly emerged Oi. alexis at Araluen begin to appear in pads in the late spring, and new emergence continues into the summer. The maturation-feeding phase is short, and oviposition starts within a week or two of emergence. Eggs laid in December/January develop to emerge as adults in late summer and autumn, eggs laid in February and later do not give rise adults until the following season. The species over-winters mainly in the larval stages, though a proportion of adults may survive the winter in some years. The species at Araluen is thus partly univoltine and partly bivoltine. The population was greatly reduced by cold, wet winters. Dung from hayed-off pasture caused some decrease in fecundity, though not as great as occurred in Ot. granulatus, and it also caused some mortality in the larvae. Follicle resorption occurred in response to rainfall.

Additionally, older Oi. alexis were more likely to resorb their follicles than were younger beetles, whereas all sexually mature Ot. granulatus were equally likely to resorb.

Temperature summation calculations showed that the predicted and observed times of emergence of Ot. granulatus arising from eggs laid in the field under experimental conditions coincided well, and they occurred at times of peaks of new emergence as determined by age-grading. Predicted times of emergence of the 1982-83 summer population of Oi. alexis always preceeded the observed emergence time, indicating the presence of a larval diapause.

Diapause in the third instar larvae of Oi. alexis was found to be facultative, and occurred in response to cold temperature and dry conditions during larval development, and to short daylength experienced by the parents. As well, progeny of young parents were more

likely to enter diapause than were the progeny of older parents.

Ot. granulatus is dependent on summer rainfall, and is unable to survive and breed well in dung produced from hayed off pastures. Temperatures above 30°C are lethal to the species. Its distribution is therefore very limited. Oi. alexis, in contrast, can survive dry summers and cold, dry winters (down to about 0°C) by entering diapause, and can breed in dung produced from dry pastures, though the survival of larvae is reduced. Its distribution appears to be limited only by cold wet winters. It has therefore spread from hot summer rainfall areas through regions of even rainfall and into temperate winter rainfall climates.

<u>Contents</u>	<u>Page</u>
Chapter 1 Introduction	1
Chapter 2 Age-grading in adult insects - a review	11
2.1 Introduction	11
2.2 Criteria used for age-grading	13
2.21 Changes in the reproductive system	13
2.211 Follicular relics	13
1 Follicular dilatations	17
11 Single follicular dilatations	21
111 Yellow body	23
2.212 Tracheolar skeins	29
2.213 Decline in fecundity	30
2.214 Other criteria	31
2.215 Changes in the reproductive tract of males	32
2.22 Somatic changes	32
2.221 Cuticle growth	32
2.222 Fluorescence	36
2.223 Colour and tergite pattern of abdomen	38
2.224 Fat body and other criteria	40
2.23 Changes associated with wear and tear	41
2.231 Wing fray	41
2.232 Cuticle wear	42
2.3 Some interpretations and applications of age- grading	43
2.4 Summary	49
Chapter 3 General methods	51
3.1 Field sites	51

	viii Page
3.2 Beetle traps	52
3.3 Dissections	52
3.4 Trap catches	54
3.5 Weather records	54
3.6 Laboratory cultures	55
3.7 Origin of dung and dung characteristics	56
3.8 Dung bioassay	57
3.9 Oviposition, developmental time and survival in the field.	58
Chapter 4 Validation of age-grading method.	60
4.1 Relationship between yellow body and tibial wear	60
4.11 Method	60
4.12 Results	61
4.121 <u>Ot. granulatus</u>	61
4.122 <u>O1. alexis</u>	61
4.2 Relationship between age and yellow body in <u>Ot. granulatus</u>	62
4.21 Method	62
4.22 Results	62
4.3 Distinction between nulliparous and parous <u>O1. alexis</u>	63
4.31 Method	63
4.32 Results	64
4.4 Conclusions.	66
Chapter 5 Age structure, incidence of resorption, trap catches and environmental quality in the field.	68
5.1 Introduction	68
5.2 Results	71
5.21 <u>Ot. granulatus</u> , Uriarra	71
5.211 Trap catches, age structure and resorption	71

5.212	Rainfall	72
5.213	Dung quality	73
5.22	<u>Ot. granulatus</u> , Armidale	74
5.221	Trap catches, age structure and resorption	74
5.222	Rainfall	75
5.23	<u>Oi. alexis</u> - Araluen	75
5.231	Trap catches, age structure and resorption	75
5.232	Rainfall	76
5.233	Dung quality.	77
5.3	Relationship between incidence of follicle resorption and other factors.	77
5.31	Statistical method	77
5.311	<u>Ot. granulatus</u> , Uriarra	79
5.312	<u>Ot. granulatus</u> , Armidale	79
5.313	<u>Oi. alexis</u> , Araluen	80
5.4	Conclusions	80
Chapter 6	Over-wintering and developmental time in the field.	83
6.1	Introduction	83
6.2	Overwintering	84
6.21	<u>Ot. granulatus</u> : survival as adults	84
6.211	Method	84
6.212	Results	84
6.22	<u>Ot. granulatus</u> : survival as immatures	86
6.221	Method	86
6.222	Results	86

6.23	<u>Oi. alexis</u> : survival as adults	87
6.231	Method	87
6.232	Results	88
6.24	<u>Oi. alexis</u> : survival as immatures	88
6.241	Methods	88
6.242	Results	88
6.3	Survival from egg to adult in the field throughout the season	89
6.31	<u>Ot. granulatus</u>	89
6.311	Method	90
6.312	Results	90
6.32	<u>Oi. alexis</u>	93
6.321	Method	94
6.322	Results	94
6.4	Conclusions	96
Chapter 7	Fecundity and longevity of adults.	98
7.1	Introduction	98
7.2	Temperature	98
7.21	<u>Ot. granulatus</u> - effect on life span and fecundity	98
7.211	Method	98
7.212	Results	99
7.22	<u>Oi. alexis</u> - effect on life span and fecundity	100
7.221	Methods	100
7.222	Results	100

7.3	Dung quality	101
7.31	<u>Ot. granulatus</u> - effect on maturation feeding period	102
7.311	Methods	102
7.312	Results	102
7.32	<u>Ot. granulatus</u> - effect on fecundity	103
7.321	Methods	103
7.322	Results	104
7.33	<u>Oi. alexis</u> - effect on fecundity	105
7.331	Methods	105
7.332	Results	106
7.4	Conclusions	106
Chapter 8	Development of immatures	107
8.1	Introduction	107
8.2	Temperature	109
8.21	<u>Ot. granulatus</u> - effect on rate of development	109
8.211	Method	109
8.212	Results	109
8.22	<u>Oi. alexis</u> - effect on rate of development	110
8.221	Method	110
8.222	Results	110
8.3	Dung quality	112
8.31	<u>Ot. granulatus</u> - survival of larvae, developmental rate and size of emerging adults	112
8.311	Method	112
8.312	Results	112

8.32	<u>Oi. alexis</u> - survival of larvae, developmental rate and size of emerging adults	113
8.321	Method	113
8.322	Result	114
8.4	Moisture	116
8.41	<u>Ot. granulatus</u> - effect on survival, developmental rate, and size of emerging adults	116
8.411	Method	116
8.412	Results	117
8.42	<u>Oi. alexis</u> - effect on survival, developmental rate and size of emerging adults	117
8.421	Method	117
8.422	Results	119
8.5	Combination of cold and high moisture	119
8.51	<u>Oi. alexis</u> - effect on survival	119
8.511	Method	119
8.512	Results	121
8.6	<u>Maternally induced diapause in larvae of <u>Oi. alexis</u></u>	122
8.61	Method	124
8.62	Results	125
8.7	Conclusions	127
Chapter 9	Discussion	129
9.1	Comparative and confirmatory aspects of this study	129
9.2	Follicular resorption as an indicator of stress	131
9.3	Diapause in <u>Oi. alexis</u>	135
9.4	Phenology of <u>Onthophagus granulatus</u>	138
9.5	Phenology of <u>Onitis alexis</u>	143
9.6	Conclusions	149

Appendix 1 References to species of insects that have been
age-graded by various techniques since Detinova's
review in 1968.

Appendix 2 Publications relating to the experimental work in
this thesis: (in pocket of back cover)

- a. Tyndale-Biscoe and Watson, 1977
- b. Tyndale-Biscoe, 1978
- c. Tyndale-Biscoe et al. 1981
- d. Tyndale-Biscoe, 1984

FIGURE CAPTIONS

- 1 a Onthophagus granulatus Boheman
- b Onitis alexis Klug
- 2 a Distribution of Ot. granulatus
- b Present distribution of Oi. alexis in Australia
- 3 Schematic representation of an insect ovary, showing the terminology used in this review
- 4 a Generalised insect ovary with follicular dilatations and
- b a single follicular dilatation
- 5 Generalised insect ovary with yellow body
- 6 Pitfall trap
- 7 Different degrees of tibial wear, and size of yellow body characteristic of Oniticellus intermedius (from Tyndale-Biscoe, 1978).
- 8 a Ot. granulatus - relationship between yellow body and tibial wear
- b Oi. alexis - relationship between yellow body and tibial wear
- 9 a Ot. granulatus ovary with yellow body
- b Oi. alexis ovary with yellow body
- c O. alexis ovary with terminal follicle extruded into haemocoel
- d Oi. alexis ovary with extruded follicle being resorbed in haemocoel
- 10 Oi. alexis with extruded follicular sheath

- 11 Ot. granulatus trap catches, percent resorption and age structure, together with seasonal variation in dung quality as measured by bushfly percent pupariation and pupal weight, and rainfall at Uriarra during the
 - a 1976-77 season
 - b 1977-78 season
 - c 1978-79 season
 - 12 Ot. granulatus trap catches, percent resorption and age structure, together with seasonal variation in rainfall at Armidale during the
 - a 1977-78 season
 - b 1978-79 season
 - 13 Oi. alexis trap catches, percent resorption and age structure, together with seasonal variation in rainfall at Araluen during the
 - a 1980-81 season
 - b 1981-82 season
 - c and including dung quality as measured by percent bushfly pupariation and pupal weights during the 1982-83 season
 - 14 a Effect of time of season on the proportion of Ot. granulatus resorbing follicles at Uriarra (adjusted for covariates) and at Armidale (unadjusted for covariates)
 - b Effect of time of season on the proportion of Oi. alexis resorbing follicles at Araluen (adjusted for covariates)
 - c Effect of age on the proportion of Oi. alexis resorbing follicles (adjusted for independents and covariates).
- 1=N₃, 2-4=parous with tibial wear 2-4.

- 15 Effect of temperature on Ot. granulatus in relation to
 - a mean lifetime fecundity
 - b mean fecundity per week
 - c longevity of females
- 16 Effect of temperature on Oi. alexis in relation to
 - a mean lifetime fecundity
 - b mean fecundity per week
 - c longevity of females
- 17 Effect of dung quality on Ot. granulatus in relation to
 - a fecundity
 - b mortality of females
- 18 The relationship of fecundity of Ot. granulatus and Oi. alexis, when fed on dung of different qualities, with
 - a bushfly puparial weights and
 - b percent bushfly survival
- 19 Effect of dung quality on Oi. alexis in relation to
 - a fecundity
 - b survival of females
- 20 Regression of the relationship between developmental rate from egg to adult and temperature in Ot. granulatus and Oi. alexis
- 21 Summary of Ot. ganulatus phenology at Uriarra, A.C.T.
 - a monthly trap catches
 - b mean monthly percent newly emerged and nulliparous beetles in samples

- c Predicted times of emergence from eggs laid at different times of the year (arrows), and actual emergence from cylinders (). Broken lines indicate failure of the beetles to emerge from the cylinders.
- d Monthly rainfall and total monthly accumulation of day degrees
- 22 Summary of Ot. granulatus phenology at Armidale, N.S.W.
a-d same as for fig. 21
- 23 Summary of Oi. alexis phenology at Araluen, N.S.W.
a-d same as for fig. 21.

Chapter 1. Introduction

Scarabaeinae are generally coprophagous beetles, which use animal excrement (in the majority of species herbivore dung) both as adult and larval food. A great amount of interest has been generated in them in recent years because of their possible use as biological control agents (Waterhouse 1974, Bornemissza 1976). Many studies assessing their potential for controlling dung breeding flies, and their ability to breed in and disperse dung under different conditions, have been reported in the literature (Blume et al. 1973, Hughes et al. 1978, Moon et al. 1980, Ridsdill Smith 1981, Tyndale Biscoe et al. 1981a, Lee and Peng 1982, Wallace & Tyndale Biscoe 1983, and others.)

The nesting behaviour amongst the different orders of Scarabaeinae is very diverse and interesting, resulting in a mass of literature on descriptions of different nesting patterns, different ways of provisioning the young, and different degrees of sexual cooperation during the nesting cycle. This has also led to speculation about the probable evolution of the different behavioural types. All these have been ably reviewed by Halffter & Matthews 1966, Halffter 1977, Halffter and Edmonds 1982 and Klemperer 1983.

However, there have only been a few studies reported in the literature on life histories of Scarabaeinae. Most of what was known up to 1966 was descriptive and was reviewed by Halffter & Matthews, and subsequent studies of dung beetle life cycles are listed in Table 1. Most of these studies were done by careful field observations, coupled with excavations of the immature stages from the soil or dung pats and

Table 1. Recent literature on aspects of life cycles of Scarabaeinae

	Species	Locality	No. of gene - rations/ year	Stage during stress period	Breeding season	Adult longevity	Fecundity	Rate of development egg-adult	Diapause	Activity index	Mortality
1	<u>Onthophagus</u> <u>compositus</u>	N.S.W. Australia	2	?	Summer autumn	?	4-6 broods per nest	?	?	2-3 beetles /pellet	?
2	<u>O.dunningi</u>	N.S.W.	1 or 2?	Pupa or teneral adult?	Summer autumn	?	2-6 broods per nest	?	?	Average 6-8 beetles/ mushroom	?
3	<u>O.gazella</u>	Niger	?	-	?	?	11±2.5 broods /♀/3days	In field: ± 41 days	?	?	35% klepto- parasitised by <u>Aphodius</u>
4	<u>O. gazella</u>	Texas USA	-	-	-	About 60 days	Up to 4.4 eggs/♀/day	At 29°C 28.9 days	-	-	?
5	<u>Onitis</u> <u>alexis</u>	Niger	?	-	?	?	7±4 eggs /nest	In field: 112 days	?	?	klepto- parasitised by <u>Aphodius</u>
6	<u>Bubas bison</u>	Sth France	1	Summer: larvae	Sept.- June	?	1.9 eggs/ brood mass	In field: 4 months	?	1.6-1.9 beetles per pat masses	Mould & parasites in brood
7	<u>Helicocopris</u> <u>dilloni</u>	Kenya	2	dry season: adults & larvae	March- April Nov-Dec	Several years	2-7 eggs ♀/nest	In field: 6 months	?	?	some predation
8	<u>Copris</u> <u>armatus</u>	Mexico	1	Winter:adults & larvae	Jul-Oct	2yrs or longer	3eggs/♀/ nest	laboratory: 224 days	?	?	?
9	<u>Cephalodesmius</u> <u>armiger</u>	Sth.Qld	1	Winter: adults	Sept- Nov.	1 year	4-5 eggs/ ♀/nest	Field : 6 months	?	20,000- 50,000 beetles /ha	none, only age

Legend to Table 1.

1. Bornemissza 1971a
2. " 1971b
3. Rougon & Rougon 1980
4. Blume & Aga 1975
5. Rongon & Rongon 1982
6. Kirk 1983
7. Kingston & Coe 1977
8. Huerta et al 1981
9. Monteith & Storey 1981

at times supported by laboratory studies. Such methods are best applied to species with non-overlapping and highly synchronised generations; more complex life systems require some method of evaluating the age structure of the adult population in the field. For example, studies on carabid beetles, which used some form of age-grading method, have frequently demonstrated the fact that although a population may be univoltine, some portion of the adult population may survive to breed again in the next season (e.g. Agonum fuliginosum Murdoch 1966; Calathus melanocephalus Vlijm & Van Dijk 1967; Van Dijk 1979; Pterostichus coerulescens, Van Dijk 1979; P. madidus and Harpalus rufipes, Luff 1973 and 1980; Carabus glabratus and C. problematicus, Houston 1981.)

Several of the studies listed in Table 1 did not provide information on such things as adult longevity, the presence or absence of diapause in some portion of the population, and whether there is a dichotomy in the population at any particular stage in the life cycle such as occurs in some Carabidae (see above). An example of such dichotomy has been observed in the melolonthine Costelytra zealandica. Eggs laid early in the summer develop through the winter and give rise to adults the following summer; but eggs laid late in the summer give rise to adults only after about 20 months (Perrott & Stockdill, 1973). Thus part of the population is univoltine while the other part is semivoltine.

Without age-grading methods it is possible to mistake population peaks as times of emergence of new generations; thus Bornemissza's (1971) claim of two probable emergences of Onthophagus compositus (Table 1) based on two such peaks in a season requires further evidence. It may be that one of these peaks consisted of activity

by older, sexually mature and breeding beetles, while only the second peak consisted of newly emerged individuals, (as was the case with Ot.granulatus Tyndale-Biscoe et al. 1981b).

Age-grading in some form has only been used in the study of three dung-beetle species to date (Table 1): Tibial and clypeal wear provided some evidence that Helicopriss dilloni may live for several years (Kingston & Coe, 1977), and that Cephalodesmius armiger has only a short period of generation overlap (Monteith & Storey 1981). Both these studies gave a highly detailed account of the life histories and how they fit into the regular climatic pattern in the region of the studies. However neither made any attempt to examine the populations for variability in the life cycles in response to variations in the environment, which may explain some of the mechanisms regulating the life cycle patterns. In the third study, age-grading of Bubas bison enabled Kirk (1981) to describe the normal life cycle at Montpellier. However, due to lack of information on day-degree requirements for development of the species, he failed to notice that a proportion of the immatures may enter diapause in the laboratory and may spend up to 12 months in the 3rd instar larval stage before emergence (CSIRO quarantine records, pers. obs.). Hence it may be a species in which some individuals take one year to develop in the field and other individuals may take nearly two years.

Age-grading of adult field populations provides quantitative as well as qualitative information on a number of aspects of the life cycle of a species. For example, it establishes the number of generations a year, and if these are not synchronised, then the proportion of the population which differs. It determines the the stage in which the species survives the stress period (winter/summer), and the proportion that does so; the timing and duration of the breeding season, adult

longevity, the developmental rate of immatures as well as that of follicles in the females. However, in order to allow interpretation of field events and their timing, this information should then be supplemented by laboratory experiments on the developmental threshold and rates of development at given temperatures. This way the duration of the immature stages in the field can be monitored for length of developmental times. Discrepancies in these times may indicate developmental delays; failure to emerge may indicate mortality. Measurement of fluctuations in abundance of the field populations is desirable, but absolute estimates are rarely possible. However, it is usually feasible to obtain some estimate of abundance by trapping techniques, although it must be recognized that such estimates are affected by variations in trappability as well as variations in abundance. Finally, a measure of different resorption rates of follicles through the season, obtained during age-grading dissections, may assist in identifying periods adverse to the beetle and in recognizing environmental factors which modify the life cycle.

One of the environmental factors that is known to vary throughout the season in a temperate climate is the physical and nutritional characteristics of the dung. Coprophagous insects have been shown to respond to these seasonal changes. One insect which breeds in cattle dung and where the influence of the dung characteristics on the larvae have been studied extensively is the Australian bushfly, Musca vetustissima. Greenham (1972) showed that seasonal change in pastures induced by climate was the major influence affecting the composition of dung, and this in turn affected the survival and subsequent size and hence fecundity of the bushflies. Characterisation of the dung, particularly by its nitrogen content was correlated with the stage of maturation

or senescence of the pasture. Pasture type also had an influence on the seasonal fluctuation of the nitrogen content. The value of the dung quality as a food resource for the bushfly could be measured by the mean weight of the puparia and mean survival from egg to adult (Sands & Hughes 1977, Matthiessen & Hayles 1983).

Subsequent work by Macqueen et al. (1984) has shown that the fecundity of a dung beetle, Oniticellus intermedius (Reiche) was directly correlated with percent survival and size of bushflies when reared in dung collected at the same time from the same place. Thus dung collected throughout two years at Rockhampton resulted in nil survival of bushflies in winter dung, minimum puparial weights of 2.4 mg in autumn when survival was around 5%, to a maximum puparial weight in the summer of 13.6 mg with 80% survival. The corresponding fecundities of the dung beetle were a minimum of 0.9 broods per week per female in the winter dung to a maximum of 11.7 in the summer collected dung.

Furthermore, it will be shown in this thesis (Section 7.3) that the response in fecundity of the dung beetle species Ot. granulatus, and to a lesser extent that of Oi. alexis, to dung of different characteristics is also directly correlated with the response of bushfly survival and size. Hence a measure of the bushfly response is a valid indicator of dung characteristics as perceived by these two beetle species and can be used to monitor seasonal changes in dung in the field in relation to the beetles' phenologies.

The aim of the project described in this thesis was to study in detail the field populations of two species of dung beetles, Onthophagus granulatus Boheman (Fig. 1a) and Onitis alexis Klug (Fig. 1b), using the age grading technique, in order to explain and compare their phenologies. The former is a native, diurnal beetle common in South

Eastern Australia, and spreads into Southern Queensland (Fig.2a). Its distribution is "in the coastal habitats, tablelands and mountains in a broad band on the wet side of the 20" isohyet from Adelaide to the McPherson range on the NSW-Qld border" (Matthews 1972). The isolated occurrence of Ot. granulatus at Carnarvon Gorge in SW Queensland is probably due to lower average temperature there associated with higher altitudes (Wallace, pers. com.). In some seasons Ot. granulatus reaches high densities in cow dung and is able to disperse or bury large quantities very rapidly (Hughes 1975).

Onitis alexis, on the other hand, is a crepuscular species (Wallace & Tyndale-Biscoe 1983) introduced into Australia in 1972, and has become established in the warmer, northern regions of the continent (Fig. 2b). Owing to the relatively short period since its introduction it probably has not yet spread into all the climatically suitable areas, and thus its final distribution on this continent is not yet certain. It originates from Africa, where it is widely distributed all through tropical and southern regions (Cambeport 1984). Apparently it is a very adaptable species, ranging over the whole of the warmer, drier regions south of the Sahara (A. Davis, pers. com.). It also penetrates into some hot Mediterranean regions such as Syria, Southern Spain (Ferreira, 1967), Tunisia and Southern Greece, but apparently not to the South West Cape of South Africa (M.M.H. Wallace, pers. com.). The exact climatically limiting factors for this species have not been documented.

In Australia it has spread along the eastern seaboard as far south as Moruya, N.S.W., and by 1977 had also become established in the Araluen Valley near Moruya, a valley with an even rainfall climate. It has not spread onto the surrounding tablelands where Ot. granulatus

is common, though the latter species does spread into Araluen and to the coast. Hence the two species coexist along the eastern part of N.S.W. and southern Queensland.

Both species belong to Halffter & Edmond's (1982) Pattern I grouping based on nesting patterns in the Scarabaeinae. This is the most primitive of all Halffter and Matthew's groupings, and many of its members are opportunistic breeders capable of several generations per year when conditions are favourable. The life cycle pattern in this group of beetles is described by Bornemissza (1976), and Halffter and Edmonds (1982) for another species belonging to the group, Onthophagus gazella Fab. Briefly, the adult beetles are attracted to a freshly dropped dung pad to feed on dung juices (adults do not have chewing mouthparts, see Halffter & Edmonds 1982) and to construct a nest. The nests are subterranean and compound, with brood masses arranged in a linear racemose fashion. Adults work in pairs to provision the nest, but only the female constructs brood masses. The eggs are laid singly in superior chambers of the dung mass; they hatch and the larvae develop and feed on the brood mass with chewing mouthparts. There are three larval instars. Upon completing the feeding phase the larva constructs a pupation chamber in the dung mass with its own faeces and pupates. The pupal stage is relatively short. The teneral adult remains in the pupation chamber until its cuticle becomes fully sclerotised; when it digs its way to the surface it spends a short time feeding before starting to breed. The adults generally only spend a short time (a few days) in and under any particular pad - when conditions become unsuitable or the dung has been used up, they move to another freshly dropped pad.

Ot. granulatus constructs individual ovoid brood masses with one egg in each mass underneath the dungpad. Oi. alexis constructs sausage-shaped masses of dung, usually ovipositing several eggs into one mass, and usually a number of such sausages are formed in tunnels in the ground underneath the pad (Rougon & Rougon 1982). In south eastern Australia both species are active in the warmer months of the year, and disappear during the winter.

In both Ot. granulatus and Oi. alexis, as in all Scarabaeinae studies so far, there is a single ovary with one ovariole (Halffter & Matthews 1966, Richter & Baker 1974). This consists of a coiled germarium, a vitellarium where the eggs develop sequentially, a calyx and an oviduct. Opening into the latter is the bursa copulatrix and receptaculum seminis. The scarabeine ovary is a modified meroistic type, telotrophic in its early stages since nutritive cords have been observed in the pre-vitellogenic stage in two species i.e. Liatongus monstrosus (Bates) and Copris armatus Harold (Halffter & Edmonds 1982).

There are no differentiated oocytes in the tassel-like ovariole of a newly emerged female of either Oi. alexis or Ot. granulatus. Egg maturation and mating occurs in the first few days after emergence, during the maturation feeding period. The eggs mature sequentially, the terminal egg being the only one ready or nearly ready to be laid at any one time. In the laboratory, once oviposition starts the females lay more or less regularly for the rest of their lives, as long as conditions necessary for oviposition are adequate.

This study first reviews the literature on age-grading methods in adult insects. It then evaluates the reproductive strategy of Ot. granulatus and Oi. alexis by monitoring the changes in the age

structure and reproductive status of the beetles in the field, and by experimental manipulation of reproduction and development in the laboratory. Specifically, age-grading will be used to obtain information on the longevity, the duration of the reproductive season, the stage at which over-wintering occurs, the time taken for the development of the immatures, and the response of adult beetles to ambient conditions i.e. humidity, dung quality and temperature.

The conclusions will then be confirmed and expanded by studies on juvenile development both in the field and in the laboratory, as well as fecundity and survival studies under varying conditions in the laboratory. Day-degree requirements obtained from laboratory data will be used to confirm developmental times obtained by age-grading methods, and the need for diapause in the over-wintering and over-summering strategy of one species will be inferred.

The results of these studies are then used to evaluate the contrasting reproductive strategies of these two species in the temperate seasonal environment of south-eastern Australia.

Chapter 2 Age-Grading Methods in Adult Insects - A Review

2.1 Introduction

It is usually not difficult to estimate the age of pre-adult individuals in a population; but to study the ecology of a species, particularly one with overlapping generations, it is desirable to determine the age structure of the adult population. Examination of changes in population age structure require "intensive", i.e. frequent and/or continual, sampling of the population in the same area (Southwood, 1978). The resulting information on the proportion of the population in successive developmental stages can be used to construct time-specific life tables or budgets, to determine factors that cause and regulate fluctuations in population size and dispersal rate, and to monitor fertility and mortality factors in the population. This information can then be used to construct population models for predicting the response of a population to some change, such as assessing the impact of a control programme (Detinova, 1968), or predicting outbreaks of pests in response to certain weather conditions (Farrow, 1979).

Any age-grading technique for adult insects should be simple and rapid, so that a reasonable sample of the population can be examined at regular intervals throughout a season. The technique should distinguish adults in certain physiological stages, either by observing their reproductive condition (e.g. nulliparous or parous), or by measuring some somatic change (e.g. the accumulation of fluorescent age pigment). Alternatively, it should provide information on chronological age (e.g. counting daily cuticular rings).

There have been many ways in which people have age-graded adult insects over the years (Southwood, 1978), and these fall into three main functional categories:

(1) Criteria associated with the reproductive system to provide a clue to the age of the adult insect. These include changes occurring in the ovaries and in the tracheoles supplying the ovaries (Detinova, 1962 p.69-77), decline in fecundity (Mokry, 1980a), as well as indices of copulation in the form of

spermatophores (Waloff, 1958) or copulation marks (Usinger, 1966).

(2) Somatic changes which occur with age. These include changes in the fat body (Waloff, 1958), the cuticle (Neville, 1983), the Malpighian tubules (Haydak, 1957; Ramirez-Perez et al., 1976), and the gut (Rosay, 1961; Laveissière, 1975), and accumulations of fluorescent compounds in specific cells (Ettershank et al., 1983; Mail et al., 1983).

(3) External changes which occur as a result of wear and tear, such as wing fray (Jackson, 1946), and tibial and mandibular wear (Luff, 1973, Tyndale-Biscoe, 1978).

Not all criteria used in the past have survived the test of time, but two have been found to be especially reliable. One is cuticle growth, first described by Neville (1963), and the other is the accumulation of follicular relics in the ovary as a result of repeated ovipositions, first described by Polovodova (1949). Some of the other criteria, such as wing fray, do not provide very accurate information on their own, but when used in conjunction with another criterion, such as reproductive age, enable a more detailed analysis of the age structure to be made.

This review covers most age-grading methods used since Detinova's (1968) comprehensive review on age structure of insects of medical importance, including new methods currently being developed. Techniques which have been reviewed in recent years (WHO 1975 a & b, Service 1976, Charlwood et al., 1980; Challier, 1982; Neville, 1983) will be discussed only in enough detail to facilitate comparisons between the different techniques and to allow assessment of their respective advantages and disadvantages. Follicular relics characteristic of the many different insect groups are described and compared and their origin, eventual fate and relative usefulness in terms of age determination are discussed. Special emphasis is given to the merits of using more than one technique in the study of one species of insect, and finally some brief examples are given of the types of studies which have utilised age-grading techniques to answer specific questions.

2.2 Criteria used for age grading

2.21 Changes in the reproductive system

2.211 Follicular relics (applicable to females only)

This method is based on irreversible changes that take place in the ovary as a result of oviposition or egg resorption. In an insect ovary each ovariole consists of an apical germarium which produces the follicles, and a basal vitellarium where the follicles grow and develop (eg. in mosquitoes, Clements, 1963). Each ovariole has an external wall or sheath, and within this lies a fine elastic membrane, the tunica propria (Bonhag & Arnold, 1961; Clements, 1963; Koch et al., 1967) or intima (Nicholson, 1921; Beklemishev et al., 1959; Detinova, 1962; Giglioli, 1965). Intima will be the term adopted in this review because of its frequent use in age-grading literature. This membrane, in some nulliparous ovarioles, is joined basally to the calyx. The portion of the intima between the most developed follicle and the calyx is called the pedicel. In some species the pedicel tears away from the calyx at the time of ovulation (Saunders, 1962) or dissection (Giglioli, 1965) resulting in a pedicel that appears to end in a point lying free in the ovariole, or is replaced in that region by more diffuse connective tissue (Bonhag & Arnold, 1961) (Fig. 3).

Each oocyte is surrounded by a follicular epithelial layer. In a polytrophic ovariole, when the follicle is nearing maturity, the nurse cells are separated from the oocyte by the follicle cell bridge (Adams, 1974). The nurse cells degenerate, and in the mosquito their nuclei, surrounded by the cell membranes, pass into the follicular epithelium (Nicholson, 1921, Clements, 1963). In Drosophila they are resorbed by the squamous cells of the epithelium (Brown & King, 1964), and in Lucilia cuprina (Wied.) their remnants are pushed into the anterior end of the follicle (Clift, 1972). In any event, just after oviposition, the intima is greatly expanded in the region where the follicle developed, and the follicular epithelial cells

together with what is left of the nurse cells remain behind in it and degenerate. As the intima contracts after oviposition, the degenerating debris also contract and form the follicular relic.

In the telotrophic ovary such as is found in some Coleoptera (Halffter & Edmonds, 1982), or the panoistic ovary found in Locusta (Singh, 1958) there are no nutritive cells in the vitellarium, and hence the follicular relic does not include their remnants. However, the remnants of the much folded intima can be seen in the relics of locusts (Singh, 1958), cockroaches (Bonhag & Arnold, 1961), and dung beetles (Tyndale-Biscoe, unpublished.). The final stages of a resorbing follicle also form a follicular relic, which in some species is indistinguishable from a relic formed after oviposition (Bellamy & Corbet, 1974). This is because after yolk resorption it is again the follicular epithelial cell and nurse cell remnants that persist in the intima.

The permanence of the follicular relics appears to differ between species. Schlottman and Bonhag (1956) state that, in Tenebrio molitor L. the "cellular degeneration (of the follicular epithelium) continues until the contents of the corpus luteum (i.e. follicular relic) have almost completely deteriorated; they are then apparently either reabsorbed or discharged". In Simulium ochraceum Wlk. the follicular relics are described as ephemeral, and are not detectable after 144 hours with current methods (Cupp & Collins, 1979). In the bees Ceratina flavipes Smith and C. japonica Cockerell, the follicular relic disappears with time due to autolysation (Kurihara et al., 1981). In Lucilia cuprina there is evidence that follicular relics formed as a result of resorption disappear completely with time (Barton Browne, pers. comm.).

However, in most species examined the follicular relics are visible in the ovary till death. In the beetle Colobopterus fossor (L.) (cited as Aphodius fossor) the degenerated follicular epithelial cells are present

in old females and have been described as being a sign of parity (Willimzik, 1930), as they are in Scarabaeus sacer L. (Heymons, 1930). In many species the number or size of the follicular relics was confirmed in the laboratory to be a good index of the known number of ovipositions, for example in the mosquitoes Anopheles and Culex (Gillies & Wilkes, 1965; Rosay, 1969a; Kay, 1979) and Mansonia perturbans Wlk. (Trueman & McIver, 1983), the muscid fly Musca vetustissima Wlk. (Tyndale-Biscoe & Hughes, 1969), and the anthomyiid fly Delia coarctata (Fallen) (cited as Leptohylemyia, Jones 1971), several acridian species (Launois Luong 1978), and the dung beetle Euoniticellus intermedius (Reiche) (Tyndale-Biscoe, 1978). Indeed, in the tabanid Hybomitra jersey (Tak.) the presence of some large isolated cells attached between the intima, the outside of the next developing follicle and the follicular relic was thought to ensure preservation of the latter for a long time (Kurihara & Hasegawa, 1978).

Not unexpectedly, the spatial distribution of the follicular relics varies in different species, and although some debris occurs in most parous insects, there is some confusion as to its exact nature and distribution. A further complication is that in some small species dissection of the ovariole to count follicular relics is almost impossible.

The sites of accumulation of the follicular relics in the ovary can be grouped into three main categories depending on the nature of the intima:

- (1) The intima forms a distinct tube behind the most developed follicle. After each oviposition a dilatation forms in it at the site of development of the follicle just laid. Thus a multiparous ovariole will have a string of dilatations, each containing some follicular debris, attached to the next developing follicle on the calyx side. The dilatations have the appearance of a string of beads (Lineva, 1953), and their number corresponds to the number of eggs laid, or partly developed and subsequently resorbed (Fig. 4a).
- (2) Only one dilatation is ever observed in the intima, even though several

eggs have been produced from the ovariole. The dilatation is situated just behind the largest developing follicle. In this group the follicular relic can only be used to distinguish a nulliparous from a parous ovary, but the number of ovarian cycles completed cannot be determined (Fig. 4b).

In this review an ovarian cycle is defined as the period between successive ovipositions in insects in which follicles in the different ovarioles develop synchronously and the eggs are normally laid at about the same time.

(3) The intima is rarely seen as a distinct empty tube beneath the next developing follicle, probably having been torn during oviposition at the junction between the terminal follicle and the next developing one. Hence there is no pedicel at the base of the most developed egg. The follicular relics remain loose in the ovariole and accumulate at its base in the calyx, or even extrude into the common oviduct. The remains of the portion of the intima which enveloped the follicle can at times be seen in the follicular relics (Singh, 1958). The follicular relics may be deposited as distinct granules or bodies after each oviposition or resorption and then their number corresponds to at least the first few ovarian cycles, or their cumulative size and density may give a clue to the number of ovipositions completed (Fig. 5).

Examples of insects showing these three categories of follicular relics are given below, but first some rationalisation must be made here about the terminology used in the literature. Most of the early publications used the term "corpus luteum" for follicular relics (Willimzik, 1930; Lewis, 1957; Singh, 1958). The mammalian corpus luteum, from which the term was borrowed, is an endocrine organ (Peters & McNatty, 1980). No endocrine function has been demonstrated for the follicular relics in insects, and thus the use of this term is misleading (Descamps & Wintrebert, 1961; Phipps, 1966). Another term that has commonly been used is "yellow body" (Kuzina, 1942; Lineva, 1953). This has been used in cases where the follicular relic was situated at

the base of the ovariole and had a yellow colour. Anderson (1964) and Miller & Treece (1968) recommended the term "follicular relic", since some of the relics they described were not yellow. But the term "follicular relic" has frequently been used for dilatations as described by Polovodova (1949) and subsequent authors have also used "follicular relic" irrespective of where it was found in the ovary. Other terms used in the literature have been "follicular residue body or FRB" (Giglioli, 1959), "residual body" (Saunders, 1967), "follicular remnants" (Smith, 1968), "normal and abnormal relics" (Ovazza et al., 1965), "serial follicular relics" (Saunders, 1962), "multiple follicle relics" (Walker, 1977), and so on.

There is no doubt that "follicular relic" is the most descriptive term and it will be used here when referring to the phenomenon in general. However, it is useful to differentiate between the various sites where these relics are found. Thus in this review, where relics are arranged in the characteristic bead-like fashion, the term "follicular dilatation" will be used. "Single follicular dilatation" will be used if only one relic is ever found in the intima; "yellow body", irrespective of colour, will be used, if the relic is found at the base of the ovariole, except in the locusts and grasshoppers where relics due to resorption may be as numerous as relics due to oviposition, and the two will be distinguished as "laying body" and "resorption body" (the terms used by most workers on Orthoptera).

(i) Follicular dilatations

Follicular dilatations have only been recorded in Diptera. Species exhibiting these characteristics include Anopheles maculipennis Meig. (Polovodova, 1949; Detinova, 1949, 1953); A. quadrimaculatus Say, A. gambiae Giles and A. funestus Giles, A. squamosus Theo. and A. coustani Lav. (Giglioli, 1959, 1965; Detinova & Gillies, 1964); Mansonia uniformis (Theo.) (Samarawickrema, 1962); Mansonioides uniformis (Theo.) and M. annulifera

(Theo.) (Bertram & Samarwickrema 1958); Culicoides grisescens Edw. (Gluckhova, 1958) and Musca domestica Degeer (Lineva, 1953).

Mosquitoes were the subjects of much age-grading after Detinova's review in 1968 because of their importance as vectors of diseases. Species of mosquitoes and other Diptera in which follicular dilatations have been observed since then are listed in column FD of appendix 1. In some species, especially of Tabanidae the intima takes several days to contract after oviposition, eventually forming the compact follicular dilatation. Thus a uniparous ovary may contain a distended sac or a dilatation, and a biparous ovary either a distended sac and a dilatation, or two follicular dilatations (Thomas, 1972; Thompson et al., 1979).

Follicular dilatations are sometimes difficult to dissect out intact as the intima is fragile and liable to break, thus in practice a number of ovarioles have to be examined and the constant maximum number of dilatations recorded (Rosay, 1969b; Kay, 1979). Bellamy & Corbet (1974) warn against acceptance of a dilatation as a definite sign of parity in epidemiological studies, since in autogenous species a proportion of follicles are resorbed in the first cycle while others continue to develop, resulting in a follicular dilatation in some ovarioles of a gravid, nulliparous female. However, the formation of dilatations due to resorption takes as long as the development of the follicles in neighbouring ovarioles, at least in species showing gonotrophic concordance, and thus they have still been found to be good age indicators in C. variipennis Coq. (Mullens & Schmidtman, 1982). Species in which dilatations occur as a result of degeneration of partly developed follicles are marked in the appendix with an asterisk.

Lange & Khok (1981) described three types of dilatations in Culex pipiens molestus Forsk, Aedes aegypti L. and Anopheles maculipennis atroparvus Thiel.:

(a) dilatations formed after oviposition, where the debris are retained at the apical end of the expanded intimal sac. In these species the sac does not contract to form a dilatation in the classical sense, and the pedicel is never reformed; there is thus only one relic visible indicating parity, even in multiparous females.

(b) Dilatations occurring when eggs degenerate in an ovariole. These form dilatations connected to each other and to the next developing follicle by stalks, and if oviposition has never occurred from that ovariole then the intima has follicular dilatations and the pedicel can be seen in a narrow contracted state. If oviposition does occur after dilatations are formed by resorption then the dilatations disappear, and the intima remains stretched with a follicular relic at its head. Since egg degeneration does occur in some ovarioles while corresponding eggs develop and are laid from others, the maximum number of dilatations corresponds to the number of gonotrophic cycles completed. Lange & Khok called these relics "gonotrophic dilatations", and for purposes of age-grading they belong in the group exhibiting follicular dilatations.

(c) Dilatations due to egg resorption caused by a carbohydrate diet; this phenomenon has also been reported in Culex spp. by Knight & Nayar (1982). Lange & Khok called these "agonotrophic dilatations", and their number does not correspond to the number of ovipositions that could be completed in the equivalent time.

Each ovary has ovarioles exhibiting any of these three types of dilatations or combinations of them, and so far it has not been possible to distinguish between gonotrophic and agonotrophic dilatations. However, an estimate of the number of ovarian cycles can be determined by counting the number of dilatations in ovarioles which occur alongside other ovarioles from which eggs had been laid. The number of ovarioles containing dilatations ending in a narrow pedicel in an ovary (termed the diagnostic index by Lange & Khok),

decreases during life because of alternations in individual ovarioles of follicle maturation and degeneration. In mosquitoes where periods of protein diet alternate with periods of carbohydrate diet it is not possible to estimate the age of the adults.

In the intact ovary of some mosquitoes the dilatations containing the relics can be seen as granulations when observed soon after oviposition (Colless, 1958). In addition, in Anopheles farauti Lav. each ovariole becomes progressively longer with successive ovulations and the intima contains increasing quantities of globules (Spencer, 1974, 1979). Spencer drew up a key for differentiating between the different parous categories of mosquitoes coming to bite (i.e. largest follicle undeveloped, gut empty) on the basis of the appearance and position of the granulation as seen in an intact ovary. Briefly, she defined the category with no granulations as nulliparous; that with diffuse or close granulation in the extended intima and no discrete mass as uniparous; that with compacted masses of single granules as biparous; that with compacted masses in pairs or triplets as triparous; that with masses of compacted granules which overlies the oocyte, and with an ovarian sheath with a light deposit of globules as quadriparous; and that similar to quadriparous but with heavy deposits of globules as quinquiparous.

In addition to separating the cohorts of flies into nulliparous, uniparous, biparous, etc., categories, several authors found it convenient to subdivide each ovarian cycle into further categories based on the stage of follicle development within the cycle (Spradbery & Sands, 1976). Thus, for example, 16 physiological stage categories were devised for Lucilia cuprina (Vogt et al., 1974), and Musca autumnalis De Geer (Moon & Kaya, 1981) from emergence to the end of the third gonotrophic cycle, consisting of follicle developmental stages 0-V (undeveloped to developed follicles in the first cycle) and I-V in the second and third cycles. Further examples are 17 categories for M. vetustissima (Tyndale-Biscoe & Hughes, 1969) and

12 for Stomoxys calcitrans L. (Scholl, 1980). Spencer (1974, 1979) found that in Anopheles farauti the process of contraction of the intima, and the appearance of the granules within it, could be divided into three categories up to 24 h after oviposition, thus enabling the subdivision of each ovarian cycle in that species.

(ii) Single follicular dilatations

Species in this group are also all Diptera, and they include Anopheles melas (Giglioli, 1965); Simulium damnosum Theo. (Lewis, 1960), Prosimulium fuscum Symes & Davies and P. mixtum Symes & Davies (Davies, 1961); Culicoides barbosai Wirth & Blanton and C. furens (Poey) (Linley, 1966), some Tabanidae (Duke, 1960), and Glossina spp. (Saunders, 1960, 1962; Challier, 1965).

Only one dilatation has ever been observed, or the number of females with more than one dilatation has been so low as to be of no consequence. The dilatation can be in the form of a distended sac before full contraction of the intima after oviposition, or in the form of a compact follicular dilatation. Species in this group, described since 1968, are shown in the column marked SFD in the appendix.

The reason for the presence of only one dilatation appears to vary between species. Successively formed dilatations may coalesce, e.g. in Culex pipiens quinquefasciatus Say and C. tarsalis Coq. (Rosay, 1969a), Anopheles melas (Giglioli, 1965), Stomoxys calcitrans (Charlwood & Lopes, 1980). Failure of the intima to contract after oviposition has been noted in some mosquitoes (Lange & Khok, 1981) thus causing a failure of previously formed dilatations to reform. It is also possible that the intima in some species might tear during oviposition at a point near the previously formed dilatation, and hence only the most recently formed dilatation would be present.

It is of interest to note that some authors only found one dilatation in a multiparous ovariole of some species, while others counted several in the same species. Examples of this have been found in Anopheles melas Theo. (Giglioli, 1965; Snow & Wilkes, 1977); Tabanus nigrovittatus Macq.

(Magnarelli & Anderson, 1977; Thompson et al., 1979; Wall & Doane, 1980; Magnarelli & Stoffolano, 1980); Hybomitra nuda (McDunn.) (Thomas, 1972; Magnarelli, 1976); H. illota (O.S.) (Thomas, 1973; Troubridge & Davies, 1975; Magnarelli, 1976); and Chrysops indus (O.S), C. univittatus Macq. and C. vittatus Wied. (Troubridge & Davies, 1975; Magnarelli, 1976). The reason for these discrepancies is not known, although it is possible that some authors are more adept at dissections than are others (Gillies & Wilkes, 1965). However, it would be interesting to examine the hypothesis that the elasticity of the intima of the same species varies in different geographical regions, causing it to break at the time of oviposition in some localities but not in others. Alternatively, of course, the duration of life of some species may vary between different climatic regions, resulting in multiparous females in some areas but only uniparous ones in others.

Although the tsetse flies accumulate only one follicular dilatation in each ovariole, age-grading can be done very accurately until just before the last of the four ovarioles has ovulated. In Glossina morsitans Westwood, at 26°C this occurs when flies are about 40 days old (Saunders, 1962). Tsetse fly ovaries contain only four ovarioles (two per ovary), and the follicles are developed one at a time from the four ovarioles in a strictly repeated sequence. There is no gonotrophic concordance. The egg hatches in the uterus. Thus the stage of egg development in each ovariole, and the presence or absence of a follicular relic, together with the stage of development of the offspring in the uterus, can be used to determine the physiological age of the females up to the ovulation of the fourth egg, e.g. G. morsitans Westw. (Saunders, 1960), G. pallidipes Aust., G. palpalis fuscipes Newst., G. brevipalpis Newst. (Saunders, 1962), G. palpalis palpalis (Robineau-Desvoidy) (Saunders, 1967). In G. palpalis gambiensis Vanderplank, the age during the second cycle (i.e. the period between ovulation of the fourth to the seventh egg) was revealed by the order in which the eggs developed

and were shed into the uterus. However, each physiological stage in the second cycle can be confused with the shedding of the fourth subsequent egg, hence the ages indicated by ovulation of the 4th to the 7th eggs are minimum ages only (Challier, 1965, and review 1982).

Thus Taylor (1979) in his study of G. m. morsitans Westw. in the Zambezi Valley, and Jaenson (1980) of G. pallidipes from Kenya, recognised the ages of the females up to the time of ovulation of the fourth egg by Saunders' method, but age-graded older females by the wing fray method (Jackson, 1946, see below).

(iii) Yellow body

Follicular relics accumulating as yellow body occur in a wide range of insects. Included amongst the Diptera are Musca domestica (Anderson, 1964; Smith, 1968); Muscina stabulans (Fallen) and Fannia canicularis (L.) (Anderson, 1964), M. autumnalis (Miller & Treece, 1968); Mansonia metallica (Theo.) (Corbet, 1964); Stomoxys calcitrans, and Haematobosca stimulans (Meig.) (cited as Haematobia stimulans) (Kuzina, 1942). Yellow body has also been noted in dragonflies (Corbet, 1962), fleas (Darskaya et al., 1962; Kosminsky, 1960), some beetles (Willimzik, 1930; Vlijm & van Dijk, 1967), and locusts and grasshoppers (Singh, 1958; Lusi, 1963). Immediately after oviposition the follicular epithelium can be seen as an empty sac loose in the stretched ovariole, at the distal end of the next developing follicle. Over the next few hours the sac contracts, and forms a follicular relic. As the follicle grows to fill the ovariole, the relic is pushed down to the region of the calyx. When the next egg is laid, another sac is formed, but the previous follicular relic can still be seen at the base of the ovariole. In some dung beetles it is firmly fixed in this position, apparently caught among the folds formed by the highly elastic wall of the calyx. In many species, the yellow bodies accumulate, and their numbers increase with increasing numbers of ovipositions (Appendix, column YB/LB.), e.g. in Delia

coarctata (cited as Leptohylemyia coarctata, Jones, 1971), Musca vetustissima (Tyndale-Biscoe & Hughes, 1969), or they may just become bigger and denser as in the case of Lucilia cuprina (Vogt et al., 1974). The relics are often yellow or brown, e.g. in Haematobia irritans L. (Schmidt, 1972), D. coarctata (Jones, 1971) and M. vetustissima (Tyndale-Biscoe & Hughes, 1969), or they are colourless and can be seen only if stained, e.g. in L. cuprina (Vogt et al., 1974), and the stable fly Stomoxys calcitrans (Scholl, 1980). Charlwood & Lopes (1980) reported that those in S. calcitrans were translucent in unipars and brown in multipars, and Sutherland (1980) described the accumulated relics in this species as yellow. Clift & McDonald (1973) noted the presence of the yellow body in L. cuprina but for lack of staining failed to notice its increased size and density in biparous compared to uniparous females.

Sometimes the presence of a yellow body does not preclude the presence of dilatations. In M. vetustissima the intima does not tear at the time of oviposition (Tyndale-Biscoe & Hughes, 1969); however, relics accumulate both in the intima, forming dilatations, and in the calyx forming a yellow body. Since yellow bodies are much easier to see than are dilatations, the former are used in routine dissections for age estimation. Similarly M. domestica was described as having only a yellow body by Anderson (1964) and Smith (1968), although Lineva (1953) observed both a yellow body and follicular dilatations in that species. In the dung beetle Oniticellus intermedius, the epithelial layer can be dissected out as an empty transparent envelope after oviposition. Within a short time, however, it breaks down, forming a mass of tissue in the calyx. With increasing pressure from the growing terminal follicle, and its subsequent exit through the calyx at the time of the next oviposition, this material is partly pushed out through the calyx wall where it forms small transparent granules or bubbles (as occurs in Onthophagus granulatus Boh., Tyndale-Biscoe, unpublished.). Some of the

material remains inside the calyx. As the epithelial cells degenerate, they form a deposit together with the crumpled intima, and become yellow or brownish in colour. The size and quantity of these deposits give a clue to the number of ovipositions completed by females of O. intermedius (Tyndale-Biscoe, 1978), and Onthophagus granulatus (Tyndale-Biscoe et al., 1981b).

In several species of Coleoptera, however, yellow body does not appear to enlarge with successive ovipositions, e.g. in Onitis alexis Klug. In that species, extrusion of the follicle into the haemocoel, as described for Euoniticellus intermedius (Tyndale-Biscoe & Watson, 1977), is a very common occurrence (Tyndale-Biscoe, unpublished). Probably as a consequence of this, most of the relics are also extruded, resulting in the presence of very little yellow body inside the calyx.

