STUDIES ON THE HELMINTH PARASITES OF SMALL MAMMALS, WITH PARTICULAR REFERENCE TO THE ECOLOGY AND PHYSIOLOGY ~~OF~~ NEMATODE NEMATOSPIROIDES DUBIUS, BAYLIS 1926

by

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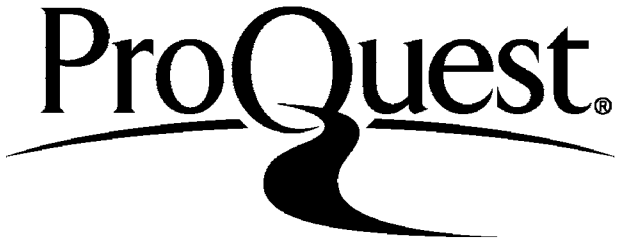

ABSTRACT

A study of the monthly incidence and intensity of infection of *Apodemus sylvaticus* and *Clethrionomys glareolus* with selected intestinal helminths has been made. The majority of the variation in these two factors was shown to be due to differences in the age and sex ratio of the host populations throughout the year. Multiple regression and correlation analyses were used to assess the effect of independent environmental variables on the incidence and intensity of infection.

A competitive interaction was found to occur between the trichostrongylid nematode *Nematospiroides dubius* and the oxyurid *Syphacia stroma* in the small intestine of *Apodemus sylvaticus*, though such an interaction did not lead to the total exclusion of either species.

The distribution of *N. dubius* in the small intestine of laboratory mice was found to be highly aggregated with respect to both the intestine and the worm population; this was thought to occur in response to a gradient in oxygen tension along the mouse gut.

Growth curves of male and female *N. dubius* in male and female mice were typically sigmoid and the daily growth rates for each sex of worm were the same in both sexes of mice. The growth of the free-living stages was negative after infectivity had been reached.

The respiration rates of male and female N. dubius increased with age, whereas those of the free-living stages decreased after infectivity was reached.

The size/metabolism relationship for free-living stages of N. dubius indicated a slow-down in metabolism as soon as infectivity was reached; this is likely to be of adaptive significance to a non-feeding stage. The size/metabolism relationship of both male and female parasitic stages indicated that aerobic metabolism may be important in vivo.

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TABLE OF CONTENTS

GENERAL INTRODUCTION

The present work was undertaken in order to study the inter-relationships between small mammals and their helminth parasites. The monthly incidence and intensity of infection of *Apodemus sylvaticus* and *Clethrionomys glareolus* with selected intestinal helminths was examined and related to the age and sex structure of the host populations. The effect of independent environmental variables, also measured on a monthly basis, on the level and degree of infection has been ascertained.

Lewis (1964) indicated that interspecific competition occurred between two nematodes, the trichostrongylid *Nematospiroides dubius* and the oxyurid *Syphacia stroma* along the small intestine of *A. sylvaticus*. A study of the spatial distribution of these two species in *A. sylvaticus* trapped during this survey verified his finding. Lewis also suggested that the limiting factor in this competition could be the availability of oxygen in the small intestine. A study of the individual oxygen requirements of the two species involved was therefore planned in order to determine whether they were likely to compete for this resource. In addition, controlled experimental infections of laboratory mice with concurrent and single species infections of *N. dubius* and *S. stroma* were to be made in order to determine the effect of different numbers of each species, and

the chronological order of infection, on the competitive situation.

Although N. dubius was easily maintained under laboratory conditions, S. stroma was found to be extremely difficult to culture, and large numbers of infective eggs of the latter species were never obtained, thus rendering a series of experiments requiring large numbers of infective stages impossible.

It was therefore decided to make a study of the physiology of N. dubius and to investigate its distribution and growth within the host, and also to gain some knowledge of its respiratory requirements throughout the entire life cycle, i.e. during both the free-living and parasitic phases. A detailed study of the life cycle and the times of moulting of N. dubius was necessary for these investigations and was accordingly carried out. It was hoped that a study of the growth/metabolism relationship of N. dubius would throw some light on the type of metabolism exhibited by this parasite in vivo.

The first two chapters deal with helminth infections in naturally infected hosts and the last four chapters deal with specific aspects of the life cycle and physiology of Nematospiroides dubius in experimentally infected laboratory mice.

GENERAL MATERIALS AND METHODS

(A) Culture of Nematospiroides dubius

The strain of Nematospiroides dubius used was obtained from the Wellcome Laboratories, Beckenham and was maintained in culture by the method employed in that laboratory. The nematode was maintained in S.P.F.ASH/CS1 mice obtained from Scientific Products Farm, Canterbury.

Fertilised eggs laid in the host intestine were voided with the faeces which were collected by placing infected mice in cages over wire grids. Short collecting periods were used in order to minimise age differences in the larvae produced. Faeces were comminated with tap water, strained through a double thickness of surgical gauze and washed through with water to a volume of 150 - 200 ml. The resulting suspension was centrifuged at 2,000 r.p.m. for 5 minutes and the supernatant discarded. Fresh water was added to the centrifuge tubes, which were respun for 5 minutes. This procedure removed urine and light debris from the faecal mass. The resulting sediment, containing the eggs, was spread on to a double layer of moistened Whatman 54 filter paper in a petri dish, and incubated in a humidity chamber at 20°C. Dechlorinated water was used to moisten the filters. After 7 days, all the larvae produced were infective.

(B) Extraction of Larvae from culture

Larvae were extracted from cultures using a Baermann

funnel containing dechlorinated water. The "filter" used consisted of a layer of bolting silk (196 meshes per sq. in.) covered with a Boots' milk filter. The larvae which migrated through this barrier were free from debris.

(C) Standardization of dose of infective larvae

The suspension of larvae in dechlorinated water was shaken to ensure even dispersion and the number of larvae per ml. was estimated using a counting chamber. The suspension was adjusted by dilution to give the correct number of larvae per ml. for the required dose. The standard dose for all experiments was 70 larvae in 0.2 mls of dechlorinated water.