In Pterostichus madidus (Fab.) (Luff, 1973), Harpalus rufipes De Geer. (Luff, 1980), Carabus glabratus Paykull and C. problematicus Herbst (Houston 1981) no change was found in the yellow body with advancing age. However these species were dissected after fixation (Carnoy's fixative and dissection in 70% alcohol, Luff, 1973 & 1980, 10-20% "Teepol" detergent, Houston, 1981) and thus any change in the density, size or colour of the yellow body, if such did occur, would have been obscured through hardening and discoloration of the tissues (see section on methods).

Many beetles resorb their ovaries when preparing to overwinter, e.g. Onthophagus granulatus (Tyndale-Biscoe et al., 1981b), and Copris armatus Harold (Huerta et al., 1981); during brood care, e.g. C. diversus Waterhouse (Tyndale-Biscoe, 1983), and C. armatus (Huerta et al., 1981); or during the breeding season when they encounter environmental stress, e.g. Oniticellus intermedius (Tyndale-Biscoe & Watson, 1977), and Phanaeus daphnis Harold (Tyndale-Biscoe & Lopez-Guerrero, 1982). As with follicular dilatations, distinction between yellow body caused by oviposition and one formed by

egg resorption has not so far been possible in routine dissections. Thus distinguishing between pauciparous and multiparous ovaries, as well as between yellow body resulting from oviposition and one resulting from resorption, necessitates the use of other age-grading criteria in combination with yellow body.

It is possible to distinguish between laying bodies and resorption bodies in some locusts and grasshoppers. In Locusta migratoria migratorioides (Reiche & Fairmaire) the follicular epithelium after oviposition is an empty, colourless envelope which gradually contracts, together with its surrounding intima (Singh, 1958, insect cited as L. migratoria). This forms a ring-like structure at the base of the ovariole which is not pigmented (i.e. the laying body), and after several ovipositions several such bodies can be seen (Uvarov, 1966). In this species, the laying body is attached at its apex where the intima is joined to the next developing follicle, and is milky white to pale yellow in colour (Launois, 1972, insect cited as L. m. capito (Saussure)). Degenerating oocytes also leave a similar, orange to red body at the base of the ovariole (i.e. the resorption body) (Singh, 1958), the colour of which is due to β -carotene (Singh, 1958; Descamps & Wintrebert, 1961; Lusi, 1963).

There appears to be colour variation in both laying and resorption bodies in some species. In Schistocerca gregaria (Forsk.) Lusi (1963) found that some empty epithelial follicles, resume lipid formation for a while before being resorbed. The lipid is a triglyceride, and has some pigment dissolved in it. During subsequent resorption of the lipids the pigment crystallises, giving a yellow colour to the laying body. Singh (1958) showed that colourless laying bodies of L. migratoria migratorioides can, on occasions, acquire a pigmentation through partial resorption of the adjacent oocyte. The intensity of the colour in a pigmented resorption body may depend on the amount of yolk present in the egg when that resorption

starts (Descamps & Wintrebert, 1961). The darker pigment may be due to oocyte resorption while paler pigment may be due to resumed lipid metabolism of the follicular epithelium undergoing resorption (Phipps, 1966). That author also speculated that in females of Austeniella cylindrica (Ramme) that had recently oviposited the red pigment may be the result of resorption of an egg from the previous ovarian cycle, while yellow pigment may be the result of either the resorption of an egg or an empty epithelial follicle from a still earlier cycle. This explanation seems unlikely since it requires the female to have undergone a minimum of three ovarian cycles, yet only one relic is shown in his figure at the base of each ovariole.

Singh (1958) demonstrated that counts of eggs laid in pods in L. migratoria migratorioides correspond closely to the number of ovarioles which have either a colourless or pigmented relic, or an unovulated "subnormal" egg present in them. Also he showed that up to three laying bodies, formed after successive ovulations, can be seen in the ovarioles of older females. This evidence was supported by work on several acridid species (Launois, 1972; Launois-Luong, 1978) where the difference between the two types of bodies was clearly recognised on the basis of colour, and the history of each ovariole could be interpreted up to the end of the third ovarian cycle. The third oviposition could not be separated from the subsequent ones. However, in a small proportion of the species it proved impossible to interpret the reproductive histories accurately. Farrow (1977) noted the presence of red pigmented relics in the pedicels of Austracris guttulosa (Wlk.) which had undergone resorption, and laying bodies in the ovarioles of Chortoicetes terminifera (Wlk.) (Farrow, 1979) whose number indicated the number of times the female had laid.

Methods: Dissection of ovaries and examination of the follicular relics are best done in freshly killed specimens, while the tissues are elastic, and their colour and opacity have not changed (Detinova, 1962).

Thus the insect must be either kept live at low temperature until dissection to prevent further egg development, or killed and kept in cold storage until dissection. Fixation hardens and discolours the tissues, making accurate diagnosis difficult.

If dissections cannot be done on freshly killed females, then deep freezing the material for storage is successful in some species: e.g. Tabanus species at -60°C (Thomas, 1972; Bosler & Hansens, 1974; Magnarelli & Pechuman, 1975); Haematobia irritans at -19°C for up to four days (Schmidt, 1972), Musca vetustissima up to three weeks in liquid nitrogen (Hughes, pers. comm.), Culex, Simulium and Culicoides species placed live in saline (0.9% NaCl with a drop of detergent) and stored on dry ice for up to five months (Davies, 1969). Deep freezing for lengthy periods sometimes desiccates the material. Thus tabanids were kept moist in sealed vials at -12 or -15°C for up to six months (Thomas, 1972; Auroi, 1982), and at the time of dissection the ovaries were injected with physiological saline to allow restoration (Thompson et al., 1979). However, eggs in deep frozen females of Oniticellus intermedius burst after thawing, making recognition of physiological stages impossible (Tyndale-Biscoe, unpublished).

Dipteran ovaries can be dissected out in physiological saline (Detinova, 1962; Cupp & Collins, 1979; Mullens & Schmidtman, 1982), in SGF solution (glycerine 10 parts, 0.65% saline 25 parts, and 3% formalin 1 part) (Shalaby, 1962; Spencer, 1979), and locust ovaries were dissected out in either physiological saline or slightly saline water (9 g NaCl/1000 ml H_2O) (Launois-Luong, 1978). Sutherland (1980) found that Pampel's fluid cleared the ovaries more effectively than did saline and used it as the dissecting medium for Stomoxys calcitrans.

Ovaries of Lucilia cuprina (Vogt et al., 1974), and Stomoxys calcitrans (Scholl, 1980), were stained for 5-30 seconds with 1 g/100 ml neutral red in saline and those of some mosquitoes with 1:8000 neutral red (Lange et

al., 1981). Mosquito ovaries have been injected with oil to separate the ovarioles, and make the pedicels more obvious (Lange et al., 1981; Lange & Khok, 1981). A dissecting solution of gentian violet, physiological saline and detergent ("Teepol") was used for Culicoides marmoratus (Skuse) (Kay, 1973). Ovarioles of Culex annulirostris Skuse were stained with Giemsa which made dilatations be more visible; they were then carefully teased apart with fine tipped needles and/or forceps, or electrolytically sharpened tungsten wire needles (Kay, 1979); the ovariole wall was carefully removed to expose the intima in the region between the most developed follicle and the calyx. Dilatations can be counted under a binocular microscope (with x12-48 magnification, Rosay, 1969b) when the intima has contracted after oviposition but before the next developed egg has grown to full size, i.e. when the egg is between Christophers' (1911) and Mer's (1936) stages II and IV. Yellow body can be seen in the region of the calyx by stretching the common oviduct, or by gently pulling apart ovarioles so that the calyx region of each ovariole is exposed. Very small dilatations may need to be examined under a phase contrast microscope (Mullens & Schmidtman, 1982). Launois-Luong (1978) suggested that without staining the ovaries of acridids, colour differences can be noted by examination for transparency under transmitted light, and for granulation under reflected light. In Chortoicetes terminifera and Austracris guttulosa the laying bodies are brown, and can be seen under ordinary reflected lighting (Farrow, pers. comm.)

2.212 Tracheolar skeins in the ovary (applicable to females only)

The small tracheoles supplying the ovary wall in a nulliparous fly are in tight skeins or bundles; once the wall has stretched to accommodate the growth of the first developing follicles the skeins uncoil and never revert back to the original tight bundles. In the shrunken ovary after the first and subsequent ovipositions they form a loose tracheal net, this being the

sign of parity (Detinova, 1962). Species which have been age-graded by this method are shown in the appendix, column TS.

This technique has only been used in Diptera; it only separates parous from nulliparous flies, and can only be used on flies whose oocytes have not developed beyond the early stages of vitellogenesis. However, it is a quick technique, as large batches can be processed at the same time (300-400 females per day, Detinova, 1962). Since only one ovary needs to be examined per female, the other ovary as well as the rest of the body is available for examination using other techniques.

Methods: The ovaries are best dissected out of a freshly killed female in a drop of water and the ovary allowed to dry out on a slide; the air in the tracheoles makes them clearly visible under low power microscopy (Detinova, 1962), or under a compound microscope with X70 or X280 magnification in blue filtered transmitted light (Corbet & Smith, 1974). The technique worked well with four South African species of Culex when they were stored in solid carbon dioxide, either dry, or in saline containing a small quantity of detergent. Prior to dissection the females were thawed, wetted if dry and dissected under distilled water (Jupp, 1973). Detinova (1962) advised against dissection in saline since this forms crystals on the surface of the ovary on drying and obscures the vision; but Kay (1979) dissected Culex annulirostris in saline and found the technique to be satisfactory, quick and accurate.

2.213 Decline in fecundity (applicable to females only)

The number of egg rudiments have been observed to decline with age in Lepidoptera (Waloff, 1958). Decline in fecundity with age was used by Mokry (1980a) to estimate the age of field collected females of Simulium damnosum. Parity was confirmed by the presence of a follicular relic, only one having been reported even in old females (Le Berre, 1966, cited by Mokry, 1980a). The egg numbers found fell into five distinct groups, the group

with the highest number being the nulliparous category and the others the parous categories. Whether these indicate successive ovarian cycles has yet to be determined, but they are assumed to indicate advancing age.

Both Simulium vittatum Zetterstedt and Prosimulium mixtum were found to be less fecund in the second ovarian cycle than in the first (Mokry 1980b). Egg numbers were not a function of body size in S. damnosum, although in the autogenous S. vittatum and P. mixtum fecundity was related to the size of the female (Mokry, 1980b), and Mokry (1980a) cautions that the examination of a larger sample of S. damnosum may highlight a wing length-fecundity relationship, especially in younger flies. Sucrose intake together with the ingestion of blood is also known to affect fecundity in some blood feeding Diptera, e.g. some mosquitoes (Nayar & Sauerman, 1975, Edman & Lynn, 1975) and the effect this factor may have on the technique needs further investigation.

Method: Simuliids were caught coming to bite, allowed a blood meal and kept until the follicles were partially developed. The ovaries could then be teased apart and the follicle numbers counted (Mokry, 1980a).

2.214 Other criteria (applicable to females only)

In the phlebotomine sandfly Lutzomyia flaviscutellata (Mangabeira) the accessory gland of the female was found to be filled with granulated material (Lewis et al., 1970). It appears that most of this material is discharged at oviposition; thus a non-granular accessory gland indicates a parous condition. The authors found good agreement between determination of parity rates using this criterion and parity rates assessed on the basis of dilatations; the instances of disagreement were thought to be due to the disappearance of the follicular relic in about 5% of females.

The shape of the bursa copulatrix, the degree of development and number of eggs were used to distinguish young, middle aged and old females of Costelytra zealandica White (Fenemore, 1971). In unmated females the bursa

copulatrix remained undistended throughout life, and eggs did not mature. In mated individuals the bursa copulatrix became distended, and initially there were many immature eggs; as these matured and were laid the ovary contained many mature eggs, and finally with successive ovipositions there were fewer eggs, both immature and mature (column marked "Other" in the Appendix).

2.215 Changes in the reproductive tract (applicable to males only)

Some grading schemes have been proposed specifically for male insects, most of which are for monitoring changes occurring in the male reproductive tract. The width of the seminal vesicle was narrow in a newly emerged male, wide in unmated young males, sharply diminished after mating and gradually distended again with advancing age in C. zealandica (Fenemore, 1971). In Pterohelaeus darlingensis Carter (Allsopp, 1979) it was found to be partly distended in young, and fully distended in middle aged and old males. The testes were found to occupy less than half the ventral part of the abdomen in newly emerged and immature males, more than half the abdomen in developing males, and the whole of the ventral part of the abdomen in mature males of Pterostichus madidus (Luff, 1973), Carabus glabratus and C. problematicus (Houston, 1981).

The ages of the males of Lucilia cuprina could be estimated up to 24 hours after emergence by the size of the lumen of the accessory gland and the width of the seminal ducts; also, the colour of the testes darkened from pale to deep reddish orange over five days at 27°C (Clift & McDonald, 1973).

2.22 Somatic changes

2.221 Cuticle growth (applicable to both females and males)

Neville (1963) discovered that paired layers of endocuticle are deposited daily in the cuticle of the legs in grasshoppers and locusts. Subsequently (1967) he found that the internal cuticular projections to which muscles are attached (variously known as apodemes, furcae, and phragmata)

also exhibit daily growth bands. Alternatively or jointly with the counting of growth bands, the measurement of the depth (Tyndale-Biscoe & Kitching, 1974) or area of the phragmata (Drew, 1969) has been found useful. The phenomenon of daily growth layers has been reviewed by Neville (1983) who points out that endopterygotes can be age-graded best by examining the apodemes, and exopterygotes by counting layers in the cuticle. Some insects which have been age-graded in these ways are shown in column CG in the appendix.

In the beetle Oryctes rhinoceros L. layers were found in the pronotum, horn and femora, but they were not deposited daily (Zelazny & Neville, 1972; Zelazny, 1975). However, using an age-layer correlation in the pronotum the age of beetles could be estimated up to 32 days.

The rate of cuticle deposition in adult insects is correlated with temperature, i.e. growth ceasing at low temperatures (Neville, 1965; Dingle et al., 1969), and increases with increasing temperatures (Zelazny & Neville, 1972; Schlein, 1972b, 1975, 1979a). The bands on the apodemes are clearest under fluctuating temperatures where the minimum temperature is below a threshold for cuticle growth (Tyndale-Biscoe & Kitching, 1974; Johnson & Ellison, 1982). Light has been shown to be implicated in clear layer formation in Sarcophaga (Schlein, 1975) and Drosophila (Johnston & Ellison, 1982), but not in Lucilia cuprina (Tyndale-Biscoe & Kitching, 1974) or O. rhinoceros (Zelazny & Neville, 1972).

Daily layers are deposited only for a short period after eclosion; e.g. the mean maximum number counted was between 10-14 in Anopheles (Schlein, 1979a), 10-11 in Culex and Aedes (Schlein & Gratz, 1972), about 12 in Sarcophaga (Schlein, 1972a), 10 in Lucilia (Tyndale-Biscoe & Kitching, 1974), 14-18 in Drosophila (Johnston & Ellison, 1982), and at least 15 in Cochliomyia hominivorax (Coq.) (Ellison & Hampton, 1982). The method is accurate until cuticle growth ceases; hence in wild-caught material

individuals possessing the maximum possible number of daily layers (as determined by laboratory studies) may be that age or older. In some insects a few cuticular bands are already present at the time of eclosion (Zelazny & Neville, 1972), and this number has to be subtracted from the total number of layers present at the time of age-grading; however in some species the layer formed at eclosion can be clearly recognised, as in Anopheles gambiae (Schlein, 1979a). The technique is therefore most accurate for young insects; in some Culicidae (Schlein, 1979a) the largest group of wild caught females had a mean of between 2-5 layers, while in the laboratory up to 14 layers could be counted. The author suggested that most of the wild mosquitoes had died before cessation of cuticular growth.

The advantage of the technique is that it gives direct chronological age under normal field conditions. However in insects inactivated by cold, cuticle deposition ceases (Tyndale-Biscoe & Kitching, 1974; Schlein 1979a), and at high temperatures maximum apodeme depth is achieved too quickly to give a useful age estimate (Tyndale-Biscoe & Kitching, 1974).

Methods: Layers of endocuticle are best seen in cross sections of the legs and wing veins, cut by hand with a razor blade and counted either under phase contrast, between crossed polaroids or with Nomarski differential interference contrast microscopy (Neville, 1983). Age-grading by counting growth rings, or measuring depth or area of apodemes involves separation of the muscles from the apodeme, staining the structure if it contains no melanin, and mounting it on slides for examination under a microscope.

Material before examination has been fixed in 3:1 ethanol and acetic acid and stored in 70% ethanol (Johnston & Ellison, 1982), or kept dry until used (Schlein & Gratz, 1979a). Separation of the muscles from the apodemes involves boiling the thorax or abdomen for between 20 s and 10 min in 7-10% KOH depending on the size of the insect; heating to boiling in 4% NaOH and then cooling; or allowing the tissue to stand in KOH or chloral hydrate solution for 24 to 72 h at room temperature.

Dissection of the apodemes is usually done in water. The tissues are then dehydrated, cleared and mounted in Canada balsam or mounted directly in Hoyer's mounting medium. The third furcae and the ejaculatory apodemes of C. hominivorax were dehydrated in ethanol, cleared in xylene and mounted in silicon immersion oil. For examination with the scanning electron microscope they were dehydrated in diethyl ether, mounted with Scotch double-sided sticky tape on to a scanning electron microscope stub, and coated with a 25 μ m gold/palladium layer using a Technics Hummer V sputter coater (Ellison & Hampton, 1982). Drosophila apodemes were mounted in Paragon water soluble mounting medium (Johnson & Ellison, 1982). Apodemes of Dacus tryoni and Anopheles sinensis Wied. have been stained with mercurochrome (Drew, 1969), and chlorazol black (Pan Shi-Qing et al., 1983) respectively; those of Culex and Aedes with haematoxylin Heidenheim followed by immersion in iron alum solution for 30 min and washed and restained for a further 30 min (Schlein & Gratz, 1972). Glossina phragmata were stained by immersion for 30 min in 0.5% aqueous aniline blue-organe G (Michrome) (Schlein, 1979b). In some Culicidae, the phragmata were fixed for 10 min in saturated picric acid, rinsed in water, oxydised in 1% potassium permanganate for 5 min, and rinsed again. Next they were soaked for 15 min in a mordant (fresh solution of 1% iron alum), rinsed in 2 changes of water for 20 min, stained in a ripe solution of 0.2% Gurr's haematoxylin in 70% ethanol for up to 1 min, rinsed in water again and then dehydrated in alcohol, cleared in xylene and mounted in Canada balsam (Schlein, 1979a).

A more recent technique for treating batches of Drosophila involves deep freezing the flies in liquid nitrogen, and sonicating them gently until the thoraxes are broken up. This is followed by immersion in a protease solution for 40 min at 37°C (0.25 mg in 2.5 ml of 1-molar phosphate buffer at a pH of 7). The tissues are rinsed in water, sonicated for 10 s and rinsed again, and then stored in 0.5% potassium hydroxide for 24-72 h

or until the thorax is clear of muscle. The tissues are bleached for 1 min in a solution of 1 ml formic acid, 1 ml of 30% peroxide and 10 ml of water, rinsed, stained for 1 min. in 1% potassium permanganate, dehydrated in 70%, 95% and absolute ethanol followed by 100% hexane. The apodemes are dissected out and mounted under silicon immersion oil and examined under x500 and x800 magnification (Johnston & Ellison, 1982).

2.222 Fluorescence (applicable to both females and males)

Fluorescence microscopy has been suggested as a technique for determining the physiological age of insects (Yurgenson & Teplykh, 1972) (Appendix, column F1). In Aedes aegypti and in the flea Ctenophthalmus orientalis (Wagn.) the size of fluorescent inclusions in the germarium, and in the yellow body, observed with an ML-2 luminescence microscope under blue-violet rays, increased with age.

Fluorescent age pigment, termed lipofuscin, accumulates in post-mitotic cells of many animals including insects (Sheldahl & Tappel, 1974). In Musca domestica it has been found in the heart (Sohal & Allison, 1971), brain (Sohal & Sharma, 1972), fat body (Sohal, 1973), and Malpighian tubes (Sohal et al., 1977). Donato & Sohal (1978) and Sohal & Donato (1979) found the highest concentrations of the fluorescent substances in the head, though the abdominal tissues contained numerous autofluorescent granules which could be observed by fluorescence microscopy. Lipofuscin was present in whole larvae and pupae, as well as in adults of Sarcophaga bullata Parker, mainly in the head but also in the thorax (Ettershank et al., 1983).

Production of lipofuscin appears to depend on physiological rather than chronological age, since it accumulates faster in short-lived high activity adults of M. domestica than in long-lived low activity ones (Sohal & Donato, 1978, 1979); the highest level reached was the same for both.

A class of fluorescent eye pigments, the pteridines, accumulates with age in Stomoxys calcitrans (Mail et al., 1983). The rate of accumulation

increases with increasing temperature between 16 and 27°C, with regression coefficients of between 0.9496 and 0.9687 for both males and females; and a threshold temperature varying with sex and strain below which pteridines do not accumulate. Pteridine levels were measured in flies of known age which had been released in the field and subsequently recaptured. The slope of the line (pteridine on age) was very similar to the slope of an expected line calculated from laboratory data and corrected for meteorological conditions during the experiment (mean daily temperature, hours of sunlight and body temperature due to solar radiation). This demonstrated the method to be accurate to within ± 1.37 days over the life span of males and ± 1.90 days over that of females.

Mail et al. (1983) also measured pteridine levels in some flies of known age, and compared the ages derived from this with ages obtained for the same flies using the dilatation technique. They claim that the pteridine technique is "emphatically more accurate". This is possibly true, but since no detail is given of the method used to calculate the reproductive age (they only quote unpublished results) it is difficult to judge the accuracy of this statement.

The simplicity of this method allows large samples to be processed rapidly, and its accuracy is encouraging. Its applicability to other insects is currently being examined (Mail et al., 1983).

Method: Lipofuscin measurement, as described by Ettershank (1983) involves roughly crushing the tissue in a centrifuge tube with a glass rod in 1 ml of spectroscopic grade chloroform-methanol 2:1 with a further one ml being used to rinse the rod. The tissue is then sonicated at 100 W for 3 min with the tube immersed in an ice-water bath to absorb the heat. Next, 0.4 ml of 100 mM magnesium chloride in double distilled water is added to the tube, and the contents mixed to cause separation of the chloroform from the methanol fraction. The tube is then centrifuged at -4°C for

20 min. at RCF 2000 g, and then transferred to a water bath at 20°C. The lower (chloroform) layer containing the lipofuscin is assayed in a Perkin-Elmer MP 204 spectrofluorimeter at an exciter wavelength of 350 nm and an analyser wavelength of 420 nm. Quinine sulphate (1 mg/litre in 1 N sulphuric acid) was used as a standard. The technique may be used with fresh or deep frozen specimens, though Ettershank et al. (1983) cautioned that a separate calibration curve with insects of known age may be necessary for each type of material. They found the extraction procedure to be simple and straightforward, and suggested that the technique lends itself to processing field caught insects, as these could be stored deep frozen for processing later.

Pteridine assays were done on fresh material (Mail et al., 1983). The flies were killed at -20°C, the head removed and homogenised in 3 ml of 0.05 M tris/HCl buffer at pH8. The homogenate, which was henceforth protected from light, was centrifuged at 6000 rev./min. for 4 min. and the supernatant measured for fluorescence in either a Farrand or a Perkins-Elmer LS5 spectrofluorimeter at an exciter wavelength of 354 nm and the analyser wavelength set at 445 nm.

2.223 Colour and tergite pattern of abdomen (applicable to females only)

Dyce (1969) found that in Culicoides victorinae Macfie a burgundy-red pigmented tissue lined the abdominal wall during the development of the ovarian follicles. The colour develops during the first ovarian cycle, becoming obvious when the follicles are in an advanced stage of vitellogenesis, and persists throughout the parous state. The colour is easiest to assess in females which have not had a blood meal but whose abdomen have been distended with a sugar meal, and gives a highly reliable count of parity. The nature of the pigment is unknown, but it is not associated with blood digestion since it develops in some autogenous species (Birley & Boorman, 1982). The pigment is confined to the depleted parietal fat body lining the abdominal wall, and thus may be a product of excretion (Dyce, 1969).

This method has been used mainly in Culicoides spp., though some abdominal pigmentation was reported in parous females of Culex annulirostris (Kay, 1979). The technique separates nulliparous from parous females, but cannot determine the number of ovarian cycles completed (Walker, 1977; Akey & Potter, 1979).

Abdominal pigmentation as a valid sign of parity was assessed against the presence of follicular dilatations in Culicoides marmoratus (Kay, 1973) and C. variipennis (Walker, 1977; Mullens & Schmidtman, 1982), and against tracheolar skeins in C. variipennis (Akey & Potter, 1979) and found to be accurate. The tiny ovarioles in the Culicoides make ovarian dissections very difficult and time consuming, but scoring empty females without dissection into nulliparous and parous categories on the basis of the colour of the abdomen is fast, more than 1000 C. victoriae being scored per hour by Dyce (1969). A big advantage is that the method can be used on living, refrigerated, deep frozen or alcohol preserved material as well as for females cleared in creosote and mounted in Canada balsam. Blood engorged specimens were difficult to score on the colour of the abdomen (Dyce, 1969; Birley & Boorman, 1982), but easier to score on pigmentation patterns of abdominal tergites 2 and 3, which also changed during the first ovarian cycle whereas they do not change in the absence of a blood meal (Potter & Akey, 1978; Mullens & Schmidtman, 1982). Species that have been age-graded both on colour of abdomen and tergite patterns are shown in the Appendix, column CTP.

Pigmentation of the abdomen was maintained even in midges whose advanced follicles had undergone resorption (Mirzaeva, 1974), and when this occurred in the first ovarian cycle the midges were mistakenly classified as parous. However widespread degeneration of follicles in field populations is thought to be unusual (Dyce, 1969). Since the pigment develops gradually during the first ovarian cycle, it could be subdivided to indicate separate physiological stages within the first cycle. Thus Kay (1973) subdivided the females of C. marmoratus into five categories according to the degree of pigmentation and

the stages of digestion. The pattern of pigmentation in the abdominal tergites, the abdominal pigmentation and blood digestion, all facilitated scoring even in dead and shrivelled flies (Potter & Akey, 1978, Mullens & Schmidtman, 1982). The C. obsoletus (Meigen) group was divided by Birley & Boorman (1982) into six categories on the basis of degree of pigmentation, blood digestion and follicle development, ranging from teneral females to females at the time of the blood meal following the first oviposition.

2.224 Fat body and other criteria (applicable to both females and males)

Larval fat body sometimes persists for a short time in the teneral adult, and has been used to recognise that state, e.g. in Musca vetustissima (Tyndale-Biscoe & Hughes, 1969) and Lucilia cuprina (Vogt et al., 1974). Adult fat body declines with age in some species, e.g. Argyroploce variegana Hub. (Waloff, 1958), Simulium ornatum Meigen (Davies, 1957), S. damnosum (Duke, 1968) and Heliothis armigera (Hub.) (Tyndale-Biscoe, unpublished observations). This criterion would probably be most reliable in insects which do not feed during adult life, for example in the beetle Callosobruchus maculatus (F.) (Sharma et al., 1983). In some insects which do feed during adult life the quantity of adult fat body is known to increase at times, such as during the first 13 days of adult life in the fly Zaprionus indianus Gupta (cited as Z. paravittiger Godbole & Vaidya) (Sharma et al. 1983), or during periods of egg resorption as in Onthophagus granulatus (Tyndale-Biscoe, unpublished), thus rendering fat-body volume inaccurate as an age grading criterion.

Wing muscles are undeveloped in newly emerged females of Pterohelaeus darlingensis (Allsopp, 1979), develop into bundles in middle age and become an amorphous mass in old age. The liquid nutritive substances in the interior of the distal part of the mid-gut of newly emerged adults of Glossina tachinoides Westw. are surrounded by a peritrophic membrane secreted by the proventriculus. This membrane grows for a period during teneral life, the

growth rate being dependent on temperature. Laveissiere (1975) described a method for extracting the membrane, and measuring its length to give an estimate of the age up to 30 h of both males and females.

In some insects the adults, on emergence, have a soft cuticle which hardens during the next few days. A soft cuticle, therefore, identifies the newly emerged adult. The rate of hardening of the cuticle was measured in Chortoicetes terminifera by counting cuticular rings (see above), and was used to estimate the total number of fledglings emerging in an experimental plot (Farrow, 1979). Soft cuticle also allowed identification of newly emerged Oniticellus intermedius (Tyndale-Biscoe 1978) and Onthophagus granulatus (Tyndale-Biscoe et al. 1981b).

Green colour was observed through the integument of the thorax and abdomen of newly emerged Culex adults in Burma. Excretion and/or egestion of the green material during the first two to three days of life causes the mosquito to assume a non-green colour, thus facilitating the recognition of the newly emerged insect without dissection (Self & Sebastian, 1971; Graham & Bradley, 1972). Species age-graded by these several ways are shown in the Appendix, column marked "Other".

2.23 Changes associated with wear and tear (applicable to both females and males)

2.231 Wing fray

The trailing margins of wings of some insects become worn and frayed with time, and Jackson (1946) proposed six wear categories for the wings of males of Glossina morsitans. Because of their relatively lower activity levels, the wings of the females did not wear so quickly. Saunders (1962) scored wing fray against the more accurate ovarian age in females of G. pallidipes and found a good relationship between the two. However, Ryan et al. (1980) working on G. palpalis gambiensis in the Upper Volta, made the

same comparison and concluded that females were more active in the dry season than the wet season, resulting in a seasonal variation in rate of wing fray, and Gruvel (1975) found that mean ages of old female G. tachinoides (Westwood) differed when assessed by the ovarian and wing fray methods.

Releases of marked laboratory-reared adults of Glossina morsitans morsitans, and of G. morsitans and G. pallidipes emerging from field-collected puparia, were made, and recaptures were examined up to 59 days after release in Zimbabwe. No difference was found in the amount of wing fray in the two types of flies, and wing fray was found to increase with age. The known age range of the released flies necessary to encompass 90% of individuals of each fray class was so large that the age of an individual could only be established approximately (Vale et al., 1976). The age of males of G. morsitans was estimated by wing fray in Zimbabwe in flies caught by various methods (Phelps & Vale, 1978). The technique was accurate enough to establish the six wear categories for both G. morsitans and G. pallidipes, but Ford (1969) and Ford et al. (1972) working on G.m. morsitans and G.m. submorsitans Newstead, grouped the six wear categories into pairs (apart from the teneral group), thus separating only three fray categories. Age-grading methods used on tsetse flies have been reviewed by Mulligan & Potts (1970), Jordan (1974) and Challier (1982). Some species age-graded this way are shown in the Appendix column WF.

2.232 Cuticle wear

Like wing fray, other external characters also wear out with time (column CW, in the Appendix). The wearing away of ventral thoracic hairs on adult Locusta migratoria migratorioides can be grouped into six categories, from no wear in the young adults to almost complete wear in the old ones. The duration in days of these categories in Madagascar was found to be three times greater in the hot-humid than in the cool-dry seasons. This technique is fast and easy and gives an approximate chronological age which

can be compared with the age obtained by the ovarian method, but care has to be taken to allow for individual variations due to differential seasonal activity (Launois & Launois-Luong, 1980).

The wearing away of hair on the head, mandibular wear and darkening of the elytra of the beetles Pterostichus caerulescens (L.) and Calathus melanocephalus (L.) enabled Van Dijk (1979) to classify individuals into young and old categories. The tibiae of dung beetles are used for digging and the teeth on them wear down with age. Four grades of wear (Tyndale-Biscoe, 1978) together with reproductive condition were used to establish the age of Onthophagus granulatus, a dung beetle which lives for one year only (Tyndale-Biscoe et al., 1981b). Houston (1981) used mandible wear to suggest that Carabus glabratus and C. problematicus in northern England lived for up to three years; however, Van Dijk (1979) stated that it was too difficult to discriminate between two-, three- and four-year-old beetles in that way. The problem with wear of cuticle and hairs is that they are dependent on the type of terrain, the activity of individuals and the climate (Tyndale-Biscoe, 1978; Launois & Launois-Luong, 1980). They do, however, help to establish relative ages of individuals in a homogeneous population.

2.3 Some interpretations and applications of age-grading

Age-grading techniques used in the study of insect populations utilize criteria which are dependent on temperature, and at times on other extrinsic factors. Thus they include techniques which categorise physiological stages in development, the duration of which is temperature dependent but also subject to variation induced by factors other than temperature. For example, delays may be induced by lack of a protein meal (Tyndale-Biscoe & Kitching, 1974), lack of oviposition sites (Whitten et al., 1977; Kitching, 1977), or diapause (Moon & Kaya, 1981); or development may be speeded up by exposure to solar

radiation (Woodburn et al., 1978; Mail et al., 1983). In addition, they include those techniques which measure a physiological process whose rate, once started, is not likely to be affected by factors other than temperature, for example accumulation of fluorescent age pigment (Ettershank et al., 1983; Mail et al., 1983), the disappearance of larval fat-body (Tyndale-Biscoe & Hughes, 1969) or adult fat body (Waloff, 1958). In all these cases the result obtained from the techniques is physiological age. The only technique which allows direct reading of chronological age is the counting of daily cuticular growth rings and bands. However, even this method is possible only while the insect is under "normal" conditions, i.e. a diel day-night temperature cycle and in some cases light cycle, and provided the temperature fluctuates above the minimum necessary for cuticle growth. Chronological age can be estimated only until cuticle growth is complete (Neville, 1983).

In population studies of poikilotherms such as insects, physiological age-grading methods are more useful than chronological methods, because it is physiological rather than chronological age which primarily determines age-specific characteristics such as fecundity, behavioural traits, and potential future life span. Conversion of physiological to chronological age may only be necessary when the information is to be used for timing of control measures and the like.

Since sampling of the population is usually done over set time intervals, most models are built around chronological time. Conversion from physiological to chronological age requires corrections to be made for varying ambient temperatures; alternatively, physiological time may be used in the model, i.e. the number of day degrees (Hughes, 1962) or hour-degrees (Atkinson, 1977), this being the cumulative product of total time and temperature above the threshold for development (Southwood, 1978). In addition, attempts should be made to correct for periods of inhibition or acceleration of development.

To convert the reproductive age indicated by follicular relics to chronological age, it is necessary to know the rate of development of the first, and if possible, subsequent ovarian cycles at ambient temperatures. This can be done in a number of ways. For Anopheles subpictus Grassi and A. culicifacies Giles it was measured by using a mark-release-recapture method (Riesen et al., 1979, 1981). Newly moulted adults of Chortoicetes terminifera were released into a field cage at different times of the season, and the rate of ovarian development was monitored by ovarian dissections. Thus the length of the intervals between ovipositions could be calculated in days (Farrow, 1979).

Tyndale-Biscoe and Hughes (1969) calculated the accumulated number of day degrees above the threshold for development necessary for each ovarian cycle in the laboratory, and then fitted sine curves to records of daily minimum and maximum temperatures in the field, to work out the probable days of emergence of the flies and hence their ages.

In Lucilia cuprina the length of the first and second ovarian cycles were measured in the laboratory both at constant and fluctuating temperatures, and the linear relationship between temperature and rate of development derived from the two sets of data was the same. A developmental threshold was estimated, and the reproductive stages could then be converted into minimum actual age in day degrees (Vogt et al., 1974). A subsequent study in a field cage showed, however, that the effective field temperature was the ambient temperature plus a temperature excess proportional to the solar radiation intensity, and this relationship gave better predictions of rates of development for field flies (Woodburn et al., 1978).

The duration of the ovarian development does not depend only on ambient temperatures. In Glossina morsitans morsitans (cited as G.m. orientalis Vanderplank), Saunders (1972) demonstrated that the inter-larval period could be increased by 30-40% when pregnant females were deprived of food in the

middle of the gestation period, i.e. when the second instar larva was being nurtured. Ovarian development was not retarded in those females that aborted. Laveissiere & Kienou (1982) found that the time taken by G. tachinoides to develop and ovulate the first egg, and the time interval between ovulations depended on the season, as well as on the availability of hosts for adult feeding. Thus, the period between ovulations as determined by rate of development at given temperatures may indicate a minimum time only, and care has to be taken in translating these results into field situations.

The availability of several age-grading methods for the same insect has been found advantageous in a number of studies. Thus when results obtained by the follicular relic method are compared with results obtained using a second method (preferably one that is independent of factors such as the need for a protein meal or to oviposit), an independent estimation of age is obtained. This may serve to confirm results obtained by reproductive age estimation, or a disagreement may highlight developmental delays which might have occurred in the field. Such comparisons have been found useful, for example in Lucilia cuprina using reproductive age-grading together with counts of cuticular bands (Tyndale-Biscoe & Kitching, 1974), in Oniticellus intermedius using reproductive age-grading together with tibial wear (Tyndale-Biscoe, 1978) and in Culicoides variipennis in which the accuracy of parity rates as scored by abdominal pigmentation was assessed by comparison with parity rates scored by the tracheolar method (Akey & Potter, 1979).

Alternatively, several age-grading methods have been used successfully in which one serves to identify a short part of the insect's life span in detail, while another roughly age-grades older individuals. For example, the age of G.m. morsitans was defined precisely in the first ovarian cycle using ovarian criteria and wing fray was used to estimate approximately the age of older flies (Taylor, 1979). For Locusta migratoria migratorioides Launois & Launois-Luong (1980) suggested that the age during the first ten

days of the adult's life can be determined by counting cuticular rings, while subsequent age-grading can be done by scoring wear of the thoracic hairs.

Another use for two techniques in the study of one species is where the more rapid one is used to make a quick separation into nulliparous and parous categories (e.g. the tracheolar method) and a smaller sample, or the parous group only, is age-graded more precisely with the dilatation technique (Reisen et al., 1979; Kay, 1979). A crude separation of age categories by wing fray can be followed up by a study of the duration of these categories using an ovarian method (Ryan et al., 1980). In this way much additional information can be gained by processing larger samples, while still retaining the accuracy derived from the slower method.

Some techniques are applicable to members of one or only a few insect genera, while others have been found to have wider application. Thus colour and tergite pattern of the abdomen has so far been a useful age criterion only in the Ceratopogonidae, whereas follicular relics and cuticle growth have been applicable to most insect orders in which they have been tried.

The undoubtedly very accurate technique of cuticle layer count applies in general only to the teneral adult phase or a little beyond it (Neville, 1983). It is therefore of great relevance to studies of preovipositional events such as the timing of the first feed, or mating or the recognition of teneral flight, etc., but it provides no information on the later life of the insect. The same limitation applies to all techniques which can only monitor up to the time of sexual maturity, such as the presence of single follicular dilatations, tracheal skeins, and the colour of the abdomen. Multiple dilatations and yellow bodies, where these represent successive ovarian cycles, remain the fastest accurate technique covering the major part of the insect's life span where large samples can be examined. Wing fray, while also covering the entire life span, is not nearly so accurate.

Fluorescence measurements, if shown to be as generally applicable as they promise to be, may provide a very accurate method of age determination for the duration of the whole life span as well as a method of calibrating other methods of age-grading. They may help to distinguish between age groups not separable with established techniques, such as the yearly age groups of insects whose lives span several years, and whose ovaries develop and regress in a seasonal pattern.

Age-grading techniques have been used for a wide variety of reasons, but the vast majority of studies have been on populations of insects of epidemiological importance. An insect which becomes infective at its first blood meal may transmit the disease at a subsequent blood feed. Hence to assess the infective potential of a population it is important to identify, and quantify, that proportion of the population which has had one or more blood meals. In cases where one blood meal is sufficient for the completion of one ovarian cycle, knowing the number of cycles completed will indicate the potentially infective nature of the insect. The proportion of potentially infective individuals in a population will in turn indicate its epidemiological danger (Rachou et al., 1973; Thomas, 1973; Jordan, 1974; Ungureanu, 1974, Walker, 1977; Magnarelli, 1977; Charlwood & Wilkes, 1979; Birley & Boorman, 1982).

Age-grading has been used to demonstrate that older, potentially infective mosquitoes fly higher and therefore are more prone to downwind displacement to uninfected areas (Snow & Wilkes, 1977). The seasonal and diurnal incidence of active parous females in airborne populations was determined (Akey & Barnard, 1983), as was an estimate of the effectiveness of spray campaigns and the timing of spray schedules (Davies, 1978). The best time for taking samples, and the optimum sample size for age-grading a population was determined by Corbet & Smith (1974), and Morris & de Foliart (1971b).

The lack of parous individuals in an overwintering population of Culicoides variipennis indicated its unsuitability as a host for overwintering arboviruses (Nelson & Scrivani, 1972).

Age-grading helps in the study of life histories (Luff, 1973, 1980; Mercer & King, 1976; Tyndale-Biscoe et al., 1981b), life-span (Okiwelu, 1976; Reisen & Aslamkhan, 1979), survival and biting rates (Birley & Rajagopalan, 1981), and population regulation mechanisms (Farrow, 1975) as well as in the construction of life tables (Taylor, 1979). It was used by Service (1969) to study biting incidence and behaviour in mosquitoes. Autogeny in some species was recognised by the absence of insects in the first ovarian cycle in the samples caught coming to feed (Anderson, 1971; Bosler & Hansens, 1974; Troubridge & Davies, 1975; Magnarelli & Pechuman, 1975; Magnarelli & Cupp, 1977; Magnarelli & Anderson, 1977; Charlwood & Rafael, 1980; Wall & Doane, 1980; Magnarelli, 1981; Auroi, 1982).

Population age structure was used as a marker to recognise long distance migration (Hughes, 1970), short distance displacement (Whitten et al., 1977), and the age of night flying insects (Lambert, 1972).

Finally, it is known that sampling methods may bias the catch in favour of certain age groups (reviewed by Gillies, 1974). Age-grading has highlighted the effectiveness of different types of traps for catching various age samples (Hughes, 1974; Phelps & Vale, 1978; Wilkes & Rioux, 1980), and it also helped to test traps and other collecting methods for catching insects in different reproductive or behavioural phases (Vale et al., 1976; Phelps & Vale, 1978; Taylor, 1979; Moon & Kaya, 1981).

2.4 Summary

The literature on age-grading techniques of adult insects, published since 1968, is reviewed. The techniques described include those which deal with changes in the reproductive system, as well as somatic changes which take place with age. They also include techniques based on changes induced by wear and tear.

Follicular relics found in different orders of insects are described,

and their origin, fate and relative usefulness in age-grading studies are discussed. Attention is drawn to the formation of anomalous, or aberrant dilatations, particularly ones formed as a result of follicle resorption.

The suitability of the various techniques to the study of different insect species is compared and the advantages of using more than one type of age-grading method in the study of one species are discussed. Enough detail of each technique is given to permit field workers to choose, and apply the technique most suitable for the study of the insect of their choice, and for the degree of accuracy required by them. Lists of species which have been studied by particular age-grading techniques, or combinations of them, are given. Finally, examples are cited of the types of problems entomologists have sought to solve, often successfully, using age-grading methods.

Chapter 3. General methods

3.1 Field sites.

Most field observations of Ot. granulatus were carried out at a field station at Uriarra, 20 km west of Canberra. The experimental area was located in an 18 ha paddock permanently grazed by cattle. The mean temperature from October to March ranges from 12.9° to 20.7° and down to 17.8°C; average annual rainfall is 979 mm and 47% of it falls during the warmer months from October to March. However, the area is prone to frequent short term droughts varying from 1 to 3 months in duration.

Other observations were carried out near Armidale, on the northern tablelands of NSW. The experimental site was within the CSIRO Experimental Station at Chiswick, 20 km south of Armidale. Mean temperature through spring and summer ranges from 13.4° to 19.2° and down to 17°C, the annual rainfall is 762 mm, and 63% of the rain falls during the warmer months from October to March.

All field observations of Oi. alexis were carried out at Araluen, 130 km south east of Canberra. The work was done in a 100 ha paddock permanently grazed by cattle. Araluen is a valley with a coastal climate, with spring/summer mean temperatures ranging from 15.1° to 21.1° and down to 19.2°C; and it is the closest area to Canberra where Oi. alexis has become established. Average annual rainfall is 928 mm, 56% of it falling during the warmer months from October to March. The area is prone to periodic droughts of several months' duration.

Because the sites were far apart, they could not be monitored

simultaneously. Hence work at Uriarra was carried out during 1976-1979, and at Araluen from 1980-1983.

3.2 Beetle traps.

Dung beetles (both species) were caught in pitfall traps, as illustrated in fig. 6. The trap consisted of a cylindrical pit 300 mm deep and 200 mm in diameter. The walls of the pit were lined by a metal cylinder. A wide-mesh (30 mm) metal grid rested on top of the pit. One litre of fresh dung wrapped in thin gauze was placed on the grid. Beetles attracted to the dung fell through the grid into a plastic funnel leading into a collecting jar.

3.3 Dissections.

For age-grading Ot. granulatus, the beetles were collected live in dry jars and dissected immediately upon return to the laboratory. Oi. alexis were collected live, directly from dung pads. Weekly dissections of a maximum of 30 female Ot. granulatus were made, or, if there were less than 30, then all females were dissected. Similarly, the first 30 female Oi. alexis, or the maximum number of females found in the first 1.5 h of searching through pads, were dissected fortnightly.

The beetles were killed in 70% alcohol and dissected in Ringer solution (Tyndale-Biscoe 1978).

I had previously developed an age-grading system for another dung beetle, Oniticellus intermedius Reiche, (Tyndale Biscoe 1978). It uses the yellow body in conjunction with cuticle hardness, tibial wear, and degree of development of the oocytes (see review, Chapter 2) to distinguish seven distinct age categories during the adult life span of the beetle. Briefly, the age-grade categories are as follows:

Nulliparous : N1 = newly emerged beetles, soft cuticle, undeveloped reproductive system, unworn tibiae (Category 1), no yellow body.

N2 = young beetles, hard cuticle, undeveloped or slightly developed reproductive system, slightly worn tibiae (category 2), no yellow body.

N3 = mature beetles, cuticle hard, fully developed reproductive system, slightly worn tibiae (category 2), no yellow body.

Parous : P1 = similar to N3, except some small amount of yellow body visible in the calyx.

P2 = older beetles, similar to N3 but a ring of yellow body visible in the calyx, tibiae moderately worn (category 3).

P3 = old beetles, similar to N3 but a solid plug of yellow body present on the calyx, tibiae moderately to severely worn (category 3 or 4)

Spent : P4 = old beetles reproductively spent, oocytes in the process of resorption or resorbed, yellow body a solid yellow plug, tibial teeth severely worn (Category 4).

In addition, at the time of dissection a score was kept of the number of females which showed signs of oocyte resorption. This manifested itself in two ways : internal resorption of the terminal follicle inside the ovariole, with an uneven appearance of the yolk, and/or opaque patches on the surface of the follicle (Bell and Bohm, 1975); or external resorption where a portion of the terminal follicle was extruded through the ovary wall into the haemocoel (Tyndale-Biscoe &

Watson 1977). Resorption could occur at any age from N₃ to P₃. P₄ beetles were by definition resorbing all their follicles.

3.4 Trap catches

In the first year of study the average number of Ot. granulatus per trap caught in 10 pitfall traps per week was taken as an index of the abundance of adult beetles. As noted previously, this index will be affected by variations in trapability as well as in abundance. Examination of the first year's data showed that three to five traps gave indices very little different from those obtained from the ten traps. Thus only three traps were used at Chiswick and Araluen, and five traps at Uriarra, for the remainder of the study. The traps were set at 1600 h on the trapping day and left out for 24 h. The 1600h setting time ensured that the pad was fresh and attractive to Oi. alexis flying that evening and the following dawn; and retained its attractiveness through to the following morning when Ot. granulatus was active.

3.5 Weather records.

A thermohydrograph in a standard Stephenson screen provided a continuous record of temperature at Uriarra and Armidale. At Araluen the temperature was measured throughout the three activity seasons, and through the winter of 1983. Rainfall was measured each week in a standard rain gauge. Information on the average yearly and monthly rainfall and temperature data for each site, was obtained from the Bioclimate prediction system (Buzby 1983).

3.6 Laboratory cultures.

Cultures of both species were maintained in a laboratory/glasshouse, at $27\pm 2^{\circ}$, with 14:10 h light:dark cycle. Beetles were placed in groups of five pairs (Ot. granulatus) and single pairs (Oi. alexis) in gauze-covered bread boxes (300x200x250 mm) filled with a mixture of moist, compacted vermiculite and sand in the proportions of 2:1 (Ot. granulatus), or soil/vermiculite mixture in the proportions 4:1 (Oi. alexis).

Different numbers were used per container for the two species because they differ in size (Ot. granulatus = 6mm in length, Oi. alexis = 15mm). The fecundity of dung beetles is affected by crowding (Hughes et al. 1978, Ridsdill Smith et al. 1982) hence for maximum brood production only a single pair of Oi. alexis, but up to five pairs of Ot. granulatus could be used in the standard bread boxes.

Different substrate mixtures were used for each species because , Ot. granulatus buries small masses of dung singly (.5 gm) in shallow excavations underneath the dung pad, and for this a moist vermiculite/sand mixture proved suitable. Oi. alexis, however, excavates a deeper burrow which it fortifies with a lining of dung before burying up to 600 gms of dung mass. The entrance needs to be firm to prevent it collapsing; hence compacted soil was used for this species. Enough vermiculite was mixed with it to make the mixture lighter without affecting its stability.

On top of this vermiculite or soil mixture was placed 0.5 l of fresh cow dung. The dung was changed twice a week, and the soil or vermiculite was sieved once a week to extract the masses of broods. Since each Ot. granulatus egg is in an individual mass of dung, these can easily be counted. Oi. alexis eggs are laid in the upper surface of the brood mass, the site of each egg being characterised by a raised

area on the surface of the mass; thus the number of eggs in a brood mass could be counted when the adhering soil mixture was carefully brushed or washed away.

The brood masses were placed in refrigerator boxes (300x300x150 mm) under a layer of moist soil and kept damp to prevent desiccation. Newly emerged adults were caught in small dung-baited pitfall traps that had been sunk into the soil in the boxes. All beetles of each species emerging in any one week were placed in large stock cages on soil or vermiculite mixture, and fresh dung was added regularly as food. This provided beetles of known age.

3.7 Origin of dung, and dung characteristics

The dung used in the maintenance of the cultures, and in most experiments except where otherwise stated, was obtained from a dairy at Fyshwick A.C.T., with an irrigated pasture of clover and rye grass. The cattle were supplementary fed with green oats and green lucerne in the winter, and green millet in the summer. Since dung characteristics, as assessed with a bioassay with bushflies (see below) depends on the proportion of actively growing compared with senescent pasture (Greenham 1972, Sands and Hughes 1976, Matthiessen and Hyles 1983), the characteristics of the dung collected in the dairy varied from medium to good for bushflies. In experiments where dung of different characteristics was needed, the dung was collected at Uriarra, A.C.T.(improved pasture of phalaris, clovers and rye grass); at Gungahlin, A.C.T.(pasture predominantly composed of kangaroo grass with some subclover in it) or from elsewhere if not available locally, as described in the relevant experiments. It was then deep frozen until needed, at which time it was thawed to room temperature and a bioassay with bushflies was done

to assess its characteristics. In addition, collections of dung were made through three seasons at Uriarra, and through one season at Araluen, to monitor the changes of dung characteristics at these two study sites.

3.8 Dung Bioassay

Freshly dropped dung was collected from several head of cattle. The mixed bulk sample was stored in an airtight container and deep frozen from two to four weeks to kill any organisms which may have been present in the dung. Prior to testing the dung was thawed, and three one-litre samples were each placed on a bed of moist vermiculite 30 mm deep in a plastic box (250x250x100 mm). A sample of ten gravid female bushflies cultured in the laboratory (for fly culture method see Hughes et al. 1972) were dissected to determine the mean number of eggs per female in the current stock. Enough gravid females to lay approximately 300 eggs were caged above the dung samples. This number of eggs was chosen because it was not likely to result in density-dependent mortality (Sands and Hughes, 1976). After 2 h the flies were collected, killed and dissected under saline and the number of eggs laid by them estimated (ie. no flies x av. no. eggs/female - no. eggs retained). The boxes were covered with a gauze lid and maintained at 27°C until all flies had pupariated. The puparia were sieved out of the vermiculite, counted and weighed. The average size of the puparia, as well as the percent survival, varied in dung of different characteristics.

Thus puparia weighing 18 mg. or over, with an 80% or higher survival rate is characteristic of spring dung or dung from an irrigated improved pasture. In contrast, puparia weighing less than 8 mg, together with a survival of 30% or less bushflies are typical of a dung produced by

cattle grazing a senescent, non-irrigated pasture. This measure of the dung characteristics using bushflies will henceforth be referred to as dung quality. As mentioned above, adult dung beetles, like bushfly larvae, feed on the dung juices (Halffter and Edmonds 1982), and thus they may react to dung of different qualities in the same way as do bushfly larvae, (Macqueen et al, 1984). Dung beetle larvae, however, have chewing mouthparts, and hence may feed on different, more solid material in the dung. Thus they may or may not be affected by the quality of the dung as assessed by bushfly larvae; this has not been tested to date.

3.9 Oviposition, developmental time and survival in the field.

To monitor oviposition and developmental times at the field study sites, gauze-bottomed cylinders were dug into the ground with their tops flush with the soil surface. The cylinders were 400 mm high, 200 mm in diameter, and were filled to within 50 mm of the top with moist, compacted vermiculite/sand mixed in a ratio of 2:1 (Ot. granulatus) or moist, compacted soil (Oi. alexis). 1.0 l of fresh dung was placed on the vermiculite and left exposed for 1 week, during which time Ot. granulatus, if they were present in the field, were attracted to it either to feed or to produce broods. At the end of the week, when most beetles had left, the vermiculite was searched for broods. These were counted but left in place in the cylinder, which was then closed with a gauze lid. The lid was to stop further oviposition and to contain any emerging beetles, but did not prevent rainfall from reaching the broods.

Because of the scarcity of Oi. alexis during the 1982-83 season, and the presence of several other species of dung beetles (including Ot. granulatus) at Araluen, the method employed with this species was

slightly different. Two pairs of Oi. alexis were collected from nearby dung pads and placed on the dung in the cylinder, which was then closed with the gauze lid. One week later the lid was removed for several days to allow the parent beetles to escape. The dung was all used up in the week hence there was no possibility of other species ovipositing into the cylinder while it was open. The soil was again searched for broods. Since Oi. alexis lays several eggs into one brood mass the eggs were not counted though their presence was noted, and the brood masses were replaced in the soil and the lid replaced in the cylinder.

After allowing several weeks for development, small petri-dishes baited with fresh dung were placed into the surface of the soil or vermiculite/sand in the cylinders. When the first beetles completed their development, they emerged and were attracted to the fresh dung. At the time of the next sampling cylinders were brought back to the laboratory and all broods were opened, the date and stage of development of all the progeny was recorded. The date of the first emergence was taken as being midway between the day beetles were found and the previous time of sampling.

To monitor overwintering survival of adults, young beetles of known age, typical of those present in the field at the time, were placed on the dung in the cylinders and the lid closed. Fresh dung was added at regular intervals until all the beetles had died, or until spring, when the species were again found to be active in the field.

To monitor survival of immatures of both species in the winter, brood masses containing eggs or larvae of known developmental stage from the laboratory culture were placed in soil in cylinders in the autumn. Survival was scored during the winter and into the spring by digging up and examining samples of broods at regular intervals.

Chapter 4. Validation of age-grading method.

Yellow body is a more accurate age-grading criterion than is cuticle wear (see review, Chapter 2), and therefore is preferable to it. Cuticle wear serves well for confirmation of age-classes obtained by other methods, but should be used as an age-grading method by itself only when other criteria are not available.

To establish whether the ovarian age-grading criteria developed for Oniticellus intermedius can be applied to the species chosen for this study it was necessary first to know whether yellow body accumulates with age in Onthophagus granulatus and Onitis alexis. This was done in two ways : first in a field trial to see whether yellow body size correlated with an alternative index of age (tibial wear) and second, in the laboratory, by examining yellow body accumulation with age and increasing amounts of oviposition.

4.1 Relationship between yellow body and tibial wear.

4.1.1 Method. In the field the ages of the beetles are unknown, but increased tibial wear was taken to indicate advancing relative age. From October to December 1977 Ot. granulatus was collected live in pitfall traps at weekly intervals. On return to the laboratory the beetles were dissected, and each beetle was scored for quantity of yellow body (on a scale of 0-3 i.e. none to a plug of yellow follicular remnants), and degree of tibial wear (on a scale of 1-4 i.e. no wear to complete wear)(Fig. 7). Oi. alexis was collected once a fortnight at Araluen through November and December 1980 and processed the same way.

4.12 Results

4.121 Ot. granulatus:- Most Ot. granulatus with unworn tibiae (category 1) had no yellow body. The arbitrary categories of increased tibial wear, taken to indicate increasing age of the beetles, correlated well with increasing quantities of yellow body (Fig. 8a, 9a). Since all beetles were collected from the same environment, so that tibial wear would be expected to occur at the same rate for all the beetles examined, it appears that in this species yellow body does accumulate with age.

4.122 Oi. alexis:- Most Oi. alexis with unworn tibial teeth also had no yellow body present in the calyx (Fig. 8b), and there was a trend for beetles to have more yellow body (Fig. 9b) with increased tibial wear. However, 29% of beetles which had a tibial wear category of 2 had no yellow body, indicating that either yellow body is not always a reliable criterion of parity, (ie. it gets lost), or that some beetles do some digging before the start of oviposition.

Many female Oi. alexis were found to be extruding the terminal follicle into the haemocoel through the calyx wall (Fig. 9c) a phenomenon already reported for Onticellus intermedius (Tyndale-Biscoe and Watson, 1977). This occurred in O. intermedius in response to an environmental stress, and enabled the beetle to resume oviposition as soon as the stress was removed. Extrusion of the terminal oocyte occurred before chorionation, and ensured that the calyx was not obstructed at the time of the next oviposition. Most of the yellow body found in Oi. alexis was in the form of granules

attached to the outside of the calyx wall, and no doubt represents the remnants of the resorbing extruded eggs (Fig.9d). The fact that so little yellow body is seen inside the calyx indicates that much of that is also extruded, probably along with the terminal follicle, or perhaps even at the time of the next oviposition.

4.2 Relationship between age and yellow body.

Ot. granulatus. Since yellow body quantity does increase with increasing tibial wear, an experiment was designed to monitor its increase with increasing age.

4.21 Method. Four groups of 50, 0-2 week old beetles from the culture were set in bread boxes with fresh dung, which was replenished twice weekly. Under these conditions the beetles bred freely. Females were dissected when they were 1-3, 4-5, 6-8 and 9-11 weeks old, and scored for yellow body category. Every two weeks the boxes were sieved to remove all brood masses to avoid newly emerged beetles becoming mixed with the experimental ones. The experiment was conducted at 27°C at 14:10 h light:dark cycle. There were two replicates.

4.22 Results. The relationship between yellow body quantity and age in Ot. granulatus is shown in table 2.

Table 2 .Relationship between quantity of yellow body and age in Ot. granulatus. (Number of beetles in each category).

Repl. 1		Age of beetles in weeks			
Yellow body category		1-3	4-5	6-8	9-11
	0	17	2	0	0
	1	1	6	2	0
	2	1	2	12	1
	3	0	0	5	11
Repl. 2					
	0	-	5	0	0
	1	-	6	6	0
	2	-	6	9	3
	3	-	0	1	18

The two replicates were homogenous (for 4-5 week old beetles $X^2 = 1.57$ with 2 d.f., for 6-8 weeks $X^2 = 4.87$ with 2 d.f). Thus the results were pooled, and the X^2 test indicated that there is a highly significant increase in yellow body with age in Ot. granulatus ($X^2 = 144.39$ with 9 df.)

4.3 Distinction between nulliparous and parous Oi. alexis.

Because yellow body was not always present in Oi. alexis with tibial wear category 2, it was important to find a reliable additional method of distinguishing nulliparous from parous beetles. The following experiment was designed to determine whether there was such a criterion.

4.31 Method. Forty pairs of newly emerged beetles from the culture were set up in bread boxes in individual pairs (♀ + ♂) with fresh dung supplied twice weekly. Ten pairs were dissected after 2, 4, 7 and 14 days; the soil was sieved and any brood masses

extracted and the eggs counted. The experiment was conducted at 27°C, with 14:10 h light:dark cycle.

4.32 Results. [Table 3.] No coloured granules of any kind could be seen in the calyx, or outside the calyx, in nulliparous female beetles. Only the prefollicular tap was sometimes visible; this is an opaque whitish plug of tissue at the base of the terminal follicle in developing ovarioles (Heurta et. al. 1981)

However, not all parous females possessed yellow granules either in or outside the calyx wall. This indicated that some parous beetles must lose their yellow body. In all non-extruding parous beetles the follicular sheath which surrounded the last laid oocyte was present in the form of a crumpled transparent bag, either inside the calyx, or partly extruded through the ovariole wall forming small, transparent bubbles on the outside of the calyx wall. [Fig.10] With care it could be dissected out whole. It appears, therefore, that since it is mainly the follicular sheath which forms the yellow body (Chapter 2), and in this species much of the sheath is extruded into the haemocoel, it is the extruded portions of it which may eventually form the yellow granules on the outside of the calyx wall, and that at times these may disappear from there. Thus initially after oviposition the follicular sheath can always be recognised, and later this may or may not be present in the form of a yellow body internally and/or a yellow granule externally. In the two beetles which extruded the terminal follicle the extrusions initially appeared large and filled with yolk, but as resorption progressed the extrusions must eventually shrink to the size of yellow

Table 3 . Difference between nulliparous and parous Oi. alexis

Age of beetles (days)	n	No. eggs per ♀ ± S.D.	Range	Size of terminal oocyte ± S.D. (mm)	Range (mm)	No. beetles with y.b.	No. beetles with epithelial sheath	Extraovari- olar resorption
2	10	0	-	1.66 ± .51	0.88-2.64	0	0	0
4	(9	0	-	2.13 ± .46	1.41-2.81	0	0	0
	(1	4	-	4.67		0	1	0
7	10	6.2 ± 3.0	2-12	3.71 ± .38	3.16-4.40	5	10	0
14	10	18.4 ± 5.0	10-28	3.47 ± 0.84	1.76-4.31	10	8	2

granules (as they do in Onticellus intermedius, Tyndale-Biscoe & Watson 1977). At that stage it would not be possible to differentiate between ovaries from which eggs has been laid and ones in which follicles had been resorbed. However, for the purposes of age-grading, a beetle which had developed a terminal follicle and resorbed it can be classified as physiologically old enough to have oviposited.

By day 7, all beetles had laid eggs. Only half of them had signs of yellow body, but they all had the follicular epithelial sheath present. The empty epithelial sheath was present in all non-extruding beetles on day 14, and the two resorbing ones had the terminal follicular extrusions.

The average size of the terminal oocyte increased through development up to day 7; the reason for the decrease in mean size on day 14 was due to the two beetles with resorbing follicles.

4.4 Conclusions

Yellow body increases with increasing tibial wear in the field in Ot. granulatus, and in the laboratory it increases significantly with increasing age.

As in Onticellus intermedius (Tyndale-Biscoe 1978) yellow body can therefore used to recognise three parous categories (see Chapter 2) even though the exact number of eggs laid by the females of Ot. granulatus in each yellow body category is not known.

In field caught Oi. alexis there is also some correlation between tibial wear and yellow body. However, in the laboratory trial it became obvious that although parous beetles could always be recognised by the

presence of the empty follicular epithelial sheath or by extruded terminal follicle, in a number of them there was no sign of yellow body. Hence only one parous category was used for age-grading sexually active mature Oi. alexis.

The three nulliparous categories, as described for Onticellus intermedius (Tyndale-Biscoe 1978) apply to both species (N_1 - N_3 , see section 3.3); spent beetles of both species have worn tibial teeth and resorbed follicles in the ovariole. Thus altogether seven age categories could be recognised in Ot. granulatus. In Oi. alexis only five age-categories were used in age-grading the females on the basis of the ovary: the three nulliparous categories, one parous category and the spent P4 category.

Chapter 5. Age-structure, incidence of resorption, trap catch and environmental quality in the field.

5.1 Introduction.

An analysis of the age structure of a population enables one to learn a great deal about the behaviour and life cycle of the insects. Thus, for example, the timing, duration and number of peaks of newly emerged individuals in a population per season give information on how many generations there are in a season; whether these are overlapping generations or not, and the generation length. Peaks of other nulliparous categories indicate the length of the maturation - feeding phase. When the nulliparous phase overlaps with the earliest parous category in a population with non-overlapping generations, it can be assumed that there is no delay in the onset of reproduction, and that the beetles only spend short periods of time burying brood masses and promptly return to fresh dung pads for further feeding and breeding. This is in contrast to brood-caring beetles which, once having buried the dung for breeding purposes remain underground with their broods. Thus the parous categories are largely missing from the samples (e.g. Copris armatus, personal obs.)

Information on the stage in which the species survives the non-reproductive and presumably unfavourable periods e.g. cold winters or hot summers, can be obtained by the age category of the beetles appearing in the field immediately following the stress period. Thus if there are newly emerged beetles (N1) in the first samples appearing in dungpads, it suggests that the species overwintered/oversummered in the larval stage. If the majority of the first samples are in the N2 stage or older, then provided one has not missed the earliest flush of emergence

(and thus missed the N_1 stage) then the species most likely spent the winter/summer in the adult stage underground, and appeared on the soil surface in response to favourable environmental conditions. It is not usually possible to obtain this information more directly since the non-breeding period is generally spent underground and individual beetles may be difficult or impossible to find.

Information on the chronology of life-cycle events can be further supplemented with knowledge of the population density in a dung pad. Since adult dung beetles depend both for feeding and for reproduction on dung, both sexes and all age groups are attracted to it. Therefore regular use of dung-baited pitfall traps on days with similar meteorological conditions (particularly temperature, Vogt et al. 1983) gives a measure of the numbers of beetles colonising the pads in any one area at different times of year. Age-grading dissections of these samples give an analysis of their age composition.

Thus new emergences are frequently accompanied by peak beetle densities in a dung pad, and breeding activity peaks are usually smaller. If the relative size of these peaks is greatly different, it may mean that conditions for the survival of the adult beetles was not favourable. If the relative sizes of the peaks are reversed, ie. a large breeding peak is followed by a small newly emerged peak, it indicates either low fecundity or poor survival of the immatures in the ground.

The aim of the work reported in this chapter was to build up a picture of the seasonal fluctuations of beetle numbers in the dung pads as well as the seasonal changes which occurred in age-structure of the field populations of both species. From this data the life cycle phenomena of each species were inferred. (In this thesis the term "season" refers to the warmer part of each year when adults can be

caught in traps i.e. October to March.)

Environmental factors, when measured concurrently with the trapping and age-grading, may help explain the mechanism of some of these phenomena. As the distribution of insect species depends to a great extent on climatic factors, temperature and rainfall were monitored throughout the study. As well, the quality of the diet is known to influence biological phenomena such as fecundity (Mokry 1980b), and this is known to vary with rainfall and temperature through the season (Greenham 1972, Sands and Hughes 1976). The third environmental factor measured during this study, therefore, was the characteristics of the current dung by obtaining an index of dung quality with the bushfly bioassay technique (see Chapter 3).

Finally, follicle resorption may be related to the age of the insect, to regular seasonal factors such as a drop in temperature in the autumn, or to incidental environmental stress such as drought (Bell & Bohm 1975). Dung beetles of nesting pattern I group (Halffter and Edmonds 1982, see Chapter 1) spend a comparatively short period of time in the maturation feeding phase, and a major part of their adult life span is in the breeding phase. Therefore, if adverse environmental conditions induce follicle resorption, there is scope to recognise this during most of the beetle's life span. For this reason, during age-grading dissections the numbers of beetles undergoing follicle resorption were counted, and the results analysed to see what factors might induce resorption in the two species. Thus the sampling programme was intended both to characterize the life cycle pattern and to identify factors favourable or inimical to beetle reproduction and population growth.

5.2 Results

5.21 *Ot. granulatus*, Uriarra

5.211 Seasonal pattern of trap catches, age structure and resorption.

Trapping and dissection results are summarised in Figure 11a, b, and c. Beetles appeared in the traps in September each year and were caught throughout the summer until April, with peak catches in the spring and early in the following year. Few, or no beetles were trapped from May through early August.

Beetle catches were exceptionally high in 1976/77 often reaching more than 1000 per trap. In the following two years, numbers were substantially lower.

Changes in the age composition of the population showed up clearly in the dissections. Thus in September 1976 when the first beetles were trapped in substantial numbers, up to 80 per cent were nulliparous and developing their eggs (N_{2-3}) and the remainder were parous in the process of producing broods (P_{1-3}). There were no newly emerged beetles in the catches. The relative numbers of parous beetles increased through October and November. The trap catch decreased sharply in November and then consisted almost exclusively of actively breeding parous beetles (P_{1-3}), with little change into December except that late in that month, some newly emerged beetles (N_1) appeared. Earlier in the month increasing numbers of beetles were resorbing their eggs and some were clearly old beetles probably incapable of further breeding.

In January and February a mass emergence of beetles occurred and the beetle numbers increased dramatically so that on February 9 over 3000 came to each trap. Dissections indicated that during

that period between 70-85 per cent of the beetles were newly emerged.

Capture rates had begun to fall again by mid-March with a decrease in new emergences, and increasing age of the population. Most beetles were developing eggs, some were actively breeding, and substantial proportion were resorbing eggs. The last beetles caught before the onset of winter were at the end of May.

Beetles reappeared in traps again in September 1977. As in the previous year the population consisted entirely of N_2 and older beetles, indicating a survival of adult beetles over the winter period. During most of January there was no emergence of new beetles as had occurred the previous year. However, small numbers of newly emerged beetles appeared in late January and February and the proportion increased in March and April. There was a high incidence of resorption during November and December, which then decreased for the remainder of the season to approximately the same levels as for the previous season.

The 1978/79 season began with small numbers of overwintering beetles which came to the traps in September and brood production proceeded until December. There was a substantial emergence of new beetles from mid-December to January. New emergences decreased during the second half of January and ceased in February and March, and an increasing proportion of beetles were resorbing their eggs. However, beetle numbers were so low at this time that dissection data must be treated with some caution.

5.212 Rainfall - (Fig 11a,b,c) The 1976/77 season was characterised by consistent rains throughout, maintaining a fairly high soil

moisture content. There were only two periods where rainfall was less than 10mm per fortnight, these were from November 23 to December 21, 1976 a period of 4 weeks, and January 18 to February 1, 1977, a period of two weeks.

During the first half of the 1977-78 season there was a prolonged drought, lasting from the end of September to the end of December. During the second half of the season there was adequate rain both in January and in March. In contrast, the 1978-79 season was characterised by regular rain up to the second half of December, but then there followed a dry period through January and up to the end of February.

5.213 Dung quality (Fig 11a,b,c) Both percent survival of bushflies, and the mean size of the puparia, indicate seasonal variation in dung quality (Sands & Hughes 1976). During the 1976-77 season only the percent survival of the bushflies was measured, and only from November 23 onwards. Survival was 50-70 percent at the end of November - early December, indicating an average quality for bushflies. The quality deteriorated through January and February with the haying off of the pastures, and then improved again by March 1 with new grass growth following the rains in February.

During the 1977-78 season both the good survival, and the large size of the puparia indicated good quality dung through October and November. There was a period of poor quality through December and into January when the pastures were haying off, but then due to abundant rain and warm weather the pasture grew again, giving an improvement in dung quality through February, March and April. In the 78-79 season the quality was again good in the

spring, though puparial size indicated that it began to deteriorate by November. January and February were very dry and this prevented the growth of new pasture, causing dung quality to remain poor until about a fortnight after the rains in early March.

5.22 *Ot. granulatus*, Armidale.

5.221 Seasonal pattern of trap catch, age-structure and resorption (Fig. 12a,b).

Large numbers of beetles were trapped throughout both seasons although the exceptional numbers seen at Uriarra in 1976/77 were not reached. The spring and summer peaks which occurred at that site were also apparent at Armidale in 1977/78 but not as marked in 1978/79.

The age structure of the population resembled closely that found at Uriarra, with most of the trap catches in spring being sexually mature, and new emergence taking place in January, February and March. However, an unusual feature of the Armidale early spring populations was the presence of a few newly emerged beetles at that time in contrast to the situation at Uriarra. This probably indicates some survival of immature stages over the winter period in that area, where mean and minimum temperatures from May to August are on average about 1° warmer than at Uriarra (Bureau of Metereology, 1975).

Fewer beetles were found to be resorbing eggs at any time during either season at Armidale. After some resorption early in the season, there was one period of resorption at the end of February in both seasons, and only in 1979 did it reach 26% of the sample. (The resorption percentage on Sept 13, 1978 was based on 2

beetles only and therefore probably unrealistically high.)

5.222 Rainfall - (Fig. 12a & b) This was consistent through both seasons, with never more than a two week period of less than 10mm of rain.

5.23 *Onitis alexis* - Araluen (Fig.13 a,b,& c).

5.231 Seasonal pattern of trap catches, age-structure, and resorption.

Trapping and dissection results are summarised in Fig.13a, b & c. Beetle captures commenced early November in 1980 and 1982, and early in December in 1981. In the season 1980-81 there were good catches from November through till February, after which time the average number coming to the traps fell to below 5. By the end of April no beetles were caught in the traps, and none were found in the dung pads. In the following season (1981-82) trap catches were low throughout the season, never exceeding ten beetles per trap. Captures ceased in April 1982. During the 1982-83 season numbers increased slightly in November-December, but dropped to three or less per trap from January onwards, ceasing altogether in March, though some were still present in the pads and these were used for dissections. No beetles came to the pitfall traps in November or December 1983, nor in January 1984, hence trapping was discontinued. However, some *Oi. alexis* were found in dungpads from 12th December to 5th January, but never more than about ten beetles in 1.5 h of searching. Therefore field work was discontinued.

Changes in age-composition of the population were fairly constant between the three seasons. All beetles caught at the beginning of the 1980-81 season were nulliparous, the vast majority being newly emerged (N_1). Newly emerged beetles continued to

appear until the end of January, by which time the proportion of parous beetles had increased. Parous individuals were dominant in the population for the rest of the season with the exception of a peak of new emergence in February and a small peak of nulliparous (N_2) beetles in early April.

Of the first females caught in the 1981-82 season 60% were nulliparous and 40% were parous. By January nearly all the females caught were parous, with a short peak of new emergence at the end of February. There may have been another peak of new emergence earlier in February which was missed due to sampling only being done once a fortnight, and this peak then gave rise to the peak of N_2 beetles on March 1st. Less than 30% of the females found after March 15 were either newly emerged or nulliparous.

During the 1982-83 season the first catch again contained 40% parous beetles, though the bulk of the population was nulliparous during November and December. The beetles became progressively older through December and into January. From early February to the end of April there was almost continuous new emergence, with peaks in mid February and at the end of March.

Follicle resorption was very common throughout the three years of observations on this species. It occurred at times in nearly all the breeding beetles, and even sometimes in nulliparous but developed ones.

5.232 Rainfall (Fig 13a,b,& c) Rainfall was very uneven over the three years of this study. There was frequent rain during the 80-81 season, but during the 81-82 summer only two lots of rain were recorded, in early January and early February respectively. The drought continued throughout the winter of 82, with only 38 mm

of rain being recorded in September - October. Thereafter there was intermittent rain but not enough to break the drought until the end of the following season.

5.233 Dung quality - (Fig 13c) This was only monitored during the 1982-83 season. It was moderately good in November when the first beetles appeared in the field, but deteriorated rapidly with the haying off of the pasture and the continuing drought in December. There was a flush of new growth of grass in January in response to some rain late in December and early January, but this dried off again through February. A little rain in February and some in March gave some pasture growth, causing dung quality to improve in March and April.

5.3 Relationship between incidence of follicle resorption and other factors.

5.31 Statistical method

The dissection data for field beetles was examined using an analysis of covariance, to determine whether the incidence of resorption was related to any of :

- (a) Site (for Ot. granulatus only, since Oi. alexis was studied at only one site.)
- (b) Time of season, counted in periods of two weeks starting from the time the beetles first appeared in the field and finishing when they disappeared.
- (c) Age of beetles. Only beetles of age N3 or more were included in the analysis. For Ot. granulatus there were therefore four age classes, from N3 to P3 (P4 beetles are excluded because they are by definition resorbing). For Oi. alexis the parous group was also divided into three age classes, but on the basis of tibial wear

since ovarian age-grading of parous beetles was not feasible in this species (Chapter 4).

- (d) Rainfall. Because it seemed likely that any effect of rainfall would probably be indirect, acting via the quality of the pasture and hence dung quality, the total rain falling during periods of 2, 4, 6 and 8 weeks prior to each catch was calculated and the effects of rain on resorption over each of these intervals was examined in the analysis.
- (e) Dung quality, as evaluated by bushfly bioassay of dung collected at the same time and in the same pastures as the beetle sample. Because bioassays were only carried out for part of the study, the effects of dung quality could be examined in only part of the data. Consequently each analysis was carried out twice, once on the whole data set without including dung quality as a variable, and once to look for dung quality effects using only that part of the data for which dung bioassays were available.

In these analyses, dung quality and the rainfall variables were treated as covariates, and the other three variables as classification variables. An initial analysis for Ot. granulatus revealed a significant interaction between site and time of season: i.e. that seasonal patterns differed between sites - so each site was therefore analyzed separately. The estimated incidence of resorption in each sample was weighted according to the sample size (Gilbert 1973). A multiple classification analysis was used to examine the effect which each level of the classification variables had on the proportion of beetles resorbing their follicles.

5.311 *Ot. granulatus* at Uriarra

Rainfall and time of season both affected the incidence of resorption. The rainfall effect was a negative correlation between the incidence of resorption and the amount of rain over the 8 weeks prior to the sample: i.e. a prolonged period without rain was associated with an increased level of follicle resorption ($F=18.07$, with 1×39 d.f., $p < 0.01$). The effect of time of season is shown in figure 14a: there is a marked increase in the level of resorption late in the season. ($F=3.75$, with 10×39 d.f. $p < 0.01$). Classification variables and covariates combined account for 79% of the variation in the proportion of individuals resorbing.

A re-analysis including dung quality as a covariate indicates a significant effect of dung quality ($F=7.942$, with 1×39 d.f. $p < 0.01$) superimposed on the rainfall and seasonal effects previously identified. This suggests that the effect of drought is not completely accounted for by its effect in reducing dung quality, since drought still explains much of the variation in resorption incidence even when dung quality is included in the analysis.

5.312 *Ot. granulatus* at Armidale

None of the variables examined had a significant effect on resorption frequency at this site, where the proportion of beetles resorbing was always relatively low. Although the trend with rainfall is in the same direction as at Uriarra (i.e. less rain associated with more resorption) it is not significant, and there is no indication of the increase in resorption late in the season which was observed at Uriarra (Fig.14a).

5.313 *Oi. alexis* at Araluen

Rainfall and time of season affect resorption in this species also, but the effects are quite different from those observed in *Ot. granulatus*. There is a correlation between resorption and the amount of rain 2 and 4 weeks prior to the sample, and the correlation is direct - i.e. rainy periods rather than drought are associated with an increase in levels of resorption. (For 2 weeks rain, $F=6.75$, with 1×26 d.f. $0.05 > p > 0.01$; for 4 weeks rain, $F=7.50$, with 1×26 d.f. $0.05 > p > 0.01$). There is a weak effect of time of season ($F=2.57$, with 11×26 d.f. $0.05 > p > 0.01$), but no obvious pattern to the variation through the season (Fig.14b).

The most striking effect on resorption incidence in this species, however, is that of the age of the beetles; older beetles are very much more likely to resorb than are young ones ($F=13.51$ with 3×26 d.f., $p < 0.01$; Fig 14c.). The classification variables and covariates accounted for 78.4% of the variation in the incidence of resorption in *Oi. alexis* at Araluen.

No significant effects of dung quality were identified, but this analysis had only a very small data set and so cannot be regarded as conclusive.

5.4 Conclusions

Ot. granulatus.

At Uriarra *Ot. granulatus* has a univoltine life cycle. Newly emerged beetles appear in mid to late summer and then feed, develop and even lay eggs in the early autumn. This time coincides with medium to poor dung quality at that time of the year. When conditions become

cool the adult beetles over-winter in the ground, to reappear again the next spring as nulliparous developing, or young parous adults. Autumn laid broods apparently do not survive the winter, since there were no N1 beetles in the samples in spring. In the spring the adults breed on the good quality dung, and eventually die off at about the same time or just before the new generation emerges.

At Armidale Ot. granulatus has basically the same life cycle, with the difference that a few newly emerged beetles appear in the spring population. This indicates that a few broods laid in the autumn survive the winter to emerge in the spring owing to the marginally milder winter temperatures at Armidale.

At Uriarra, during periods of drought lasting 8 weeks adult beetles tended to resorb their oocytes. There was a tendency for increased resorption later in the season, and to some extent beetles tended to resorb their follicles more frequently when fed on poor quality dung (as calculated by bushfly bioassay) than when fed on good dung quality.

At Armidale, where rainfall was more regular and seasonal changes are less marked, resorption levels remained low throughout the period of the study.

Oi. alexis

The presence of about 40% parous beetles in the initial catches of the 1981-82 and 1982-83 seasons might mean that some beetles survive the winter in the adult stage in some years, possibly depending on winter temperatures. However, the large proportion of newly emerged and nulliparous beetles in the spring of each year indicated that most overwintering occurs in the larval stages. Beetles continued to emerge

throughout the first half, and sometimes even into the second half, of the season. The population gradually became composed of older, parous individuals and the parous component of the population remained present for the remainder of each season, though each year there were one or two extended peaks of new emergence in February and March. These were probably the progeny of beetles laying eggs during the first half of the season. It therefore appears that eggs laid early in the season have time to emerge early that summer and autumn, but eggs laid later in the summer do not complete development in the same season, and have to overwinter and emerge the following spring. The dung quality in February and March of 83 was medium to good, coinciding with the time when eggs destined to overwinter were laid.

Thus at Araluen the data suggest that this species is partly univoltine and partly bivoltine, depending on whether the eggs are laid early or late in the season. Whether the over-wintering strategy of the larvae involves a diapause, or whether it is just a case of slowed development due to cool temperatures cannot be known from these data alone. However, it does appear probable from these results that most adults die with the advent of cold weather in April and May.

Follicle resorption in this species at Araluen was a very common occurrence. Resorption increased after rain, contrary to what occurred with Ot. granulatus. Older beetles were more likely to resorb their follicles than were younger beetles, also contrary to what occurred with Ot. granulatus. There were temporal changes in resorption frequencies but no clear seasonal pattern.

CHAPTER 6 Over-wintering and developmental times

6.1 Introduction

In the previous chapter hypotheses of the life histories of the two species were proposed, derived from results obtained by the use of age-grading dissections of beetles collected in the field. However, age-grading methods have not previously been used to elucidate quantitatively the phenology of any dung beetles, and seldom of other Coleoptera. Hence in the following three chapters, certain salient aspects of the life cycles proposed by the hypothesis, and the responses of the beetles to certain changes in their environment, will be tested by independent experiments in order to establish three things. Firstly, to check that the explanation of the life cycle is correct; secondly to evaluate the mechanisms which determine the life history pattern; and thirdly to establish the validity of the age-grading technique as a means of studying life cycles. The experiments will include testing in the field:

- a. The stage in which the beetles over-winter
- b. The time taken in the field, at different times of the season, for development from egg to adult.

Further experiments will be conducted in the laboratory to determine for adults:

- a. Effect of temperature on fecundity and longevity
- b. Effect of dung quality on the duration of maturation feeding
- c. Effect of dung quality on fecundity and longevity

for juveniles:

- a. Rate of larval development at different temperatures
- b. Effect of dung quality on the survival of larvae and size of emerging adults

- c. Effect of moisture on the survival of larvae and the size of the emerging adults.

6.2 Over-wintering

Age-grading dissections indicated that Ot. granulatus overwinters mainly in the adult stage, and Oi. alexis in the immature stages. The following experiments tested the ability of adults and immatures of both species to survive the winter at the study sites.

6.21 Ot. granulatus. - survival as adults.

6.211 Method - 25 newly emerged (N₁) and 25 developing (N₂) females from the laboratory culture were placed in the field at Uriarra in April 1978, in each of two cylinders dug into the ground as described in section 3.9. About an equal number of males were added, but these were not counted. Fresh dung was placed on top of the vermiculite/sand every two weeks. At intervals throughout the winter a variable sample of beetles was found (in one of the two cylinders in alternate order, by searching for them by hand, without disturbing the whole of the cylinder) and were dissected.

6.212. Results - During the first two sampling occasions (Table 4) it was noted that there were small numbers of dead and decomposing beetles on the surface of the vermiculite, indicating that some mortality had taken place. 56 of the 100 female beetles were found alive and most were at the N₂ stage during the course of the experiment up to and including September 19. At the last sampling occasion (October 13), when a maximum of only 44 females could have been left in the cylinders, 18 females were found alive (41%) and thus 26 had died (59%) during the course of the winter.

Females surviving in the N₂ stage had hard cuticles, some fat body but no visible development of the ovary. However, by October 1978 several beetles had begun to develop their eggs. On three occasions (August 9, August 30 and October 13) one female in each sample had a small quantity of yellow body visible, which may indicate that some beetles had started ovarian development earlier during a warm spell, but underwent resorption due to recurring cold weather.

Thus a significant proportion of adult females were able to survive the winter at Uriarra in 1978. A number of males had also survived the winter in the cylinders, but they were not counted.

TABLE 4

Survival of newly emerged and developing Onthophagus granulatus at Uriarra between April and October 1978 and their physiological ages

Date examined	No. females examined	Physiological age category of female			
		N ₁	N ₂	N ₃	P ₁
18 Apr	4	-	4	-	-
1 Jun	3	-	3	-	-
20 Jul	11	4	7	-	-
9 Aug	13	-	12	-	1
30 Aug	12	-	11	-	1
19 Sept	13	-	13	-	-
13 Oct	18	-	13	4	1

6.22 Ot. granulatus - survival as immatures

6.221 Method : Four categories of broods were taken from laboratory cultures in May 1979. These were 0-3 days old containing eggs (n=38) 1-7 days old containing eggs or early instar larvae (n=44); 8-14 days old containing early and late instar larvae (n=47) and 14-28 days old containing late instar larvae, pupae and teneral adults (n=34). Each of these categories of broods were placed in separate gauze-bottomed cylinder and placed in the ground at Uriarra. Samples of broods were removed and examined at monthly intervals from May onwards until the end of winter just before the beetles again became active in the field. Any broods which contained white, healthy looking eggs at the time of sampling (from the 0-3, and 1-7 day old groups) were placed at 27°C in the laboratory to check for hatching.

6.222 Results - (Table 5) - The oldest group of larvae and pupae survived until July, but by August they were all dead. All the younger larvae had died by June. The small size of the egg chamber in the empty broods indicated that death had probably occurred either in the egg stage or during the first larval instar, leaving no trace of a head capsule in the brood. All eggs were dead from June onwards.

Thus no immatures of Ot. granulatus survived in the cylinders at Uriarra during the winter of 1978.

TABLE 5

The survival of immatures of O. granulatus in the field at Uriarra between May and September 1978.

Age in days, and date when placed in field	Date examined	No examined	Brood contents		
			Living	Dead	Empty
0-3 days	23 May	10	10	-	-
23 May	6 Jun	4	-	4	-
	3 Jul	5	-	5	-
	31 Jul	9	-	9	-
	28 Aug	10	-	1	9
1-7 days	8 May	10	10	-	-
8 May	6 Jun	4	-	2	2
	3 Jul	10	-	10	-
	31 Jul	10	-	8	2
	28 Aug	10	-	2	8
8-14 days	8 May	10	10	-	-
8 May	6 Jun	7	-	5	2
	3 Jul	10	-	9	1
	31 Jul	10	-	9	1
	28 Aug	10	-	8	2
14-28 days	8 May	10	10	-	-
8 May	6 Jun	6	6	-	-
	3 Jul	8	7	1	-
	28 Aug	10	-	9	1

6.23 Oi. alexis - survival as adults

6.231 Method - In April 1982, at a time when there were still some beetles found in dung pads in the field, 25 newly emerged (N_1) and 25 developing (N_2) beetles (all laboratory reared) were placed in each of two cylinders on damp compacted soil, and supplied with a fresh dung pad. The dung was replaced with fresh dung a week later, at which time the original pads were still moist and were showing some signs of beetle activity, indicating that the beetles had fed. One week after that the new pads showed no signs of beetle activity,

and thereafter the pads were renewed once a month.

Early in November 1982, when the first beetles reappeared in the field, the cylinders were brought back to the laboratory and the soil in them was sieved to find any surviving beetles.

6.232 Results. - There were no surviving adult beetles in either cylinder; the remains of the beetles were found in the form of pieces of dry cuticle.

6.24 Oi. alexis - survival as immatures.

6.241 Method - Two soil filled cylinders each containing approximately *25 eggs and first instar larvae in brood masses 0-7 days old were dug into the ground at Araluen in April 1982. The broods were examined at two-monthly intervals throughout the winter by removing one mass at a time and examining it for the presence of live larvae. All sampled broods were discarded.

6.242 Results - At no time up to September did more than five brood chambers have to be opened to find a live larva (Table 6). On November 17, 1982, at the time when beetles began to appear in dung pads in the field, the soil in the cylinders was sieved, and all brood masses were examined. Ten live larvae, late third instar, were found in the two cylinders. Thus altogether 28 and 23 broods were recovered from cylinders 1 and 2 respectively. Excluding the broods sampled during the winter, on November 17 cylinder 1 contained 7 live larvae of a possible 17 immatures

* The exact number of eggs and larvae was not counted for fear of damage to the eggs while removing excess soil from the brood masses (see methods section 3.6).

(41%), while cylinder 2 contained 3 live larvae of a possible 9 immatures (33%). This indicated that survival of the immature stages of Oi. alexis was possible at Araluen during the winter of 1982, though overwintering mortality may be high.

TABLE 6

Survival of eggs and first instar larvae of Oi. alexis at Araluen between April and November 1982.

Cylinder	Date examined	No. examined	Brood contents	
			living	dead
1	May 18	1	1	0
	July 13	5	1	4
	Sept 21	5	1	4
	Nov 17	17	7	10
2	May 18	4	1	3
	July 13	5	1	4
	Sept 21	5	1	4
	Nov 17	9	3	6

6.3 Survival from egg to adult in the field throughout the season.

6.31 Ot. granulatus.

The hypothesis of the life cycle formulated in Chapter 5 suggested that newly emerged adults of Ot. granulatus present in the field in summer and/or autumn originated from eggs laid in the spring. The following experiment tested this hypothesis, and monitored the time at which immature mortality and the appearance of the new generation occurred in relation to the incidence of rainfall.

6.311 Methods : Every two weeks throughout both 1977/78 and 1978/79 summer seasons cylinders filled with vermiculite/sand were dug into the ground at Uriarra. One litre of fresh, locally produced dung was placed on the vermiculite in each cylinder and left exposed for one week, as described in the methods chapter (section 3.9). In order to monitor the time at which mortality occurred through development, subsamples of broods were examined at fortnightly intervals until there was no longer any living material remaining in the cylinder, or the beetles had pupated or emerged.

6.312 Results :- In 1977/78 brood production commenced in September and continued through until early January, (Table 7a), the numbers of broods depending upon the number of beetles colonizing the pad, and the mean number of eggs laid by the females. No more broods were produced for the remainder of the season apart from some in February. During the period between September and January there was a drought. Most eggs laid during this period survived and developed for some weeks but subsequent mortality was high, and none of the progeny survived past the pupal stage. Few broods were laid during the drought, and few were laid after the drought broke in January. However, a proportion of eggs laid after December 21 developed to the adult stage by March, and their development coincided with the period when some soil moisture was maintained by the rains in January.

In 1978/79 (Table 7b) beetle numbers were exceptionally low and no broods were produced from experimental dung pads until early November. There was regular rainfall until December and good numbers of broods were produced throughout November and into December; however their numbers declined in January/February when

Table 7

Survival of immature Onthophagus granulatus laid naturally in the field at Uriarra during the active seasons of a) 1977/78 and b) 1978/79. No superscript=larva, p=pupa, a=adult.

a) Date pad exposed	Rainfall in mm.	No. Broods	Sampling Date	Brood contents	
				Live	Dead
<u>1977</u>					
14 Sept	22	21	28 Sep	10	-
			12 Oct	2	2
			26 Oct	2	1
			9 Nov	-	4
28 Sep	30	13	12 Oct	3	-
			26 Oct	2	-
			9 Nov	2	1
			23 Nov	1P	1
			7 Dec	-	3
12 Oct	2	50	26 Oct	7	3
			9 Nov	1	-
			7 Dec	-	39
28 Oct	3	3	9 Nov	2	-
			7 Dec	-	1
9 Nov	3	6	23 Nov	2	-
			7 Dec	2	-
			21 Dec	1P	-
23 Nov	2	7	7 Dec	3	-
			31 Dec	1	-
			4 Jan	1P	-
			1 Feb	2	-
7 Dec	4	1	21 Dec	-	1
21 Dec	2	3	18 Jan	2	-
			15 Feb	-	-
			1 Mar	1 ^a	-
<u>1978</u>					
4 Jan	53	9	14 Jan	3	-
			1 Feb	2	-
			15 Feb	-	2
			1 Mar	1	-
18 Jan	48	0			
1 Feb	35	10	15 Feb	3	-
			1 Mar	3pa	3
			15 Mar	1a	-
15 Feb	1	0			
1 Mar	2	0			
15 Mar	57	0			

Table 7

b)	Date pad exposed	Rainfall in mm.	No. Broods	Sampling Date	Brood contents	
					Live	Dead
<u>1978</u>						
	13 Sep	19	0			
	27 Sep	29	0			
	10 Oct	18	0			
	24 Oct	41	0			
	7 Nov	47	21	28 Nov	1	-
				12 Dec	-	-
				8 Jan	20pa	-
	21 Nov	33	38	28 Nov	2	-
				12 Dec	-	-
				8 Jan	36pa	-
	6 Dec	22	44	8 Jan	2p	-
				30 Jan	42 ^a	-
	18 Dec	21	6	8 Jan	1	2
				30 Jan	3pa	-
<u>1979</u>						
	9 Jan	4	0			
	23 Jan	3	2	30 Jan	1	-
				20 Mar	-	1
	6 Feb	10	0			
	21 Feb	4	0			
	1 Mar	0	0			
	7 Mar	22	5	20 Mar	1	-
				3 Jul	-	4
	20 Mar	58	0			

drought conditions prevailed. There was a late production of small numbers of broods in March following the breaking of the drought on March 4.

Many of the broods deposited in November and December developed successfully to emerge as adults in January. The few eggs deposited in early March hatched and developed for a short time before temperatures fell, but died during the early winter months.

The contrasting degree of survival, and the different timing of the emergence of the adults during the two seasons indicated that rainfall during development was of great importance. From October to mid-December 1977, a period of drought, the few eggs which were laid did not develop past the pupal stage. Only later-laid eggs, whose subsequent development coincided with some rainfall (from late December 1977 onwards) completed development to give rise to the new generation appearing in the field in February and March (Fig. 11b). In the equivalent period in 1978, a period with frequent rains, most progeny in the cylinders survived to give rise to the new generation from early January onwards; consequently the newly emerged peak observed in December 1978 (Fig. 11c) would have originated from eggs laid in the previous October.

6.32 *Oi. alexis*

The hypothesis tested here suggests that eggs laid in the early part of the season develop to give rise to adults later in the same season, and that eggs laid later in the season over-winter to give rise to the new generation early in the following season. Rainfall was monitored to correlate it with survival of the immatures.

6.321 Method. Two closed cylinders were placed in the field at Araluen at fortnightly intervals throughout the 1982/83 season, each with two pairs of adults collected from a nearby dung pad. This was done to prevent other species of beetles ovipositing in the cylinder (see section 3.9). The beetles were kept in the cylinders for one week, and supplied with fresh dung. At the end of this period the adult beetles were allowed to leave, and the cylinders were examined for the presence of brood masses. However the number of eggs was not counted due to the danger of damaging the eggs. Periodic destructive sampling of developing larvae was also not attempted because in the equivalent experiment with Ot. granulatus (section 6.311), when only few eggs were laid, very few or none were left to complete development. The first emerging progeny in each cylinder was trapped with a small bait, as described in section 3.9, the date of emergence was noted, and the contents of the rest of the brood masses were destructively sampled.

6.322 Results:- Brood masses were buried in the cylinders on every occasion, indicating that oviposition had taken place right through the season, (Table 8). Eggs which were laid from November 1982 through till January 4, 1983, began emerging in mid-February and continued emerging until April 14. Eggs laid on January 18 and later did not emerge that autumn, nor did anything emerge the following spring. On December 12, 1983, all cylinders were sieved, and the broods were found to be water logged due to heavy rains in September and October 1983 (191mm). All the brood balls were soggy, and all but one larva was dead and decomposing indicating that death had occurred not long ago. The single live larva, from the

Table 8

Survival of eggs & larvae in brood masses of Oi. alexis laid in each of two cylinders at Araluen during the active season of 1982/83.

Date cylinders set	Rainfall in mm.	Date eggs laid	Date of first emergence	No. of Brood contents			
				Adults	Pupae	III	Dead
10 Nov.82	0	10 -	14 Feb.-	12 #	2	1	6
		24 Nov.	2 Mar.	6 #	2	-	16
24 Nov.	0	24 Nov.-	2 Mar. -	9	2	1	2
		9 Dec.	14 Mar.	5	5	3	3
9 Dec.	0	9 -	2 Mar. -	5	-	-	8
		20 Dec.	14 Mar.	14	3	-	1
20 Dec.	13	20 Dec. -	14 Mar. -	4	5	2	5
		4 Jan.	30 Mar.	1	2	-	5
4 Jan.83	59	4 -	30 Mar. -	1	10	3	6
		18 Jan.	14 Apr.	1	9	-	-
18 Jan	0	18 Jan -	-	-	-	-	-
		1 Feb					
1 Feb	7	1 -	24 Jan.	1	-	-	-
		14 Feb.	1984*				
14 Feb.	20	14 Feb. -	-	-	-	-	-
		2 Mar.					
2 Mar.	0	2-14 Mar.	-	-	-	-	-
14 Mar.	9	14-30 Mar.	-	-	-	-	-
30 Mar.	193	30 Mar. -	-	-	-	-	-
		13 Apr.					
13 Apr.	0	13-27 Apr.	-	-	-	-	-

* All cylinders were sieved on 12/12/83, and only one live larva was found. This was allowed to complete its development in the laboratory and it emerged on 24/1/84.

The two lines of data for each fortnight are the brood contents from the two cylinders.

cylinder set on February 1, 1983, was brought into the laboratory and maintained at 27°C in slightly moist soil; it metamorphosed into an adult on January 24, 1984.

Thus the hypothesis derived from the dissection data suggesting different lengths of developmental times for eggs laid early and later in the season was supported by the results. Drought during November and December 1982 did not appear to cause large-scale mortality, nor did rainfall in January 1983. Rain in the winter of 1983 did, however, have an adverse effect on the survival of the over-wintering immatures.

6.4 Conclusions

The over-wintering strategies of the two species are quite different. Ot. granulatus over-winters in the adult stage at Uriarra, as well as at Armidale, though some larvae managed to survive the winter at Armidale possibly owing to the slightly higher winter temperatures. Oi. alexis, on the other hand, survives the winter mainly in the larval stages, though some adults may have survived the winters of 1981 and 1982.

Eggs produced by Ot. granulatus in brood balls made in the spring developed, and provided there was some rainfall during their development, the new generation emerged in the summer and autumn. Few eggs were laid in March and these did not survive the winter. Eggs laid in late spring and early summer by Oi. alexis also emerged late that summer and autumn, but eggs laid after the second half of January did not emerge that season. Provided they do not get too wet, probably late in their development, they over-winter to form the new generation

in the following spring/summer. Adults of Ot. granulatus appear to need moisture for oviposition, since in most of October, November and December 1977, when there was hardly any rain, very few broods were laid in the cylinders (Table 7a). In contrast, Ot. alexis appears to be able to breed in dry weather, since in November and December 1982, a dry period, (Fig. 13c), good numbers of eggs must have been laid judging from the emergence which took place from the cylinders in February and March 1983, (Table 8).

CHAPTER 7. Fecundity and longevity of adults.

7.1 Introduction

Temperature and nutritional quality vary in the field during the season, and are likely to have an influence on the fecundity and longevity of the adults (Engelmann 1970, Dempster 1975). The main oviposition period, as determined by dissections, lasts from October to December for Ot. granulatus, and from November/December to March for Oi. alexis. The following experiments examined the influence of a) temperature on fecundity and longevity of the two species in order to gain information on how the range of temperatures experienced by the insects during the breeding season at the study sites relate to the optimum temperature for reproduction; and b) dung quality on fecundity of Ot. granulatus, to see whether periods of resorption caused by poor dung quality translate into decreased fecundity. In Oi. alexis not enough data was available to relate poor dung quality in the field with increased incidence of resorption, hence the direct effect of dung quality on fecundity and mortality was examined.

7.2 Temperature

7.21 Ot. granulatus - effect on life span and fecundity.

7.211 Methods:- Fifteen pairs (♀ & ♂) of 0-1 day old beetles were set in individual containers at 14:10 light: dark cycle.

Half a litre of fresh dung was placed on the soil and changed weekly.

There were four treatments at the following temperature 15, 20, 25 and 30°C. Broods were counted weekly, and the experiment was continued until all the females had died, except at 15°C, where the experiment was stopped after 8 weeks. Any broods found in containers in which the female was dead were not included in the

calculations. Dead males were replaced by spare males from the culture.

7.212 Results - The mean number of broods over the lifetime was clearly highest at 25°C, and the Tukey multiple range test (Zar 1984) indicated that it was significantly different from the mean numbers produced at both 20°C ($q=8.29$, $P<.001$) and 30°C ($q=7.48$, $P<.001$. Fig. 15a). The mean number of broods per female fluctuated from week to week, but there was a peak during the third week at 30°C, and between the seventh and 13th weeks at 25°C. There was no distinct peak of oviposition at 20°C, (Fig. 15b). No broods were produced in 8 weeks at 15°C. Cuticle hardening did take place, but there was no ovarian development after 8 weeks even though that temperature is above the calculated threshold of 11.4°C. (See Chapter 8).