(D) Infection of mice

Male and female mice were infected when they were 6 - 8 weeks old - just prior to sexual maturity. They were infected orally by means of a tuberculin syringe fitted with a blunt-ended wide gauge needle, which was inserted into the oesophagus.

(E) Storage of infective larvae

Infective larvae were stored in dechlorinated water at $4^{o}C$ in a refrigerator in loosely capped universal bottles and larvae remained infective for up to 35 days without loss of infectivity.

(F) Removal of worms from the host

Mice were killed by cervical dislocation, and the

small intestine removed and placed in Tyrodes' saline. The mesenteries were cut away and the intestine cut into convenient lengths, which were slit open and laid flat on a glass slide. Encysted third and fourth stage larvae were teased out of the submucosal region using fine Tungsten dissecting needles. Adult worms were lifted out of the lumen using fine forceps.

(G) Histology

Sections of the duodenum, approximately 5 cms. from the pylorus were fixed in Bouins solution, embedded in paraffin wax, sectioned and stained with haematoxylin and eosin. The positions taken up by the larvae in the mucosa were determined on examination of the mounted sections.

Free-living larvae were stained for fat using oil Red O (Lee, 1960b). Larvae were placed in a drop of distilled water in a crystallizing dish and transferred to an oven at 60°C. A hot saturated solution of oil Red O in 70% alcohol was added to the larvae and they were incubated for 4 minutes. After staining they were washed with distilled water and mounted on slides for microscopic examination.

CHAPTER I

THE ECOLOGY OF THE HELMINTH PARASITES OF APODEMUS SYLVATICUS AND CLETHRIONOMYS GLAREOLUS IN GREAT WOOD

Introduction

In 1939, Elton, Ford and Baker studied the incidence and intensity of parasitism in Apodemus sylvaticus and Clethrionomys glareolus in Bagley Wood, Oxford. They indicated that the intensity of infection with parasites was correlated with age, young animals in general harbouring smaller worm burdens than adults. They did not, however, link this seasonal variation with the biology of the parasites, as little information was available about their life cycles at that time. More recently, Thomas (1953)made a comparative survey of the nematode and trematode parasites of small mammals from the Inner Hebrides; although he examined the incidence and intensity of parasitism on Raasay, Ulva and Scalpay, he did not relate his findings to the ecology and biology of the hosts or parasites. Sharpe (1964) and Lewis (1968 a and b) both carried out comprehensive studies on the parasite populations of A. sylvaticus and C. glareolus in which the importance of the density and ecology of the host population in determining the degree of

parasitism, was stressed. Both these authors correlated the age structure of the host population with seasonal changes in the level of parasitism, and they also showed that the types of life cycle exhibited by the different species of parasites were important in determining incidence and intensity of infection.

The present survey is an extension of this work, in that the incidence and intensity of infection of A. sylvaticus and C. glareolus with helminth parasites in Great Wood have been studied on a seasonal basis. These variations have been related to the sex, age and feeding habits of the host population, and the types of life cycles exhibited by the most commonly occurring species have also been considered. In addition, the effect of environmental factors, such as rainfall, temperature and relative humidity on the parasite population has been estimated using a multiple regression and correlation analysis. Pike (1968) and Kennedy (1969) have both reported that environmental temperature was one of the major factors influencing the incidence and distribution of certain larval and adult trematode and cestode parasites. Kennedy showed that changes in the population of the cestode Caryophyllaeus laticeps in the dace Leuciscus leuciscus bore no relationship to the availability of infective larvae nor probably to the feeding behaviour of the fish, but were closely correlated with water temperature changes. Furthermore, the most important factor controlling the dynamics of

the C. laticeps-dace system appeared to be a temperature dependent rejection response to the parasites by the dace, the parasite establishing itself most successfully at low temperatures. Pike, in his investigations on the distribution and incidence of larval trematodes in the fresh water fauna of a coastal plain in South Wales, concluded that the main factors affecting seasonal variation were the life cycle of the intermediate host and the environmental temperature, the latter acting on the rate of development of the trematodes. Rainfall and patterns of egg production of adult trematodes in the definitive host were also found to be important.

Those stages of parasites that inhabit a poikilothermic host are more susceptible to fluctuations in environmental factors than those inhabiting homoiothermic hosts which in general provide a more stable environment than cold-blooded animals. It therefore seems likely that environmental variables are most likely to act upon the free-living and intermediate stages of the helminths found in this survey which parasitize small mammals. Kisielewska (1964) showed this to be the case with cestodes infecting shrews. She found that the incidence of cestodes requiring an insect as an intermediate host (a rainy year was unfavourable for the latter) dropped after a year that was characterized by extremely heavy and prolonged rainfall whereas those

requiring a snail as an intermediate host (a rainy year was favourable for the latter) occurred more frequently.

Intensity of infection has been measured in terms of the mean number of parasites per infected host. Monthly means have been calculated for each species which included male, female and larval stages of parasites. It must, however, be borne in mind that this is not a true measure of intensity of infection but rather a reflection of it. There was no measure of the mortality of the parasites within the host, nor was it possible to know exactly when the infection was incurred. Most of the parasites encountered during this survey are mature within nine days of entering the host, and once mature, they are impossible to age. For this reason, little information can be obtained about the dynamics of the infections, that is whether the parasites entered the hosts continuously or whether the worm burdens represented several discreet infections. The nature and extent to which the host intestine imposes limitations on the different parasite species inhabiting it is unknown.

Materials and Methods

(a) The study area

Trapping was carried out in an area of unmanaged woodland known as Great Wood near Egham, Surrey. It consists of an old larch plantation with a narrow hard wood fringe which

leads into an extensive area of oak woodland at the southern end. The ground cover is mainly bracken and brambles and there is also a reasonable amount of fallen timber and rotting tree stumps, both of which provide good habitats for small mammals.