The life span of beetles was very variable within each temperature treatment (Fig.15c), however the mean life span (in weeks $\pm$ SE) decreased with increasing temperatures (20°=21.7 $\pm$ 3.7, 25°=16.8 $\pm$ 1.5, 30°=7.8 $\pm$ 0.9).

The mean air temperatures from October to December at Uriarra range from 12.9 to 18.5°C, and at Armidale from 13.4 to 18.1°C (Buzby 1983). Mean soil temperatures during these months at 5cm depth under pasture are always 1°C warmer, and under bare soil they are 7-10° warmer (Mottershead 1971). Therefore the temperatures experienced by the beetles at Uriarra and Armidale are likely to be within the temperature ranges necessary for oviposition at least on warmer days in October, and most of the time in November and December. However, the equivalent of the optimum constant 25°C is probably seldom, if ever, achieved at either site.

7.22 *Oi. alexis* - effect on life span and fecundity.

7.221 Methods:- Ten pairs of 0-3 day old beetles were set in individual containers at 16:8 h light:dark. One l of fresh Fyshwick dung collected weekly was added to the container twice a week, the brood masses were sieved out once a week and all excess soil/vermiculite was washed off them under a tap. This exposed the raised areas where the eggs are situated, and these were counted. (The whole dung mass was broken apart on three separate occasions to check for additional eggs, but none were found). The temperatures tested were 18, 22, 25, 27 and 30°C, and the experiment was continued until all of the females had died.

Dead beetles were not replaced. A white fungus grows inside and outside a beetle which has been dead for several days in the pots. Hence dead females with the fungus were considered to have died early in the week, and any broods in the pots were not used for the calculations of average numbers of eggs per beetle. Fresh looking dead females with no fungal growth were considered to have been alive for most of the week and their broods were used in the calculations, particularly since the number of eggs in their pots were within the average for that age-cohort.

7.222 Results:- The mean number of eggs produced during their lifetime was highest at 30°C, and it decreased with decreasing temperatures (Fig 16a.) The mean number at 30°C was not significantly different from the mean number produced at 27°, ($q=2.30$, $P>.1$), but was significantly different from those produced at 25° ($q=6.377$, $P<.001$) and below. The peaks of oviposition occurred between the second and sixth weeks of life at all four of the higher temperatures tested (Fig. 16b). Only three beetles produced a few broods at 18°C, even though this is above the threshold for development of 12.8°C.

The life-span of Oi. alexis was very variable within each temperature regime (Fig.16c), and there was no clear cut increase or decrease of the mean, within the temperature range tested. (Mean lifespan in weeks $\pm$ S.E at $18^{\circ} = 6.9 \pm 1.61$, $22^{\circ} = 7.5 \pm 1.42$, $25^{\circ} = 4.0 \pm 0.83$, $27^{\circ} = 5.7 \pm 0.45$, $30^{\circ} = 8.7 \pm 0.73$). 18°C is clearly close to the lower limit of their temperature tolerance for fecundity though not for survival, but 30°C was not yet past the upper limit.

The mean air temperatures from December to March at Araluen range from 19.3° to 21.1°C . Mean soil temperatures at 20cm depth under pasture are 2°C , and under bare ground 4°C , higher than mean air temperatures (Mottershead 1971). Thus the temperatures experienced by the beetles at the study site are well within the range for possible breeding, though the optimum conditions are probably seldom, if ever, present.

7.3 Dung quality.

The characteristics of the dung clearly varied in the field during the course of the seasons, both at Uriarra (section 5.213) and at Araluen (section 5.233). It was therefore relevant to know what effect these differences had on the rate at which the beetles develop their ovaries, and the oviposition rate. In order to have available dung with the different characteristics at the same time for the several experiments, locally produced dung, collected from different pasture types, was used either fresh, or collected earlier and kept deep frozen until use. On two occasions dung had to be imported deep frozen from other parts of Australia in order to have available the range of qualities needed for the experiments.

7.31 Ot. granulatus - effect on maturation feeding period.

7.311 Methods:- Ten containers, each with ten pairs of 0-1 day old beetles were set in October/November 1978. Half were fed twice a week on 0.5 l of dung collected in October from an improved pasture at Uriarra (bushfly survival 91.7%, weight of puparia = 22.6 mg); the other half with an equal quantity of dung collected at the end of the dry season at Rockhampton, Queensland (bushfly survival = 4.7%, puparial weights = 2.85 mg). Samples of ten females and ten males were dissected each week and the stage of ovarian development in females, and the presence or absence of fat body in males was noted.

7.312 Results:- Beetles fed on Uriarra dung developed rapidly; the first beetle with yellow body was present in the sample at 2 weeks and nine out of ten sampled females were parous within three weeks. Beetles fed on Rockhampton dung failed to produce broods and 70 percent had died within four weeks (Table 9).

Males responded in a similar way with large quantities of fat body being produced when fed on Uriarra dung, and none being visible in those fed on Rockhampton dung, and all dying within four weeks.

Thus dung collected from a temperate climate spring pasture, on which bushfly larvae had a good survival and produced large puparia, induced beetles to develop their eggs rapidly; in contrast dung from a senescent pasture, which supported very little bushfly survival, was equally not satisfactory for egg development or survival of Ot. granulatus. This suggests that the bushfly bioassay gives an adequate evaluation of nutritional quality for Ot. granulatus, as is also supported by effects on fecundity outlined below.

Table 9. The influence of dung quality on the maturation feeding period of newly emerged female Onthophagus granulatus, and on the accumulation of fat body (FB) in the males. (+ Fat body present, - fat body absent)

Time (weeks)	Number and physiological age category of beetles			
	Good quality dung from Uriarra		Poor quality dung from Rockhampton	
	♀	♂	♀	♂
0	10N ₁	10FB -	10N ₁	10FB -
1	6N ₂ 3N ₃ 1 dead	9FB + 1 dead	8N ₁ 2 dead	9FB - 1 dead
2	5N ₃ 1 parous 4 dead	10FB +	6N ₁ 4 dead	8FB - 2 dead
3	1N ₃ 9 parous	10FB +	5N ₁ 6N ₂	8FB - 2 dead
4	2N ₃ 9 parous	Not sampled	3N ₂ 7 dead	10 dead

7.32 Ot. granulatus - effect on fecundity

7.321 Methods:- Twenty pairs of 1-2 week old beetles were set in each of three containers and each was fed twice weekly half a litre of dung of one of three different types (as described below).

The experiment was carried out at $27^{\circ} \pm 2^{\circ}\text{C}$, and spare beetles of the same age were kept on type II dung at the same temperature as replacements for dead beetles in the test groups. The broods were sieved out once a week for five weeks, and counted. There were three replicates.

The dung types were as follows:

I. Dung from Uriarra improved pasture collected in November 1976

and frozen for eight months until use.

(Bushfly survival = 91.7%, mean puparial weight = 22.6mg.)

II. Fresh dung from Fyshwick irrigated, improved pasture collected in July 1977.

(Bushfly survival 76.0%, mean puparial weight = 17.6 mg.)

III. Dung from a partially hayed off native pasture in West Australia, collected in December 1976 and kept deep frozen for 7 months until the experiment.

(Bushfly survival = 58.1%, mean puparial weight = 7.8 mg.)

7.322 Results :- There was a clear-cut difference between the numbers of broods produced on the different dung types, at least during the five week period of the experiment (Fig.17a)(mean total number of eggs per female = Uriarra 101.2, Fyshwick 68.7, West Australia 17.8). Beetles produced on the average more than five times as many broods on the dung from Uriarra than they did on the dung from West Australia, a response to quality parallel to the response of the bushfly larvae to the dung types. Except for a few isolated broods during the first week, brood production in all treatments began in the second week, increasing to a peak in the fourth week. Mortality of females was also correlated with the dung quality, being highest in the West Australian dung and lowest in the Uriarra dung (Fig 17b).

Since replacement beetles were previously fed on type II dung, they must have oviposited for a while at a rate determined by that dung even after transfer to the respective treatments. This would have acted to decrease brood numbers in the treatments fed on Uriarra dung, and increase brood numbers in the treatments

fed the West-Australian dung. The differences between the treatments are therefore conservative estimates of the dung quality effects. The results indicate that periodic poor quality dung in the field during the breeding season, causing follicle resorption, does result in reduced fecundity.

The relationship between beetle fecundity and dung quality as measured by bushfly survival ($r^2 = 0.9888$ with 7d.f.) and pupal weights ($r^2 = 0.9909$ with 7d.f.), both gave highly significant positive correlations ($P < .001$, Fig. 18).

7.33 *Oi. alexis* - effect on fecundity.

7.331 Method:- Three treatments of ten pairs of 0-3 day old beetles were set in individual containers at 27°C, 14:10 light:dark cycle, and fed one l of dung twice a week. Each treatment was given a different type of dung, as described below. The brood masses were sieved out, carefully cleaned of all excess soil and broken apart to expose the egg chambers and the eggs were counted once a week for eight weeks. Broods from containers with dead females were treated the same way as described in section 7.221.

The three dung types were as follows:-

- I. Dung collected at Uriarra from an irrigated pasture in August 1983 and deep frozen until used in March 1984 (Bushfly survival = 89.3%, mean puparial weight = 16.2 mg).
- II. Dung collected from an improved, irrigated pasture at Fyshwick in February 1984 and kept frozen until used (bushfly survival 78.6%, mean puparial weight = 10.62 mg).
- III. Dung collected at Gungahlin from an improved pasture in October 1983 (the end of the drought) and kept deep frozen until used (bushfly survival 8.3%, mean puparial weight 3.5 mg).

7.332 Results:- Dung quality clearly does not have as great an effect on the fecundity of Oi. alexis as it does on Ot. granulatus though the mean total number of eggs produced per female was highest on the Uriarra dung (103.4 eggs) intermediate on the Fyshwick dung (76.2 eggs) and lowest on the Gungahlin dung (67.0 eggs, Fig 19a). Brood production commenced in the first week in all three treatments, hence there was no major difference in the rate of maturation feeding when fed different types of dung. Mortality of beetles was similar in all three treatments over the eight-week period of the trial. (Fig.19b).

The correlation between beetle fecundity and dung quality as measured by bushfly survival was not significant ($r^2 = 0.298$ with 28 d.f.), however when measured against pupal weights there was a weak relationship ($r^2 = 0.4165$ with 27 d.f., $P < .02$, Fig.18).

7.4 Conclusions

Ot. granulatus is most fecund at 25°C, and has both a lower fecundity and shorter life span at 30°C. It responds to good quality dung by producing significantly more eggs than it does on the poorer qualities.

Oi. alexis was most fecund at 30°C in the range of temperatures tested; however its fecundity did not begin to drop significantly until the temperature was 25°C and below. Longevity was also not decreased at 30°C. There was a trend towards higher fecundity with increasing dung quality, but the effect was much weaker than in Ot. granulatus.

Thus the differences in dung quality as perceived by bushfly larvae were reflected strongly in the fecundity responses of Ot. granulatus, and were also reflected by Oi. alexis fecundity, though to a much lesser extent.

Chapter 8 Development of immatures.

8.1 Introduction

Developmental rates and survival rates of immature insects are known to be affected by temperature and at times by other extrinsic factors. Data on the effect of temperature on developmental rate were needed to calculate the day-degree requirements for each species. There are a number of functions which have been used to describe the relationship between the rate of development of an insect and temperature (Logan et al 1976, Sharpe et al, 1977, Welch et al. 1978). Over the middle part of the range of temperatures where development is possible, the relationship can be expressed by a straight line (Campbell et al. 1974). If the line is extrapolated downwards, the point at which it intersects the temperature axis is then considered the threshold temperature for development. However in most cases this linear model underestimates developmental rates when average daily temperatures remain close to the threshold for lengthy periods. In cold climates, or cold seasons, this defect may necessitate the use of a more complex model (Moon 1983).

At the other extreme, if mean temperatures approach the insects' upper limit of tolerance, then the rate of development may decline. Hence the straight line will overestimate rate of development when temperatures are very high. In practice this has not often proved a serious problem.

In spite of its defects, in circumstances where the precise form of the relationship is not of central importance, the use of a linear model to represent the relationship between rate and temperature has the advantages of both simplicity, and a capacity for clear and meaningful comparisons (Nealis et al. 1984). From it, developmental times can be estimated very accurately if the ambient range of temperatures experienced

by the insect are within the middle of the range. In practice, errors in estimating the ambient temperatures experienced by the insect are likely to be of much greater importance in most cases than the errors arising from the assumptions of linearity, at least in warmer climates. It therefore seems sensible to use the linear model unless it proves to be clearly inappropriate in the particular case being examined, and it has consequently been used in this study.

Daily records of maximum and minimum temperatures at the study sites were used to calculate the rate of accumulation of day degrees in the field, and from this work out when eggs laid at a particular time were likely to emerge (predicted emergence time). This was then compared with actual times of emergence in the field from broods laid down at known times (i.e. in cylinders, section 6.3). Large discrepancies in these two dates may indicate the presence of a factor other than temperature, such as diapause, which may influence the rate of development and thus suggest directions for further analysis. As well, the periods of predicted emergence calculated from the times of peak oviposition in the field were compared with the periods when dissections indicated the presence of newly emerged beetles in the field, thus providing a check on developmental time postulated by the life cycle hypothesis.

In addition, temperature, dung quality and moisture are likely to affect survival rates of larvae in the ground. Hence in this chapter the role of temperature, dung quality and moisture on the rate of development and survival of immatures of Ot. granulatus and Oi. alexis, and the size of emerging adults will be investigated. The next chapter will deal with the comparisons of estimated and actual emergence times in the field and discuss the likelihood of the presence of a diapause.

8.2 Temperature

8.21 *Ot. granulatus* : effect on rate of development.

8.211 Method:- Fifty 0-2 day old broods were placed in each of four containers in moist vermiculite/sand, and each container was placed at one of the following temperatures : 15, 20, 25 and 30°C. The cabinets had a 14:10 h light:dark cycle. The contents of the boxes were watered to a constant weight three times a week to maintain a constant moisture. Dates of emergence of beetles were recorded three times a week. After no more beetles had emerged during a two week period, broods from which nothing had emerged were carefully opened to see whether their contents were still alive, or whether they had died.

8.212 Results (Table 10) - The mean rate of development from egg to adult increased with increasing temperatures. However, the low survival at 30°C suggested that the rate at this temperature may be above the linear range. It was therefore excluded from the calculations. The regression line drawn through the rates of development at the other three temperatures intersects the base line at $11.4^{\circ}\text{C} \pm 0.16^{\circ}\text{C}$ (Fig.20). This is considered to be the threshold temperature for development. It was calculated that to complete development from egg to adult, *Ot. granulatus* requires a total of 494 ± 8.06 (SE) day degrees above this threshold temperature i.e. $494 = D(T - 11.4)$. (Estimation of thresholds, physiological times and their standard errors are calculated as described by Campbell et al. 1974).

Table 10. Rate of development of broods, survival and size of emerging adults of Onthophagus granulatus at different temperatures.

Temp.°C	No broods	Day of emergence Mean $\pm$ SD	No. Surviving	Mean thoracic width (mm)
15°	50	139 $\pm$ 3.40	29	3.71 $\pm$ 0.19
20°	50	56 $\pm$ 2.05	44	4.08 $\pm$ 0.22
25°	50	36 $\pm$ 3.02	44	4.00 $\pm$ 0.18
30°	50	30 $\pm$ 0.00	4	3.58 $\pm$ 0.07

Temperatures at both extremes tested (15° and 30°C) had an adverse effect not only on the survival of the eggs and/or larvae but also on the size of the emerging adults.

8.22 O1. alexis : effect on rate of development.

8.221 Method: Seventy, 0-3 day old eggs in brood masses, obtained from field-collected females on Feb. 20, 1981, were set in moist soil in each of four refrigerator boxes. These were counted by brushing away all excess soil and exposing the raised egg chambers. Great care had to be taken not to damage any in the process. The boxes were placed at each of the following temperatures: 21, 25, 28 and 30°C. The boxes were watered to constant weight once a week. Because the C.T. rooms were shared with other people, the light regimes could not be standardised; hence the three higher temperatures were kept at 16:8 h light:dark, while the 22°C CT room was kept at 14:10 h light:dark. Dates of emergences were scored three times a week.

8.222 Results : The mean rate of development increased with increasing temperatures (Table 11). The linear regression line drawn through the rates of development at the four temperatures

tested intersects the base line at $12.8 \pm 0.64^{\circ}\text{C}$, and this is considered the threshold for development(Fig.20). Unlike Ot. granulatus, 30°C does not appear especially adverse for this species, and the 30° data are included in estimating the threshold temperature and the developmental times. It was calculated that to complete development from egg to adult, Oi. alexis requires a total of 896 ± 39.06 day degrees above this threshold temperature i.e. $896 = D(T-12.8)$, day degrees = No. of days x temperature above the threshold.

The temperatures tested had no effect on the size of the surviving adults, and best survival was at 28°C . Mortality was highest at 21°C , and the survivors at that temperature fell into two categories: a small number (9) emerged within 150 days of being laid, these were the beetles used in the regression calculations. A larger number were still third instar larvae (21), pupae (3), and 1 teneral adult, 270 days after oviposition. This was the first indication that these larvae had gone into diapause and were beginning to emerge 9 months later even though they had been kept at a constant 21°C . Even the nine beetles which did emerge within the 150 days took longer than would be expected from the developmental rates at higher temperatures (Fig. 20).

Table 11 - Rate of development of broods, survival and size of emerging adults of Onitis alexis at different temperatures.

Temp. $^{\circ}\text{C}$	No. eggs	Times of emergence (days) Mean $\pm$ SD	No. emer. within 150 days	Thoracic width $\pm$ S.E.	Live but not yet emerged after 270 days.
21	70	139 ± 4.55	9	$8.68 \pm .13$	25
25	70	70 ± 12.80	51	$8.38 \pm .12$	-
28	70	60 ± 3.97	68	$8.39 \pm .15$	-
30	70	52 ± 2.63	47	$8.60 \pm .12$	-

8.3 Dung quality

8.31 *Ot. granulatus* : Survival of larvae, developmental rate and size of emerging adults.

8.311 Method: Ten pairs (♀ & ♂) of two to three week old beetles were set in a breadbox at $27^{\circ} \pm 1^{\circ}\text{C}$, 14:10 h light:dark cycle with 0.5 litre of fresh Fyshwick dung collected in June 1984, (bushfly survival 78.3%, puparial weights 17.2 mg). Another ten pairs were supplied with 0.5 l dung collected at Gungahlin in October 1983 and kept frozen (bushfly survival 8.3%, puparial weights 3.5 mg). All broods produced from each type of dung during one week were set in moist vermiculite/sand and kept moist by watering to a constant weight three times a week. The number of emerging beetles was counted and their thoracic width was measured. There were two replicates.

8.312 Results :- There was no significant difference in survival rate between replicates, which were therefore pooled to compare survival in the two dung types. Survival of larvae was better in broods made of Fyshwick dung than in Gungahlin dung ($\chi^2 = 12.36$ with 1 d.f. $P < .001$), but the sizes of the surviving progeny from the two dung types were not significantly different (Table 12). The mean rate of development was significantly faster in the Fyshwick dung than in the Gungahlin dung ($t=49.19$ with 43 df, $P < .001$).

Table 12 - Survival and developmental rate of immature Ot. granulatus in broods made of two different dung qualities, and the size of the emerging adults.

	Fyshwick dung		Gungahlin dung	
Replicate	1	2	1	2
No. of broods set	25	27	35	35
No. of beetles emerged	19	17	12	14
% emergence	76	63	34	37
Mean days to emerge $\pm$ SD	34.79 $\pm$ 1.357		41.27 $\pm$ 0.827	
Thoracic width (mm) $\pm$ SE	3.88 $\pm$ 0.078		3.92 $\pm$ 0.037	

8.32 Ot. alexis : Survival of larvae, developmental rate and size of emerging adults

8.321 Method :- The immatures used in this experiment were the progeny of the females fed on I Uriarra (good) and III Gungahlin (poor) quality dung (section 7.33). Because for that experiment it was important to know the egg numbers exactly in each brood mass, these were carefully cleaned of all soil and broken apart. In the process a number of eggs got damaged, and hence the exact number of viable eggs in each treatment for this experiment was not known (see below). However, the third instar larvae form a distinct faecal shell, and these could easily be counted when the brood mass was examined about 4 weeks after the start of the experiment. Hence in this experiment mortality from egg to early third larval instar was not known; only mortality from the faecal shell stage to the teneral adult. In a separate trial with three

replicates using the same dung, egg numbers were estimated by cleaning the brood mass of all excess soil, counting the egg chambers, and subsequently counting the faecal shells. It was calculated that in Uriarra dung there was 77, 55 and 60% survival from egg to faecal shell stage in the three replicates, and in Gungahlin dung the survival rates were 86, 59 and 71%.

The brood masses were placed in refrigerator boxes covered with moist soil at 27°C and watered to a constant weight three times a week until emergence started. Half the broods produced from each dung treatment were placed at 14:10h light:dark, and the other half were placed at 16:8 h light:dark. Emerging beetles were counted, sexed and their head width was measured. Checking whether a faecal shell, from which no emergence had taken place, contained a live larva or a dead one, was done in the following way. The faecal shell was shaken very gently. A live larva or pupa could be felt to rattle inside the faecal shell, whereas there was no movement inside a faecal shell in which the larva or pupa had died. This is because a fungus grows on the dead tissue and binds it to the faecal shell wall.

8.322 Results :- There was a significantly higher proportion of larvae dying at the faecal shell stage in the Gungahlin dung than in the Uriarra dung ($X^2=65.65$ with 6 d.f. $P<.001$, Table 13). This difference reflected the dung quality as indicated by the bushfly bioassay. A very small number of larvae were still live after 137 days, indicating that these may have gone into diapause.

There was no significant difference between the size of newly

Table 13

Effect of dung quality on survival and developmental rate of immature *Onitis alexis*, and size of emerging adults. B.S.= bushfly survival, P.wt.= pupal weight. Percentage shown in brackets.

Light/Dark cycle	Uriarra dung (B.S.= 89.3%, P.wt=16.2mg)		Gungahlin dung (B.S.=8.3%, P. wt.=3.5 mg)	
	14/10	16/8	14/10	16/8
No. faecal shells	64	73	48	52
No. emerged from 53-71 days	40(62.5)	54(74.0)	5(10.4)	15(28.9)
Live immatures after 137 days	1(1.6)	3 (4.1)	0	1(1.9)
Dead	23(35.9)	16 (21.9)	43(89.6)	36(69.2)
Mean days to emerge $\pm$ SD (non-diapausing)	59.37 $\pm$ 6.563		59.35 $\pm$ 4.580	
♀	5.41 $\pm$.04	5.35 $\pm$.05	5.06 $\pm$.04	5.27 $\pm$.04
Size of emerging adults n	25	30	2	7
♂	5.32 $\pm$.04	5.22 $\pm$.04	4.96 $\pm$.26	5.19 $\pm$.04
n	15	20	3	8

emerged beetles which developed in broods made of Uriarra dung held at the different daylengths (for ♀ $t=1.11$ with 53 d.f., ♂ $t=1.65$ with 33 d.f.) nor was there any difference in size between the sexes (at 14:10 h light:dark $t=1.70$ with 38 d.f.; at 16:8 light dark $t=1.84$ with 48 d.f.). Hence the sexes were pooled, as were the daylength treatments, for size comparisons between the two dung types. The size of beetles emerging from broods made of the two types of dung did not differ ($t=2.1724$ with 38 d.f.), nor did the mean developmental time of the non-diapausing progeny ($t=.013$ with 112 d.f.).

8.4 Moisture

8.41 *Ot. granulatus* - effect on developmental rate, survival and size of emerging adults.

8.411 Methods:- Ten pairs (♀ & ♂) of 3-4 week old beetles were set in a container at 27°C, with 14:10 h light:dark cycle, and supplied with 0.5 l of fresh current Fyshwick dung (bushfly survival 78.3%, puparial weights 17.2 mg). The broods produced in 1 week were divided into three groups and each was placed in a separate container and covered with moist vermiculite (180g of oven dried vermiculite mixed with 100 cc water). Each container of broods plus vermiculite was weighed, and given one of the following treatments:

- I Wet treatment : covered with a loose fitting petri dish lid, and a standard quantity of water (5cc) was added three times a week.
- II Moist treatment : watered to a constant weight three times a week

III Dry treatment : allowed to dry out through development

These treatments continued until emergence started, by which time the contents of container I had gained on average 33.9% of their original weight; those in II had retained the same weight, and those in III had lost a mean of 22.5% of their original weight. Counts were kept of the numbers of beetles emerging from each treatment, and the time of emergence and the thoracic width of the teneral beetles was measured.

8.412 Results:- The best emergence took place from broods in treatment II, which were kept moist throughout development (Table 14). Survival was marginally worse in the wet (I) treatment ($F = 11.32$ with 1×3 df, $P < .05$), but there was no survival in any broods which were allowed to dry out during development (treatment III). There was no difference between treatments I and II in the size of the surviving progeny, but there was a significant, though very small, difference in mean developmental time ($t = 2.94$ with 47 d.f., $P < .01$); animals in the wet treatment developed slightly slower than those in the moist treatment.

8.42 *Oi. alexis* - effect on survival, developmental rate and size of emerging adults

8.421 Method:- Ten pairs (φ & σ) of 3-4 week old beetles were set in individual containers on 20/3/84 and supplied with *Uriarra* dung collected in August 1983, deep frozen until use as in section 8.321 (*Bushfly* survival 89.3%, puparial weights 16.2 mg.) The brood masses made within 1 week were divided into three moisture treatments as described for *Ot. granulatus* (Section 8.41), only the standard amount of water added in treatment I was 20 cc to 1500 gm of brood masses. Both the parent beetles and the brood masses

Table 14

Effect of moisture on survival and developmental rates of immature Onthophagus granulatus, and size of emerging adults. Percentage shown in brackets.

	I Wet		II Moist		III Dry	
Replicates	1	2	1	2	1	2
No. of brood masses	30	9	25	27	20	19
Original wt in g.	110.0	73.4	94.1	103.5	99.9	67.2
Final wt in g.	134.1	90.3	94.1	103.5	59.3	48.9
Percent of original wt in g.	122	123	100	100	59.3	72.8
No emerged	10(33.3)	6(66)	19(76)	17(63)	0	0
No. dead	20(66.6)	3(33.3)	6(24)	10(37)	20(100)	19(100)
Mean developmental time in days $\pm$ SD	37.31 $\pm$	1.93	35.5 $\pm$	1.89		
Mean thoracic width (mm)	3.77*	3.87*	3.64*	4.14*		
SE	.076	.148	.089	.101		

* These values are not significantly different from each other.

were kept at 27°C in a 14:10 h light: dark cycle. Counts were kept of the numbers of beetles emerging, the dates of emergence, and their head width was measured.

8.422 Results: (Table 15). Some beetles emerged at the expected time (at 27° this is 63 days from rate of development section 8.22) in both the moist and the wet treatments; but none did so in the dry treatment. Many of the larvae took over 100 days to develop, the proportion being highest in the dry treatment and lowest in the wet treatment ($\chi^2 = 21.38$ with 4 d.f., $p < .001$). The Tukey multiple range test (Zar 1984) on a one way analysis of variance showed that there was no significant difference in size between beetles emerging from the wet and moist broods ($q = 1.894$, $P < .5$), but there was a difference between both the wet and dry broods ($q = 4.945$, $P < .005$) and between the moist and dry broods ($q = 6.108$, $P < .001$). Survival was not significantly different in the three treatments ($\chi^2 = 3.923$ with 2 d.f. $P > .1$).

8.5 Combination of cold and high moisture.

8.51 *Oi. alexis* - effect on survival.

The high mortality in the over-wintering progeny in the cylinders (Section 6.322) may indicate that immatures of *Oi. alexis* can survive the winter temperatures at Araluen provided conditions remain relatively dry. The following experiment tested this idea.

8.511 Method:- Ten pairs (♂ & ♀) of three to four week old beetles were set in individual bread boxes at $27^\circ \pm 1^\circ\text{C}$, 14/10 h L:D cycle, with fresh Fyshwick dung collected in early May, 1984. More fresh dung was added three days later. Half the broods produced by the end of the week were placed in a breadbox with holes drilled at

Table 15

Effect on moisture on survival and developmental rate of immature Oi. alexis, and size of emerging adults. Percentage shown in brackets.

	Wet	Moist	Dry
No. faecal shells	37	36	29
Weight change as a percentage of original wt.	121.0	100.0	80.9
No. emerged within 80 days	13 (35)	10 (28)	0
No. emerged between 80-100 days	1 (3)	2 (5)	0
No. emerged between 100-200 days	6 (16)	4 (11)	17 (59)
No. emerged between 200-250 days	4 (11)	16 (44)	7 (24)
Live immatures after 250 days	4 (11)	2 (6)	1 (3)
Dead	9 (24)	2 (6)	4 (14)
Head width of emerging adults (mm) ♀ & ♂ pooled (up to day 127)	5.27 ± .06	5.37 ± .036	4.91 ± .076

the bottom to allow excess water to drain away, and the broods were covered with moist soil to a depth of 180 mm. The other half of the broods were placed into a second bread box and similarly covered, but with dry soil. Both boxes were kept at 27°C for three weeks, by which time the larvae had reached late second or early third instar. On the 30/5/84 they were placed outside under the laboratory eaves to prevent them being rained upon. They were covered with a gauze lid and were left outside all winter, and once a month about 0.5 l of water was poured evenly over the surface of the soil in the moist treatment. Thus the soil in one box was kept moist, in the other dry, but both were subjected to Canberra winter temperatures. A max-min thermometer showed that the temperature near the boxes never fell below freezing point.

On the 27/8/84 all faecal shells in the dry treatment were examined either by gentle shaking, or by opening the faecal shells (see section 8.321), and all containing live progeny were replaced in the soil for another month. None were sampled from the wet treatment because moist faecal shells are very fragile and are liable to damage if disturbed.

On the 27/9/84 the contents of both boxes were sieved, the faecal shells were counted and examined for live progeny.

8.512 Results:- By the end of winter (27/8) the majority of the faecal shells in the dry treatment contained live progeny (Table 16). All faecal shells with no movement inside were opened, and found to contain dead larvae. Eleven faecal shells with some movement inside when shaken were found to contain live pupae.

On 27/9 67% of faecal shells in the dry treatment still

contained live progeny, however, of those in the wet treatment only 7% were alive (Table 16).

Table 16. Survival of Oi. alexis larvae through the winter (30/5-27/9/84) under dry (58 faecal shells) and wet (59 faecal shells) conditions.

Date	Dry soil		Wet soil	
	Live	Dead	Live	Dead
27/8	41 (11 pupae)*	17	-	-
27/9†	39	2	4 (4 pupae)	55

*Subsample of faecal shells opened and progeny found to be alive

8.6 Maternally induced diapause in larvae of Oi. alexis

Many species of beetles with soil dwelling larvae regulate their life cycles by the presence of a dormancy period. In Coleoptera the most common type of documented diapause occurs in the larval or adult stages, more rarely in eggs or pupae. The factors which initiate or trigger it may be daylength changes, changes in temperature and humidity, or possibly changes affecting food. (Saunders 1976, Crowson 1981). The diapause sensitive phase may be earlier in the same stage of development of the insect which subsequently enters diapause, or the sensitive and diapause phases may be separated by one or several moults. Examples of the former are Coccinella semipunctata (Hodek 1962) and Agonum assimile (Thiele 1966), and the carabid beetles Patrobus atrorufus and Nebria brevicollis (Thiele 1977), where photoperiod on the adult causes it to go into reproductive diapause; an example of the latter is the influence of low temperature in the early instar larvae of Anomala cuprea, A. rufocuprea and Maladera castanea, all of which enter facultative

diapause in the late third larval instar (Fujiyama 1983). Further examples of species where the induction and diapause phases are well separated are cases where it is the photoperiod experienced by the mother which determines the proportion of diapausing larvae e.g. Nasonia vitripennis (Saunders 1966), Coeloides brunneri (Ryan 1965), the blowflies Lucilia caesar and L. sericata (Ring 1967) and the horn fly Haematobia irritans (quoted as Lyperosia irritans Depner 1962). However, maternal induction of diapause in its progeny has never been demonstrated in a beetle to date.

Low temperatures and dry conditions acting directly on the larvae have already been implicated in diapause induction in Oi. alexis. (Sections 8.22 and 8.42). The effect of low temperature appears to be similar to that found in the Anomala and Maladera species mentioned above. However, not much is known about the importance of moisture in diapause of any insect and there has been no conclusive evidence for its direct participation either in its induction nor its termination (Masaki 1980). In the hessian fly Mayetiola destructor a higher proportion of individuals entered summer diapause in hot dry years than in cool wet years, and contact moisture was required for diapause-free development at high temperatures (Zhukovsky 1950). Thus the influence of moisture in the induction of diapause in Oi. alexis requires further investigation, and this is currently in progress.

Photoperiod acting directly on soil dwelling larvae has never been shown to be implicated in larval diapause induction, nor has it been found to influence diapause in Oi. alexis (Section 8.32). This is not surprising, since larvae are seldom if ever exposed to light (Fujiyama 1983). However, the effect of photoperiod acting through the mother on the progeny appears to be a common phenomenon in insects, (Saunders 1976, Beck 1980),

and although it has never been documented as a factor inducing diapause in soil dwelling larvae, this may well be because no one has looked for it. A crepuscular species like Oi. alexis which has a short flight period at dusk and dawn (Houston, pers. com.) is obviously sensitive to light intensity, and it seemed reasonable to suppose that the diapause in the larva of this species might be controlled, at least partially, by daylength experienced by the mother. Additionally, maternal age is also known to influence the offspring in some insects. Thus, for example, the early apterous progeny of the apterous form of the pea aphid, Acyrtosiphon pisum, are more likely to produce alate offspring when crowded, than do their later-born siblings (MacKay & Wellington 1977). Maternal age has also been implicated in diapause-induction in the larvae of the parasitic wasp Nasonia vitripennis. The females tended to produce non-diapausing progeny early in their reproductive life, and diapausing ones later in life. The effect of age, however, was modified in that species by temperature and photoperiod (Saunders 1965).

Oi. alexis is a species whose distribution ranges from warm summer rainfall to mediterranean winter rainfall areas and it has also become established at Araluen which is a temperate even rainfall area. At Araluen there is a reduction in daylength between the periods of natural twilight from 16.5 h in December to 12.5 h in April, the months during which the adults are active above ground. The mechanisms which enable it to survive in such climatically diverse environments must be very plastic, and may well include parental sensitivity to daylength as well as parental age. Hence these two factors are examined in this section.

8.61 Method:- Adult beetles emerging in the laboratory at 27°C were placed immediately upon emergence into one of three daylength

regimes i.e. 12/12, 14/10 and 16/8 L:D cycle, all at 27°C. They were maintained under these conditions throughout life, were fed on freshly collected Fyshwick dung twice a week, and the broods were sieved out once a week. Each week's brood batch from each lot of parents were reared separately at 27°C, 14/10 L:D cycle under moist conditions. The length of the developmental period, survival and mortality of the immatures in each batch was recorded.

When a proportion of larvae were in diapause, emergence at 27°C ranged from 50 to more than 250 days, and the emergence pattern was continuous without any peaks at defined periods (Table 15). This was in contrast to the emergence pattern when there was no delay, which ranged from 53 to 71 days (Table 13). Hence the length of the developmental period in this experiment was divided into those that developed in more or less than 100 days.

8.62 Results:- There was a progressive and highly significant increase in the proportion of diapausing larvae with decreasing daylength on the parents ($\chi^2=47.45$ with 4 d.f., $P<0.001$). There was also a significant and gradual decrease in the proportion of larvae in delayed development with increasing age of the parents subjected to 12/12 L:D ($\chi^2=13.06$ with 3 d.f., $P<0.01$). (Table 17). Age was also significant at the longer daylengths, but the decrease in the incidence of diapause was sudden from parents older than 2-3 weeks, (14/10 L:D $\chi^2= 19.93$ with 1 d.f., $P<0.001$; 16/8 L:D $\chi^2= 8.78$ with 1 d.f., $P<0.01$).

Table 17. Effect of daylength on parents, and age of parents, on length of development of larvae of Oi. alexis. All larvae reared at 27°C, 14/10 L:D cycle. Figures in the table represent percentages, those in brackets are total numbers of faecal shells in each treatment.

Daylength on parents	% emergence in days	Age of parents in weeks					
		2-3	3-4	4-5	5-6	6-7	8-9
12/12		(96)	(85)	(29)	(57)	(55)	(20)
	<100	64	67	58	63	89	95
	>100	34	25	21	16	2	0
	dead	2	8	21	21	9	5
14/10		(78)	(87)	(20)	(67)	(69)	(18)
	<100	62	94	100	92	80	89
	>100	20	5	0	4	7	11
	dead	18	1	0	4	13	0
16/8		(49)	(68)	(21)	(45)	(25)	(11)
	<100	86	93	100	96	84	100
	>100	14	3	0	4	0	0
	dead	0	4	0	0	16	0

Comparisons of proportions of diapausing larvae from the different aged parents kept at the three light regimes are shown in table 18.

Table 18. Levels of significance for differences between proportions of live larvae taking more than 100 days to develop in the three light treatments, for each parental age group.

Age of parents	χ^2	d.f.	P
2-3 weeks	7.16	2	<.01
3-4 weeks	26.08	2	<.001
4-6 weeks	19.67	2	<.001
6-9 weeks	6.59	2	<.05

The progeny from the youngest parental age groups had a high proportion of diapausing larvae at all three daylength treatments, hence the difference between daylength treatments was not great. Because the proportion of diapausing larvae from parents kept at 14/10 and 16/8 L:D fell rapidly after the first weeks' progeny, the difference between the proportion at these daylengths and those from the 12/12 L:D parents was highly significant from the ages of 3-6 weeks. After 6 weeks of oviposition even the progeny of parents kept at 12/12 L:D produced mainly non-diapausing progeny, and hence the difference between the three daylength treatments is again only marginally significant.

There are small differences in the proportion of dead larvae in the three treatments ($\chi^2 = 8.29$ with 2 d.f., $P < .05$), but there is no uniform trend with parental age. The lowest survival occurs at the shortest daylength, but the small magnitude of the effect and the high (but not consistent) variation in survival between broods suggests that the apparent daylength effect on survival may not be real.

8.7 Conclusions.

Ot. granulatus has a threshold temperature for development of $11.4 \pm 0.16^\circ\text{C}$, and requires 494 ± 8 day-degrees above this threshold to develop from egg to adult. Survival was best at 20 and 25°C , and the size of the emerging adults was greatest at these temperatures.

Oi. alexis has a threshold temperature of $12.8 \pm 0.64^\circ\text{C}$, and for non-diapause development requires 896 ± 39.06 day degrees above the threshold to develop from egg to adult. Best survival was at 28°C , and

temperatures between 21 and 30°C had no effect on the size of the emerging beetles.

Larvae of Ot. granulatus survived better and developed faster in brood masses made of Uriarra dung (good quality as assessed by bioassay with bushflies), compared to their survival in brood masses made of Gungahlin dung (poor quality). Their survival at 27°C was also better in moist and wet conditions than in dry ones. The size of the emerging adults, however, was the same from broods made of both the different dung qualities and kept at the different humidities. Oi. alexis larvae also suffered higher mortality in brood masses made of poor compared to good quality dung but their rate of development was the same; and there was no size difference in the emerging progeny. In this species survival appeared to be worse in the wet treatment at low temperatures, and beetles emerging under dry conditions were significantly smaller. Diapause in larvae was induced by cold temperatures (21°C) and at higher temperatures (27°C) if the parents were subjected to a short daylength or the larvae to dry conditions. As well, progeny from younger parents were more likely to enter diapause than do progeny from older parents.

CHAPTER 9. Discussion

9.1 Comparative and confirmatory aspects of this study.

Physiological age criteria developed by Tyndale-Biscoe (1978) were applied to a field study of a native Australian dung beetle, Onthophagus granulatus, and to an introduced African dung beetle, Onitis alexis, two species whose range of distribution overlap in the temperate seasonal environment of south-eastern Australia. Through regular dissections of the reproductive organs, along with an assessment of cuticle wear of beetles caught in the field, it was possible to outline in detail the changes in composition of the population during the year, and to examine the influence of varying seasonal factors on the life cycle of the beetles. Additionally, by means of experiments, it was possible to confirm and/or explain aspects of their phenologies, thus demonstrating clearly that the two species have different reproductive strategies for survival in similar environments.

The rate of development of the immatures in the field is determined by the ambient temperature which dictates the rate at which day-degrees accumulate throughout the season (Campbell et al. 1974, Taylor 1981). This can be calculated approximately by drawing sine curves through the fluctuating daily temperatures, and summing the number of degrees each day which accumulate above the threshold for development of each species. Knowledge of the number of day-degrees necessary for the development from egg to adult allows the computation of the predicted days of emergence of adults arising from eggs laid at any particular time of the season. Thus, in this study estimates of this kind can be compared with actual lengths of developmental periods obtained from the brood masses laid in the cylinders, and predicted emergence dates can also be

compared with periods when peaks of newly emerged beetles were observed in the field by means of age-grading dissections.

To do this accurately for an insect which develops in the soil, it is desirable to calculate the day degrees from either soil temperatures or corrected air temperatures. For the immature stages of Musca autumnalis linear corrections from soil temperatures applied to daily minimum and maximum air temperatures gave good predictions of developmental times (Moon, 1983). Such corrections were calculated for soil under pasture at Uriarra and Armidale at 5 cm depth, and at 20 cm depth at Araluen, NSW (Mottershead, 1971).

The results of these calculations showed the predicted emergence dates for Ot. granulatus of both Uriarra and Armidale to coincide with times when dissections showed there to be peaks of newly emerged beetles in the field (Fig. 21 b & c, 22 b & c). As well, the predicted emergence dates coincided well on five out of six occasions at Uriarra with times of actual emergences from the cylinders (Fig. 21c).

At Araluen the predicted times of emergence of Oi. alexis also coincided with peaks of newly emerged beetles in the field, thereby separating the cohort of newly emerged beetles originating from eggs laid in the previous season from eggs laid in the early part of the current season, (fig. 23 b & c). The time when the first beetles emerged from the cylinders during 1982-83 were uniformly delayed by about 5 - 6 weeks, with larvae and pupae still present in some of the brood masses, (fig. 23c). This indicates that development had been delayed during the dry summer of 1982-83.

As well, mortality in the cylinders and the lack of newly emerged beetles in the field at times of predicted emergence highlighted unfavourable environmental conditions such as drought in spring of

1977, and autumn of 1979 (in the case of Ot. granulatus, Fig. 21c) and excess moisture (191 mm) in Sept./Oct. of 1983 (Oi. alexis).

The influence of environmental stresses in the form of moisture and dung quality were tested on both the adult and the immature stages in the laboratory. In the case of adults this confirmed the recognition of stress factors obtained by analysis of incidence of resorption in the field. In the case of larvae it isolated the factors adversely affecting survival, confirming and explaining the field observations on the variability in the time of appearance of the new generation from season to season, and the fluctuations in the numbers of beetles in dung pads within and between seasons.

9.2 Follicular resorption as an indicator of stress.

"Oosorption is a transitory, variable phenomenon that occurs in some or all follicles in response to factors which may be ecological, behavioural or physiological. When the influence of these factors is alleviated oosorption may cease, and the ovary again become productive" (Bell and Bohm, 1975). Factors which were analysed in an attempt to isolate the causes of resorption in the two species under consideration were 1) moisture, b) dung quality and c) season, all of which could be classed under Bell and Bohm's ecological factors. These, when they induce oosorption, are an indication of stress since they cause the female to reduce its fecundity. The effect of d) age, on resorption, was also analysed, however this is not an externally induced stress factor but rather an expression of the inherent physiological state of the female, whose level of resorption could be regarded as "normal" for the particular age group.

a) Effect of moisture. - Lack of moisture for periods of six to eight weeks significantly increased the proportion of Ot. granulatus undergoing resorption at Uriarra. This could be due to drought directly affecting the beetles by rapid desiccation in and under the dung pads, causing moisture stress to the beetles and making conditions for brood burial difficult. Another explanation may be that drought affects resorption indirectly by causing the pasture to hay off and this in turn causes the cattle to produce dung of poor quality as indicated by the bushfly bioassay, (Greenham, 1972), and which in turn reduces the reproductive performance of Ot. granulatus females. Although dung quality did not seem to account for the effects of rain or season in the covariance analysis, more experimental data are needed to evaluate these effects.

At Armidale the trend of increased incidence of resorption with less rain was also present, though it was not significant owing to adequate rainfall through most of the two seasons.

Moisture stress had a totally opposite effect on Oi. alexis at Araluen. Resorption occurred immediately after the rain, and its incidence diminished as conditions dried out. This suggests that excess moisture affects the females of this species unfavourably, and may help to explain their distribution in the drier summer rainfall regions of South Africa (A.L.V. Davis, pers. com.)

b) Nutritional characteristics. - Nutritional deficiency can be induced in insects by both quantitative and qualitative deprivation (Bell and Bohm, 1975). Quantitative deprivation due to crowding may have been a factor causing oosorption in Ot. granulatus at Uriarra during the 1976-77 season, and at Armidale during the 1977-78 and 78-79

seasons, since at both sites beetle numbers per pad were high. Such quantitative deprivation causing egg resorption has also been reported in the Coccinelidae (Kurihara 1968). However, quantitative deprivation is unlikely to have been a factor at Uriarra during 1977-78 or 1978-79, and here qualitative deficiencies are more likely to have played a part. This is supported by the results of experiments in sections 7.31 and 7.32. These indicated that maturation feeding in Ot. granulatus is much protracted, that fecundity is much reduced, and beetles often die when they are fed on dung collected from dry, hayed-off pastures. Reduced fecundity is likely to be at least partly caused by egg resorption. Such deprivation (in the form of protein shortage in the presence of carbohydrate and water ad lib.) has been shown to cause decreased fecundity in the sheep blowfly Lucilia cuprina (Barton Browne et al. 1979) i.e. the maturation of less than the full complement of eggs at the expense of the remainder, which were resorbed.

This correlation indicates, furthermore, that the response of two insects which feed on the liquid fractions of dung of different qualities are similar, i.e. the fly larvae experience higher mortality and a reduction in size, and Ot. granulatus undergoes increased incidence of resorption on dung produced from hayed off pasture.

Dung quality was not shown to have an effect on the incidence of resorption in Oi. alexis, but the data set for dung quality at Araluen is too small to allow positive conclusions to be made. However results from experiments (section 7.33) which tested the effect of dung quality on Oi. alexis suggest that poor dung quality as measured by bushfly

puparial weights, does reduce fecundity, though the effect is not as great as it is in Ot. granulatus.

c) Temporal effects - Time of year was found to be significant in Ot. granulatus both at Uriarra and at Armidale, but there was a strong seasonal trend of increased resorption late in the season at Uriarra, while there was no such trend at Armidale. Similarly, the effect of time of year on resorption in Oi. alexis was also significant, but as at Armidale, it showed no clear seasonal pattern. Bell and Bohm (1975) discuss a number of end-of-season factors which have been shown to induce egg resorption, such as a drop in temperature (Dacus tryoni, Fletcher 1975), the shortening of daylength (Coccinelidae, Kurihara 1968), and resorption prior to seasonal induction of diapause (some Carabidae, Joly 1950). The variable occurrence of a late-season factor in this study does not allow any conclusions to be drawn regarding the nature of the influence which seasonality exerts on egg resorption.

d) Age. - In Ot. granulatus there is no relationship between incidence of resorption and increasing age up to P₃, and all sexually active and mature beetles are equally likely to resorb when conditions become unfavourable.

In Oi. alexis, however, increasing age was highly correlated with increased incidence of resorption - furthermore the effect was progressive. This suggests that the older the female, the more likely she is to resorb her follicles. Increased incidence of resorption in Tenebrio molitor is known to be related to increasing age (Mordue 1965), but no detailed quantitative data is available on this topic. (Bell and Bohm 1975).

The factors analysed above explained 79% of the variation in the

proportion of individuals resorbing follicles at Uriarra and at Araluen. The low incidence of resorption in Ot. granulatus at Armidale indicates that conditions there must have been very favourable for them during the two years of this study. Consistent rains would be the main reason for this, possibly because the quality of the dung would never deteriorate to the extent to which it had deteriorated after drought at Uriarra.

Bell and Bohm (1975) list a number of species in which low levels of resorption were found in females kept under apparently optimal conditions, quoting 10% of all females in some Blattidae, 20 and 23% of all basal oocytes in some Culicidae and Acrididae respectively. They also mention that resorption is known to occur just before and just after ovulation in some Labiduridae, and just after oviposition in some Lepidoptera. Apparently resorption is a "normal" phenomenon in a number of insects. Therefore, recognition of stress by the incidence of resorption may have to be made against a background resorption level which is usual for the species - and whose causes have not yet been identified.

9.3 Diapause in *Oi. alexis*

The experiments reported here indicate that there may be four different cues which influence the proportion of third larval instar of *Oi. alexis* to enter into facultative diapause. Two of these appear to act directly on the larvae, and the sensitive stage is probably in the earlier larval instars. These are low temperatures, and dry conditions during development (see sections 8.22 and 8.42). The low temperatures in the autumn and winter, experienced in seasonal climates in Araluen, would allow many of the larvae to over-winter in diapause. This is not unusual in soil-dwelling larvae and has been reported in the literature

on a number of occasions for temperate climate soil-dwelling insects (Thiele 1977). Dry conditions during the winter enhanced survival (section 8.5); however dry conditions at higher temperatures also produced a high incidence of diapause as well as low mortality. It thus seems that this is a mechanism whereby the species can become established in areas with hot dry summers, such as mediterranean climates. During the drought of 1982-83 (Fig. 23c) the expected emergence on day degree calculations always preceeded the observed emergence by several weeks, suggesting that indeed the larvae in the cylinders underwent delayed development.

The other two diapause inducing factors identified in this work appear to act through the mother. When the parent beetles are exposed to a short daylength of 12:12 L:D they produce a higher proportion of diapausing larvae than do parents exposed to longer daylengths. At Araluen the adults are present in the field from mid November to April, and from December 21 to April the daylength decreases by four hours. Thus it is possible that those larvae which had developed beyond the temperature induction stages at the time of the drop in temperature in the autumn were already predisposed to enter diapause by the shorter daylengths experienced by their mothers from January onwards. Such a strategy would ensure that few or none of the larvae could develop past the third instar before the end of winter.

Finally, maternal age does appear to influence the proportion of larvae entering diapause. Offspring from young parents were more likely to enter diapause than offspring from older parents (section 8.6). This effect is directly opposite to what was found in Nasonia vitripennis

where it was the progeny of older parents which were more prone to enter diapause. The advantage of this mechanism to the beetles at Araluen is not immediately apparent, though it may be a "bet-hedging" mechanism for an insect living under unstable environmental conditions (Waldbauer 1978). It may allow the female to ensure early in life that at least part of her progeny will be able to survive unfavourable conditions, particularly since later produced eggs are more likely to be resorbed and thus may never be laid. A possible example at Araluen, where such a strategy may be useful, is when the emergence of the summer generation of beetles is delayed by diapause due to drought. Even if conditions allow rapid egg development, these adults will only lay a relatively few eggs before the advent of the cold weather. The ability of these first laid larvae to enter diapause will enhance their chance of survival through the winter period.