(b) Trapping

Trapping of small mammals was carried out every month from October, 1968 to November, 1969, in an attempt to obtain a monthly sample which would represent the main age/sex groups present in the population throughout the year. It was not possible to initially estimate the small mammal population in this area using the mark and release method of Chitty (1937), as all the animals caught were required for examination for internal parasites. Trapping was carried out for five nights a week for the first fortnight of each month; a minimum of thirty-six Longworth traps were used each night, and their positions recorded on a map. Hay was used as bedding in the nesting boxes and a mixture of crushed cats and corn was used as bait. As very few dead animals were found in the traps, it was only necessary to visit them every twenty-four hours, that is early each morning. When a trap had been sprung, it was removed and taken into the laboratory, having been replaced by a freshly baited trap. The traps were laid at random within the selected sites, each trap being a minimum of six feet from its nearest neighbour.

The trapping area was changed each week, usually to an adjacent area not less than 100 yards from the previous site. Although this method is probably not truly random, subsequent analysis of the age of animals caught indicated that they did represent a typical cross-section of the population that would be expected in Great Wood over the sampling period (Twigg - personal communication).

Weekly weather data for the study period was obtained from the Meteriological Station at Silwood Park, Imperial College Field Station. It was felt that the weather conditions prevailing there, approximately five miles distant, would be similar to those at Great Wood.

(c) Examination of hosts for Parasites

Longworth Traps were opened in the laboratory and the small mammals were removed and killed using an ether/air mixture. This method causes little disruption to the positions of intestinal parasites to be studied. The animals were weighed and the sex and breeding condition of each animal was noted. The alimentary canal and associated organs were then removed for further examination and the eviscerated body weight of each animal recorded. The small intestine from pyloric sphincter to caecum was separated from the surrounding fat tissue and mesentery, and cut into twenty equal sections,using a graduated scale. Each section was slit open in Tyrodes saline and examined under a binocular

microscope for parasites. The rectum was treated in the same way, after being divided into four sections. The liver, stomach and large intestine were examined separately. Detailed records of the occurrence and distribution of each helminth parasite were made. Permanent preparations were made of certain specimens for identification (Lewis, 1968a).

(d) Ageing of small mammals

The most accurate method of separating different generations of small mammals involves detailed examination of the pelvic girdle (Cleveden Brown and Twigg, 1969). This was not possible in the present work, and frequency distributions based on weight were used to age the animals trapped in each month, as this is the most practical and convenient criterion to age small mammals (Baker, 1930). The occurrence of pregnant or lactating females was recorded, although it is possible that very early pregnancies were not detected, as histological examinations of the female reproductive system were not carried out.

(e) Analysis of results

The incidence and intensity of infection of the mammalian hosts with parasites were recorded. Incidence has been defined as the percentage of the sample in which a parasite occurs and intensity of infection has been defined

as the mean number of parasites per infected host. The means quoted have all been transformed using the Log. (N + 1) transformation. On an arithmetic scale it is often not possible to see small changes if large changes are to fit on to the same scale. The logarithmic scale makes the large means appear relatively smaller and so makes large and small changes more easily comparable. If one sample contains an abnormally high number of worms, the arithmetic mean may be higher than all the other worm counts; the geometric mean calculated using the Log. (N + 1) transformation, gives a mean that falls near the median, and is a much better description of what normally happens (Lewis and Taylor, 1967). Most statistical tests should only be applied to data that has a normal distribution, and the distribution of all the parasites found in this survey were skewed, that is most mice carried a small number of worms, large burdens being relatively rare. The Log. (N + 1) transformation also converts skewed distributions to more normal ones, which increases the validity of the statistical tests applied.

A multiple regression and correlation analysis has been used to evaluate the importance of weather factors in determining the variation in the incidence and intensity of parasite infections. These analyses have been carried out relating the monthly incidence and intensity of parasitism and the environmental variables for the concurrent and previous month, for each species of nematode and the single

digenean found in this survey. The environmental variables used were temperature, rainfall and relative humidity, an average monthly value being obtained for each. The multiple regression equation is $Y = a + bx_1 + bx_2 + bx_3$ where x_1 = temperature, x_2 = relative humidity and x_3 = rainfall. The following values were derived:- B weights which are the multiple regression coefficients for each independent variable, β^2 values which give a ranking of importance of the individual variables. The multiple R^2 value x 100 is called the "ccefficient of determination" and expresses the percentage of variation that may be accounted for by the environmental variables. The correlation coefficients for each variable and the incidence and intensity of infection were also derived.

As it was considered that the free-living and intermediate stages of the helminth parasites were most vulnerable to external influences, analyses were performed using the previous month's weather data as there may have been a time lag between population changes of the infective stages and consequent reflection in the infection rates in their hosts.