The extremely wide distribution of this species, from summer rainfall, through even rainfall and into winter rainfall (Mediterranean) climatic types, and from the hot tropics to the temperate latitudes, is undoubtedly made feasible by its ability to use several and varied cues for diapause induction. In addition, the different lengths of developmental periods under the same conditions (from 100 - >250 days, Table 15) indicate that larvae have entered diapause of different intensities, and therefore emergence is not synchronised. This is also supported by the results of the dissections, which show a continuous emergence of over-wintering larvae from the time of the first catches in November through to mid-summer. Diapause has often been regarded as a mechanism to ensure synchronisation of emergence at a time suitable for adult maturation and breeding, so that the ensuing progeny can

hatch and develop under favourable conditions. The non-synchronised emergence pattern of diapausing Oi. alexis may equally be an adaptation to survival in variable climatic environments, ensuring that at least some of the progeny will emerge at times suitable for their survival and breeding.

9.4 Phenology of Onthophagus granulatus.

At Uriarra, Ot. granulatus basically has a univoltine life-cycle. Newly emerged beetles first appear in mid-summer and then go through a maturation feeding period which may continue until autumn. The beetles then cease activity, and remain throughout the winter either in the soil or beneath ageing dung pads. In the spring, as temperature rise, the young overwintering adult beetles become active again. The population then is composed of females of a range of physiological ages ($N_2 - P_3$). In the spring dung quality is generally good as assessed by the bushfly bioassay (Greenham 1972) and the beetles require only a brief feeding period before producing their first broods. Depending upon seasonal breeding conditions brood production then continues at a rapid rate on good quality dung through until December or even early January, ceasing at about the time when the first new beetles of the next generation begin to emerge. Usually some broods are deposited in late summer or early autumn by the new generation, but these would be much fewer in number since they are produced at a time when dung quality is no longer at its peak. These broods are unable to survive the winter at Uriarra (as was shown in Section 6.22).

The numbers of beetles in pads, and their age composition, fluctuated greatly during and between seasons (Fig.21a). In the spring of 1976 all beetles possessed either developing ovaries ($N_2 - N_3$), or were parous

(Fig. 21b). Overwintering of adults can take place in the N_2 , N_3 and P_1 categories, as demonstrated in Section 6.21. Large numbers of broods were deposited during November and December. Rainfall, and total accumulation of day degrees each month at Uriarra above the threshold for development for Ot. granulatus are shown in Fig. 21d. Both were adequate throughout this period and there was a substantial emergence of new beetles in January and February 1977 when between 70-90% of the population consisted of newly emerged and developing beetles ($N_1 - N_3$). This is about the time when the temperature summation calculations showed the newly emerged adults developing from eggs laid from September through to January should take place (Fig. 21c). The pasture hayed off by this time and the dung was classified as poor on the basis of the bushfly bioassay (Fig. 11a) and laboratory studies suggest that the consequence of this nutritional decline should be an extended maturation feeding period and high mortality of beetles, (Section 7.31). Furthermore, crowding in dung pads is known to adversely affect brood production in dung beetles (Hughes et al. 1978, Ridsdill-Smith et al. 1982). Thus conditions for rapid development of the ovaries and the onset of oviposition were undoubtedly made worse in the autumn of 1977 by the extreme crowding of the dung pads, which on the 15th February reached over 3000 beetles per trap.

By early April 1977 the abundance of beetles had declined sharply and in May hardly any beetles were caught in the field.

In the spring of 1977-78 most of the newly active beetles were again in the $N_2 - P_3$ categories (Fig. 21b). Good brood production on good dung quality (Fig. 11b) continued through September and early October as confirmed by broods laid in cylinders (Table 7a); but the drought which began in early October severely restricted brood production

as indicated by the high proportion of beetles resorbing their oocytes (Fig. 11b, Section 5.31) and the decline in brood numbers in the cylinders (Table 7a). In addition, dry conditions killed a great many of the broods produced in September and October (experiment on effect of moisture on larval survival, Section 8.41 and effect of 8-week drought Section 5.31). Temperature summation calculations showed that those broods produced in September and October, had they survived, should have emerged in November and early December (broken lines, Fig. 21c). That peak in emergence did not occur, and this was confirmed by high mortality of larvae in the cylinders. The small numbers of old beetles present in December and January were able to take advantage of the rainfall in January to produce a late deposition of broods. Again temperature summation calculations showed that those broods could be expected to emerge in January and February and that is, in fact, what occurred although numbers caught were small. Of the eggs laid in the cylinder on Dec. 21, 1977, one emerged near the end of February 1978; and from cylinder of 1st Feb. emergence occurred at the same time (Fig. 21c).

To estimate survival from autumn of 1977 to the following spring, trap catches may be compared during the two periods provided the temperature ranges on the trapping days were approximately similar. On March 16, 1977, 650 beetles were caught per trap, (Fig. 11a), the temperature on that day ranged from a maximum of 19°C to a minimum of 6.5°C. On September 27 a mean of 230 beetles were caught, the maximum temperature on that day was 18°C and the minimum was 9°C. On October 11, with a similar temperature range (9° - 18°C), 160 beetles came to a trap. Thus there was substantial survival of beetles over the 1977 winter. However, during the 1977-78 season, severe drought

at the time when brood production is normally at its peak (Oct./Nov.), drastically limited population increase. Only a minor recovery was possible late in the season. (Temperature fluctuation on trap days: Feb. 28 = 6 - 26°C, March 14 = 18 - 27°C, March 28 = 8 - 19°C.)

The small numbers of mainly nulliparous beetles overwintered in 1978 and gave rise to the small population of newly active beetles in the spring. A favourable spring, with evenly-spaced falls of rain enabled those beetles to breed, and their progeny emerged, as predicted, in December and January. Unfortunately, owing to the low beetle numbers, no broods were laid during this period in the cylinders; however broods laid on Nov.7 1978 and later emerged as adults in late December (Table 7b). There was a severe drought in January and February 1979, and the anticipated emergence through February and March did not occur, although a few beetles survived in the cylinders to emerge at the end of January. A few broods did survive in the field to yield a small number of newly emerged beetles following the rains which fell in early March 1979. Again, the moisture stress of January and February, as well as feeding on very poor quality dung present in the field at the time caused a high proportion of beetles to resorb their oocytes (Fig. 11c).

At Armidale (Fig. 22a) the population fluctuations between seasons varied much less than at Uriarra, and within each season they showed a peak of breeding beetles in the spring, followed by a peak of newly emerged beetles in the late summer and autumn. The newly emerged beetles appeared in the samples at the predicted times (Fig. 22b & c). This is probably due to the fairly constant and even rainfall at Armidale throughout the two seasons (Fig. 22d). There were some newly emerged beetles present in the sample in Aug./Sept. of 1977

and in October 1978. This suggests that during the winters of 1977 and 1978 there must have been some survival of larvae as well as adult beetles, due perhaps to the marginally milder winter temperatures experienced there.

Ot. granulatus has thus evolved a life cycle designed for maximum brood production in the spring at times when the dung quality is at its peak. The medium to poor quality dung present in the field in late summer and autumn (Greenham 72) over a large part of its distribution range coincides with the maturation feeding period. The few broods which were produced in autumn failed to survive the winter at Uriarra, although some did survive in Armidale, but the proportion was so low that it did not greatly alter the univoltine nature of the population. This study also highlighted the extreme dependence of this species on fairly regular rainfall pattern through the summer. Droughts at strategic times in two successive seasons reduced the population drastically at Uriarra and it would probably take a number of good seasons for the beetles to build up to the numbers seen during the 1976-77 season. It is thus very much an opportunistic species.

Since pre-adult Ot. granulatus suffer heavy mortality during hot dry periods, (section 6.3) the amount of rain falling during the summer months may be a major factor limiting the distribution of the species into the low summer rainfall areas of southern and inland Australia. In fact, the southern and western distribution into the Mediterranean type winter rainfall regions of South Australia is well defined by the 75mm summer (December-February) isohyet (Fig. 2a). The specimen from near Euston was probably from a population sustained by additional moisture from either irrigation or natural seepage associated with the Murray River.

Experiments in Section 7.212 and 8.21 showed that life span, fecundity of adults and survival of immatures was severely restricted by temperatures of 30° and above. (At a constant 35°C all newly emerged beetles died within 3 weeks, J. Walker, pers. com.) Thus it is unlikely that Ot. granulatus is able to establish a population in the field if temperatures between 30 and 35°C are encountered for prolonged periods during the summer months. In fact, the northern limits of the species in Queensland, and the inland western limits in Northern N.S.W. are defined reasonably well by the average summer maximum temperature from December to February of 32.5°C (Fig. 2a). Lower average temperatures at high altitudes (>600m) may explain its presence at Carnarvon Gorge, Queensland.

Finally, the apparent absence of a diapause at any stage during the life cycle of the species, together with its inability to breed in dung produced on dry pastures enforces its very limited distribution to the south-eastern corner of Australia.

9.5 Phenology of Onitis alexis

Oi. alexis at Araluen has a partly univoltine and partly bivoltine life cycle. At the commencement of the season the majority of the beetles are nulliparous (Fig. 23b), though in two years there were some parous adults in the earliest samples, which may have overwintered in the adult state. The newly emerged adults go through a short maturation feeding period and then brood production commences and continues through summer and into autumn. Emergence of the new generation in the spring was not synchronised, newly emerged beetles appearing during each year of study throughout the summer months, and occasionally even into the autumn.

Progeny laid early in the summer usually have time to develop and emerge in the same season, producing a new emergence late in the summer and autumn. Eggs laid later in the summer do not finish development that season, the immatures overwinter to give rise to the new generation the following season. The adult beetles emerging late in the summer and autumn have time to develop and lay some eggs, but most do not survive the winter as do the new generation of Ot. granulatus. In contrast to that species, it is mainly the immature stages of Oi. alexis which overwinter, being facilitated in this by the presence of a facultative diapause.

The first beetles were collected in traps in early November 1980. Many adults were present in dungpads that season, with an average of over 160 beetles being caught per trap at the end of December (Fig. 13a & 23a). The population consisted of increasing proportions of parous beetles, indicating good oviposition during this period. The 1980-81 summer was relatively cool, with never more than 240 day-degrees being accumulated in any one month, (Fig. 23d) and hence only eggs laid up to mid January would have accumulated sufficient day-degrees to emerge from February onwards (Fig. 23c). There was indeed a peak in the proportion of newly emerged and nulliparous beetles during February and April (Fig. 13b) which reflected this second generation. The numbers of beetles caught in traps had decreased markedly by early March, but this was coincidental with a drop in temperature and hence trap catches may not be strictly comparable (Max.-min. temperature January 22, 1981 = 36 - 23°C, Feb. 10 = 33 - 18°C, Feb. 24 = 21 - 12°C, Mar. 10 = 28 - 8°C). The first generation had died out and there was very little recruitment from the second generation. This was supported by the presence of old (P₄) beetles from January through to March (Fig. 13a).

Survival of larvae through the winter of 1981 was poor, probably due to the heavy rains in May, June and November. This was suggested by the poor winter survival under cold, wet conditions in the experiment in section 8.5. Prepupae, or diapausing 3rd instar larvae of another Scarabaeid, Aphodius tasmaniae, are also known to undergo high mortality in the presence of excess water, by drowning and by promotion of infections with pathogenic organisms (Maelzer 1961). Thus the numbers of beetles caught in December of 1981 was never more than 10 beetles per trap.

The new generation developed, and most beetles were parous by January 1982. Their eggs laid from December up to early March started emerging from mid February onwards owing to the rapid accumulation of day degrees in the 1981/82 season (Fig. 23d). Thus the second peak of beetle activity through from February to April 1982 was composed increasingly of beetles which had developed from eggs laid in December 1981 and January 1982. Eggs laid from mid-February onwards did not finish development that season, instead the immatures overwintered to give rise to the first generation the following spring.

The winter of 1982 was dry and thus survival was good (confirmed by experiments in section 8.5), resulting in an increase in the numbers of beetles caught in the traps the following spring, (max.-min. temperatures on trap days : March 1, 1982 = 33-20°C, Nov. 24 = 36-27°C, Dec.9 = 19-15°C.) However, the drought continued throughout the summer of 1982-83, and this caused the dung quality to be poor in December 1982 and into January 1983 (Fig. 13c). Ot. alexis also responds to dung from a pasture at the end of a drought period by somewhat reduced fecundity (section 7.33) and greatly increased larval mortality (section 8.32), but the response is not as strong as that of Ot. granulatus. Thus there were some survivors of this generation, and they emerged in February,

March and April 1983. This was confirmed by emergences from the cylinders as well as the dissection results (Fig. 23b & c), and the number caught in the traps was lower than the numbers in the first generation. (Max.-min. temperatures on trap days : Feb. 1 1983 = 39-25°C, Feb. 14 = 32-25°C). The uniform delay in actual emergence from the cylinders, compared to the predicted emergence from day degree calculations indicated that the rate of development that summer was slower than it should have been had they developed at a normal rate under ambient conditions. This strongly suggests the presence of a summer diapause in the larvae. The 100% incidence of delayed development in the dry treatment at 27°C of experiment 8.422 supports this result. The diapausing cohort began emerging from late February 1983 onwards, coincident with peaks of newly emerged beetles at about the same time in February and March.

No beetles were caught in traps up to the end of December 1983 in spite of adequate summer temperatures, though some were found in dung pads in late December . This again indicated poor over-wintering survival, possibly due to very wet soil conditions in the spring of 1983. Brood masses in cylinders were found to be very soggy and partially decomposed.

Oi. alexis, therefore, survives in the temperate climate at Araluen by producing some eggs sufficiently early in the season to allow the progeny to emerge in the same season, and by later produced progeny which over-winter to give rise to the new generation in the following season. At least some of the over-wintering progeny may have to enter diapause development to facilitate their survival. In an even-rainfall area subject to periodic droughts, summer dung quality would be subject to fluctuations (as was the case at Araluen 1982-83 season, Fig. 13c); this does not greatly influence the fecundity of the

species, however larval survival is reduced when dung originates from a dried off pasture. The species was introduced from African summer rainfall areas, and since on average Araluen has 56% of its rainfall during the summer months of October to March and only 44% during the rest of the year, the species was able to become established there. However there were large population fluctuations caused by drought in the summer of 1982-83 which prevented most of the early summer generation of broods from emerging in the autumn of 1983, probably by causing them to enter diapause. Many of these larvae, however, were subsequently killed in the winter of 1983 by heavy rainfall, which in the heavy soils of Araluen caused flooding to occur.

Table 18 shows the mean minimum temperatures in the coldest month (July), and total mean rainfall during the four coldest months (June-Sept.) at Uriarra (a site on the Tablelands of N.S.W. where Oi. alexis does not occur) and three localities with summer rainfall, even rainfall and mediterranean climate (where Oi. alexis does occur). (From Buzby, 1983).

Table 18

Climate type	Summer	Mediterranean	Even rainfall	
Site	Rainfall Katherine	(winter rainfall) Geraldton	colder Uriarra	warmer Araluen
Mean min. temp°C				
Coldest month (July)	12.28	8.81	-.02	4.60
Mean precipitat ⁿ (mm) June-Sept.	10.4	324.1	355.0	269.5
Soil type	Heavy	Sandy	Heavy	Heavy

It appears from this that the limiting factors to the distribution of Oi. alexis are a) winter temperatures with a lower threshold of approximately 0°C, and b) excessive winter moisture. Of 25 sites

around Australia where Oi. alexis has been recovered, the lowest mean monthly (July) minimum temperature was 2.35°C at Stanthorpe, and the wettest winter months were at Eneabba, sandy coastal W.A., with 340mm between June and September. Heavy winter rain would drain away faster in sandy than in clay soil, ensuring that the broods do not become excessively soggy in sandy soil. Hence the species may be able to tolerate higher winter rainfall in sandy than in clay-loam localities.

This makes it tempting to speculate further that the species is not well adapted to the Araluen climate, and its presence there indicates the coldest and wettest winter limit to its possible southern distribution in Australia. The ability of Oi. alexis to go into diapause both at high and low temperatures under dry conditions, as well as its ability to breed in dung of widely differing characteristics, has enabled it to radiate out from dryer summer-rainfall areas to colonise some Mediterranean as well as even rainfall climatic zones. In the areas with Mediterranean climate they may survive the wet winters provided drainage is good; and it is likely that the dry summers cause diapause to occur, which delays the emergence of a second summer generation. In the even rainfall areas the species survives and builds up in numbers during years when there is some summer rainfall, and the winters are relatively dry (or the localities have good drainage). However, the population would be periodically reduced by heavy winter rainfall, particularly in non-draining soils, and periodic summer drought would cause the summer larvae to enter diapause, thus delaying the emergence of the second generation the same way as occurs in the mediterranean climate.

9.6 Conclusions

The reproductive strategies of the two species of dung beetles which co-exist in the temperate seasonal environment of south-eastern Australia are quite different. Both species are dependent on moisture for survival, but not nearly to the same extent. Ot. granulatus is wholly dependent on summer rainfall, both directly for larval survival, and indirectly for larval and adult utilisation of dung produced by cattle grazing on growing pasture. Adverse conditions such as dry conditions during larval development and dung from senescent pasture cause mortality. Winter rainfall is not harmful to the species since it over-winters mainly in the adult stage, presumably adults being able to evade areas of excessive moisture. The temperature threshold for development and optimum temperature for breeding together with the moisture requirements restrict this species to the south-eastern regions of Australia.

Oi. alexis, in contrast, is not nearly as dependent on summer rainfall in that the larvae survive adverse dry summers by entering diapause. The adults can survive and breed in dung of widely differing characteristics. The temperature requirements for development and breeding are higher than those for Ot. granulatus, thus enabling the species to survive in most of the northern regions of Australia. They survive the cool winters in south-eastern Australia as diapausing larvae, and the limiting factor there is excessive winter moisture, since the larvae are not able to escape from their brood masses.

Thus this study demonstrates that even two species committed to exploiting the same ephemeral and seasonally variable environment

may solve the problems associated with exploiting the resource in quite different ways, and these different tactics may have profound consequences for the species' geographic distribution and population dynamics.

Furthermore, the study establishes the relevance of the age-grading technique as a tool for studying life histories and phenologies of beetles, and demonstrates the type of information which can be obtained with it. The technique is particularly useful when it is used in conjunction with con-current monitoring of population size and of selected environmental factors, particularly temperature, rainfall and the characteristics of the food resource.

Appendix I

Some species of insects which have been age-graded since 1968 using various techniques: C.G.=cuticle growth; CTP.= colour and tergite pattern of abdomen; C.W.= cuticle wear; Fl.= fluorescent compounds; FD = follicular dilatations; Other = fat body and other techniques; S.F.D.= single follicular dilatation; TS = tracheolar skeins; WF = wing fray; YB/LB = yellow body/laying body.

The number in brackets after a species name indicates the maximum number of follicular dilatations recorded for that species. Species names are those used by the authors. The numbers in the columns indicate authors who have published data on that species. Key: 1. Aboul-Nasr 1970; 2. Akey & Potter; 3. Akey & Barnard; 4. Allsopp 1976; 5. Anderson 1971; 6. Auroi 1982; 7. Bellamy & Corbet 1973; 8. Bellamy and Corbet 1974; 9. Birley & Boorman 1982; 10. Birley & Rajagopalan 1981; 11. Bosler & Hansens 1974; 12. Challier 1982; 13. Charlwood 1980; 14. Charlwood & Lopes 1980; 15. Charlwood & Rafael 1980; 16. Charlwood & Wilkes 1979; 16a. Clarke, 1969; 16b. Clift & McDonald 1973; 17. Corbet & Smith 1974; 18. Croset et al. 1982; 19. Cupp & Collins 1979; 20. Davies 1969; 21. Davies 1978; 22. Davies et al. 1971; 23. Donato & Sohal 1978; 24. Drew 1969; 25. Duke 1975; 26. Dyce 1969; 27. Ellison & Hampton 1982; 28. Ettershank et al. 1983; 29. Farrow 1975; 30. Farrow 1977; 31. Farrow 1979; 32. Fenemore 1971; 32a. Ford, 1969; 32b. Ford et al. 1972; 33. Garms 1975; 34. Graham & Bradley 1972; 34a. Gruvel, 1975; 35. Houston 1981; 36. Hunter 1982; 37. Isaev 1972; 38. Jaenson 1980; 39. Johnston & Ellison 1982; 40. Jones 1971; 41. Jupp 1973; 42. Kay 1973; 43. Kay 1979; 44. Kniepert 1980; 45. Knight & Nayar 1982; 46. Krafsur & Ernst 1983; 47. Kurihara & Hasegawa 1978; 48. Lambert 1972; 49. Lange & Khok 1981; 50. Launois 1972; 51. Launois & Launois-Luong 1980; 52. Launois-Luong 1978; 53. Launois-Luong 1980; 54. Laveissiere 1975; 55. Lewis et al. 1970; 56. Luff 1973; 57. Luff 1980; 59. Magnarelli 1975; 60. Magnarelli 1976; 61. Magnarelli 1981; 62. Magnarelli & Anderson 1977; 63. Magnarelli & Anderson

1979; 64. Magnarelli & Cupp 1977; 65. Magnarelli & Pechuman 1975; 66. Magnarelli & Stoffolano 1980; 67. Mail et al. 1983; 68. Menzel et al. 1969; 68a. Mercer & King, 1976; 69. Miller & Treece 1968; 70. Mirzaeva 1974; 71. Mirzaeva 1980; 72. Moon & Kaya 1981; 73. Morgan 1973; 73a. Mokry 1980; 74. Morris & De Foliart 1971a; 75. Morris & De Foliart 1971b; 76. Mullens & Schmidtman 1982; 77. Nelson & Scrivani 1972; 78. Oda et al. 1978; 79. Okiwelu 1976; 80. Pajot 1976; 81. Pan Shi-Qing et al. 1983; 82. Phelps & Vale 1978; 83. Potter & Akey 1978; 84. Rachou et al. 1973; 85. Rafael & Charlwood 1980; 86. Rajagopalan et al. 1981; 87. Ramirez-Perez et al. 1976; 88. Reisen et al. 1979; 89. Reisen et al. 1981; 90. Rockel 1969; 91. Rosay 1969a; 92. Rosay 1969b; 93. Ryan et al. 1980; 94. Schlein 1972a; 95. Schlein 1972b; 96. Schlein 1975; 97. Schlein 1979a; 98. Schlein 1979b; 99. Schlein & Gratz 1972; 100. Schmidt 1972; 101. Schmidtman & Washino 1982; 102. Scholl 1980; 103. Self & Sebastian 1971; 104. Service 1969; 104a. Service 1973; 105. Shalaby 1972; 106. Sheldahl & Tappel 1974; 107. Snow & Wilkes 1977; 108. Sohal & Donato 1978; 109. Spencer 1979; 110. Spencer & Christian 1969; 111. Spradbery & Sands 1976; 112. Taylor 1979; 113. Thomas 1972; 114. Thomas 1973; 115. Thompson et al. 1979; 116. Troubridge & Davies 1975; 117. Trueman & McIver 1983; 118. Tyndale-Biscoe 1978; 119. Tyndale-Biscoe & Hughes 1969; 120. Tyndale-Biscoe & Kitching 1974; 121. Tyndale-Biscoe et al. 1981; 122. Vale et al. 1976; 123. Van Dijk 1979; 124. Vogt et al. 1974; 125. Walker 1977; 126. Wall & Doane 1980; 127. Wilkes 1968; 128. Wilkes & Charlwood 1979; 129. Wilkes & Rioux 1980; 130. Yajima 1970; 131. Yurgenson & Teplykh 1972; 132. Zelazny 1975; 133. Zelazny & Neville 1972; 134. Zharov 1980.

APPENDIX I References to species of insects that have been age-graded by various techniques since 1968.

	FD	SFD	YB/LB	TS	CG	F1	CTP	WF	CW	Other
COLEOPTERA										
<u>Carabidae</u>										
Calathus melanocephalus (Linnaeus)									123	
Carabus glabratus Paykull			35						35	
C. problematicus Herbst			35						35	
Harpalus rufipes (De Geer)			57							57
Pterostichus coerulescens (Linnaeus)									123	
P. madidus (Fabricius)			56							56
<u>Scarabaeidae</u>										
Costelytra zealandica (White)										32
Euoniticellus intermedius (Reiche)			118						118	118
Heteronychus arator (Fabricius)										68a
Onthophagus granulatus Boheman			121						121	121
Oryctes rhinoceros (Linnaeus)					132,133					
<u>Tenebrionidae</u>										
Pterohelaeus darlingensis Carter										4
DIPTERA										
<u>Anthomyiidae</u>										
Leptohylemyia coarctata (Fallen) (4)			40							
<u>Caliphoridae</u>										
Calliphora erythrocephala (Meigen)					99					
Chrysomya bezziana Villeneuve (5)	111									
Cochliomyia hominivorax (Coquerel)					27					
Lucilia cuprina (Wiedemann) (3)	120	16b	124		120					16b,124
<u>Ceratopogonidae</u>										
Culicoides cornutus de Meillon		125					125			
C. fascipennis (Staeger) (3)	37									
C. furens (Poey)		26								
C. grisescens Edwards (4)	37						26			

Appendix I/2

	FD	SFD	YB/LB	TS	CG	F1	CTP	WF	CW	Other
<i>C. hollensis</i> (Melander & Brues)		61								
<i>C. marmoratus</i> (Skuse) (2)	42	26,42					26,42			
<i>C. melleus</i> (Coquillett)		61								
<i>C. obsoletus</i> (Meigen) group							9			
<i>C. pallidipennis</i> Carter, Ingram & Macfie		125					125			
<i>C. pulicaris punctatus</i> Meigen (3)	37									
<i>C. schultzei</i> (Enderlein)		125					125			
<i>C. sinanoensis</i> Takunaga (2)	70,71						70,71			
<i>C. species</i> (3 & 4)	70,106a									
<i>C. variipennis</i> (Coquillett) (3)	76*			2			2,3,76 77,83 26			76
<i>C. victoriae</i> Macfie		26								
<i>Leptoconops carteri</i> Hoffman		101								
<u>Culicidae</u>										
<i>Aedes aegypti</i> (Linnaeus) (5)	49*,91	49		17	99	131				
<i>A. canadensis</i> (Theobald)				75						
<i>A. cataphylla</i> Dyar (3)	18									
<i>A. cinereus</i> Meigen				75,104						
<i>A. communis</i> (De Geer) group				75						
<i>A. detritus</i> (Haliday)				104						
<i>A. diantus</i> Howard, Dyar & Knab		49								
<i>A. nigromaculis</i> (Ludlow) (3)	91,92*	92								
<i>A. punctor</i> (Kirby)				104						
<i>A. serratus</i> (Theobald)				22						
<i>A. sierrensis</i> (Ludlow) (2)	91									
<i>A. simpsoni</i> (Theobald)		80		80	80					
<i>A. stimulans</i> (Walker)				75						
<i>A. triseriatus</i> (Say)				75						
<i>A. trivittatus</i> (Coquillett)				75						
<i>A. vexans</i> (Meigen) (4)	134			75						
<i>Anopheles albimanus</i> Wiedemann (6)	84				97					
<i>A. annularis</i> Wulp (3)	105									
<i>A. balabacensis</i> Baisas					97					
<i>A. claviger</i> (Meigen)					97,99					
<i>A. coustani</i> Laveran					99					

Appendix I/3

[illegible]

FD	SFD	YB/LB	TS	CG	F1	CTP	WF	CW	Other
----	-----	-------	----	----	----	-----	----	----	-------

Drosophilidae

Glossinidae

Full review				
Glossina austeni Newstead	12		12	12
G. fuscipes Newstead		98,99		
G. fuscipes fuscipes Newstead		98		
G. morsitans Westwood	93		93	
	93	98	93,122	

FD	SFD	YB/LB	TS	CG	F1	CTP	WF	CW	Other
----	-----	-------	----	----	----	-----	----	----	-------

Muscidae

Psychodidae

Sarcophagidae

Simuliidae

<i>Simulium callidum</i> (Dyar & Shannon)	33		
<i>S. damnosum</i> Theobald	73a		25, 73a
<i>S. gonzalezi</i> Vargas & Diaz Najera	33		
<i>S. metallicum</i> Bellardi	33	87	
<i>S. ochraceum</i> Walker	19, 33		87
<i>S. tuberosum</i> (Lundstrom) (2)	64		
<i>S. venustum</i> Say (2)	64		

Appendix I/6

	FD	SFD	YB/LB	TS	CG	Fl	CTP	WF	CW	Other
<u>Tabanidae</u>										
<i>Atylotus fulvus</i> (Meigen)		44								
<i>A. incisularis</i> (Macquart)		113								
<i>A. loewianus</i> (Villeneuve)		44								
<i>A. rusticus</i> (Linnaeus)		44								
<i>Chrysops aberrans</i> Philip		116								
<i>C. ater</i> Macquart		60								
<i>C. atlanticus</i> Pechuman		5,62								
<i>C. caecutiens</i> (Linnaeus)		44,104a		104a						
<i>C. callidus</i> Osten Sacken		60,116								
<i>C. cincticornis</i> Walker (2)	116	60								
<i>C. cuclux</i> Whitney (2)	116									
<i>C. frigidus</i> Osten Sacken		60,116								
<i>C. fuliginosus</i> Wiedemann		5,62, 63,90								
<i>C. geminatus</i> Wiedemann		60								
<i>C. indus</i> Osten Sacken (2)	116	60								
<i>C. lateralis</i> Wiedemann		60								
<i>C. macquarti</i> Philip		60								
<i>C. mitis</i> Osten Sacken		60								
<i>C. moechus</i> Osten Sacken		116								
<i>C. niger</i> Macquart		116								
<i>C. relictus</i> Meigen		44								
<i>C. shermani</i> Hine		60								
<i>C. univittatus</i> Macquart (2)	116	60								
<i>C. vittatus</i> Wiedemann (2)	116	60								
<i>Haematopota crassicornis</i> Wahlberg		6,44								
<i>H. italica</i> Meigen		44								
<i>H. pluviialis</i> (Linnaeus) (2)	44	6,37a								
<i>H. subcylindrica</i> Pandalle		44								
<i>Heptatoma pellucens</i> (Fabricius)		44								
<i>Hybomitra affinis</i> (Kirby) (2)	113,114									
<i>H. arpadi</i> (Szilady)		113,114								
<i>H. aterrima</i> (Meigen)		6								
<i>H. bimaculata</i> (Macquart)		6,44								
<i>H. ciureai</i> (Seguy)		44								
<i>H. distinguenda</i> (Verrall)	44	6,								
<i>H. epistates</i> (Osten Sacken) (2)	60	116								

Appendix I/7

	FD	SFD	YB/LB	TS	CG	F1	CTP	WF	CW	Other
H. frontalis (Walker) (2)	114,									
H. illota (Osten Sacken) (2)	113,114	60								
	116									
H. itasca (Philip) (2)	113									
H. jersey (Takahasi)		47								
H. kaurii Chvala & Lyneborg		6								
H. lasiophthalma (Macquart) (2)	60,113									74
	114,116									
H. liorhina (Philip)		113,114								
H. lundbecki Lyneborg		6, 44								
H. lurida (Fallen) (2)	113,114									
H. micans (Meigen)		6,44								
H. microcephala (Osten Sacken)		60								
H. montana (Meigen)		44								
H. muehlfeldi (Brauer)		44								
H. nuda (McDunnough) (2)	113,114	60								
H. rhombica osburni (Heine) (2)	113,114									
H. rupestris (McDunnough) (2)	113,114									
H. sodalis (Williston)		60,116								
H. tropica (Linnaeus)		44								
H. typhus (Whitney) (2)	113,114	60								
Lepiselaga crassipes (Fabricius) (3)	15									
Phaeotabanus cajannensis (Fabricius) (2)	85									
Stenotabanus (Stenochlorops) sp. (3)	15									
Tabanus abactor Philip		115								
T. acutus (Bigot) (2)	115									
T. atratus Fabricius		116								
T. autumnalis Linnaeus		44								
T. bromius Linnaeus (2)	44	6								
T. cordiger Meigen		44								
T. dorsiger var. dorsovittatus Macquart (2)	85									
T. dorsiger var. modestus Wiedemann (2)	85									
T. fairchildi Stone		60								

Appendix I/8

	FD	SFD	YB/LB	TS	CG	F1	CTP	WF	CW	Other
T. glaucopis Meigen		44								
T. importunus Wiedemann (3)	85									
T. lineola Fabricius		60,116								
		126								
T. lineola var. hinellus Philip		115								
T. lineola lineola Fabricius		115								
T. maculicornis Zetterstedt		6,44								
T. marginalis Fabricius	60									
T. melanocerus Wiedemann (2)	115									
T. mularis Stone		115								
T. nigrovittatus Macquart (3)	115	5,11,								
		62,66								
		126								
T. proximus Walker		115								
T. pumilus Macquart		60								
T. quinquevittatus Wiedemann (2)	65,116	60								
T. reinwardtii Wiedemann		116								
T. similis Macquart (2)	60	116								
T. sparus milleri Whitney		60								
T. subsimilis subsimilis Bellardi		115								
T. sudeticus Zeller		44								
T. sulcifrons Macquart		60,115								
		116								
T. superjumentarius Whitney		60								
T. trimaculatus Palisot de Beau		115								
T. vivax Osten Sacken		60								

Tephritidae

Strumeta tryoni (Froggatt)

24

HYMENOPTERA

Xylocopidae

Ceratina flavipes Smith

47a

C. japonica Cockerell

47a

Appendix I/9

FD	SFD	YB/LB	TS	CG	F1	CTP	WF	CW	Other
----	-----	-------	----	----	----	-----	----	----	-------

Apidae

Apis mellifica (Linnaeus)

68

ORTHOPTERA

Acridoidea

Austracris guttulosa (Walker) (2)

30

Chortoicetes terminifera (Walker) (3)

31

36,48

31

Locusta migratoria (Linnaeus)

51

L. migratoria capito (Saussure) (3)

50,52

L. migratoria migratorioides (Reiche
& Fairmaire)

29

Oedaleus senegalensis (Krauss) (3)

53

SIPHONAPTERA

Hystrihopsyllidae

Ctenophthalmus orientalis (Wagner)

131

REFERENCES

- ABOUL-NASR, A.E. (1970). Determination of the physiological age in Anopheles gambiae Giles, main vector of malaria in eastern Nigeria. (Diptera, Culicidae, Anophelinae).- Bull. Soc. ent. Egypte 53, 89-96.
- ADAMS, T.S. (1974). The role of juvenile hormone in housefly ovarian follicle morphogenesis. - J. Insect Physiol. 20, 263-276.
- AKEY, D.H. & BARNARD D.R. (1983). Parity in airborne populations of the biting gnat Culicoides variipennis (Diptera: Ceratopogonidae) in north-eastern Colorado. - Environ. Entomol. 12, 91-95.
- AKEY, D.H. & POTTER H. (1979). Pigmentation associated with oogenesis in the biting fly Culicoides variipennis (Diptera: Ceratopogonidae) application as an age grouping method. - J. med. Entomol. 16, 67-70.
- ALLSOPP, P.G. (1979). Determination of age and mated state of adult Pterohelaeus darlingensis Carter (Coleoptera: Tenebrionidae). - J. Aust. entomol. Soc. 18, 235-239.
- ANDERSON, J.F. (1964). Methods of distinguishing nulliparous from parous flies and for estimating the ages of Fannia canicularis and some other cyclorrhaphous Diptera. - Ann. ent. Soc. Am. 57, 226-236.
- ANDERSON, J.F. (1971). Autogeny and mating and their relationship to biting in the saltmarsh deer fly Chrysops atlanticus (Diptera: Tabanidae). - Ann. ent. Soc. Am. 64, 1421-1424.

- ATKINSON, P.R. (1977). Preliminary analysis of a field population of citrus red scale, Aonidiella aurantii (Maskell) and the measurement and expression of stage duration and reproduction for life tables. - Bull. ent. Res. 67, 65-87.
- AUROI, C. (1982). Physiological age of tabanid (Diptera) populations in Switzerland. - J. med. Entomol. 19, 281-284.
- BARTON BROWN, L., VAN GERWEN, A.C.M. & WILLIAMS, K.L. (1979). Oocyte resorption during ovarian development in the blowfly Lucilia cuprina. - J. Insect Physiol. 25, 147-153.
- BECK, D.S. (1980) - Insect Photoperiodism - 387pp. New York, Academic Press.
- BEKLEMISHEV, V.N., DETINOVA, T.S., & POLOVODOVA, V.P. (1959). Determination of physiological age in anophelines and of age distribution in anopheline populations in the U.S.S.R. - Bull. Wld Hlth Org. 21, 223-232.
- BELL, W.J. & BOHM, M.K. (1975). Oosorption in insects. Biol. Rev. 50, (4) 373-396.
- BELLAMY, R.E. & CORBET, P.S. (1973). Combined autogenous and anautogenous ovarian development in individual Culex tarsalis Coq. (Diptera, Culicidae). - Bull. ent. Res. 63, 335-346.
- BELLAMY, R.E. & CORBET, P.S. (1974). Occurrence of ovariolar dilatations in nulliparous mosquitoes. - Mosquito News 34, 334.
- BERTRAM, D.S. & SAMARAWICKREMA, W.A. (1958). Age determination for individual Mansonioides mosquitoes. - Nature, Lond. 182, 444-446.

- BIRLEY, M.H. & BOORMAN, J.P.T. (1982). Estimating the survival and biting rates of haematophagous insects, with particular reference to the Culicoides obsoletus group (Diptera: Ceratopogonidae) in southern England. - J. Anim. Ecol. 51, 135-148.
- ==
- BIRLEY, M.H. & RAJAGOPALAN, P.K. (1981). Estimation of the survival and biting rates of Culex quinquefasciatus, (Diptera: Culicidae). - J. med. Ent. 18, 181-186.
- BLUME, R.R., MATTER J.J., & ESCHLE J.L. (1973). Onthophagus gazella : Effect on survival of hornflies in the laboratory. Environ. Entomol. 2 (5), 811-3.
- BONHAG, P.F. & ARNOLD. W.J. (1961). Histology, histochemistry and tracheation of the ovariole sheaths in the American cockroach Periplaneta americana (L.) - J. Morph. 108, 107-129.
- BORNEMISSZA G.F. (1971). A new variant of the paracopric nesting type in the Australian dung beetle, Onthophagus compositus. Paedobiologia 11, 1-10.
- BORNEMISSZA G.F. (1976). The Australian dung beetle project 1965-1975. Aust. Meat Res. Comm. Rev. No. 30, 1-30.
- BOSLER, E.M. & HANSENS, E.J. (1974). Natural feeding behaviour of adult greenheads, and its relation to oogenesis. Ann. ent. Soc. Am. 67, 321-324.
- BROWN, E.H. & KING, R.C. (1964). Studies on the events resulting in the formation of an egg chamber in Drosophila melanogaster. - Growth 28, 41-81.
- BUREAU OF METEOROLOGY, Canberra (1975). Climatic averages, Australia. Aust. Gov. Pub. Service.

- BUZBY J.R. (1983) Bioclimatic prediction system; user's manual.
Bureau of Fauna and Flora, Canberra.
- CHALLIER, A. (1965). Amelioration de la methode de determination de l'age physiologique des glossines. Etudes faites sur Glossina palpalis gambiensis Vanderplank 1949. - Bull. Soc. Path. exot. 58, 250-259.
- CHALLIER, A. (1982). The ecology of the tsetse (Glossina spp.) (Diptera, Glossinidae): a review (1970-1981) - Insect Sci. Applic. 3, 97-143.
- CAMBEFORT, Y. (1984) Travaux des chercheurs de la Station de Lamto. Étude écologique des Coléoptères Scarabaeida de cote d'Ivoire. Programme MAB savanes 3, 1-294.
- CAMPBELL, A., FRAZER, B.D., GILBERT N., GUITERREZ, A.P. & MACKAUER, M. (1974). Temperature requirements of some aphids and their parasites. J. appl. Ecol. 11 :431 - 438.
- CHARLWOOD, J.D. (1980). Observations on the bionomics of Anopheles darlingi Root (Diptera: Culicidae) from Brazil. - Bull. ent. Res. 70, 685-692.
- CHARLWOOD, J.D. & LOPES, J. (1980). The age structure and biting behaviour of Stomoxys calcitrans (L.) (Diptera: Muscidae) from Manaus, Brazil. - Bull. ent. Res. 70, 549-555.
- CHARLWOOD, J.D. & RAFAEL, J.A. (1980). Autogeny in the River Negro horsefly, Lepiselaga crassipes, and an undescribed species of Stenotabanus (Diptera: Tabanidae) from Amazonas, Brazil. - J. med. Entomol. 17, 519-521.

- CHARLWOOD, J.D., RAFAEL, J.A. & WILKES, T.J. (1980). Methods to determine the physiological age of Diptera of medical importance. A revision, with special reference to disease vectors in South America. [in Portuguese] - Acta Amazonica 10, 311-333.
- CHARLWOOD, J.D. & WILKES, T.J. (1979). Studies on the age-composition of samples of Anopheles darlingi Root (Diptera: Culicidae) in Brazil. - Bull. ent. Res. 69, 337-342.
- CHRISTOPHERS, S.R. (1911). Development of the egg follicle in anophelines. - Paludism 2, 73-88.
- CLARKE, J.E. (1969). Trypanosome infection rates in the mouthparts of Zambian tsetse flies. - Ann. trop. Med. Parasit. 63, 15-34.
- CLEMENTS, A.N. (1963). The physiology of mosquitoes. - Int. Ser. Monogr. pure appl. Biol. (Zool.) Vol.17, - 393 pp. Oxford, London, New York, Paris, Pergamon Press.
- CLIFT, A.D. (1972). The nutritional and endocrine control of reproduction of the Australian sheep blowfly Lucilia cuprina (Wied.). - 286 pp. Ph.D. Thesis, Univ. Sydney.
- CLIFT, A.D. & McDONALD, F.J.D. (1973). Morphology of the internal reproductive system of Lucilia cuprina. (Wied.) (Diptera, Calliphoridae) and a method of determining the age of both sexes. - Int. J. Insect Morphol. Embryol. 2, 327-333.
- COLLESS, D. (1958). Recognition of individual nulliparous and parous mosquitoes. - Trans. R. Soc. trop. Med. Hyg. 52, 187.
- CORBET, P.S. (1962). Age determination of adult dragonflies. - Proc. XI. Int. Congr. Ent. 3, 287-289.
- CORBET, P. (1964). The ovarian condition of certain sylvan mosquitoes in Uganda. - Bull. ent. Res. 55, 367-382.

- CORBET, P.S. & SMITH, S.M. (1974). Diel periodicities of landing of nulliparous and parous Aedes aegypti (L.) at Dar es Salam, Tanzania. - Bull. ent. Res. 64, 111-121.
==
- CROSET, H., ARNAUD, D., GUILVARD, E. & GABINAUD, A. (1982). Dispersion, age chronologique, et age physiologique d'une population marquée d' Aedes (O.) cataphylla Dyar 1916 (Diptera, Culicidae). - Vie Milieu 30, 65-73.
==
- CROWSON, R.A. (1981). The biology of the Coleoptera. - 802pp London, Academic Press.
- CUPP, E.W. & COLLINS, R.C. (1979). The gonotrophic cycle in Simulium ochraceum. - Am. J. trop. Med. Hyg. 28, 422-426
==
- DARSKAYA, N.F., BRJUKHANOVA, L.V. & KUNITSKAYA, N.T. (1962). [Corpora lutea in ovaries and spermatozoa in receptacula seminis of fleas as signs of life span in these insects.] - Voprosy Obshchei Zoologii i Meditsinskoj Parasitologii Medgiz, Moscow, 423-446.
- DAVIES, J.B. (1969). Field preservation and storage of mosquitoes for laboratory studies. - Mosquito News 29, 259.
==
- DAVIES, J.B., CORBET, P.S., GILLIES M.T. & McCRAE A.W.R. (1971). Parous rates in some Amazonian mosquitoes collected by three different methods. - Bull. ent. Res. 61, 125-132.
==
- DAVIES, J.E. (1978). The use of ageing techniques to evaluate the effects of aerial spraying against Glossina morsitans centralis Machado (Diptera, Glossinidae) in Northern Botswana. - Bull. ent. Res. 68, 373-383.
==
- DAVIES, L. (1957). A study of the age of Simulium ornatum Mg. (Diptera) attracted to cattle. - Bull. ent. Res. 48, 535-552.
==

- DAVIES, L. (1961). Ecology of two Prosimulium species (Diptera) with reference to their ovarian cycles. - Can. Ent. 93, 1113-1140.
- DEMPSTER J.P. (1975). Animal population ecology. - 155 pp.
London. Academic Press.
- DEPNER, K.R. (1962) Effects of photoperiod and of ultraviolet radiation on the incidence of diapause in the hornfly, Haematobia irritans L. Int. J. Bioclimatol. Biometeorol. 5, 68-71.
- DESCAMPS, M. & WINTREBERT, D. (1961). Quelques remarques et recherches préliminaires a propos des criteres de maturite sexuelle et de ponte chez les femelles d'acridiens migrants. Revue Path. veg. Ent. agric. Fr. 40, 131-143.
- DETINOVA, T.S. (1949). Physiological changes of ovaries in females of Anopheles maculipennis [in Russian]. - Medskaya Parazit. 18, 410-420.
- DETINOVA, T.S. (1953). Age composition and epidemiological importance of the population of Anopheles maculipennis in the Moscow oblast [in Russian]. - Medskaya Parazit. 22, 486-495.
- DETINOVA, T.S. (1962). Age-grouping methods in Diptera of medical importance with special reference to some vectors of malaria. - Monograph Ser. W.H.O. No.47, 216 pp.
- DETINOVA, T.S. (1968). Age structure of insect populations of medical importance. - A. Rev. Ent. 13, 427-450.
- DETINOVA, T.S. & GILLIES M.T. (1964). Observations on the determination of the age composition and epidemiological importance of populations of Anopheles gambiae Giles and Anopheles funestus Giles in Tanganyika. - Bull. Wld Hlth Org. 30, 23-28.

- DINGLE, H., CALDWELL, R.L. & HASKELL, J.B. (1969). Temperature and circadian control of cuticle growth in the bug, Oncopeltus fasciatus. - J. Insect Physiol. 15, 373-378.
- DONATO, H & SOHAL, R.S. (1978). Age-related changes in lipofuscin-associated fluorescent substances in the adult male housefly. - Exp. Gerontol. 13, 171-179.
- DREW, R.A.I. (1969). Morphology of the reproductive system of Strumeta tryoni (Froggatt) (Diptera: Trypetidae) with a method of distinguishing sexually mature adult males. - J. Aust. entomol. Soc. 8, 21-32.
- DUKE, B.O.L. (1960). Studies on the biting habits of Chrysops. VII. The biting cycles of nulliparous and parous C. silacea and C. dimidiata (Bombe form). - Ann. trop. Med. Parasit. 54, 147-155
- DUKE, B.O.L. (1968). Studies on factors influencing the transmission of onchocerciasis IV. The biting cycles, infective biting density and transmission potential of 'forest' Simulium damnosum. - Ann. trop. Med. Parasit. 62, 95-106.
- DUKE, B.O.L. (1975). The differential dispersal of nulliparous and parous Simulium damnosum. - Tropenmed. & Parasitol. 26, 88-97.
- DYCE, A.L. (1969). The recognition of nulliparous and parous Culicoides (Diptera: Ceratopogonidae) without dissection. - J. Aust. entomol. Soc. 8, 11-15.
- EDMAN, J.D. & LYNN, H.C. (1975). Relationship between blood meal volume and ovarian development in Culex nigripalpus (Diptera: Culicidae). - Entomologia exp. appl. 18, 492-496.

- ELLISON, J.R. & HAMPTON, E.N. (1982). Age determination using the apodeme structure in adult screw worm flies (Cochliomyia hominivorax). - J. Insect Physiol. 28, 731-736.
- ENGELMANN, F. (1970). The physiology of insect reproduction. - 307pp Oxford. Pergamon Press.
- ETTERSHANK, G. (1983). Age structure and cyclical annual size change in the Antarctic krill, Euphausia superba Dana. - Polar Biol. 2, 189-193.
- ETTERSHANK, G., MACDONNELL, I. & CROFT, R. (1983). The accumulation of age pigment by the fleshfly Sarcophaga bullata Parker (Diptera: Sarcophagidae). - Aust. J. Zool. 31, 131-138.
- FARROW, R.A. (1975). The African migratory locust in its main outbreak area of the middle Niger: quantitative studies of solitary populations in relation to environmental factors. - Locusta. 11, 1-198.
- FARROW, R.A. (1977). Maturation and fecundity of the spur-throated locust, Austracris guttulosa (Walker) in New South Wales during the 1974/75 plague. - J. Aust. entomol. Soc. 16, 27-39.
- FARROW, R.A. (1979). Population dynamics of the Australian plague locust, Chortoicetes terminifera (Walker) in central western N.S.W. I. Reproduction and migration in relation to weather. Aust. J. Zool. 27, 717-745.
- FENEMORE, P.G. (1971). The internal condition of adult Costelytra zealandica (White) of known age and mated state. - N.Z. J. Sci. 14, 77-88.
- FERREIRA, M.C. (1967) Os escarabádeos de Mocambique. Revista de Entomol. de Mocambique 10 (1,2), 5 - 778.

- FLETCHER, B.S. (1975) Temperature - regulated changes in the ovaries of overwintering females of the Queensland fruitfly Dacus tryoni. Aust. J. Zool. 23, 91-102.
- FORD, J. (1969). Feeding and other responses of tsetse flies to man and ox and their epidemiological significance. - Acta trop. 26. 249-264.
- FORD, J., MAUDLIN, I. & HUMPHRYES, K.C. (1972). Comparisons between three small collections of Glossina morsitans morsitans (Machado) (Diptera: Glossinidae) from the Kilombero River Valley, Tanzania. Part I. Characteristics of flies exhibiting different patterns of behaviour. - Acta trop. 29, 231-249.
- FUJIYAMA, S. (1983). The larval diapause of three scarabaeid beetles and its function in their life cycles. In : Diapause and Life cycle Strategies in Insects (V.K. Brown & I. Hodek, Eds.), 55 - 66, Junk, The Hague, 283p.
- GARMS, R. (1975). Observations on filarial infections and parous rates of anthropophilic blackflies in Guatemala with reference to the transmission of Onchocerca volvulus. Tropenmed. & Parasitol. 26, 169-182.
- GIGLIOLI, M.E.C. (1959). Observations on the structure of the ovariole and the follicular residue body or corpus luteum in Anopheles gambiae. - Trans. R. Soc. trop. Med. Hyg. 53, 310-311.
- GIGLIOLI, M.E.C. (1965). The problem of age determination in Anopheles melas Theo. 1903 by Polovodova's method. - [1st int.Congr.Parasitol., Rome 1964.] Cah. ORSTOM Ser. Entomol. med. Parasitol. No 3 & 4, 157-177.