Results

(1) The host population

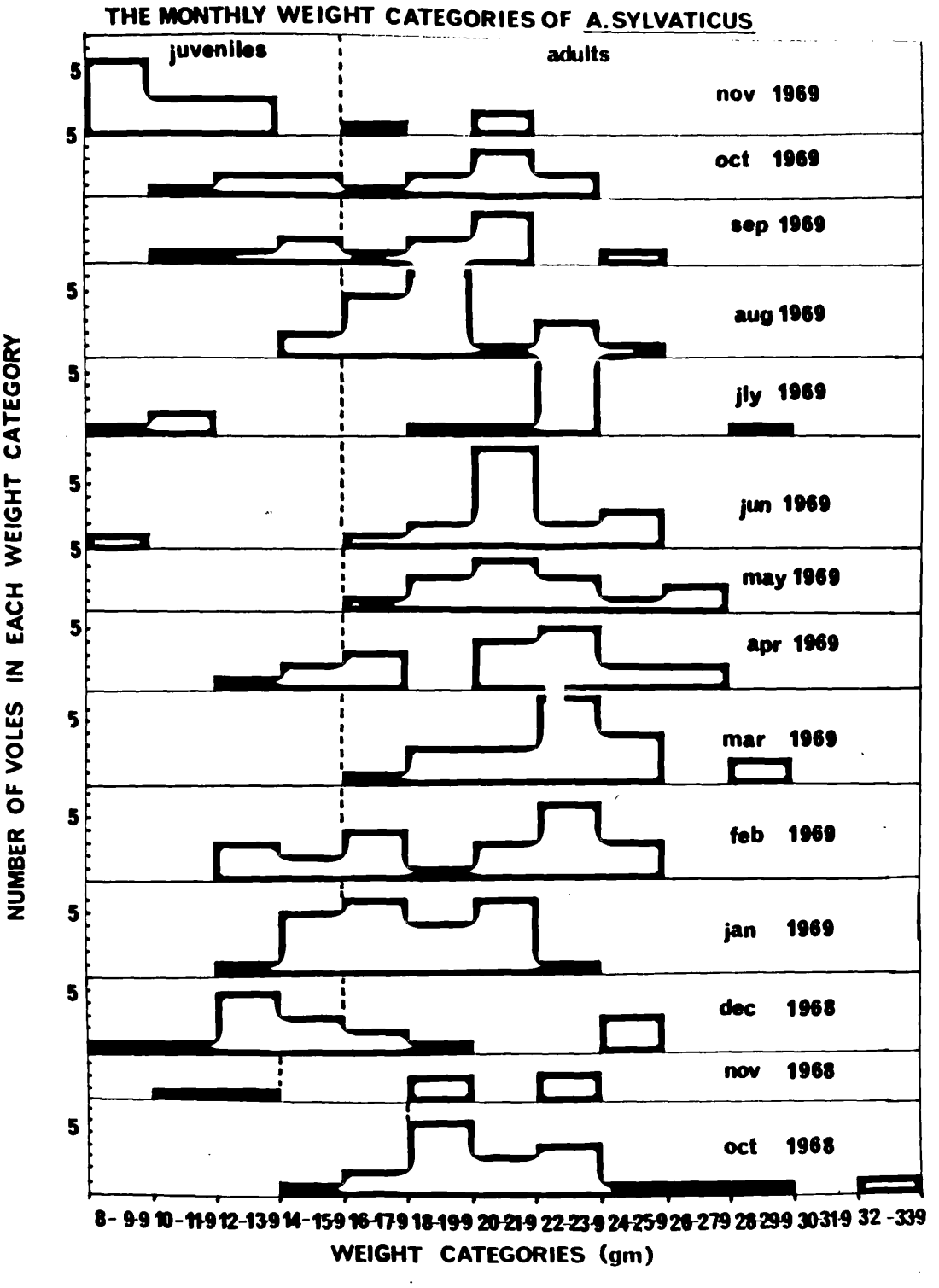
A total of 232 Apodemus sylvaticus and 101 Clethrionomys glareolus were trapped during the period October, 1968 to

November, 1969. Nine Sorex araneus, one Sorex minutus and two Microtus agrestis were also trapped, but were not examined for parasites. The shrews were always found dead in the traps and had started to decompose making examination for helminths impractical.

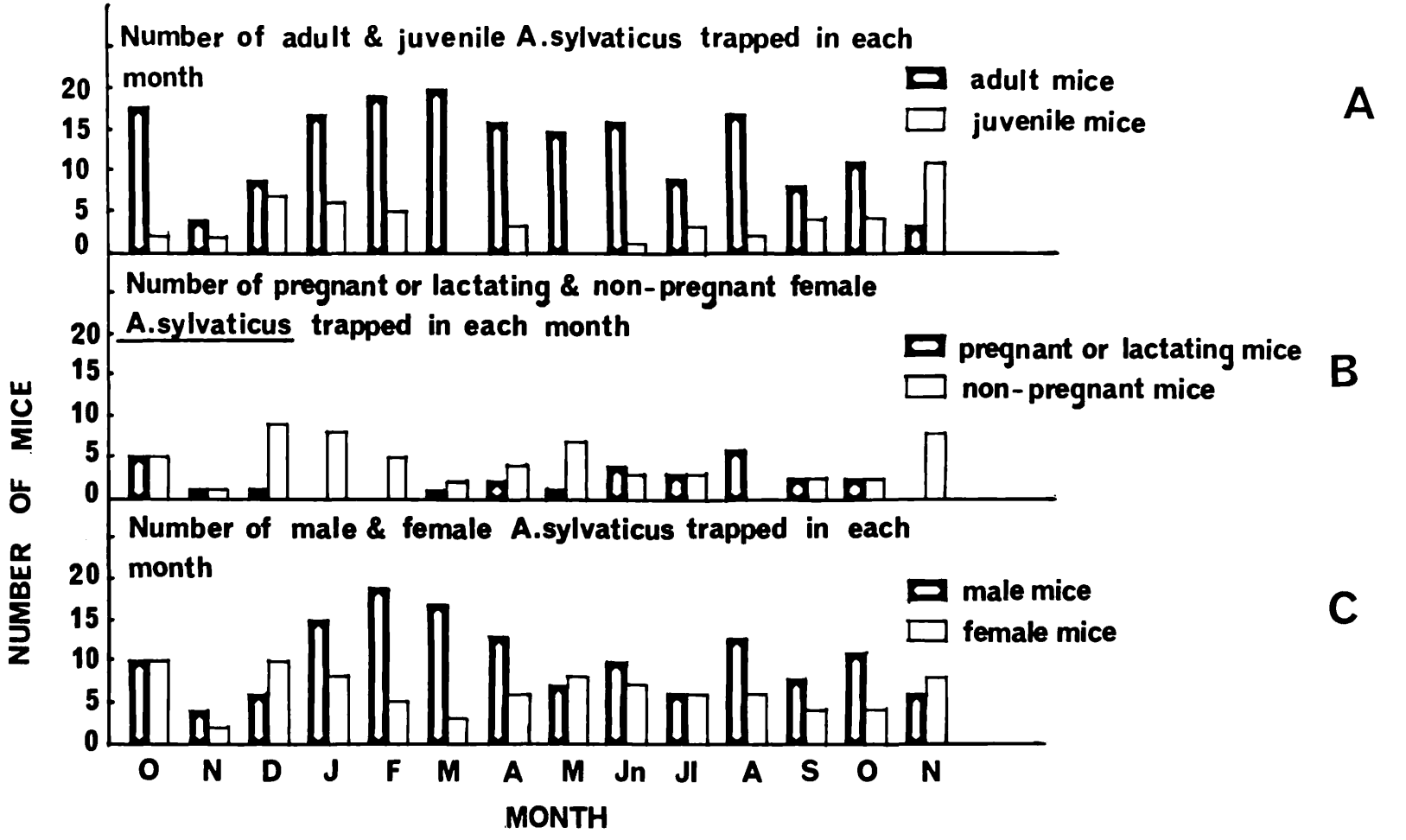
Apodemus sylvaticus

(a) Age analysis of the population

The frequency distributions of the number of animals in different weight categories trapped in each month were plotted (Figure 1.) A study of the distributions enabled an arbitrary weight to be selected which could be used to divide animals in to adults and juveniles. In October, 1968, animals of 18 g. and above were considered adult; mice which weighed 14 g. and over in November and December were considered to be adult. Juveniles were trapped in October, November and December, 1968, and pregnancies were also recorded in these months. It is clear that the overwintering population was composed of juveniles produced in the late litters of 1968 together with adults which were born in the first litters of 1968. This overwintering population is typical for Apodemus sylvaticus (Southern, 1964). It is possible that some of the overwintering adults may have been produced in 1967 or earlier as the potential life span of A.sylvaticus is four years (Ashby, 1967). In January and February, 1969, individuals of 16 g. and over were considered adult; at


THE MONTHLY WEIGHT CATEGORIES OF A.SYLVATICUS
juveniles
adults
nov 1969
oct 1969
sep 1969
aug 1969
jly 1969
jun 1969
may 1969
apr 1969
mar 1969
feb 1969
jan 1969
dec 1968
nov 1968
oct 1968
NUMBER OF VOLES IN EACH WEIGHT CATEGORY
8- 9 9 10- 11 9 12- 13 9 14- 15 9 16- 17 9 18- 19 9 20- 21 9 22- 23 9 24- 25 9 26- 27 9 28- 29 9 30- 31 9 32 - 33 9
WEIGHT CATEGORIES (gm)

this time of year adult and juvenile mice are very difficult to distinguish, but it is unlikely that any very young mice would have been recruited into the population at this time as no evidence of breeding was recorded during these months. According to Baker (1930) and Southern (1964) breeding rarely occurs during these months. The entire sample was considered to be mature in March, 1969 as no animals under 16 g. were trapped. The whole population was probably coming into breeding condition at this time and the first pregnancies were recorded in March (Figure 2b.). Sixteen grams was taken as the arbitrary dividing line between adult and juvenile mice from April until November, 1969. In April, three imperforate juveniles were trapped. No juveniles were trapped in May, although pregnancies were recorded in March and April. Presumably this was because the young had not yet left the nest, as they do not do so until they are fifteen - sixteen days old (Lowe, 1957). Juveniles were trapped in June and July which weighed between eight and twelve grams; they were probably recently weaned as young mice rarely leave the nest before they reach 7 g. weight (Hacker and Pearson, 1946). Juvenile and adult mice were trapped in every month from August until November; the latter sample consisted almost entirely of juvenile mice (Figure 2a). Pregnancies were recorded in all months from March until October; however, no adult females were trapped


Number of adult & juvenile A.sylvaticus trapped in each month
adult mice
juvenile mice
A
Number of pregnant or lactating & non-pregnant female A.sylvaticus trapped in each month
pregnant or lactating mice
non-pregnant mice
B
Number of male & female A.sylvaticus trapped in each month
male mice
female mice
C
NUMBER OF MICE
20
15
10
5
0
O N D J F M A M Jn Jl A S O N
MONTH

in November, so breeding may have continued into this month.

(b) The sex ratio of the host population

A total of 145 male and 87 female Apodemus sylvaticus were trapped giving an overall ratio of 1.67 - 1. The numbers of male and female mice occurring in each month are shown in Figure 2 c. Many workers have found that more male mice are caught than females and this may be due to a greater abundance of males or to their more bold and aggressive behaviour leading them to enter traps more frequently than females. According to Ashby (1967), male mice are more successful in intraspecific competition; this could account for the larger number of them entering traps to take food. The ratio of male to female A. sylvaticus trapped changed throughout the year. During the period October, 1968 to January, 1969, the ratio of males to females was in the region of equality - 1.17 - 1, which rose to 3.5 - 1 from February to April. This ratio dropped from May to August to 1.37 - 1 and started to rise again to 1.5 - 1 during the period September to November. A similar variation in the ratio of male to female A. sylvaticus present in a population was found by Baker (1930). He suggested that the fluctuations may in part be due to the different behaviour of the sexes at the various seasons. The rise in sex ratio in spring may be caused by the males wandering further under the promptings of sexual instinct which arise earlier in the year than

those of females, and the rise in late summer may be due to males being very active in storing food for winter, while the females only become more active later in the year when fewer pregnancies occur.

(c) General habits

Apodemus sylvaticus characteristically lives in runways in and below litter. Nests are constructed below ground of finely shredded grasses and are used for resting as well as breeding (Southern, 1964). The social organisation is loosely based on the family unit, but the home ranges may overlap. The average home range for female mice is 1,344 sq. yds and 2,171 sq. yds for male mice (Kikkawa, 1964).