- GILBERT, N. (1973). Biometrical interpretation. - 125 pp. Oxford, Clarendon Press.
- GILLIES M.T. (1974). Methods of assessing the density and survival of blood sucking Diptera. - A. Rev. Ent. 19, 345-362.
- GILLIES, M.T. & WILKES, T.J. (1965). A study of age composition of populations of Anopheles gambiae Giles and A. funestus Giles in north-eastern Tanzania. - Bull. ent. Res. 56, 237-262.
==
- GLUKHOVA, V. (1958). On the gonotrophical cycle of the midges genus Culicoides (Diptera, Heleidae) of the Karelian ASSR [in Russian] - Parazit. Sb. 18, 239-254.
==
- GRAHAM, J.E. & BRADLEY, I.E. (1972). Changes in the age structure of Culex pipiens fatigans Wied. populations in Rangoon, Burma, after intensive larviciding. - J. med. Entomol. 9, 325-329.
=
- GREENHAM, P.M. (1972). The effects of the variability of cattle dung on the multiplication of the bushfly (Musca vetustissima, Walker.) J. anim. Ecol. 41 : 153-165.
- GRUVEL, J. (1975). Structure des populations de Glossina tachinoides W. à la réserve de Kalamaloué (VI). - Revue Elev. Méd. vet. Pays trop. 28, 195-215.
==
- HALFTER, G. (1977). Evolution of nidification in the scarabaeinae (Coleoptea, Scarabaeidae). Quaestiones Entomologicae 13: 231-253.
- HALFTER, G. & EDMONDS, W.D. (1982). The nesting behavior of dung beetles (Scarabaeinae) An ecological and evolutive approach. - 176 pp. Mexico, Instituto de Ecologia.
- HALFTER, G. & MATTHEWS, E.G. (1966). The Natural History of Dung Beetles of the subfamily Scarabaeinae (Coleoptera, Scarabaeidae.) Folia entomol. Mex. 12-14, 1-312.
== ==

- HAYDAK, M.H. (1957). Changes with age in the appearance of some internal organs of the honeybee. - Bee Wld 38, 197-207.
- HEYMONS, R. (1930). Über die Morphologie des Weiblichen Geschlechtsapparats der Gattung Scarabaeus L. - Z. Morph. Okol. Tiere 18, 536-574.
- HODEK, I. (1962) Experimental influencing of the imaginal diapause in Coccinella semipunctata L. (Col., Coccinellidae). 2nd. part. Acta Soc. ent. Cechoslov. 59, 297-313.
- HOUSTON, W.W.K. (1981). The life cycle and age of Carabus glabratus Paykull and C. problematicus Herbst (Col: Carabidae) on moorland in northern England. - Ecol. Entomol. 6, 263-271.
- HUERTA, C., ANDUAGA, S. & HALFFTER, G. (1981). Relaciones entre nidificacion y ovario en Copris (Coleoptera, Scarabaeidae, Scarabaeinae). - Folia ent. Mex. no.47, 139-170.
- HUGHES, R.D. (1962). A method for estimating the effects of mortality on aphid populations. - J. Anim. Ecol. 31, 389-396.
- HUGHES, R.D. (1970). The seasonal distribution of bushfly (Musca vetustissima Walker) in south east Australia. - J. Anim. Ecol. 39, 691-706.
- HUGHES, R.D. (1974). Variation in the proportion of different reproductive stages of female bushflies (Musca vetustissima Wlk. (Diptera, Muscidae)) in bait catches as a cause of error in population estimates. - Bull. ent. Res. 64, 65-71.
- HUGHES, R.D. (1975) Introduced dung beetles and Australian Pasture Ecosystems. J. Appl. Ecol. 12: 819-837.
- HUGHES, R.D., GREENHAM, P.M., TYNDALE-BISCOE, M. & WALKER, J.M. (1972). A synopsis of observations on the biology of the Australian bushfly (Musca vetustissima Walker). J. Aust. ent. Soc. 11: 311-331.

- HUGHES, R.D., TYNDALE-BISCOE, M., & WALKER, J. (1978). Effects of introduced dung beetles (Coleoptera : Scarabaeidae) on the breeding and abundance of the Australian bush fly Musca vetustissima Walker (Diptera : Muscidae). Bull. ent. Res. 68 : 361-372.
- HUNTER, D.M. (1982). Adult development of the Australian plague locust Chortoicetes terminifera (Walker) (Orthoptera: Acrididae) - Bull ent. Res. 72, 589-598.
- ISAEV, V.A. (1972). Seasonal changes in the abundance and physiological age of blood sucking midges of the genus Culicoides (Diptera, Ceratopogonidae) [in Russian]. - Medskaya Parazit. 41, 614-617.
- IVANISHCHUK, P.P. (1977). Autogenous development of ovarian follicles in some species of blood-sucking horseflies [in Russian]. - Medskaya Parazit. 46, 15-19.
- JACKSON, C.H.N. (1946). An artificially isolated generation of tsetse flies (Diptera). - Bull. ent. Res. 37, 291-299.
- JAENSON, T.G.T. (1980). Mating behaviour of females of Glossina pallidipes Austen (Diptera: Glossinidae). - Bull. ent. Res. 70, 49-60.
- JOHNSTON, J.S. & ELLISON, J.R. (1982). Exact age determination in laboratory and field-caught Drosophila. - J. Insect Physiol. 28, 773-779.
- JOLY, P. (1950). Fonctionnement ovarien des carabes. Comptes rendus des séances de la Société de biologie 144, 1217-1220.
- JONES, M.G. (1971). Observations on changes in the female reproductive system of the wheat bulb fly Leptohylemyia coarctata (Fall.). - Bull. ent. Res. 61, 55-68.

- JORDAN, A.M. (1974). Recent developments in the ecology and methods of control in tsetse flies (Glossina spp.) (Diptera: Glossinidae) - a review. - Bull. ent. Res. 63, 361-399.
- JUPP, P.G. (1973). Distinguishing nulliparous from parous females by the ovarian tracheation technique in four S. African species of Culex (Diptera: Culicidae). J. ent. Soc. sth. Afr. 36, 271-273.
- KAY, B.H. (1973). Seasonal studies of a population of Culicoides marmoratus (Skuse) (Diptera, Ceratopogonidae) at Deception Bay, Queensland. - J. Aust. ent. Soc. 12, 42-58.
- KAY, B.H. (1979). Age structure of populations of Culex annulirostris (Diptera: Culicidae) at Kowanyama and Charleyville, Qld. - J. med Ent. 16, 309-316.
- KITCHING, R.L. (1977). Time, resources and population dynamics in insects. - Aust. J. Ecol. 2, 31-42.
- KINGSTON, T.J. & COE, M. (1977) The biology of a giant dung-beetle (Helicopris dilloni) (Coleoptera : Scarabaeidae). J. Zool. Lond. 111: 243-263.
- KIRK, A.A. (1983). The biology of Bubas bison (L.) (Coleoptera: Scarabaeidae) in southern France and its potential for recycling dung in Australia. Bull. ent. Res. 73 : 129-136.
- KLEMPERER, H.G. (1983). The evolution of parental behaviour in Scarabaeinae (Coleoptera : Scarabaeidae) : an experimental approach. Ecol. entomol. 8, 49-59.
- KNIEPERT, F.W. (1980). Untersuchungen zur Atilitatsdynamic und Autogenie imaginaler Bremsenpopulationen (Diptera: Tabanidae) im Volksberg. - Z. angew. Zool. 67, 399-411.

- KNIGHT, J.W. & NAYAR, J.K. (1982). Evaluation of ovarian age grading techniques for Florida Culex mosquitoes. - J. Fla. Anti-mosq. Ass. 53, 8-12.
==
- KOCH, E.A., SMITH, P.A. & KING, R.C. (1967). The division and differentiation of Drosophila cystocytes [in Russian]. - J. Morph. 121, 55-70.
==
- KOSMINSKY, R.B. (1960). On the methods to determine the age of fleas Leptopsylla segnis and Leptopsylla taschenbergi and the age analysis of the population of L. segnis. - Medskaya Parazit. 29, 590-594
==
- KRAFSUR, E.S. & ERNST, C.M. (1983). Physiological age composition and reproductive biology of horn fly populations, Haematobia irritans irritans (Diptera: Muscidae) in Iowa, U.S.A. - J. med. Entomol. 20, 664-669.
==
- KURIHARA, M. (1968). Histochemical observations on periodic acid-schiff positive substances and nucleic acids in the normal and degenerated ovary of the lady beetle Epilachna vigintioctomaculata Motschulsky. J. Fac. Agric., Iwate University 9, 1-15.
=
- KURIHARA, M. & HASEGAWA, T. (1978). Oogenesis in the horsefly, Hybomitra jersey (Diptera, Tabanidae) with special reference to the formation of corpus luteum [in Japanese]. - J. Fac. Agric. Iwate Univ. 14, 1-9.
==
- KURIHARA, M., MAETA, Y., CHIBA, K., & SAKAGAMI, S.F. (1981). The relation between ovarian conditions and life cycle in two small carpenter bees, Ceratina flavipes and C. japonica (Hymenoptera, Anthophoridae). - J. Fac. Agric. Iwate Univ. 15, 131-153.
==

- KUZINA, O.S. (1942). On the gonotrophic relationships in horseflies (Stomoxys calcitrans and Haematobia stimulans) [in Russian].
- Medskaya Parazit. 11, 70-78.
==
- LAMBERT, M.R.K. (1972). Some factors affecting flight in field populations of the Australian plague locust Chortoicetes terminifera (Walker) in New South Wales. - Anim Behav. 20, 205-217.
==
- LANGE, A.B., KHOK CHUNG KUANG & SOKOLOVA. M.I. (1981). The method for intraovarial oil injection and its use in the determination of the physiological age of females of blood sucking mosquitoes (Dipt: Culicidae) [in Russian]. - Medskaya Parazit. 50, 51-53.
==
- LANGE, A.B. & KHOK CHUNG KUANG (1981). Abortive oogenesis and physiological age in blood sucking mosquitoes (Diptera, Culicidae) [in Russian].
- Medskaya Parazit. 50, 48-56.
==
- LAUNOIS, M. (1972). Contribution à l'étude du fonctionnement ovarien du criquet migrateur Locusta migratoria capito (Sauss.) dans la nature. - Ann. Zool. Ecol. anim. (Hors Ser.), 55-116.
- LAUNOIS, M. & LAUNOIS-LUONG M. H. (1980). Datation des ailes du criquet migrateur Locusta migratoria dans la nature par examen de la pilosité thoracique ventrale (Orthoptera, Acrididae).
- Annls Soc. ent. Fr. 16, 233-247
==
- LAUNOIS-LUONG, M.H. (1978). Methode pratique d'interpretation de l'etat des ovaires de acridiens du Sahel. - Ann. Zool., Ecol. anim. 10, 569-587.
==
- LAUNOIS-LUONG, M.H. (1980). Etude de la production des oeufs d' Oedaleus senegalensis (Krauss) au Niger (Region Maradi) - Bull. Inst. Fondam. Afr. Noire. A 41, 128-148.
==

- LAVEISSIERE, C. (1975). Determination de l'age des glossines tenebrales (Glossina tachinoides Westw.) - Cah. ORSTOM Ser. Entomol. méd. Parasitol. 13, 3-11.
==
- LAVEISSIERE, C. & KIENOU, J.P. (1982). Écologie de Glossina tachinoides Westwood 1850, en savane humide d'Afrique de l'Ouest IX. Relations entre l'age physiologique et l'age chronologique. - Cah. ORSTOM Ser. Entomol. med. Parasitol. 20, 19-28.
==
- LE BERRE, R. (1966). Contribution a l'etude biologique et ecologique de Simulium damnosum Theobald 1903 (Diptera: Simuliidae). Mem. ORSTOM. No.17, 204 pp.
==
- LEE, J.M. & PENG, Y-S. (1982) Influence of manure availability and nesting density on the progeny size of Onthophagus gazella. Environ. Ent. 11, 38-41.
==
- LEWIS, D.J. (1957). A method of recognizing individual nulliparous and parous mosquitoes. - Trans. R. Soc. trop. Med. Hyg. 51, 561.
- LEWIS, D.J. (1960). Observations on Simulium damnosum in the southern Cameroons and Liberia. - Ann. trop. Med. Parasit. 54, 208-223.
- LEWIS, D.J., LAINSON, R. & SHAW, J.J. (1970). Determination of parous rates in Phlebotomine sandflies with special reference to Amazonian species. - Bull. ent. Res. 60, 209-219.
- LINEVA, V.A. (1953). Methods of assessment of the physiological age in females of Musca domestica [in Russian]. - Medskaya Parazit. 22, 69-75

- LINLEY, J.R. (1966). The ovarian cycles of Culicoides barbosa
Wirth & Blanton and C. furens (Poey) (Diptera, Ceratopogonidae).
- Bull. ent. Res. 57, 1-17.
==
- LOGAN, J.A., WOLKIND, D.J., HOYT, S.C., & TANIGOSHI, L.K. (1976).
An analytical model for description of temperature-dependent
rate phenomena in arthropods. Environ. Entomol. 5, 1133-1140.
=
- LUFF, M.L. (1973). The annual activity pattern and life cycle of
Pterostichus madidus (F.) (Coleoptera, Carabidae). -
Entomol. Scand. 4, 259-273.
==
- LUFF, M.L. (1980). The biology of the ground beetle Harpalus rufipes
in a strawberry field in Northumberland. - Ann. appl. Biol.
94, 153-164.
=
- LUSIS, O. (1963). The histology and histochemistry of development
and resorption in the terminal oocytes of the desert locust
Schistocerca gregaria. Q. Jl microsc. Sci. 104, 57-68.
==
- LUZ, E., CONSOLIM, J., VIEIRA, A.M. & BORBA, A.M. (1979).
Some epidemiological aspects of the persistence of the
transmission of malaria on the Parana coast. I. Physiological
age of Anopheles cruzii (Diptera, Culicidae) [in Portuguese].
- Archos Biol. Tecnol., Curitiba 22, 63-88.
=
- MACKAY, P.A. & WELLINGTON, W.G. (1977). Maternal age as a source of
variation in the ability of an aphid to produce dispersing
forms. Res. Popul. Ecol. 18, 195-209.
==
- MACQUEEN, A., WALLACE, M.M.H. & DOUBE, B.M. (1984). Seasonal
changes in favourability of cattle dung in central Queensland
for three species of dung breeding insects. J. Aust. ent.
Soc. in press.

- MAELZER, D.A. (1961) The effect of temperature and moisture on the immature stages of Aphodius tasmaniae Hope (Scarabaeidae) in the lower south-east of South Australia. Aust. J. Zool. 9, 173-202.
- =
- MAGNARELLI, L.A. (1975). Life history and physiological age studies of Anopheles quadrimaculatus Say in central New York State. - Proc. N.J. Mosq. Control Assoc. 62, 83-89.
- MAGNARELLI, L.A. (1976). Physiological age of Tabanidae (Diptera) in eastern New York State, U.S.A. - J. med. Entomol. 12, 679-682.
- MAGNARELLI, L.A. (1977). Physiological age of mosquitoes (Diptera, Culicidae) and observations on partial blood feeding. - J. med. Entomol. 13, 445-450.
- MAGNARELLI, L.A. (1981). Parity, follicular development and feeding in Culicoides melleus and C. hollensis. - Environ. Entomol. 10, 807-811.
- =
- MAGNARELLI, L.A. & ANDERSON, J.F. (1977). Follicular development in salt marsh Tabanidae (Diptera) and incidence of nectar feeding with relation to gonotrophic activity. - Ann. ent. Soc. Am. 70, 529-533.
- MAGNARELLI, L.A. & ANDERSON, J.F. (1979). Oogenesis and oviposition in Chrysops atlanticus (Diptera, Tabanidae) - Ann. ent. Soc. Am. 72, 350-352.
- =
- MAGNARELLI, L.A. & CUPP, E.W. (1977). Physiological age of Simulium tuberosum and S. venustum in New York State, U.S.A. - J. med. Entomol. 4-5, 621-624.
- MAGNARELLI, L.A. & PECHUMAN, L.L. (1975). Ovarian studies of Tabanus quinquevittatus (Diptera: Tabanidae). - J. med. Entomol. 11, 687-690.
- =

- MAGNARELLI, L.A. & STOFFOLANO, J.G. (1980). Blood feeding, oogenesis and oviposition by Tabanus nigrovittatus in the laboratory.
- Ann. ent. Soc. Am. 73, 14-17.
- MAIL, T.S., CHADWICK, J. & LEHANE, M.J. (1983). Determining the age of adults of Stomoxys calcitrans (L.) (Diptera: Muscidae).
- Bull. ent. Res. 73, 501-525.
- MASAKI SINZO (1980). Summer Diapause. Ann. Rev. Entomol. 25, 1-25.
- MATTHIESSEN, J.N. & HYLES, L. (1983) Seasonal changes in characteristics of cattle dung as a resource for an insect in southwestern Australia. Aust. J. Ecol. 8, 9-16.
- MATTHEWS, E.G. (1972). A revision of the Scarabaeine dung beetles of Australia 1. Tribe Onthophagini. Aust. J. Zool. suppl. 9, 1-330.
- MENZEL, R., MOCH, K., WLADARZ, G. & LINDAUER M. (1969). Tageperiodische Ablagerungen in der Endokutikula der Honigbiene. -
Biol. Zbl. 1, 61-67.
- MER, G.G. (1936). Experimental study on the development of the ovary in Anopheles elatus Edw. (Diptera: Culicidae) -
Bull. ent. Res. 27, 351-359.
- MERCER, C.F. & KING, P.D. (1976). Ovarian development in black beetle, Heteronychus arator (Coleoptera: Scarabaeidae). -
N.Z. Ent. 6, 165-170.
- MILLER, T.A. & TREECE, R.T. (1968). Gonadotrophic cycles in the face fly, Musca autumnalis. - Ann. ent. Soc. Am. 61, 690-696.
- MIRZAEVA, A.G. (1974). The age composition of females of Culicoides sinanoensis Tok. from coniferous broad-leaved forests of south Maritime Territory [in Russian]. - Parazitologiya 8, 524-530.

- MIRZAEVA, A.G. (1980). Age structure and duration of the life cycle of Culicoides sinanoensis Tok. (Ceratopogonidae) [in Russian]. - Ekologiya 6, 87-90.
- MONTEITH, G.B. & STOREY, R.I. (1981) The biology of Cephalodesmus, a genus of dung beetles which synthesizes "dung" from plant material (Coleoptera : Scarabaeidae: Scarabaeinae.) Mem. Qld. Mus. 20 (2), 253-277.
- MOON, R. (1983). Simulating developmental time of preadult face flies (Diptera: Muscidae) from air temperature record. Environ. Entomol. 12 (3), 943-948.
- MOON, R.D. & KAYA, H.K. (1981). A comparison of methods for assessing age structure and abundance of populations of non-diapausing female Musca autumnalis (Diptera, Muscidae) - J. med. Entomol. 18, 289-297.
- MOON, R.D., LOOMIS, E.C. & ANDERSON J.R., (1980). The influence of two species of dung beetles on larvae of face fly. Environ. Entomol. 9, 607-612.
- MORDUE, W. (1965). Studies of oocyte production and associated histological changes in the neuroendocrine system in Tenebrio molitor L. J. Insect Physiol. 11, 493-503.
- MORGAN, P.B. (1973). Effect of aging and population density on wing fragmentation in houseflies. J. econ. Ent. 66, 993-995.
- MORKY, J.E. (1980a). A method for estimating the age of field-collected female Simulium damnosum s.l. (Diptera: Simuliidae) - Tropenmed. & Parasitol. 31, 128-130.
- MORKY, J.E. (1980b). Laboratory studies in blood feeding of blackflies (Diptera: Simuliidae). (2) Factors affecting fecundity . - Tropenmed. & Parasitol. 31, 374-380.

- MORRIS, C.D. & DEFOLIART G.R. (1971a). Seasonal parous rates in Hybomitra lasiophthalma (Diptera: Tabanidae). - J. med. Entomol. 8, 207-208.
- MORRIS C.D. & DEFOLIART, G.R. (1971b). Parous rates in Wisconsin mosquito populations. - J. med. Entomol. 8, 209-212.
- MOTTERSHEAD, B.E. (1971) Soil temperature at various depths under pasture and bare soil at Armidale, NSW. Fld. Stn. Rec. Div. Pl. Ind. CSIRO (Aust) 10, 5-12.
- MULLENS, B.A. & SCHMIDTMANN, E.T. (1982). The gonotrophic cycle of Culicoides variipennis (Diptera, Ceratopogonidae) and its implications in age-grading field populations in central New York State, U.S.A. - J. med. Entomol. 19, 340-349.
- MULLIGAN, H.W. & POTTS, W.H. (Eds.) (1970). The African trypanosomiasis. - 950 pp., London, Allen & Unwin.
- MURDOCH, W.W. (1966). Aspects of the population dynamics of some marsh carabidae. J. anim. ecol. 35, 127-156.
- NAYAR, J.K. & KNIGHT J.W. (1981). Ovarian development in Culex nigripalpus Theobald (Diptera: Culicidae) and its implication in disease transmission. - Entomologia exp. appl. 29, 49-59.
- NAYAR, J.K. & SAUERMAN, D.M. (1975). The effects of nutrition on survival and fecundity in Florida mosquitoes III. Utilisation of blood and sugar for fecundity. - J. med. Entomol. 12, 220-225.
- NEALIS, V.G., JONES, R.E. & WELLINGTON W.G. (1984). Temperature and development in host-parasite relationships. Oecologia (Berlin) 61, 224-229.
- NELSON, R.L. & SCRIVANI, R.P. (1972). Isolation of arboviruses from parous midges of the Culicoides variipennis complex, and parous rates in biting populations. - J. med. Entomol. 9, 277-281.

- NEVILLE, A.C. (1963). Daily growth layers for determining the age of grasshopper populations. - Oikos 14, 1-8.
- NEVILLE, A.C. (1965). Circadian organisation of chitin in some insect skeletons. - Q. Jl. microsc. Sci. 106, 315-325.
- NEVILLE, A.C. (1967). Daily growth layers in animals and plants. - Biol. Rev. 42, 421-441.
- NEVILLE, A.C. (1983). Daily cuticular growth layers and the teneral stage in adult insects: a review. - J. Insect Physiol. 29, 211-219.
- NICHOLSON, A.J. (1921). The development of the ovary and ovarian egg of a mosquito, Anopheles maculipennis Meig. - Q. Jl. microsc. Sci. 65, 395-448.
- ODA, T., WADA, Y. & MORI, A. (1978). Follicular degeneration in unfed nulliparous females of Culex tritaeniorhynchus. - Trop. Med. (Nagasaki) 20, 113-122.
- OKIWELU, S.N. (1976). Seasonal variations in age-composition and survival of a natural population of Glossina morsitans morsitans Westwood at the Chakwenga Game Reserve, Republic of Zambia. - Zambia J. Sci. Technol. 1, 48-58.
- OVAZZA, M., COZ, J., & OVAZZA, L. (1965). Etudes des populations de Simulium damnosum Theobald 1903 (Diptera, Simuliidae) en zones de gites non permanents: I. Observations sur les variations de quelques-uns des caractères utilisés dans l'estimation de l'âge physiologique. - Bull. Soc. Path. exot. 58, 938-950.

- PAJOT, F.X. (1976). Aspects physiologiques impliqués dans l'étude
écologique des femelles d'Aedes (Stegomyia) simpsoni
(Theobald, 1905) (Diptera, Culicidae): age physiologique,
cycle gonotrophique, fécondité, longévité. -
Cah. ORSTOM Ser. Ent. med. Parasit. 14, 271-291.
==
- PAN SHI-QING, HO KWEI-MING & LI SHU-TAO (1983). Determination of
calendar age of Anopheles sinensis by cuticular daily growth
line. - Acta ent. sin. 26, 188-192.
==
- PERROTT, D.C.F., & STOCKDILL, S.M.J. (1973). Variation in the life
cycle of the Scarabaeid Costelytra zealandica. N.Z. Entomologist
5, 216-21.
=
- PETERS, H. & McNATTY, K.P. (1980). The ovary. - 175 pp.
London, Toronto, Sydney, New York, Paul Elek Granada Pub.
- PHELPS, R.J. & VALE, G.A. (1978). Studies on populations of
Glossina morsitans morsitans and G. pallidipes (Diptera, Glossinidae)
in Rhodesia. - J. appl. Ecol. 15, 743-760.
==
- PHIPPS, J. (1966). Ovulation and oocyte resorption in Acridoidea
(Orthoptera). - Proc. R. ent. Soc. Lond. (A) 41, 78-86.
==
- POLOVODOVA, J.P. (1949). Determination of the physiological age of
female Anopheles [in Russian]. - Medskaya Parazit. 18, 352-355.
==
- POTTER, H.W. & AKEY, D.H. (1978). Pigmentation associated with
oogenesis in the biting midge Culicoides variipennis:
changes in abdominal tergite patterns. Mosquito News 38,
499-504.
==

- RACHOU, R.G., SCHINAZI, L.A. & LIMA, M.M. (1973). An intensive epidemiological study of the causes for the failure of residual DDT spraying to interrupt the transmission of malaria in Atalaya and Falla, two villages on the coastal plain of El Salvador, Central America. - Revta bras. Malar. Doenc. trop. 25, 1-293.
- RAFAEL, J.A. & CHARLWOOD, J.D. (1980). Physiological age, seasonal variation and daily periodicity in four populations of Tabanidae (Diptera) on the University Campus, Manaus, Brazil [in Portugese]. - Acta Amazonica 10, 907-927.
- RAJAGOPALAN, P.K., BAI, M.G. & ARUNACHALAM, N. (1981). Age determination of man-biting populations of Culex pipiens fatigans with particular reference to transmission of Wuchereria bancrofti in Pondicherry. - Indian J. med Res. 73, 739-745.
- RAMIREZ-PEREZ, J., RASSI, E., CONVIT, J. & RAMIREZ, A. (1976). Epidemiological importance of age groups in colonies of Simulium metallicum (Diptera: Simuliidae) in Venezuela [in Spanish]. - Boln Of. sanit. pan-am. 80, 105-122.
- REISEN, W.K. & ASLAMKHAN, M. (1979). A release-recapture experiment with the malaria vector, Anopheles stephensi Liston, with observations on dispersal, survivorship, population size, gonotrophic rhythm and mating behaviour. - Ann. trop. Med. Parasit. 73, 251-269.

- REISEN, W.K., MAHMOOD, F. & AZRA, K. (1981). Anopheles culicifacies Giles: adult ecological parameters measured in rural Punjab Province, Pakistan, using capture-mark-release-capture and dissection methods, with comparative observations on An. stephensi Liston and An. subpictus Grassi. - Researches. Popul. Ecol. 23, 39-60.
- REISEN, W.K., MAHMOOD, F. & PARVEEN, T. (1979). Anopheles subpictus Grassi: observations on survivorship and population size using mark-release capture and dissection methods. - Researches. Popul. Ecol. 21, 12-29.
- RICHTER, P.O. AND BAKER, C.W. (1974). Ovariole numbers in Scarabaeoidea (Col : Lucanidae, Passalidae, Scarabaeidae). Proc. Ent. Soc. Washington 76 (4), 480 - 494.
- RIDSDILL SMITH, T.J.(1981). Some effects of three species of dung beetles (Coleoptera : Scarabaeidae) in south-western Australia on the survival of the bush fly Musca vetustissima Walker (Diptera: Muscidae) in dung pads. Bull. entomol. Res. 71, 425-433
- RIDSDILL SMTIH, T.J., HALL, G.P. and GRAIG, G.F. (1982) Effect of population density on reproduction and dung dispersal by the dung beetle Onthophagus binodis in the laboratory. Ent. Exp. & Appl. 32, 80-85.
- RING, R.A. (1967). Maternal induction of diapause in the larvae of Lucilia caesar L. (Diptera, Calliphoridae). J. exp. Biol. 46, 123-136.
- ROCKEL, E.G. (1969). Autogeny in the deer fly Chrysops fuliginosus (Diptera: Tabanidae). - J. med. Entomol. 6, 140-142.

- ROSAY, B. (1961). Anatomical indicators for assessing the age of mosquitoes: the teneral adult (Diptera: Culicidae). - Ann. ent. Soc. Am. 54, 526-529.
- ROSAY, B. (1969a). Anatomical indicators for assessing the age of mosquitoes: changes in ovarian follicles. - Ann. ent. Soc. Am. 62, 605-611.
- ROSAY, B. (1969b). Anatomical indicators for assessing the age of mosquitoes: dissection techniques and field application of methods. Mosquito News 29, 419-423.
- ROUGON, D. and ROUGON, C. (1982) Le comportement nidificateur d'Onitis alexis Klug en region Sahélienne. Bull. Soc. entomol. France 87, 15 - 19.
- RYAN, L., MOLYNEUX, D.H. & KUZOE, F.A.S. (1980). Differences in rate of wing fray between Glossina species. Tropenmed. & Parasitol. 31, 111-116.
- RYAN, R.B. (1965). Material influence on diapause in a parasitic insect, Coeloides brunneri Vier (Hymenoptera, Braconidae) J. Insect Physiol. 11, 1331 - 1336.
- SAMARAWICKREMA, W.A. (1962). Changes in the ovariole of Mansonia (Mansonioides) mosquitoes in relation to age determination. - Ann. trop. Med. Parasit. 56, 110-126.
- SANDS, P. AND HUGHES, R.D. (1976) A simulation model of seasonal changes in the value of cattle dung as a food resource for an insect. Agric. Meteorology 17, 161-183.
- SANDS, P. AND HUGHES, R.D. (1977) Mathematical model of the survival rates of female bushflies (M. vetustissima Walker)(Diptera: Muscidae) as inferred from field populations. Bull. ent. Res. 67, 675 - 683.

- SAUNDERS, D.S. (1960). The ovulation cycle in Glossina morsitans Westwood (Diptera, Muscidae) and a possible method of age determination for female tsetse flies by an examination of the ovaries. - Trans. R. ent. Soc. Lond. 112, 221-238.
- SAUNDERS, D.S. (1962). Age determination for female tsetse flies and the age compositions of samples of Glossina pallidipes Aust., G. palpalis fuscipes Newst. and G. brevipalpis Newst. - Bull. ent. Res. 53, 579-595.
- SAUNDERS, D.S. (1965). Larval diapause of maternal origin: Induction of diapause in Nasonia vitripennis (Walk.)(Hymenoptera: Pteromalidae). J. exp. Biol. 42, 495-508.
- SAUNDERS, D.S. (1966). Larval diapause of maternal origin - II. The effect of photoperiod and temperature on Nasonia vitripennis. J. Insect Physiol. 12, 569-581.
- SAUNDERS, D.S. (1967). Survival and reproduction in a natural population of tsetse fly Glossina palpalis palpalis (Robineau-Desvoidy). - Proc. R. ent. Soc. Lond. (A) 42, 129-137.
- SAUNDERS, D.S. (1972). The effect of starvation on the length of the interlarval period in the tsetse fly Glossina morsitans orientalis Vanderplank. - J. Entomol. (A) 46, 197-202.
- SAUNDERS, D.S. (1976) Insect Clocks. - 279pp. Oxford, Pergamon Press.
- SCHLEIN, Y. (1972a). Postemergence growth in the fly Sarcophaga falculata initiated by neurosecretion from the ocellar nerve. - Nature New Biol. 235, 217-219.
- SCHLEIN, Y. (1972b). Factors that influence the post-emergence growth in Sarcophaga facultata. - J. Insect. Physiol. 18, 199-209.

- SCHLEIN, Y. (1975). Effect of u.v. light and temperature on the melanization and the formation of daily growth layers of Sarcophaga falculata. - J. Insect Physiol. 21, 1859-1863.
- SCHLEIN, Y. (1979a). Age grouping of anopheline malaria vectors (Diptera: Culicidae) by the cuticular growth lines. - J. med. Entomol. 16, 502-506.
- SCHLEIN, Y. (1979b). Age grading of tsetse flies by the cuticular growth layers in the thoracic phragma. - Ann. trop Med. Parasit. 73, 297-298.
- SCHLEIN, Y. & GRATZ, N.G. (1972). Age determination of some flies and mosquitoes by daily growth layers of skeletal apodemes. - Bull. Wld Hlth Org. 47, 71-76.
- SCHLOTTMAN, L.L. & BONHAG, P.F. (1956). Histology of the ovary of the adult mealworm Tenebrio molitor L. (Coleoptera: Tenebrionidae) - Univ. Calif. Publs Ent. 11, 351-394.
- SCHMIDT, C.D. (1972). Classification of the physiological development of laboratory-reared female hornflies Haematobia irritans (L.) - Ann. ent. Soc. Am. 65, 695-701.
- SCHMIDTMANN, E.T. & WASHINO, R.K. (1982). Gonotrophic age structure of host-seeking Leptoconops carteri (Diptera: Ceratopogonidae) populations in North-Central California. Ann. ent. Soc. Am. 75, 507-512.
- SCHOLL, P.J. (1980). A technique for physiologically age-grading female stable flies Stomoxys calcitrans (L.) - Res. Bull. Neb. agric. Exp. Stn no.298, 1-28.
- SELF, L.S. & SEBASTIAN, A. (1971). A high incidence of green colouration in newly emerged adult populations of Culex pipiens fatigans in Rangoon, Burma. - J. med Entomol. 8, 391-393.

- SERVICE, M.W. (1969). Observations on the ecology of some British mosquitoes. - Bull. ent. Res. 59, 161-194.
- SERVICE, M.W. (1973). Observations on the flight activities of Chrysops caecutiens L. - Ann. trop. Med. Parasit. 67, 445-454.
- SERVICE, M.W. (1976). Mosquito ecology: field sampling methods. - 583 pp. London, Applied Science Publishers.
- SHALABY, A.M. (1962). An alternative fluid medium for age grading dissections. - WHO/Mal/348, Suppl. 2, 8-9.
- SHALABY, A.M. (1972). Age grouping investigations on Anopheles subpictus subpictus Grassi and A. annularis Wulp. (Diptera, Culicidae). Bull. Soc. ent. Egypte 55, 297-304.
- SHARMA, S.P., JIT, I. & SHARMA G. (1983). Age-related lipid changes in Callosobruchus maculatus (Coleoptera) and Zaprionus paravittiger (Diptera). - Acta ent. bohemoslov. 80, 336-340.
- SHARPE, P.J.H., CURRY, G.L., DE MICHELE, D.W. AND COLE, C.L. (1977). Distribution model of organism development times. J. Theor. Biol. 66, 21-38
- SHELDAHL, J.A. & TAPPEL, A.L. (1974). Fluorescent products from aging Drosophila melanogaster: an indicator of free radical lipid peroxidation damage. - Exp. Gerontol. 9, 33-41.
- SINGH, T. (1958). Ovulation and corpus luteum formation in Locusta migratoria migratorioides Reiche & Fairmaire and Schistocerca gregaria (Forsk.) - Trans. R. ent. Soc. Lond. 110, 1-20.
- SKIERSKA, B. (1974). Ceratopogonidae de la cote de la Pologne. - Annls Parasit. hum. comp. 49, 641-643.

- SMITH, T.A. (1968). Comparison of known age with physiological aging in the adult female housefly Musca domestica L. - J. med. Entomol. 5, 1-4.
- SNOW, W.F., & WILKES, T.J. (1977). Age composition and vertical distribution of mosquito populations in the Gambia, West Africa. - J. med. Entomol. 13, 507-513.
- SOHAL, R.S. (1973). Fine structural alterations with age in the fat body of the adult male housefly Musca domestica. - Z. Zellforsch. 140, 169-175.
- SOHAL, R.S. & ALLISON, V.F. (1971). Senescent changes in the cardiac myofiber of the housefly, Musca domestica: an electron microscope study. - J. Gerontol. 26, 490-496.
- SOHAL, R.S. & DONATO, H. (1978). Effects of experimentally altered life spans on the accumulation of fluorescent age pigment in the housefly Musca domestica. - Exp. Gerontol. 13, 335-341.
- SOHAL, R.S. & DONATO, H. (1979). Effect of experimental prolongation of life span on lipofuscin content and lysosomal enzyme activity in the brain of the housefly Musca domestica. - J. Geront. 34, 489-496.
- SOHAL, R.S., PETERS, P.D. & HALL, T.A. (1977). Origin, structure, composition and age-dependence of mineralised dense bodies (concretions) in the midgut epithelium of the adult housefly, Musca domestica. - Tissue Cell 9, 87-102.
- SOHAL, R.S. & SHARMA, S.P. (1972). Age-related changes in the fine structure and number of neurons in the brain of the housefly, Musca domestica. - Exp. Gerontol. 7, 243-249.
- SOUTHWOOD, T.R.E. (1978). Ecological methods. - 524 pp. London. Chapman & Hall.

- SPENCER, M. (1974). A simple field method for assessing the proportion of older parous females in an anopheline sample (Anopheles farauti). - Trans. R. Soc. trop. Med. Hyg. 68, 15.
- SPENCER, M. (1979). Age grouping of female Anopheles farauti populations (Diptera, Culicidae) in Papua New Guinea. - J. med. Entomol. 15, 555-569.
- SPENCER, M. & CHRISTIAN, S.H. (1969). Cyclic ovariole changes in Anopheles farauti Laveran (Diptera: Culicidae) in Papua New Guinea. - J. Aust. entomol. Soc. 8, 16-20.
- SPRADBERY, J.P. & SANDS, D.P.A. (1976). Reproductive system and terminalia of the Old World screw-worm fly, Chrysomya bezziana Villeneuve (Diptera: Calliphoridae). - Int. J. Insect Morphol. & Embryol. 5, 409-421.
- SUTHERLAND, B. (1980). Physiological age determination in female Stomoxys calcitrans Linnaeus (Diptera: Muscidae). - Onderstepoort J. vet. Res. 47, 83-88.
- TAYLOR, F. (1981) Ecology and evolution of physiological time in insects. Am. Nat. 117, 1-23.
- TAYLOR, P. (1979). The construction of a life table for Glossina morsitans morsitans Westwood (Diptera, Glossinidae) from seasonal age measurements of a wild population. - Bull. ent. Res. 69, 553-560.
- THIELE, H.U. (1966). Einflüsse der Photoperiode auf die Diapause von carabiden. Z. angew. Ent. 58, 143- 149.
- THIELE, H.U. (1977). Carabid beetles in their environments. - 369pp., Berlin, Springer-Verlag.

- THOMAS, A.W. (1972). Physiological age structure of adult tabanid populations (Diptera: Tabanidae) in Alberta, Canada. - J. med. Entomol. 9, 295-300.
- THOMAS, A.W. (1973). Follicle developmental stages in blood-seeking horseflies (Diptera: Tabanidae) in Alberta, Canada. - J. med. Ent. 10, 325-328.
- THOMPSON, P.H., HOLMES, J.W. Jr. & ARAUJO, T.I. (1979). Determination of parity in populations of dominant Tabanus species, (Diptera: Tabanidae) in southeast Texas U.S.A. - Southwest Ent. 4, 181-191.
- TROUBRIDGE, D.A. & DAVIES, D.M. (1975). Seasonal changes in physiological age composition of tabanid (Diptera) populations in southern Ontario. - J. med Entomol. 12, 453-457.
- TRUEMAN, D.W. & McIVER, S.B. (1983). Seasonal incidence of single and multiple parity in Mansonia perturbans (Diptera: Culicidae). - Can. Ent. 115, 435-438.
- TYNDALE-BISCOE, M. (1978). Physiological age-grading in females of the dung beetle Euoniticellus intermedius (Reiche)(Coleoptera: Scarabaeidae). - Bull. ent. Res. 68, 207-217.
- TYNDALE-BISCOE, M. (1983). Effects of ovarian condition on nesting behaviour in a brood-caring dung beetle Copris diversus Waterhouse (Coleoptera: Scarabaeidae). - Bull. ent. Res. 73, 45-52.
- TYNDALE-BISCOE, M. & HUGHES, R.D. (1969). Changes in the female reproductive system as age indicators in the bushfly Musca vetustissima Wlk. - Bull. ent. Res. 59, 129-141.
- TYNDALE-BISCOE, M. & KITCHING R.L. (1974). Cuticular bands as age criteria in the sheep blowfly Lucilia cuprina (Wied.) (Diptera: Calliphoridae). - Bull. ent. Res. 64, 161-174.

- TYNDALE-BISCOE, M. & LOPEZ-GUERRERO, Y. (1982). Egg resorption in Phanaeus daphnis Harold (Coleoptera: Scarabaeidae). - Folia ent. Mex. 52, 28-39.
- TYNDALE-BISCOE, M., WALLACE, M.M.H. AND MORTON, R. (1981a). Arthropod-induced mortality in immature stages of the bushfly, Musca vetustissima Walker (Diptera: Muscidae) Bull. ent. Res. 71, 681-690.
- TYNDALE-BISCOE, M., WALLACE M.M.H. & WALKER, J.M. (1981b). An ecological study of an Australian dung beetle, Onthophagus granulatus Boheman (Coleoptera: Scarabaeidae), using physiological age-grading techniques. - Bull. ent. Res. 71, 137-152.
- TYNDALE-BISCOE, M. & WATSON, J.A.L. (1977). Extra-ovariolar egg resorption in a dung beetle Euoniticellus intermedius. - J. Insect Physiol. 23, 1163-1167.
- UNGUREANU, E.M. (1974). Population dynamics and age-grading of mosquitos. - Bull. Wld Hlth Org. 50, 317-321.
- UVAROV, B. (1966). Grasshoppers and locusts. A handbook of general acridology. Vol. 1. Anatomy, development phase polymorphism, introduction to taxonomy. - 481 pp. Cambridge, Univ. Press.
- USINGER, R.L. (1966). Monograph of Cimicidae. (Thomas Say Foundation, Vol. 7). - 585 pp. College Park, Maryland, Ent. Soc. Am.
- VALE, G.A., HARGROVE, J.W., JORDAN, A.M., LANGLEY, P.A. & MEWS, A.R. (1976). Survival and behaviour of tsetse flies (Diptera: Glossinidae) released in the field: a comparison between

wild flies and animal fed and in-vitro fed laboratory reared
flies. Bull. ent. Res. 66, 731-744

VAN DIJK, T.S. (1979). On the relationship between reproduction,
age and survival in two carabid beetles, Calathus melanocephalus L.
and Pterostichus coerulescens L. (Coleoptera, Carabidae) -
Oecologia (Berl.) 40, 63-80.

VLIJM, L. & VAN DIJK, T.S. (1967). Ecological studies on carabid
beetles. II. General pattern of population structure in
Calathus melanocephalus (L.) at Schiermonnikoog. -
Z. Morph. Okol. Tiere 58, 396-404.

VOGT, W.G., WOODBURN, T.L., MORTON, R. & ELLEM, B.A. (1983). The
analysis and standardisation of trap catches of Lucilia cuprina
(Wiedeman) (Diptera: Calliphoridae). - Bull. ent. Res. 73,
609-617.

VOGT, W.G., WOODBURN, T.L. & TYNDALE-BISCOE, M. (1974). A method
of age determination in Lucilia cuprina (Wied.) (Diptera:
Calliphoridae) using cyclic changes in the female reproductive
system. - Bull. ent. Res. 64, 365-370.

WALDBAUER, G.P. (1978) Phenological adaptation and the polymodel
emergence patterns of insects. In: Evolution of Insect
Migration and Diapause ed. H. Dingle pp. 127-144. New York-
Heidelberg - Berlin : Springer 284 pp.

WALKER, A.R. (1977). Adult lifespan and reproductive status of
Culicoides species (Diptera: Ceratopogonidae) in Kenya, with
reference to virus transmission. - Bull. ent. Res. 67, 205-215.

- WALL, W.J. & DOANE, O.W. Jr. (1980). Large scale use of box traps to study and control saltmarsh greenhead flies (Diptera, Tabanidae) on Cape Cod, Massachusetts. - Environ. Entomol. 9, 371-375.
- WALLACE, M.M.H AND TYNDALE-BISCOE, M. (1983). Attempts to measure the influence of dung beetles (Coleoptera: Scarabaeidae) on the field mortality of the bush fly Musca vetustissima Walker (Diptera: Muscidae) in south-eastern Australia. Bull. ent. Res. 73, 33 - 44.
- WALOFF, N. (1958). Some methods of interpreting trends in field populations. - Proc. X Int. Congr. Ent. Montreal 1956, 2, 675-676.
- WATERHOUSE, D.F. (1974). The biological control of dung. Sci. Am. 230, 100-109.
- WELCH, S.M., CROFT, B.A., BRUNNER, J.F. AND MICHELS, M.F. (1978). PETE : an extension phenology modeling system for management of multi-species pest complexes. Environ. Entomol. 7, 487-494.
- WHITTEN, M.J., FOSTER, G.G., VOGT, W.G., KITCHING, R.L., WOODBURN, T.L. & KONOVALOV C. (1977). Current status of genetic control of the Australian sheep blowfly Lucilia cuprina (Wiedemann) (Diptera: Calliphoridae). - Proc. XV Int. Congr. Ent., 129-139.
- (WORLD HEALTH ORGANIZATION) (1975a). Manual on practical entomology in malaria. WHO Offset Publ. 3 (I), 160 pp.
- (WORLD HEALTH ORGANIZATION) (1975b). Manual on practical entomology in malaria. WHO Offset Publ. 3 (II), 191 pp.
- WILKES, T.J. (1968). The application of Polovodova's technique to the study of Anopheles melas. - Trans. R. Soc. trop. Med. Hyg. 62, 470.

- WILKES, T.J. & CHARLWOOD, J.D. (1979). A rapid gonotrophic cycle in Chagasia bonneae from Brazil. - Mosquito News. 39, 137-139.
- WILKES, T.J. & RIOUX J.A. (1980). The application of Polovodova's technique for the age determination of Phlebotomus (Larrousius) ariasi. - Trans. R. Soc. trop. Med. Hyg. 74, 119
- WILLIMZIK, E. (1930). Über den Bau der Ovarien verschiedener Coprophager Lamellicornier und ihre Beziehung zur Brutpflege. - Z. Morph. Okol. Tiere 18, 669-700.
- WOODBURN, T.L., VOGT, W.G. & KITCHING, R.L. (1978). Estimation of age of females in field populations of Lucilia cuprina (Wiedemann) (Diptera: Calliphoridae) using ambient temperatures and solar radiation. - Bull. ent. Res. 68, 251-261.
- YAJIMA, T. (1970). A note on the formation of the 'false' dilatation in Culex tritaeniorhynchus summorosus Dyar. - Jap. J. sanit. Zool. 21, 224-225.
- YURGENSON, I.A. & TEPLYKH, V.S. (1972). The application of luminescence microscopy to the determination of the physiological age of insects (Aedes aegypti (L.) and Ctenophthalmus orientalis (Wagn.)). - Proc. XIII Int. Congr. Ent. Moscow 1968, 3, 277-278.
- ZAR, J.H. (1984). Biostatistical Analysis. 2nd. ed. - 718 pp, New York, Prentice-Hall, Inc.
- ZELAZNY, B. (1975). Behaviour of young rhinoceros beetles, Oryctes rhinoceros. - Entomologia exp. appl. 18, 135-140.
- ZELAZNY, B. & NEVILLE A.C. (1972). Endocuticle layer formation controlled by non-circadian clocks in beetles. - J. Insect. Physiol. 18, 1967-1979.

- ZHAROV, A.A. (1980). On a method for determining the actual fertility of females of blood sucking mosquitoes as exemplified by Aedes vexans Meigen (Diptera, Culicidae) [in Russian]. - Medskaya Parazit. 49, 19-24.
- ZHUKOVSKY, A.V. (1950). Diapause of the larvae of the Hessian fly. Dokl. Vses. Akad. Skh. Nauk. 15, 26-29.



a I



b I

Fig. 1 a. *Onthophagus granulatus* Boheman and b. *Onitis alexis* Klug.
Scale shows actual size.