Apodemus sylvaticus is almost entirely nocturnal with dusk and dawn peaks of activity, and a single peak in summer (Miller, 1955). They are most active on dark nights and are inhibited even by moonlight. Rainfall can delay the appearance of A. sylvaticus above ground but does not inhibit its activity under shelter, although it will be much reduced in exposed areas (Brown, 1956a). Female A. sylvaticus generally have less time for exploration or even to come above ground, being concerned with the rearing of their young

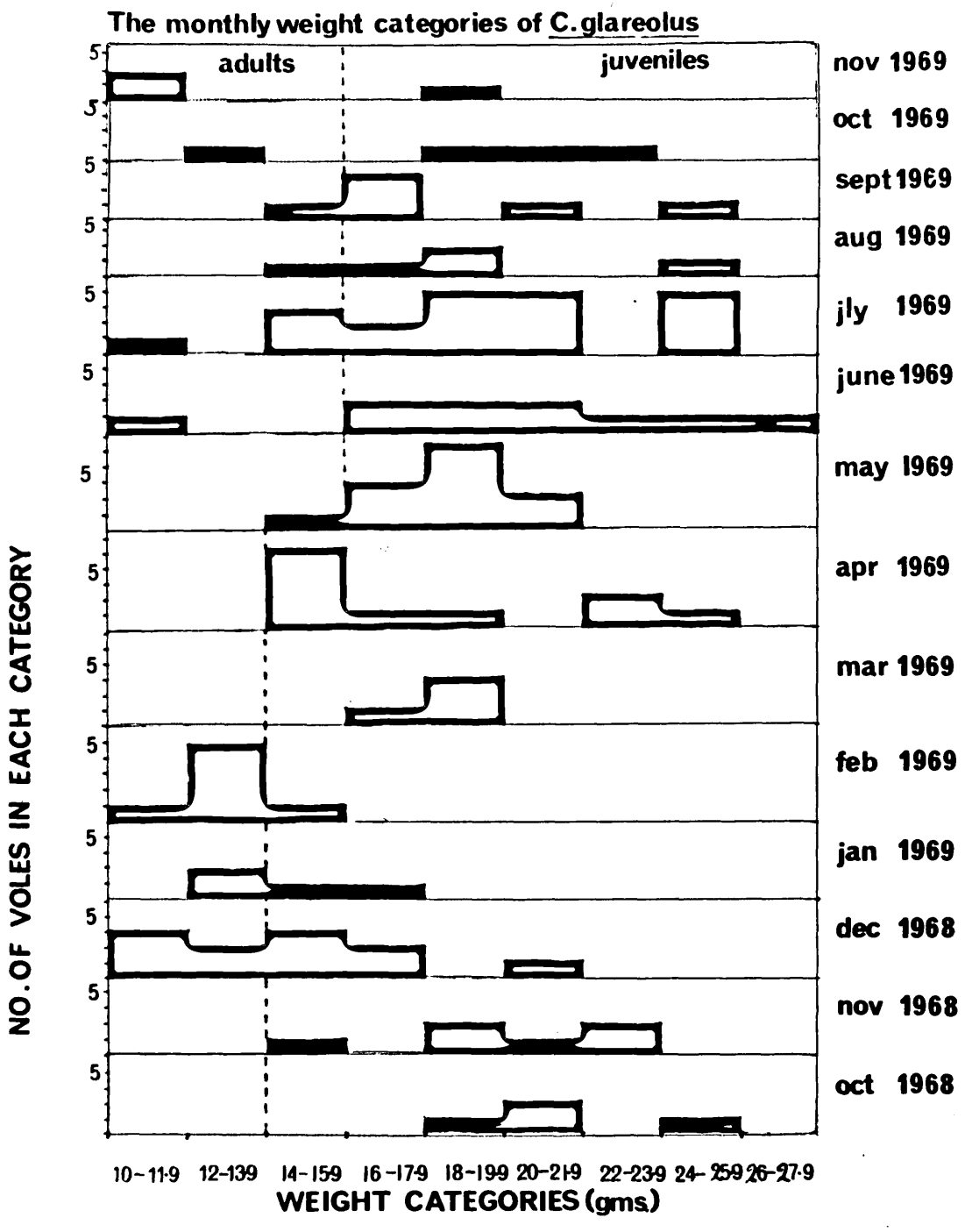
Casual observation of the stomach contents during this survey showed that larval and adult insects, grain, seeds and berries were commonly eaten. Faeces were frequently found

in the stomach indicating that refraction was common. Young mice generally eat less insects and more buds and fungi than adults, and food is stored in winter below ground (Southern, 1964).