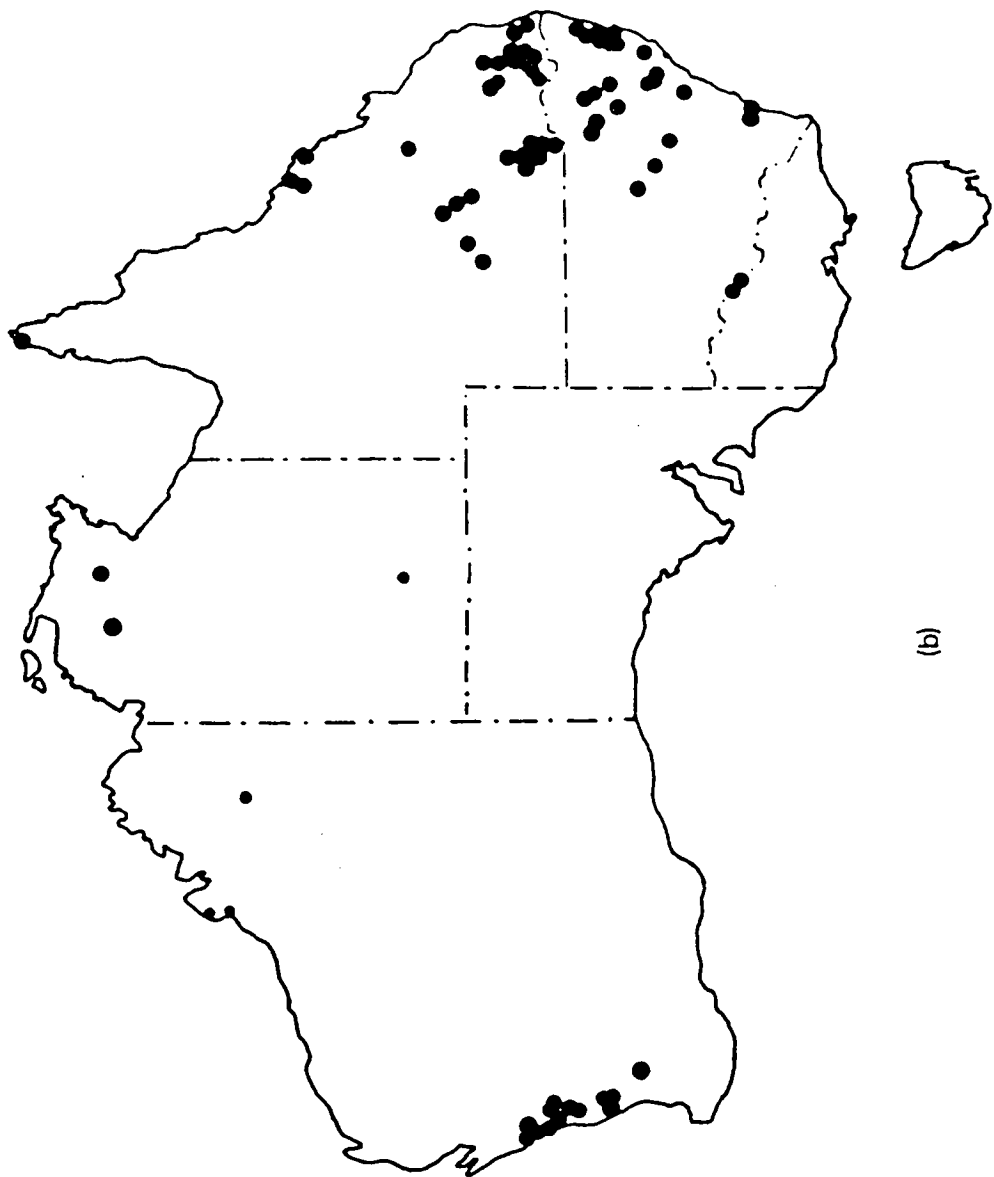
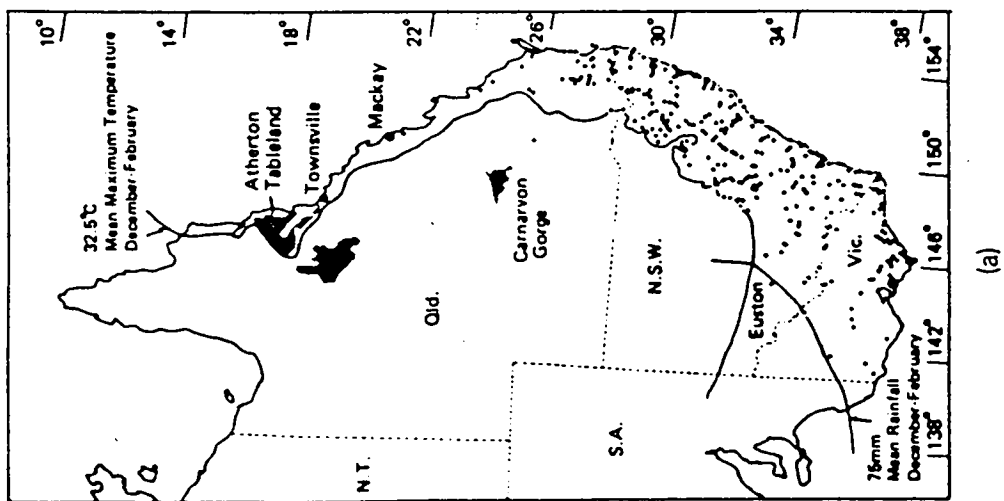


Fig. 2 a) Distribution of *Ot. granulatus* ; b) Present distribution of *Ol. alexis* in Australia

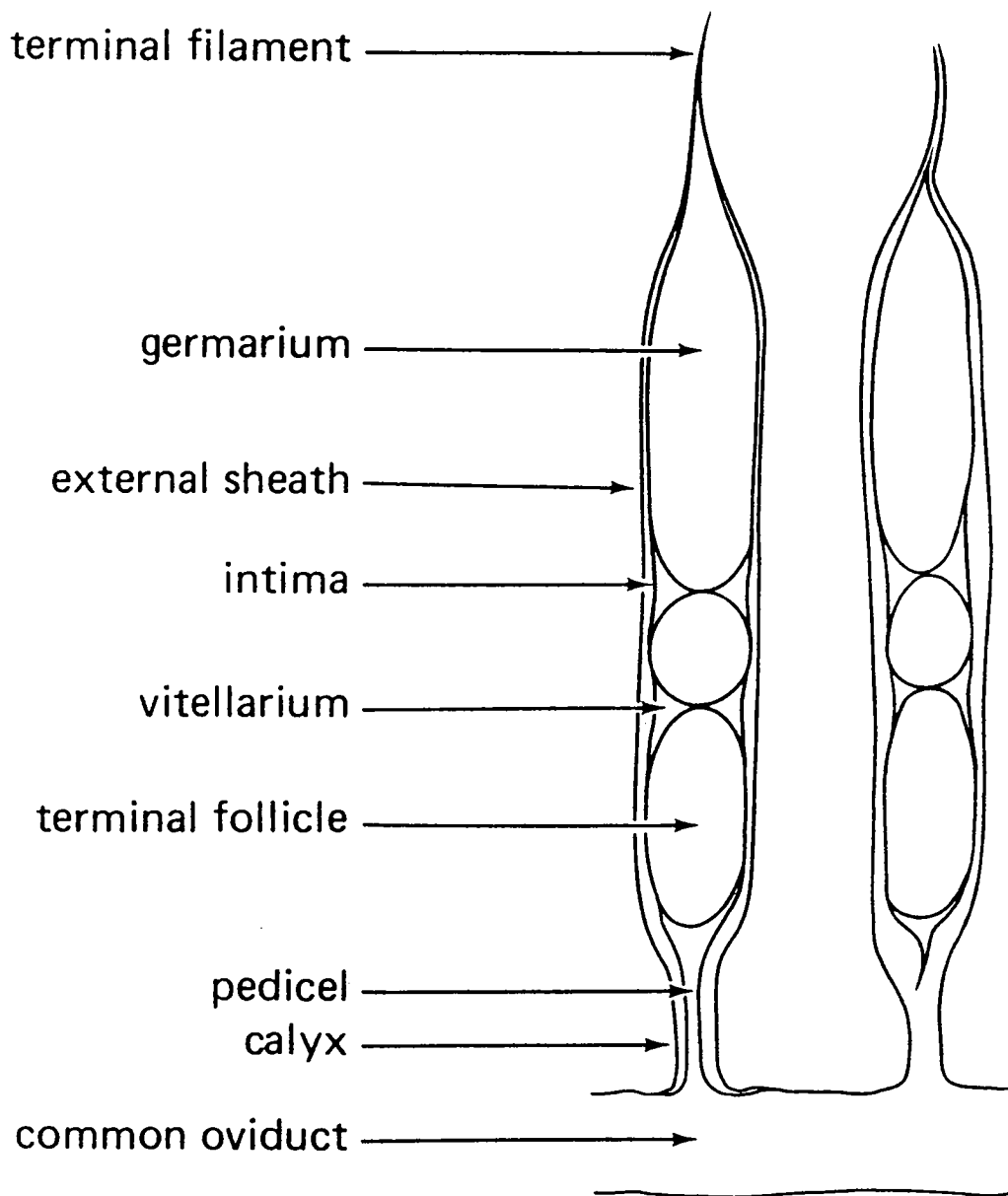


Fig. 3 Schematic representation of an insect ovary, showing the terminology used in this review.

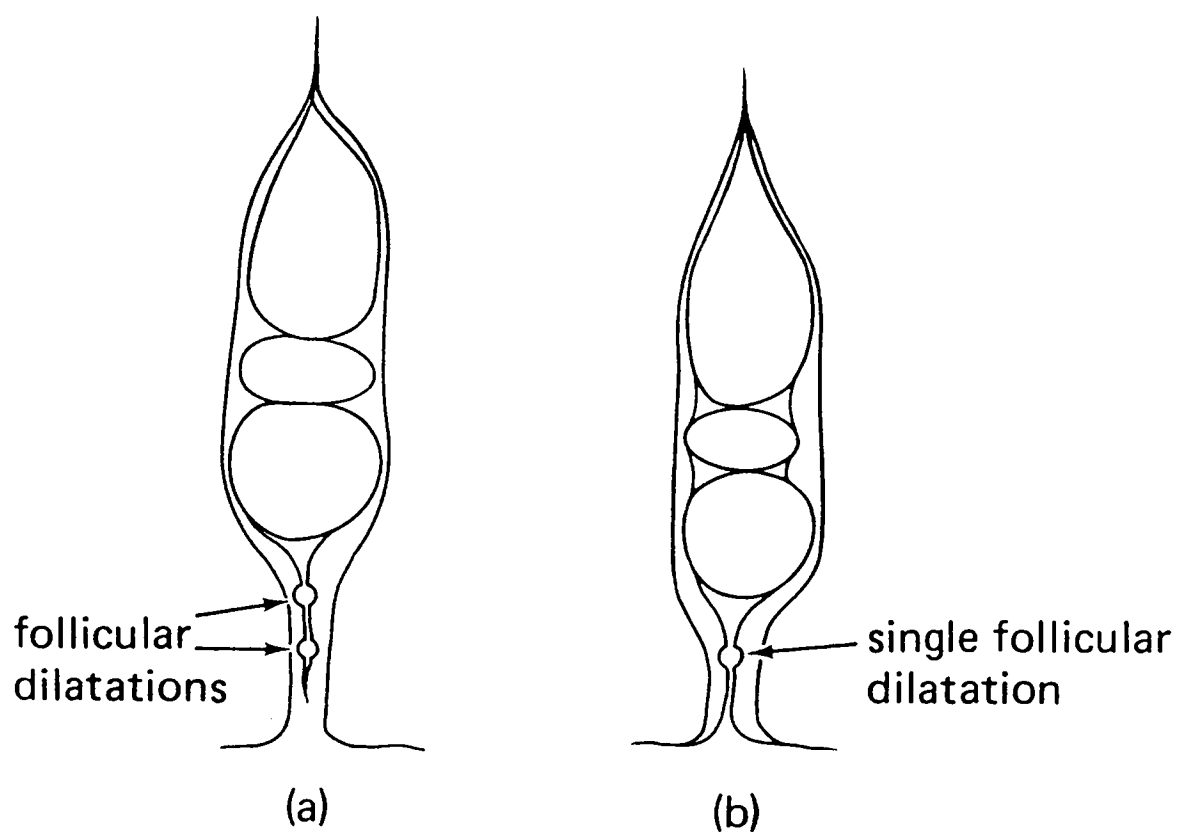


Fig. 4 Generalised insect ovary with a) follicular dilatations and b) a single follicular dilatation.

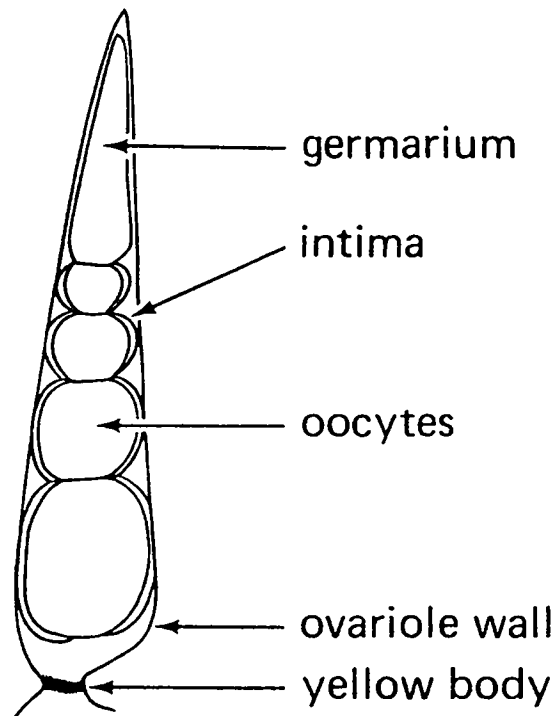


Fig. 5 Generalised insect ovary with yellow body

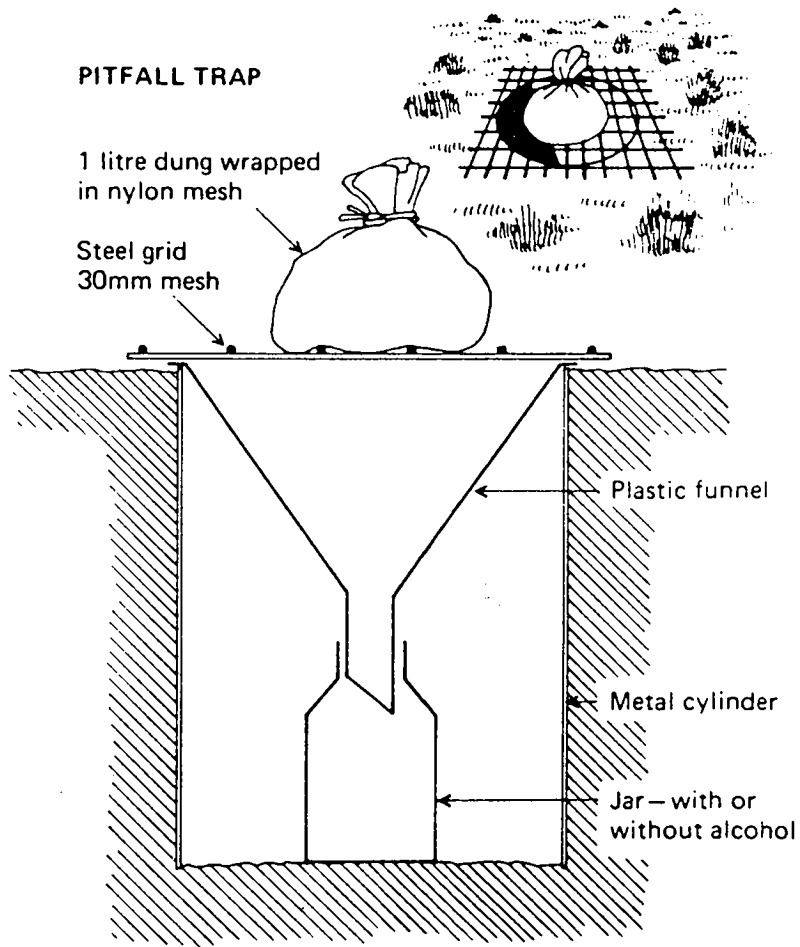


Fig. 6 Pitfall trap

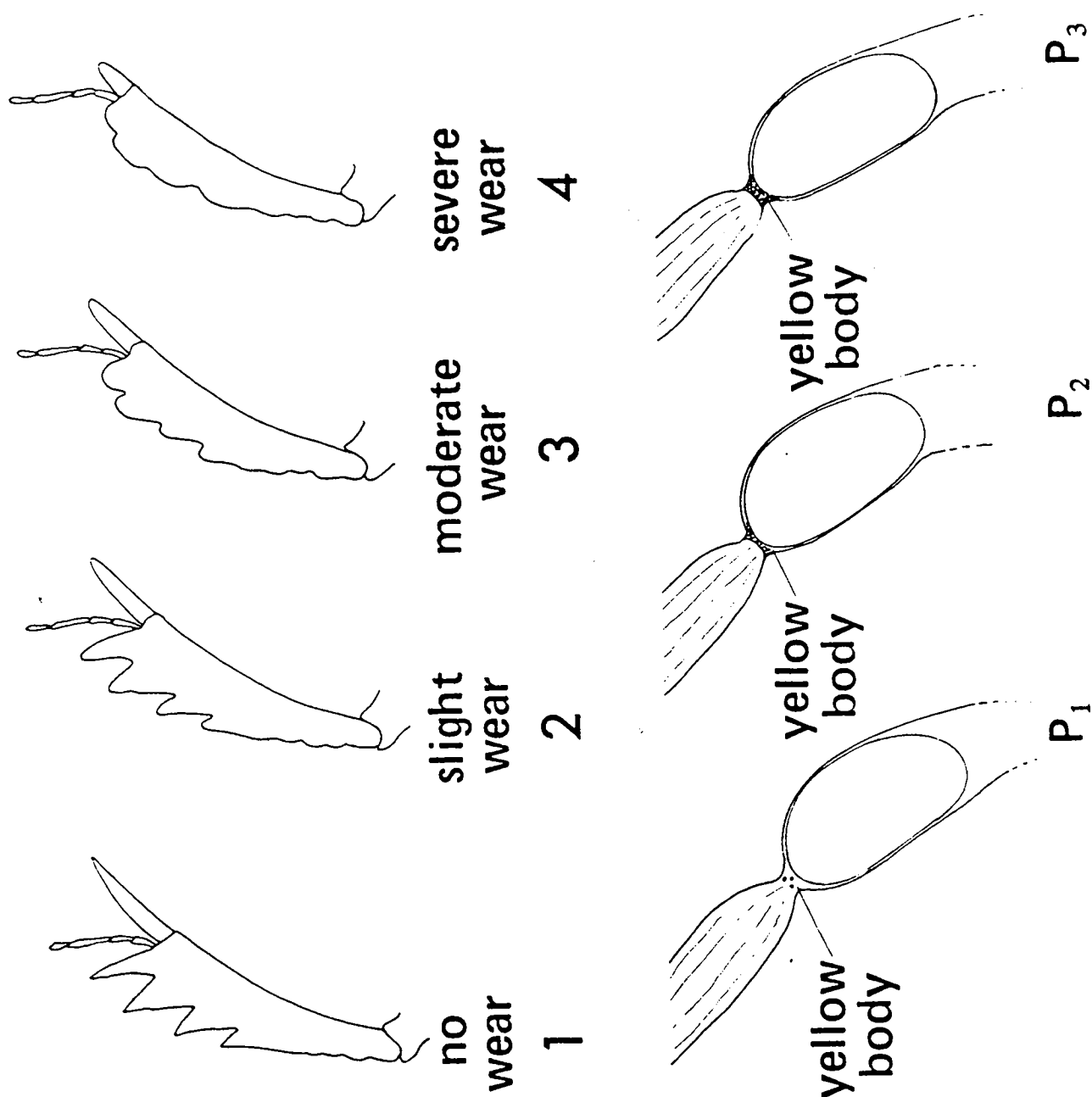


Fig. 7 Different degrees of tibial wear, and size of yellow body characteristic of Oniticellus intermedius (from Tyndale-Biscoe 1978).

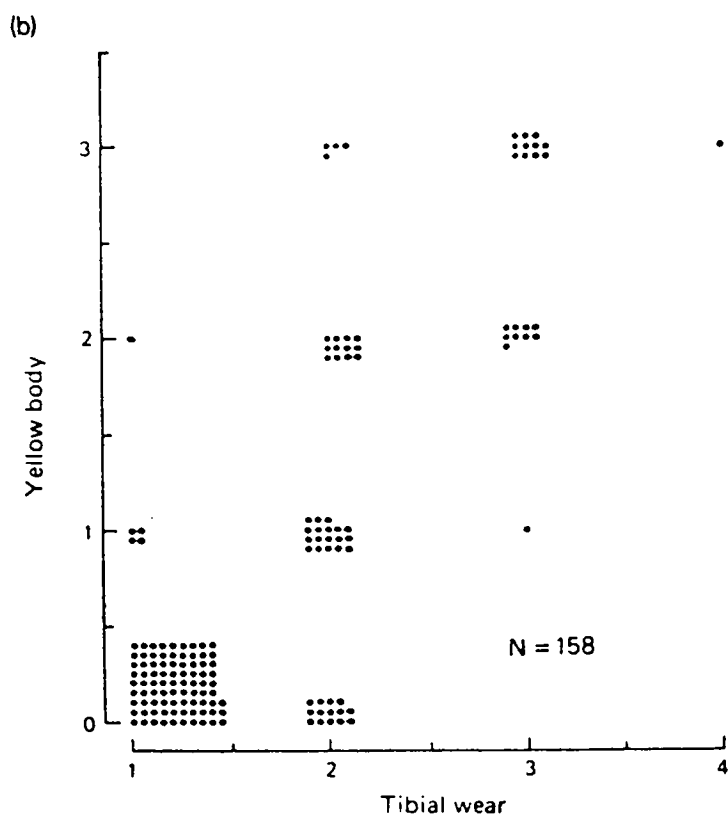
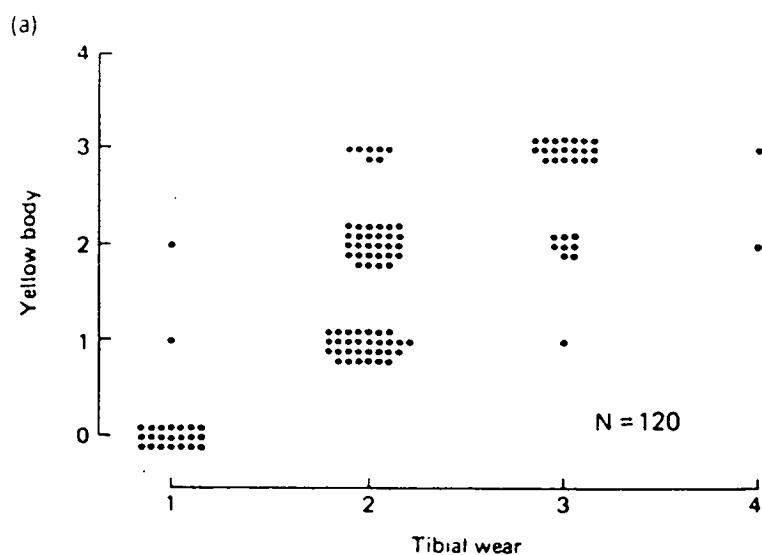
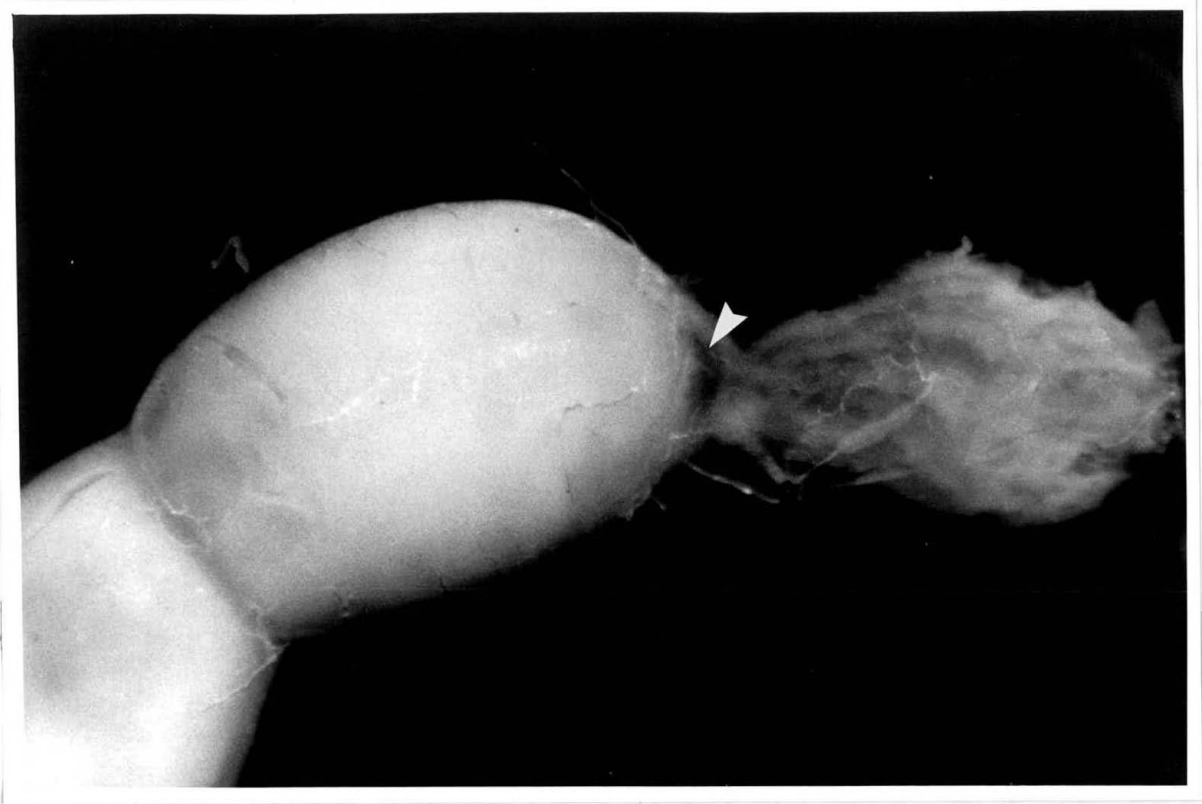
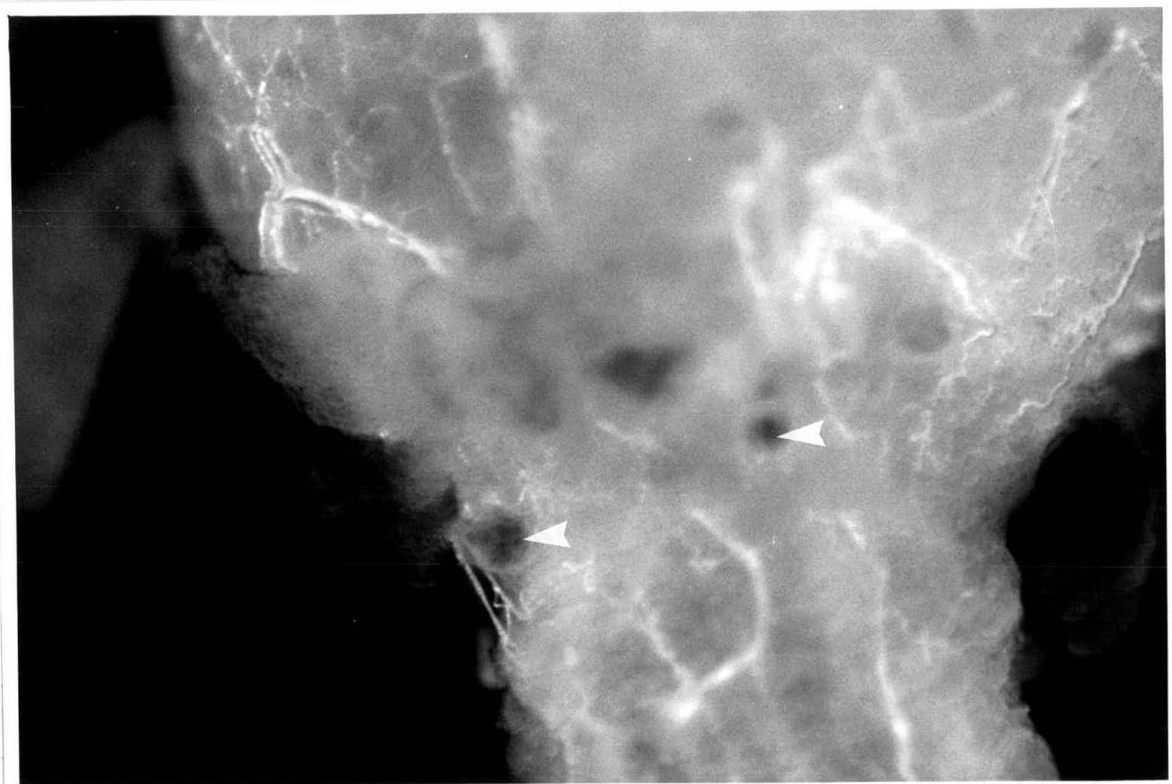


Fig. 8 Relationship between yellow body and tibial wear in
a) Ot. granulatus and b) Ol. alexis.

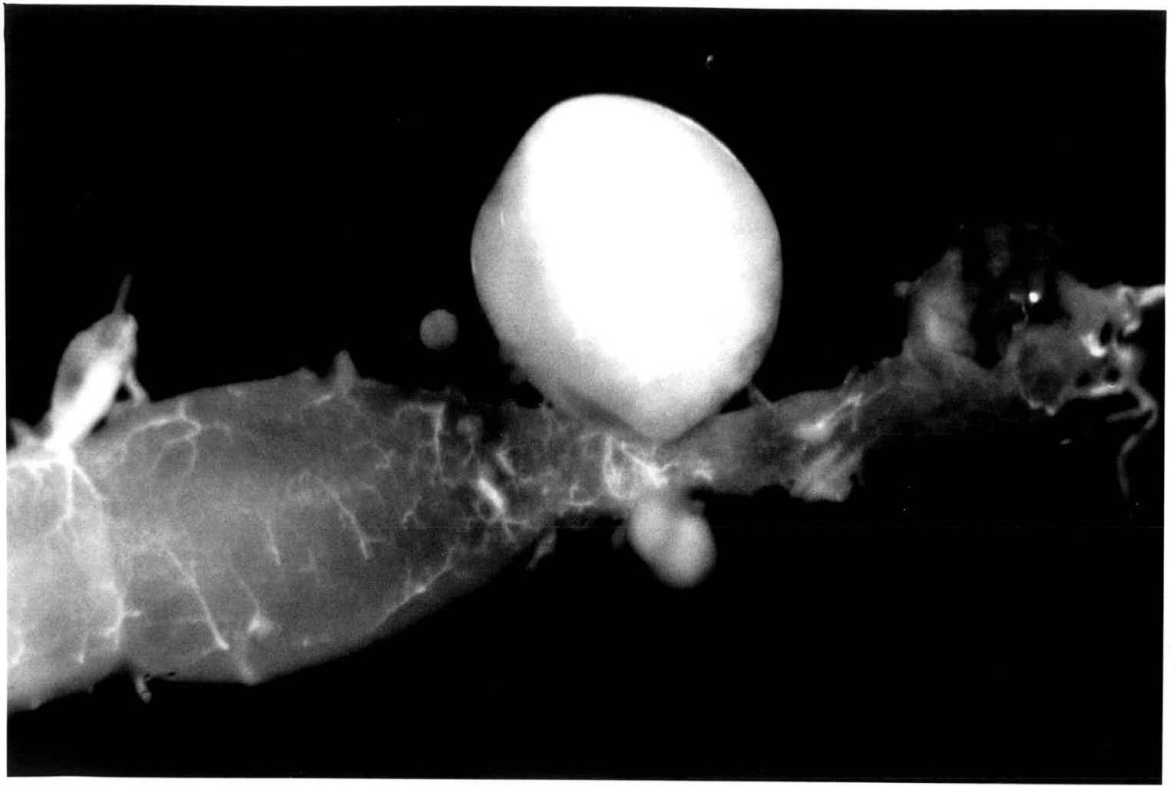


a.

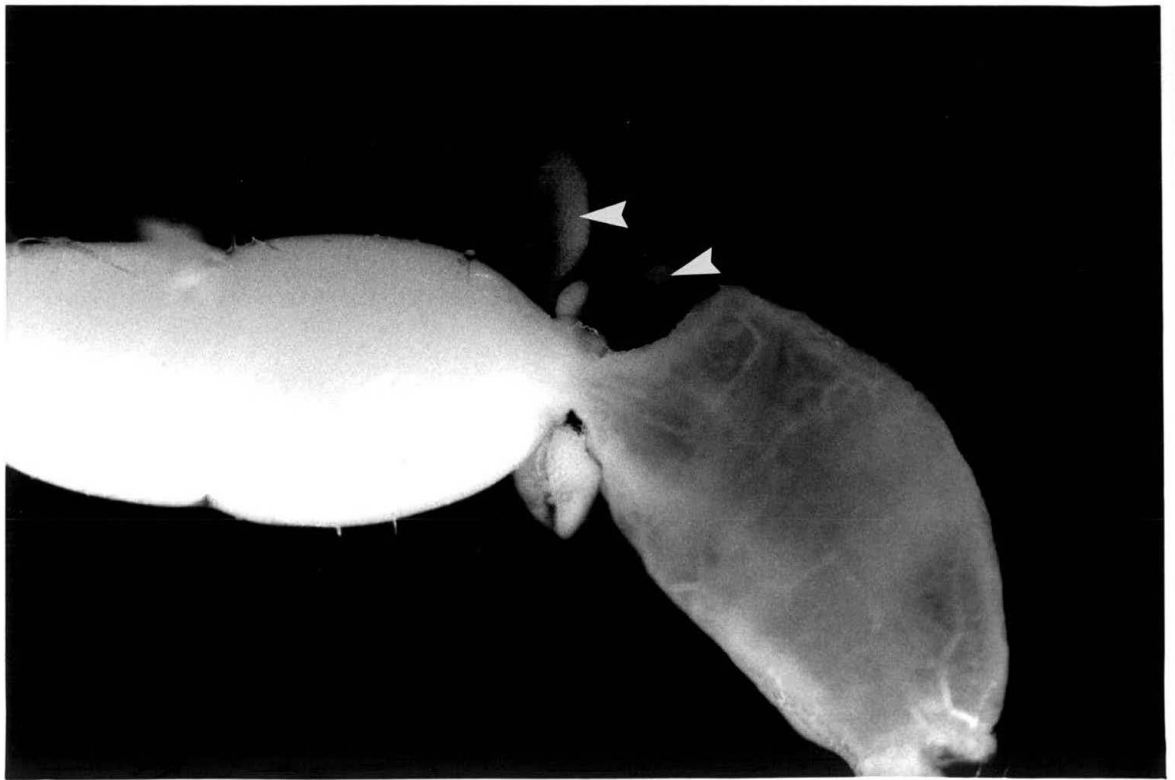


b.

Fig. 9 a. Ot. granulatus ovary showing yellow body as a solid plug
 b. Oi. alexis ovary with some yellow body granules
 c. Oi. alexis ovary extruding terminal follicle into haemocoel
 d. Oi. alexis ovary with degenerated, extruded follicles



c.



d.

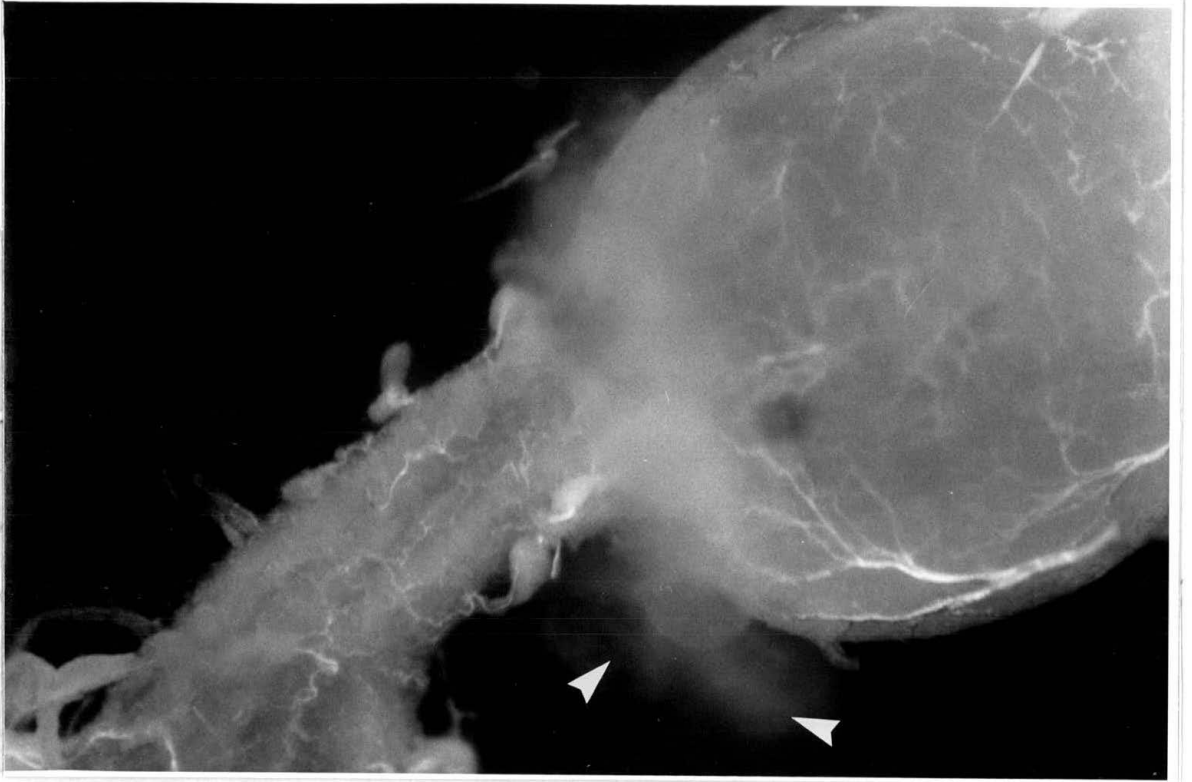


Fig. 10 Oi. alexis ovary with extruded follicular sheath.

URIARRA 1976-77

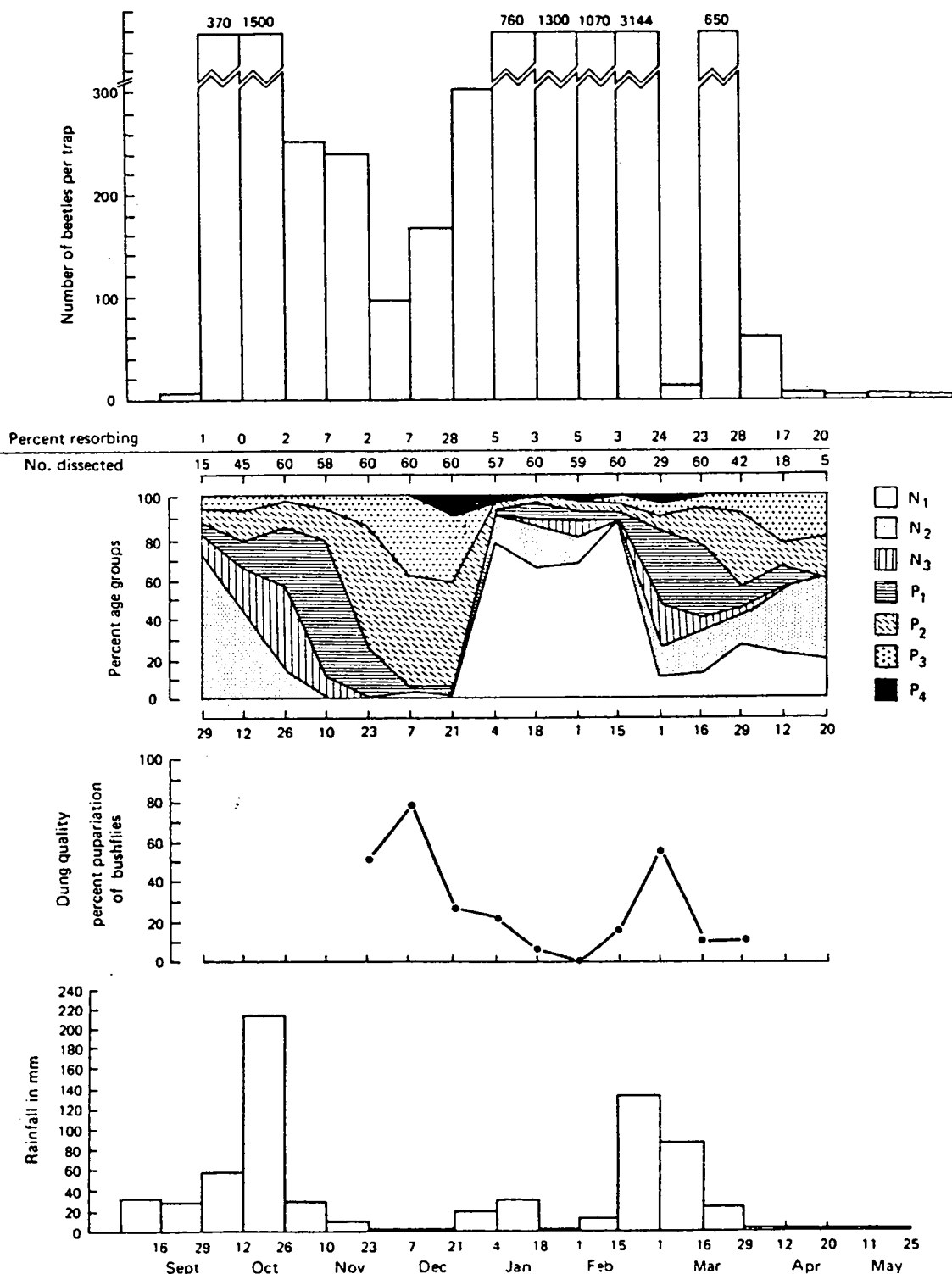


Fig. 11 a) *Ot. granulatus* trap catches, percent resorption and age structure, together with seasonal variation in dung quality as measured by bushfly percent pupariation, and rainfall.

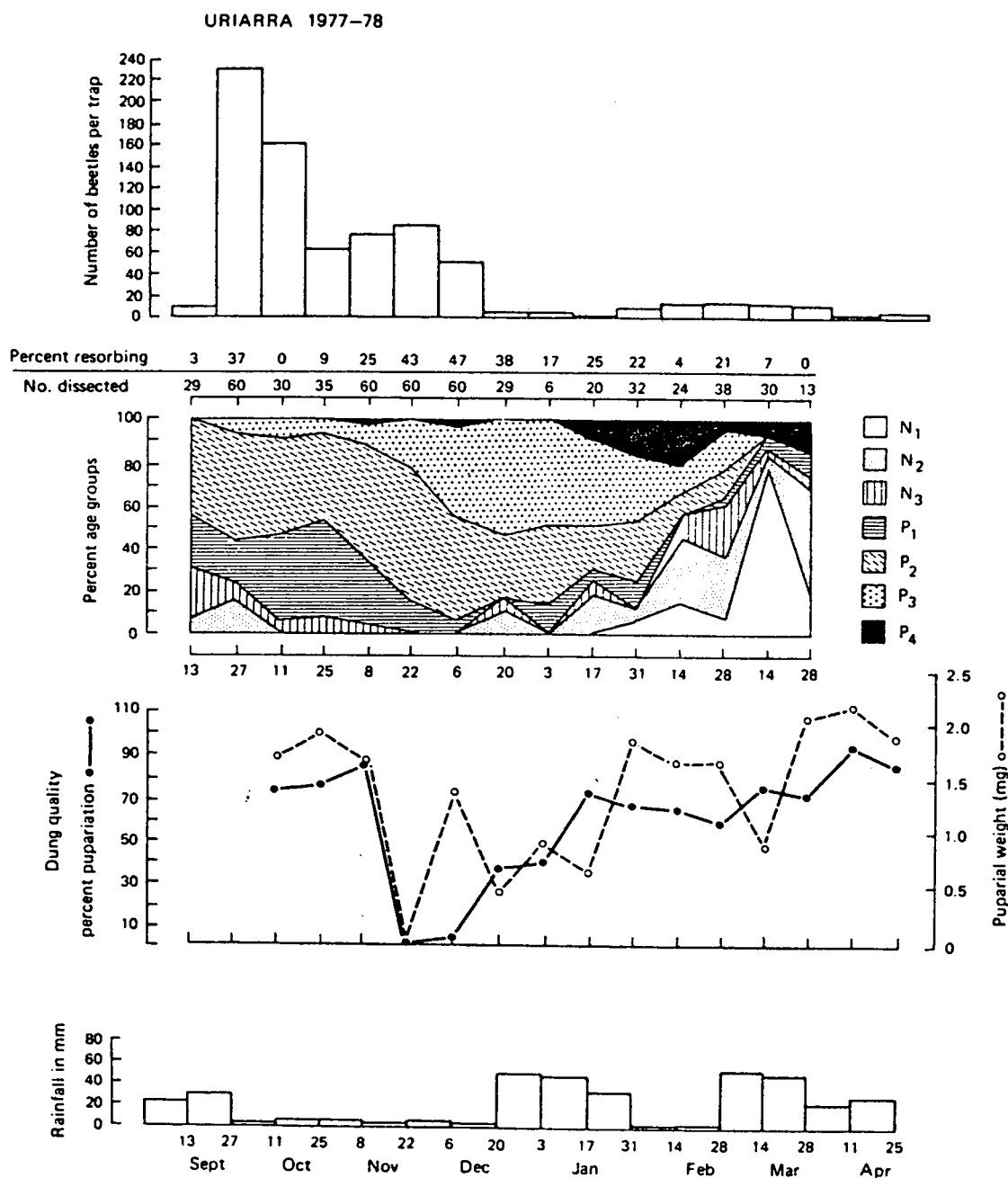


Fig. 11 b) *Ot. granulatus* trap catches, percent resorption and age structure, together with seasonal variation in dung quality as measured by bushfly percent pupariation pupal weight, and rainfall.

URIARRA 1978-79

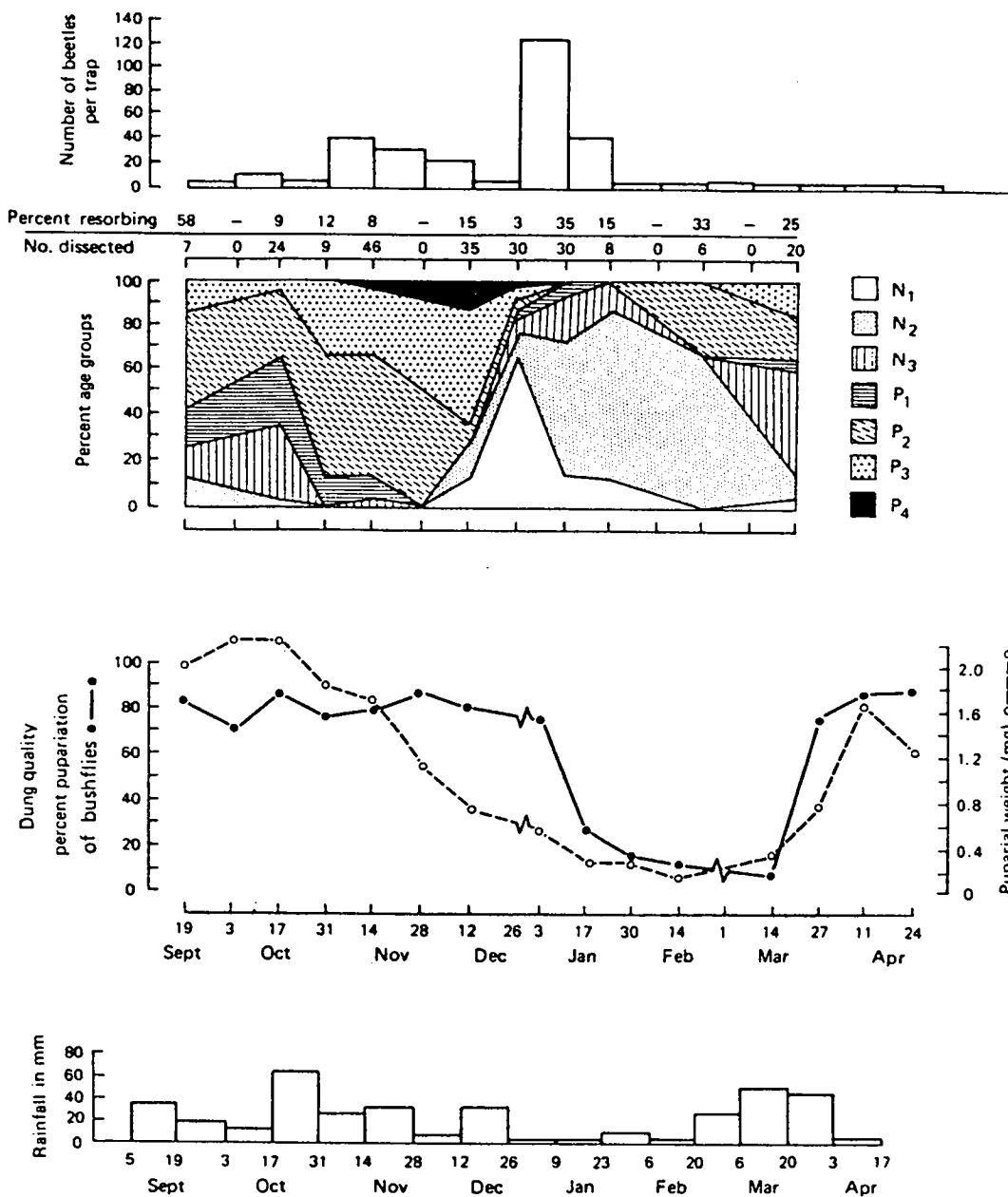


Fig. 11 c) *Ot. granulatus* trap catches, percent resorption and age structure, together with seasonal variation in dung quality as measured by bushfly percent pupariation pupal weight, and rainfall.

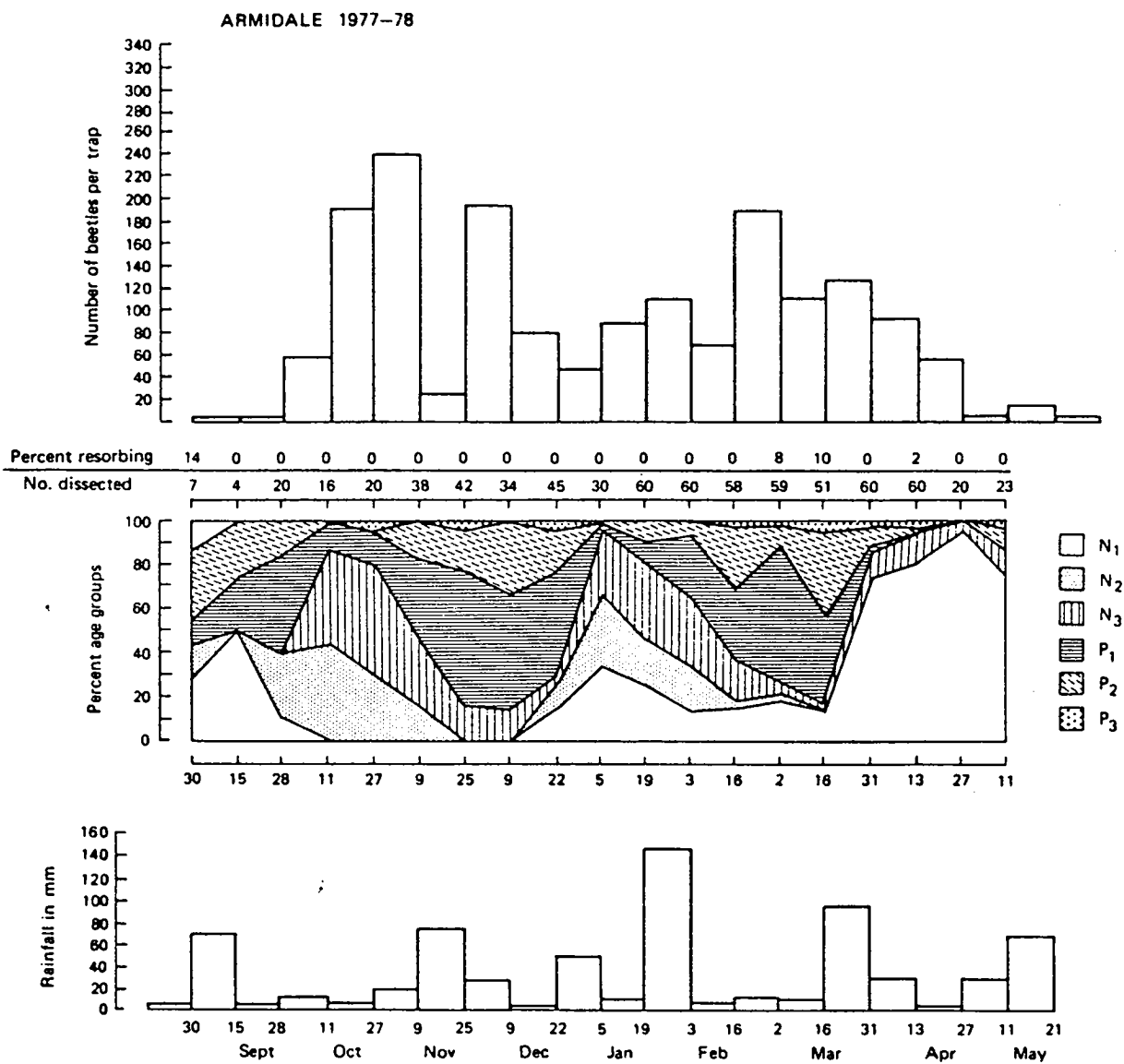


Fig. 12 a) *Ot. granulatus* trap catches, percent resorption and age structure, together with rainfall.