Clethrionomys glareolus

(a) Age analysis of the host population

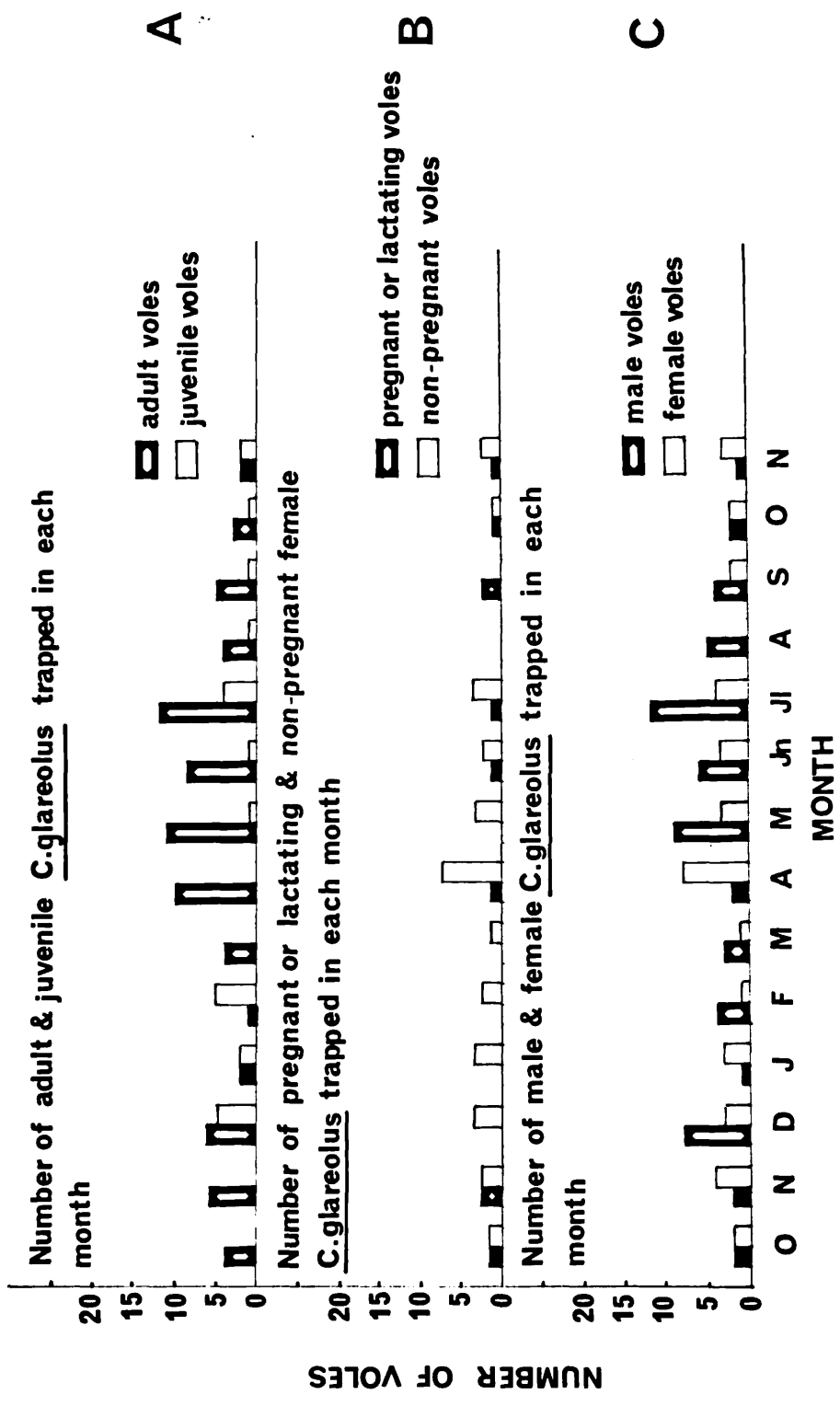
The monthly frequency distributions of the number of Clethrionomys glareolus in the different weight categories are shown in Figure 3. The arbitrary weight below which all animals were considered juvenile was 14 g. for the months October, 1968 until April, 1969. No juveniles were trapped in October or November, 1968, which was unexpected as pregnancies were recorded in these months and five juveniles were trapped in December. As was found with A. sylvaticus, the overwintering population of Clethrionomys glareolus consisted of a mixture of juveniles produced late in 1968 and adults born early in 1968 or earlier. Both adult and juvenile voles were trapped during January and February - but as was found with A. sylvaticus, they were very similar in weight and consequently difficult to distinguish; however, many of the lighter females were imperforate which indicated that they were truly juvenile. No lactating or pregnant females entered the traps from December, 1968 until March, 1969 - indicating that breeding ceased over the winter months. Only adult voles were caught in March and April. The first


The monthly weight categories of C.glareolus
adults
juveniles
nov 1969
oct 1969
sept 1969
aug 1969
jly 1969
june 1969
may 1969
apr 1969
mar 1969
feb 1969
jan 1969
dec 1968
nov 1968
oct 1968
5
NO.OF VOLES IN EACH CATEGORY
10-11·9 12-13·9 14-15·9 16-17·9 18-19·9 20-21·9 22-23·9 24-25·9 26-27·9
WEIGHT CATEGORIES (gms.)

pregnancies were recorded in April and the first juveniles of the breeding season were caught in May. From May to November, all adults which weighed 16 g. or more were considered to be adult. A heavier arbitrary dividing weight was used after April as adult animals are known to increase in weight at the onset of the breeding season.(Ashby, 1967). Both adult and juvenile voles were trapped in every month from May to November; pregnancies occurred in these months except May and August, no females being caught in the latter month. The numbers of adult, juvenile, and pregnant voles caught in each month are shown in Figures 4 a and b.

(b) The sex ratio of the host population

A total of 101 Clethrionomys glareolus were trapped, 61 males and 40 females, giving an overall ratio of 1.53 - 1. The numbers of male and female voles occurring in each month are shown in Figure 4c. Although the total sample was small, the ratio of males to females has been calculated for the four seasons, and as was found with Apodemus sylvaticus, the ratio of male to female voles changed throughout the breeding season. During the months October to January, substantially more males were trapped; the ratio was 1.3 - 1. More females were trapped from February to April, the ratio being 1 - 1.22. During the summer months of May to August, more males were caught, the ratio being 4.6 - 1, and equal numbers of each sex were trapped


A
Number of adult & juvenile C.glareolus trapped in each month
adult voles
juvenile voles
B
Number of pregnant or lactating & non-pregnant female C.glareolus trapped in each month
pregnant or lactating voles
non-pregnant voles
C
Number of male & female C.glareolus trapped in each month
male voles
female voles
0
5
10
15
20
O N D J F M A M Jn Jl A S O N
MONTH
NUMBER OF VOLES

during the period September to November. Whether these changes in the sex ratio reflect seasonal behavioural differences between the two sexes over the sampling period as was suggested for Apodemus sylvaticus, or whether they are merely an artefact due to the small sample size, is difficult to determine.

(c) General habits

Clethrionomys glareolus characteristically inhabits woodland with a dense ground cover, the extent of ground cover being much more important in determining the distribution than it is in Apodemus sylvaticus. The home range is similar to that of A. sylvaticus with an average of 2,641 sq. yds for male voles and 1,344 sq. yds for female voles (Kikkawa, 1964). C. glareolus forages both above and below ground and burrows actively making a runway system centred on a nest at 2 - 10 cms depth (von Wrangel, 1939). According to Miller (1955) and Osterman (1956), C. glareolus is active throughout twenty-four hours with more nocturnal activity in the summer; activity is, however, markedly diurnal in thick cover (Brown, 1956 a). C. glareolus is more colonial in habit than A. sylvaticus, many family units living in a burrow system. Voles take more vegetable than animal food, eating a higher proportion of green plants than A. sylvaticus. Larval and adult insects form approximately a third of their diet (Southern, 1964) but they also eat fungi and other bank voles (Türcek,1953; Miller, 1954).