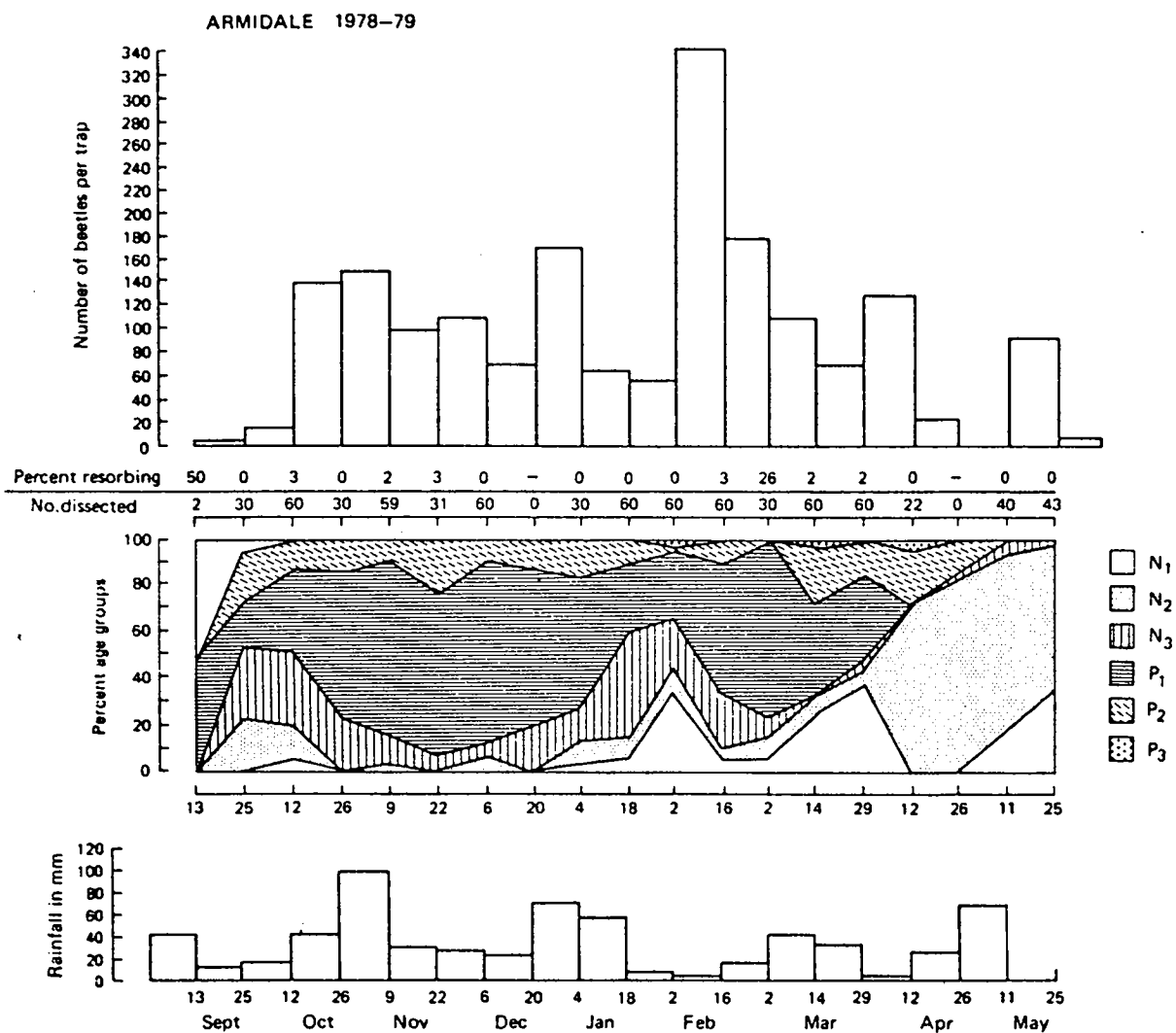


Fig. 12 b) *Ot. granulatus* trap catches, percent resorption and age structure, together with rainfall.

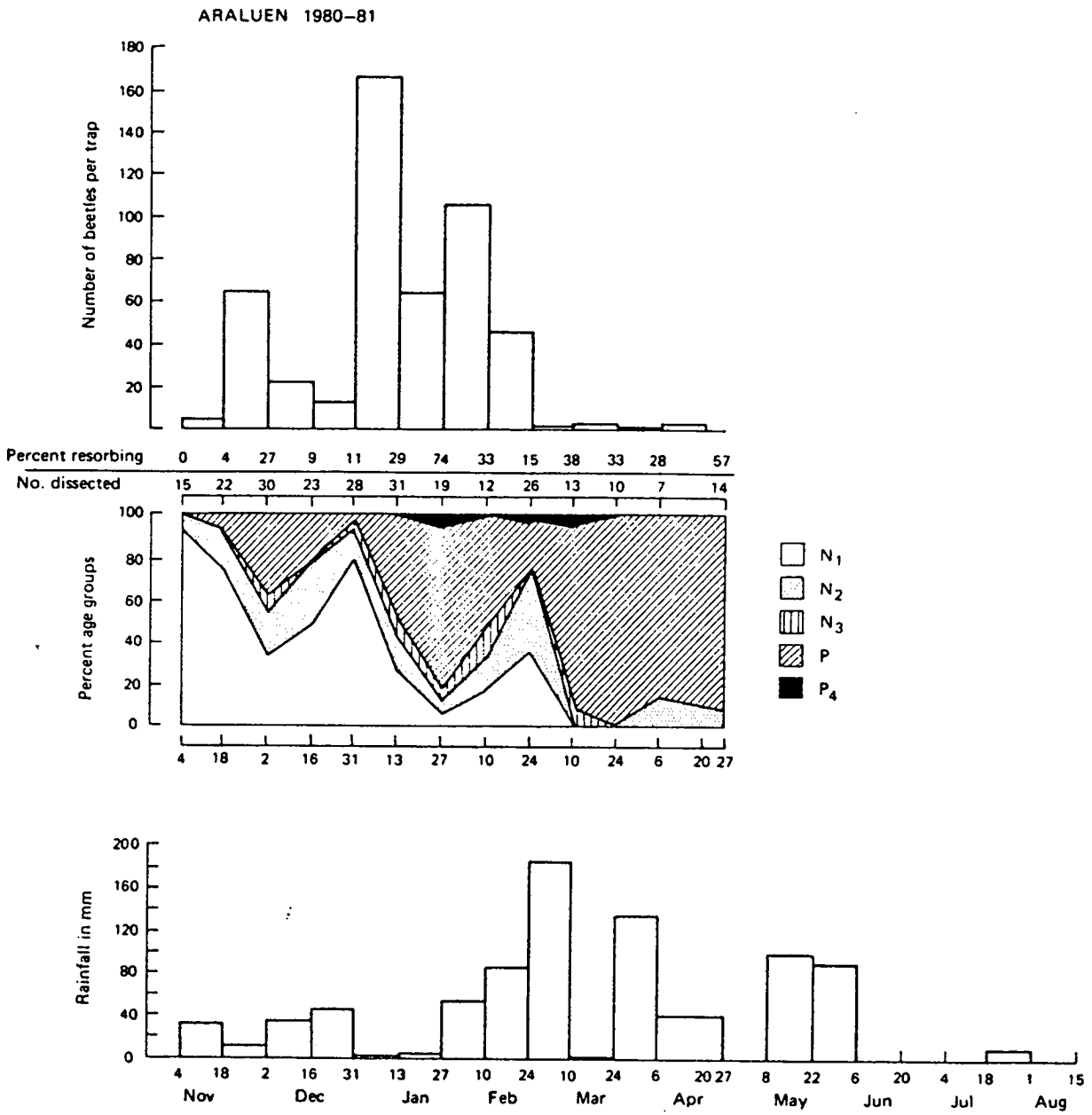


Fig. 13 a) *Oi. alexis* trap catches, percent resorption and age structure, together with seasonal variation in rainfall.

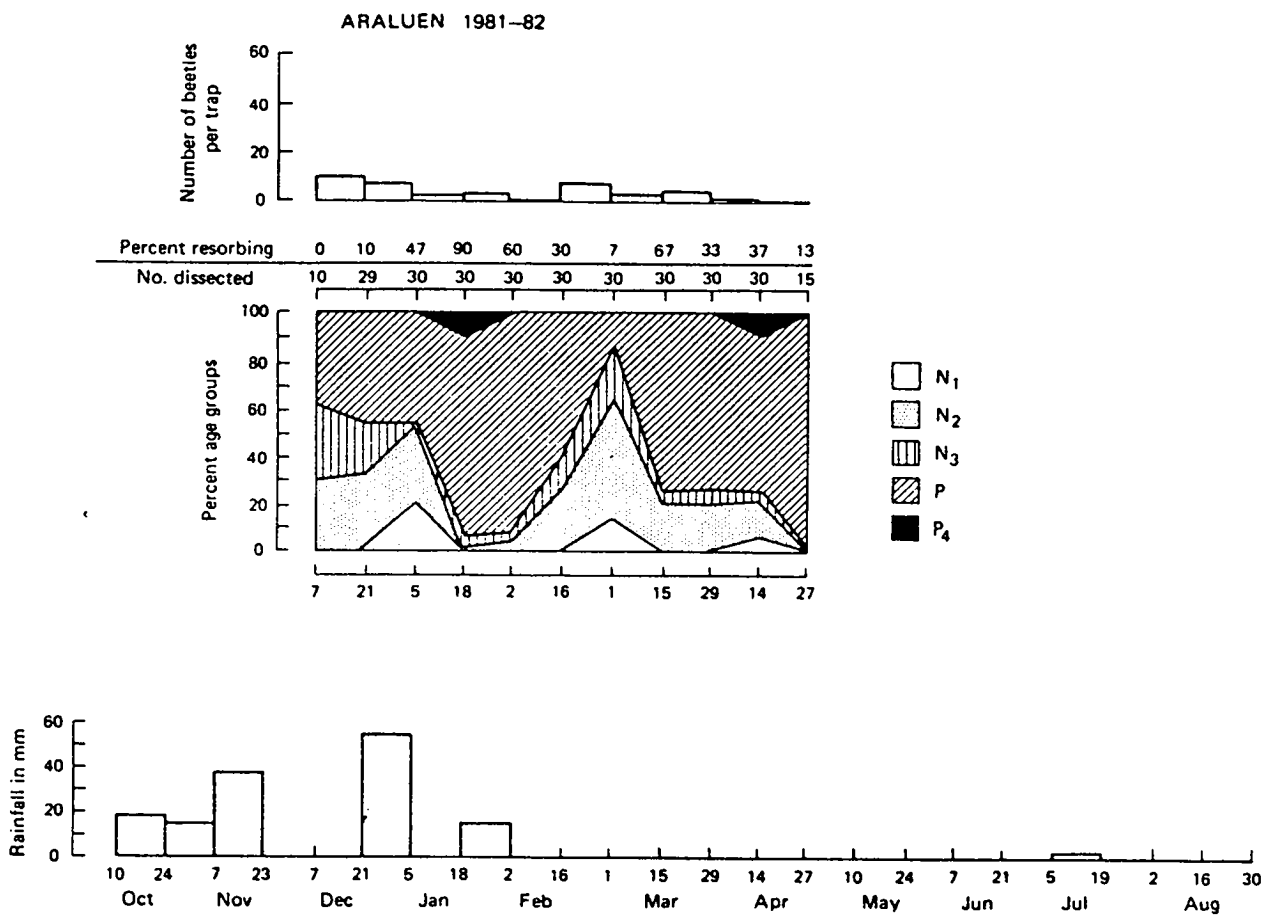


Fig. 13 b) *Oi. alexis* trap catches, percent resorption and age structure, together with seasonal variation in rainfall.

ARALUEN 1982-83

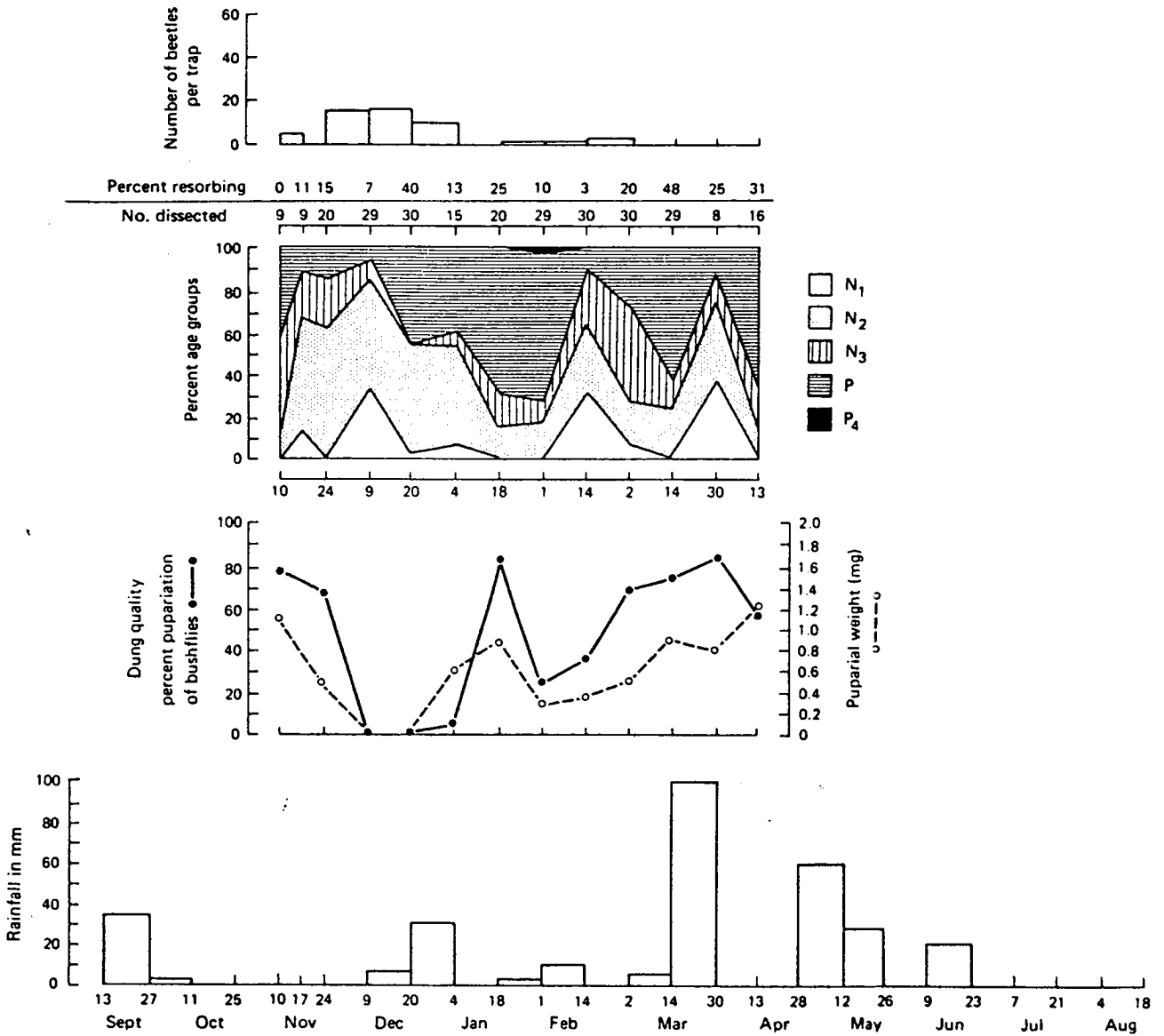


Fig. 13 c) *Oo. alexis* trap catches, percent resorption and age structure, together with seasonal variation in dung quality as measured by bushfly percent pupariation and pupal size, and rainfall.

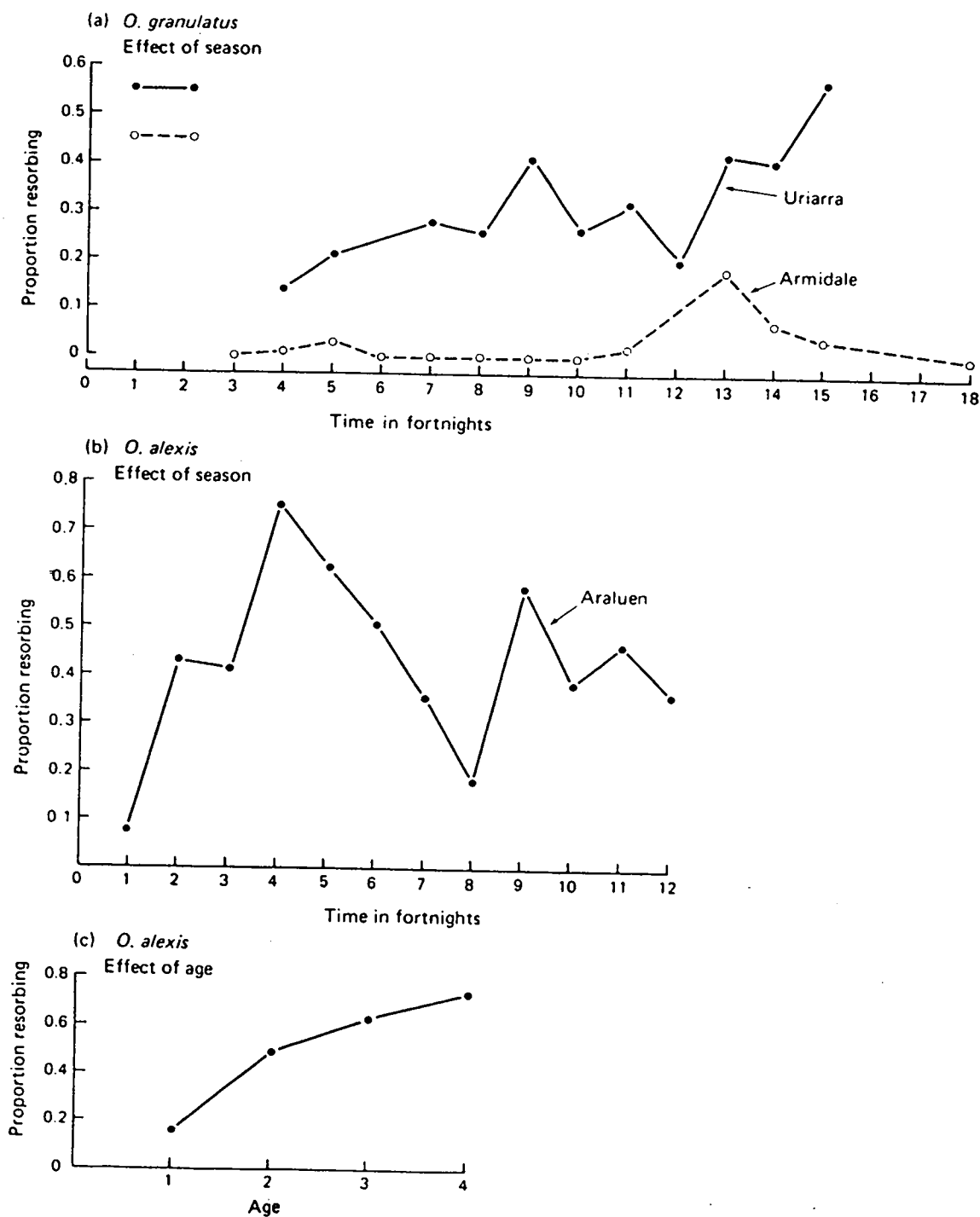


Fig. 14 Effect of time of season on resorption in a) *Ot. granulatus* at Uriarra (adjusted for covariates) and Armidale (unadjusted for covariates); b) *Oi. alexis* (adjusted for covariates); c) effect of age on resorption in *Oi. alexis* (adjusted for independents and covariates). 1=N₃, 2-4=parous with tibial wear 2-4.

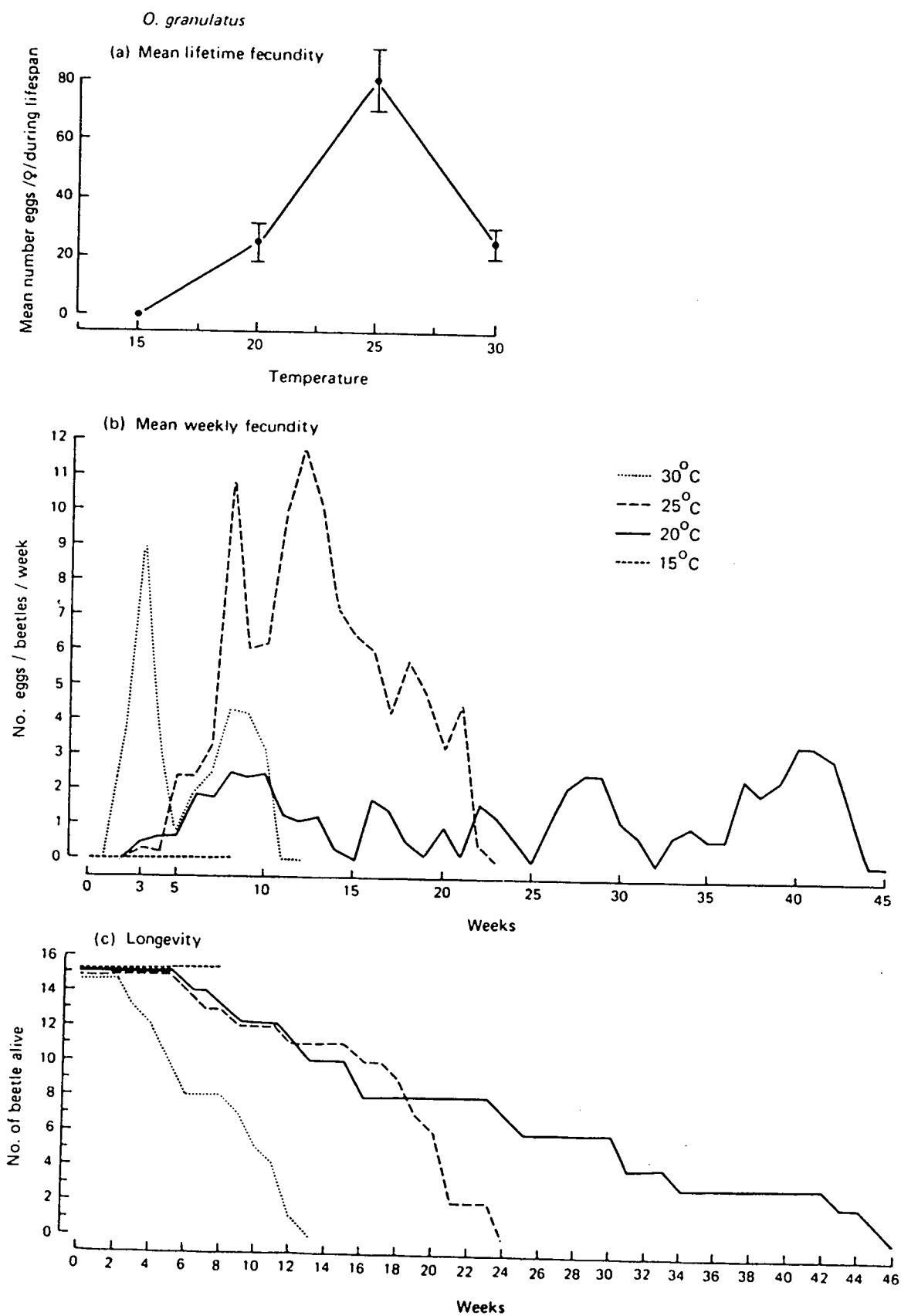


Fig. 15 Effect of temperature on fecundity and longevity of *Ot. granulatus*

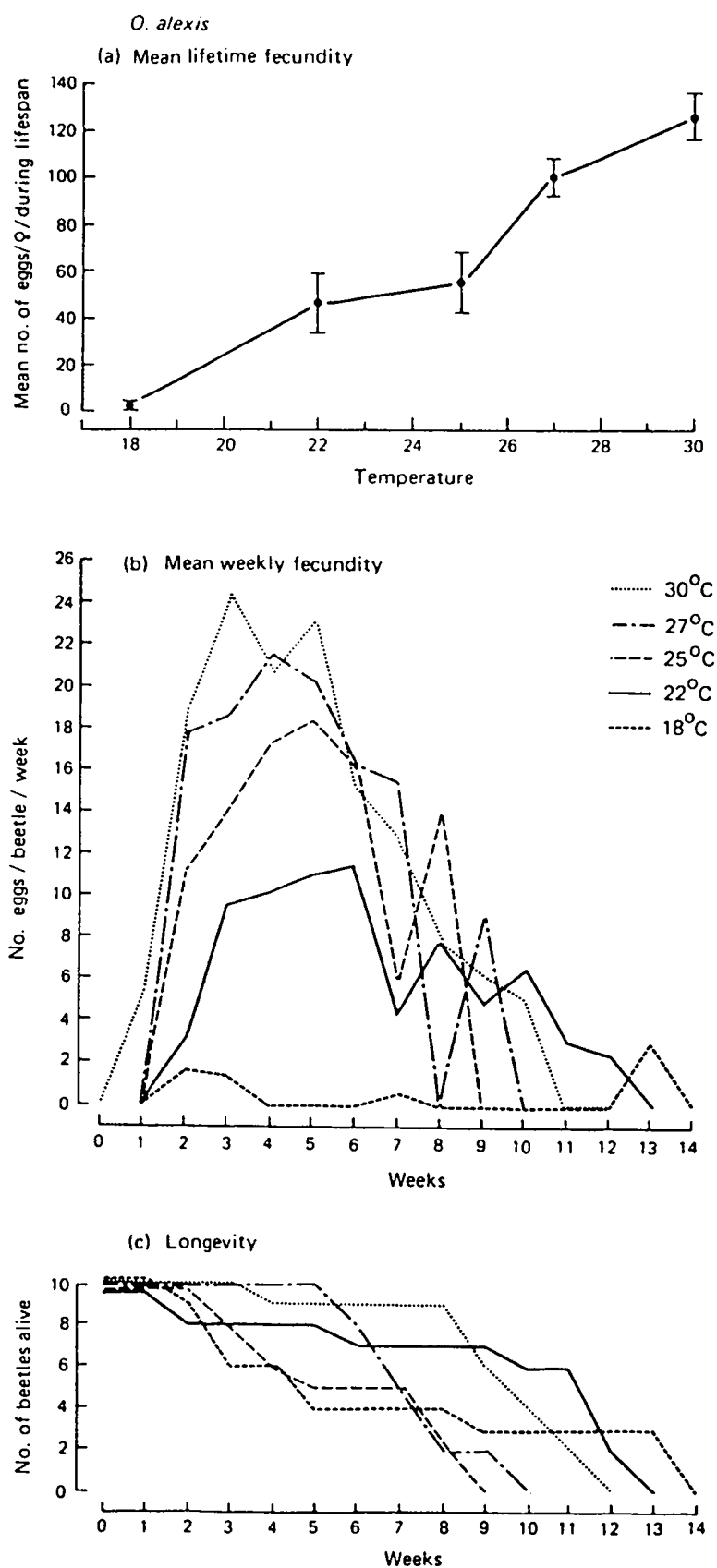


Fig. 16 Effect of temperature on fecundity and longevity of *Oi. alexis*

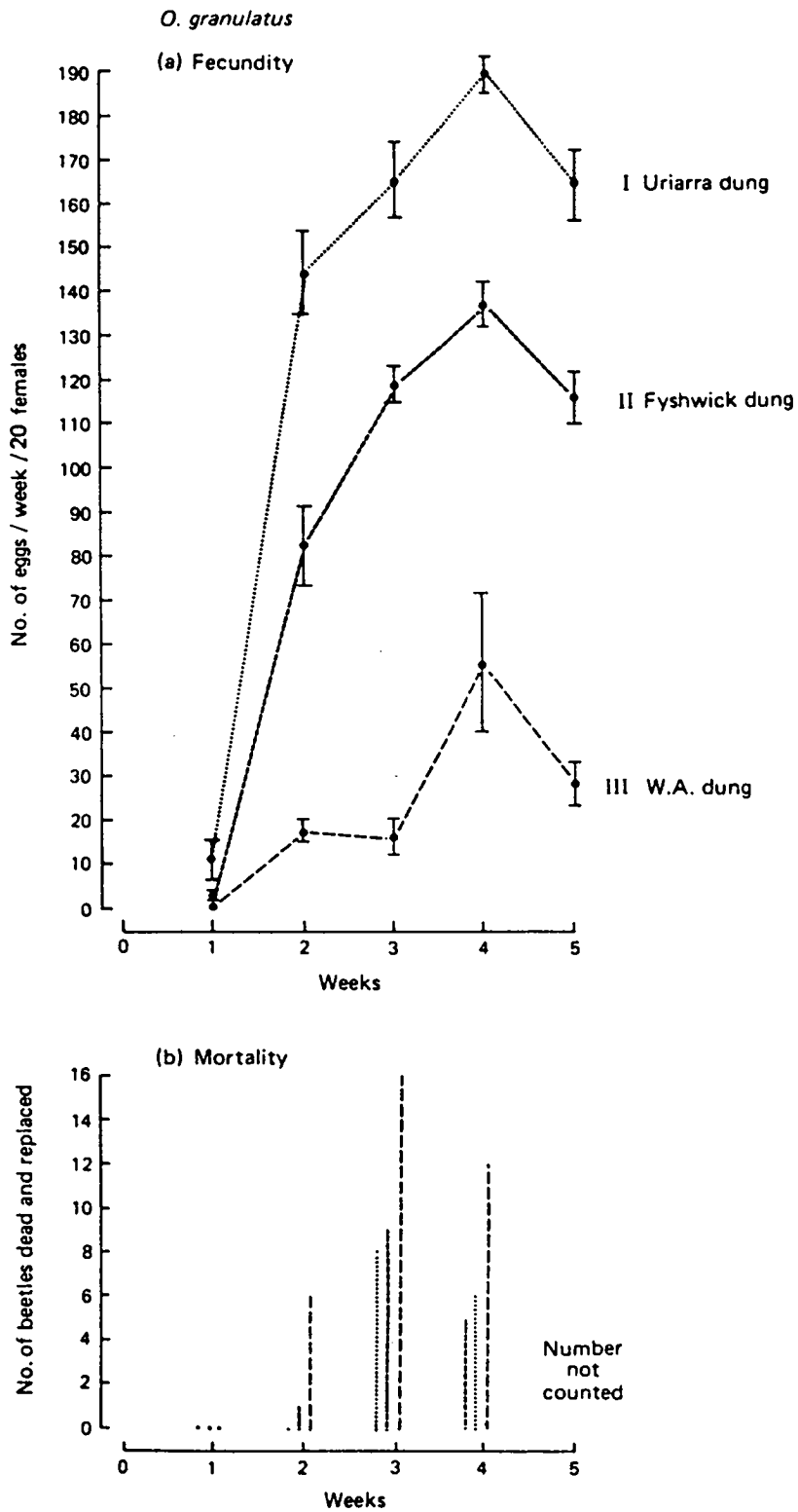


Fig. 17 Effect of dung quality on *Ot. granulatus* in relation to a) fecundity and b) mortality of females.

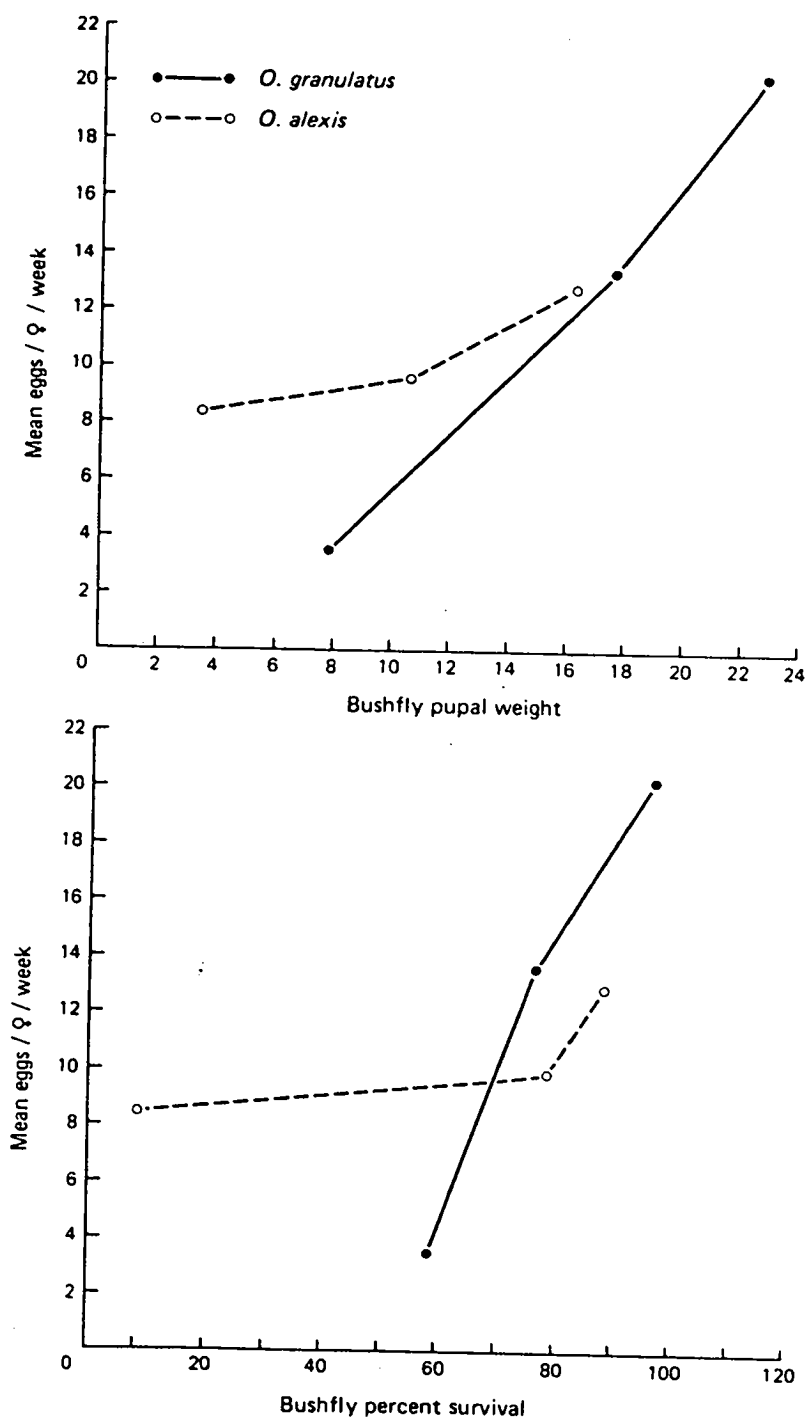


Fig. 18 The relationship of beetle fecundity and a) bushfly pupal weights and b) percent bushfly survival in the two beetle species when fed on dung of differing qualities.

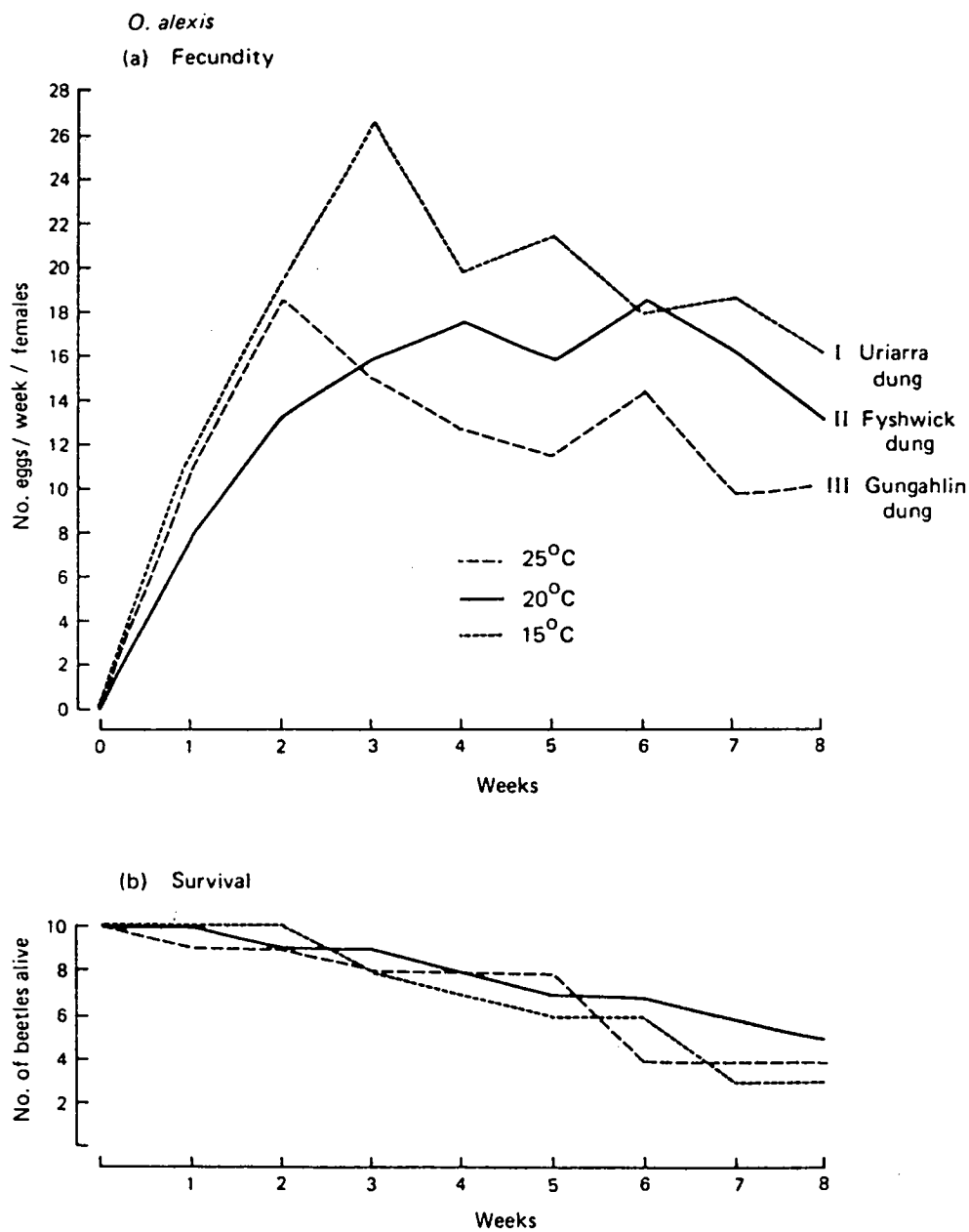


Fig. 19 Effect of dung quality on *Oi. alexis* in relation to a) fecundity and b) survival of females.

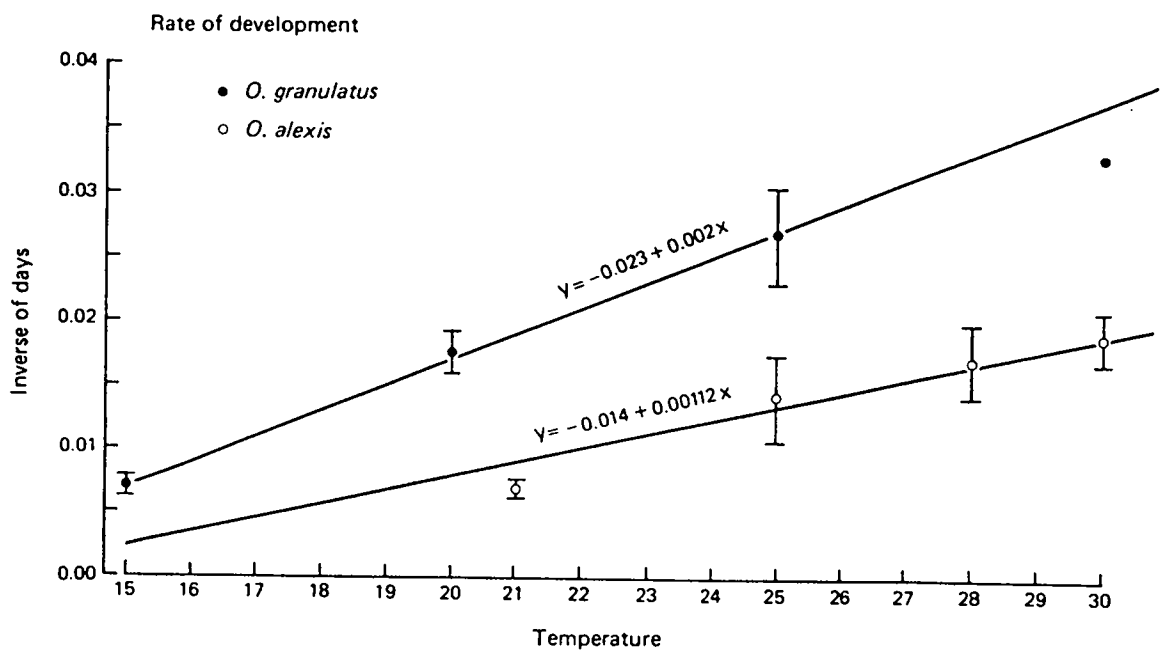


Fig. 20 Regression of the relationship between developmental rate from egg to adult and temperature in Ot. granulatus and Oi. alexis.

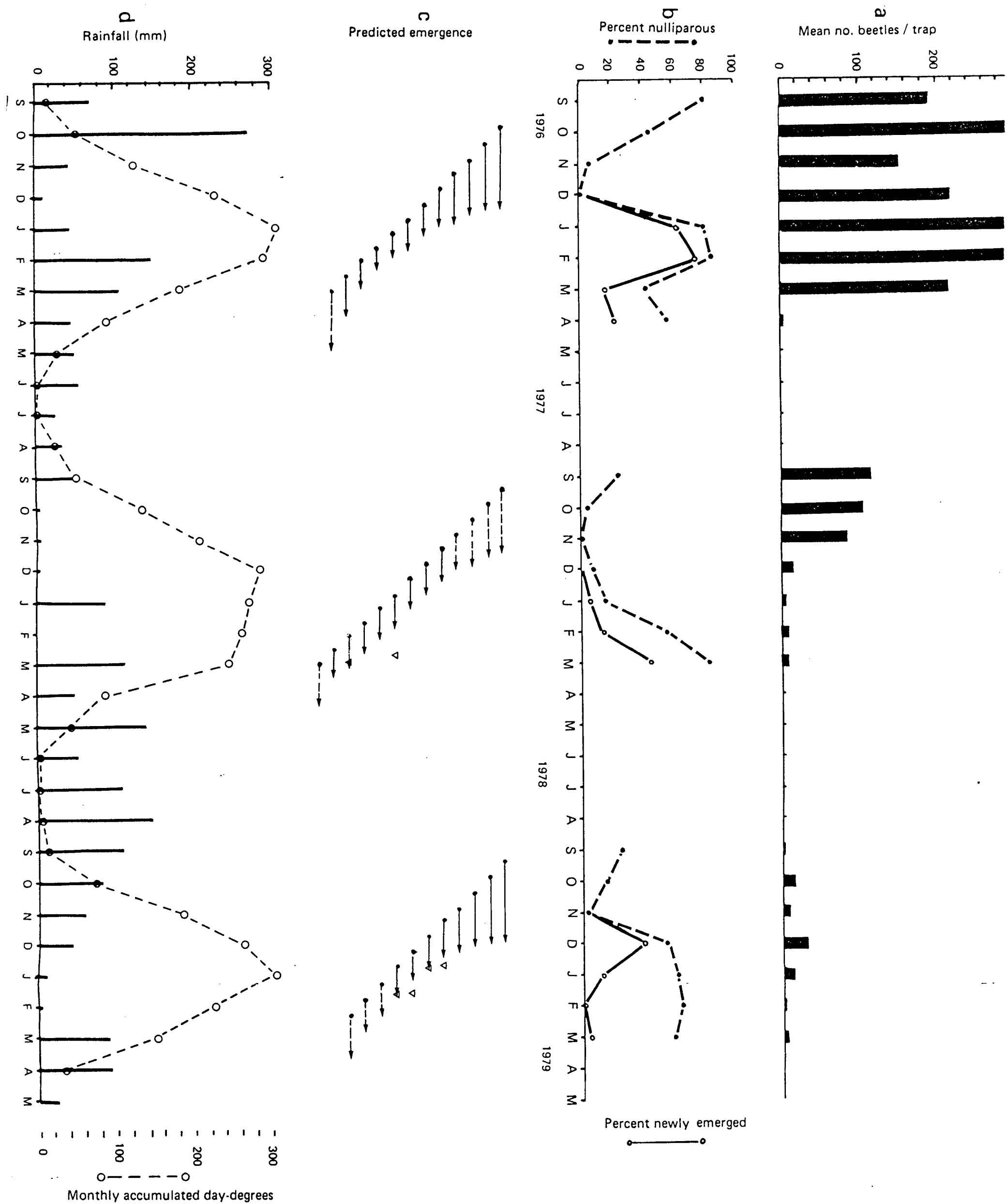


Fig. 21 Summary of *Ot. granulatus* phenology at Uriarra a. mean monthly trap catches; b. mean monthly percent newly emerged (solid line) and nulliparous (broken line); c. predicted times of emergence from eggs laid at different times of year (arrows) and actual emergence from cylinders (▼); d. monthly rainfall and accumulated day-degrees.

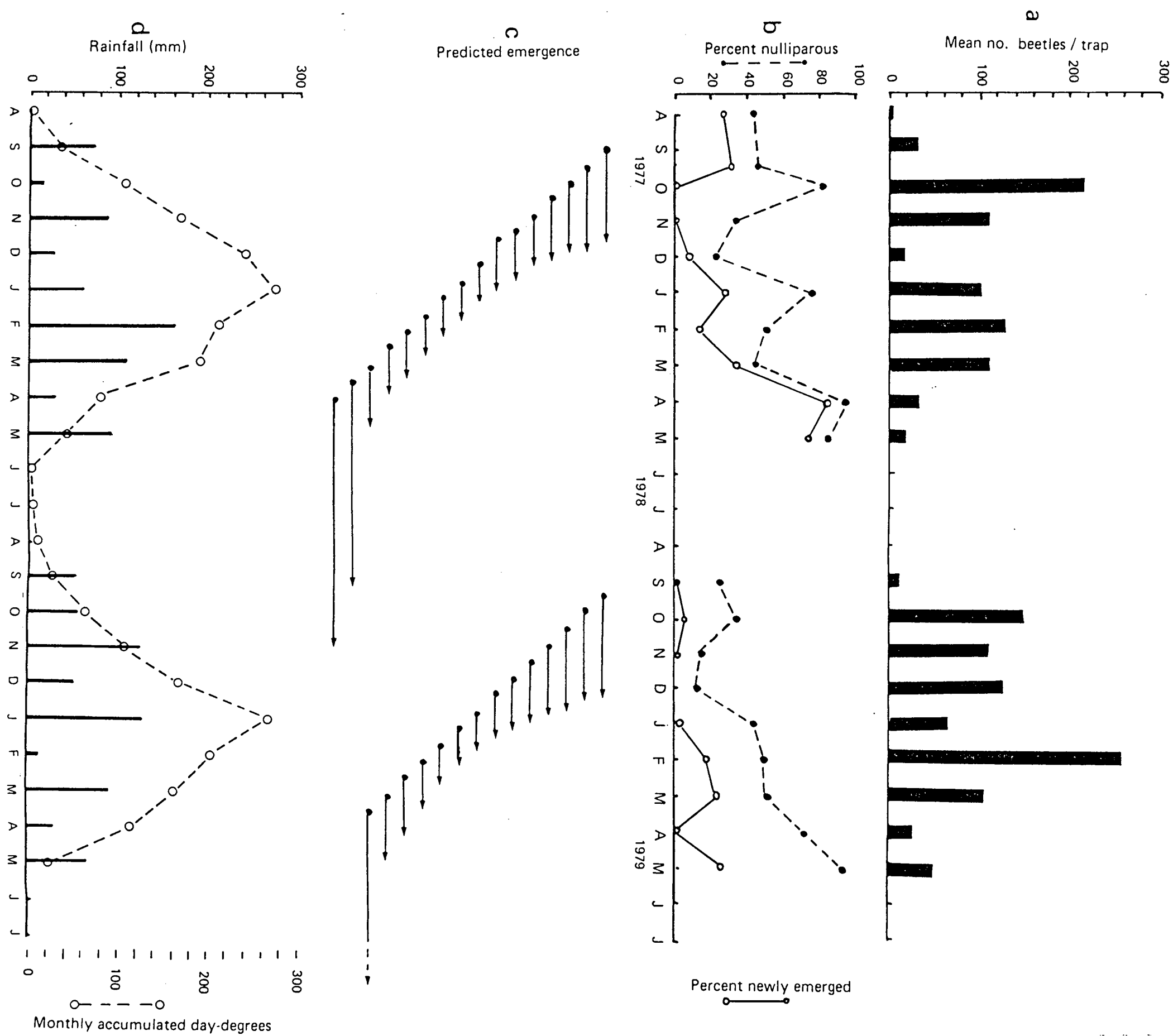


Fig. 22 Summary of *Ot. granulatus* phenology at Armidale a. mean monthly trap catches; b. mean monthly percent newly emerged (solid line) and nulliparous (broken line); c. predicted times of emergence from eggs laid at different times of year (arrows); d. monthly rainfall and accumulated day-degrees.

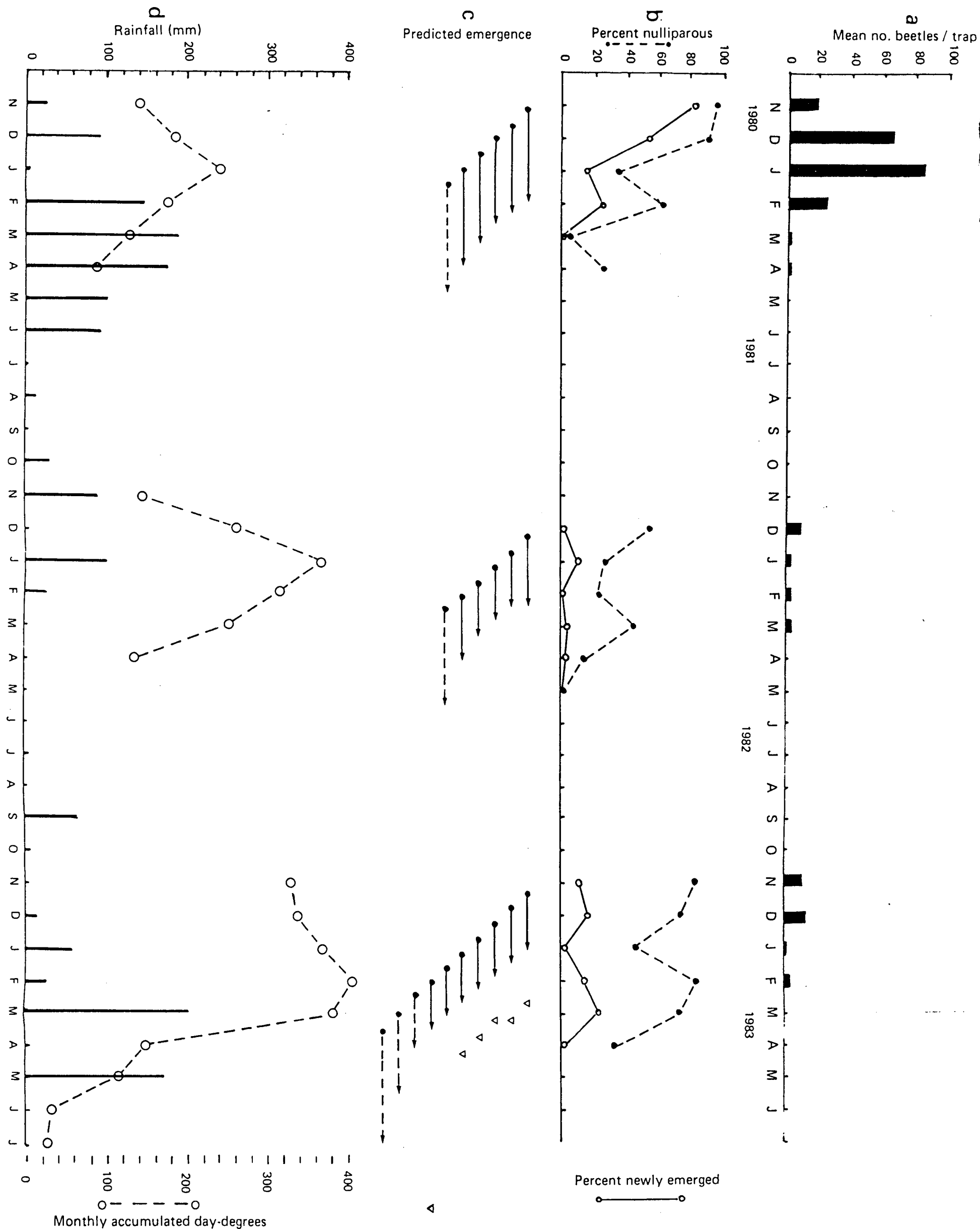


Fig. 23 Summary of *O. alexis* phenology at Araluen a. mean monthly trap catches; b. mean monthly percent newly emerged (solid line) and nulliparous (broken line); c. predicted times of emergence from eggs laid at different times of year (arrows) and actual emergence from cylinders (∇); d. monthly rainfall and accumulated day-degrees.