(2) The parasite population:-

List of helminth parasites recovered.

(a) Host - Apodemus sylvaticus

Parasite	Microhabitat
Nematoda:-	
Capillaria muris sylvatici, (Diesing, 1851)	Duodenum
Nematospiroides dubius, (Baylis, 1926)	Duodenum and small intestine
Syphacia stroma, (von Linstow, 1884)	Duodenum and small intestine
Trematoda (Digenea)	
Corrigia vitta, (Dujardin, 1845)	Interlobary canals of the pancreas and duodenum
Cestoda	
Cysticercus taeniae taeniaeformis, (Batsch, 1786)	Liver
Hymenolepis muris sylvatici, (Rudolphi,1819)	Small intestine

(b) Host - Clethrionomys glareolus

Parasite	Microhabitat
Nematoda:-	
Aspiculuris tetraptera, (Nitzch, 1821)	Colon and Rectum
Capillaria muris sylvatici, (Diesing, 1851)	Stomach
Nematospiroides dubius (Baylis, 1926)	Duodenum Small intestine
Pelodera strongyloides (Schneider)	Lachrymal fluid of ocular orbit.
Cestoda:-	
Catenotaenia lobata, (Baer, 1925)	Small intestine

Life Cycles

A brief description has been given of the life cycles of the most commonly occurring parasites. Very few cestodes were found during the survey and they have therefore not been considered in detail. The nematode classification is that of Chitwood (1969).

Nematoda:-

Capillaria muris sylvatici

Capillaria muris sylvatici is a member of the sub-order Trichinelloidea, family Capillariidae. It has a direct life cycle and is found in various small mammals. It has been found in Apodemus sylvaticus and Clethrionomys glareolus in the present survey: in the former it is found in small numbers in the duodenum and in the latter it is abundant in the stomach. The males and females copulate within the host intestine and eggs are passed out with the faeces at an early stage of embryonation; the egg is the infective agent in this species. Further development of the egg is dependent upon the presence of moisture and oxygen, and the rate of development depends upon environmental temperature. They become embryonated in nine days at 25°C and take correspondingly longer to develop to infectivity at lower temperatures (Lewis, 1968 b). He observed no moults within the eggshell and concluded that four moults occurred within the host after ingestion. There is no account of the degree of resistance

of these eggs, but they may be similar to the infective eggs of the oxyurid Aspiculuris tetraptera, which can withstand long periods of near freezing (Chan, 1953). Prolonged viability is advantageous for an infective stage which does not rely on the host for transmission and develops outside the host, as it may have to wait for considerable periods of time before being picked up by a new host.

Nematospiroides dubius

Nematospiroides dubius belongs to the sub-order Trichostrongylina and is a member of the family Heligmosomatidae. The life cycle is a direct one (Ehrenford, 1945; Fahmy, 1953) no intermediate host being required. The adult and post infective larval stages live in the duodenum and small intestine of small rodents. The mature worms copulate in the host intestine and fertile eggs are passed out in the host's faeces at the 8 - 16 cell stage of embryonation. A first stage larva develops within the egg, the time taken for this being dependent on environmental temperature and humidity; the egg hatches to produce a free-living first stage larva which is motile. A period of feeding and growth ensues and two moults occur giving rise to an infective ensheathed third-stage larva which is capable of infecting a new host. The cuticle of the L2 is retained as a sheath for the L3. The length of time for the infective stage to be reached is again dependent on environmental

temperature, but at 20°C infective stages are produced after four days. The infective stages are ingested by the host species and the worms undergo two further moults within the host intestine; the worms are mature on the ninth day after infection. Egg production starts on the tenth day. The minimum generation time for this species is therefore fifteen days. However, as the third-stage larvae are resistant and can remain infective for long periods, the generation time could be considerably lengthened. A more detailed account of this life cycle is given in Chapter 4.

Pelodera strongyloides

This is a free-living soil dwelling rhabditoid nematode which under certain conditions is able to utilise murids as hosts. Osche (1963) considers the murids to be paratenic hosts. In this survey, Pelodera strongyloides larvae were frequently found in the lachrymal fluid of the orbits of Clethrionomys glareolus and very occasionally in Apodemus sylvaticus. It has been suggested that these "infective" stages are produced in response to dry conditions and that they leave the host during wet conditions. The life history was studied by Poinar (1965), who found that the "infective" stages are picked up on the feet of the host animals and transferred to the eyes during grooming. He maintained that the larvae underwent a developmental change whilst in the orbits, returning to the soil two to three weeks later,

to moult to the adult stage.

Syphacia stroma

Syphacia stroma belongs to the sub-order Oxyurina and is of the family Syphaciidae. The life cycle is a direct one and the adult and larval worms live in the small intestines of small rodents. Mature worms copulate in the host intestine and it would appear that the males die and pass out of the host soon afterwards (Morgan, 1932). Gravid females pass down the rectum to the anus and lay their eggs on the perianal skin of the host. This was not observed in the present study, but eggs were found in the perianal area; females become gravid 11 - 12 days after infection. Chan (1951) reported that Syphacia oblevata could remain in the rectum when gravid and repeatedly discharge eggs on to the perianal area of the host. In the present study, females with no eggs were occasionally found bound into a faecal pellet passing out of the host. Mature female worms with a few or no eggs were frequently found in the intestine, so they may re-invade the small intestine once they have laid their eggs and await refertilisation by a male worm, and then produce another batch of eggs. S. stroma eggs were rarely found in the faeces indicating that the females did not release their eggs inside the host. According to Lewis (1968 a) the contact with air and reduction of temperature experienced by the females are a necessary stimulus for egg deposition.

The eggs when laid contain a fully developed larva and within a few hours the egg becomes infective (Sharpe, 1961); the time taken for infectivity to be reached is dependent on environmental temperature. Unlike Nematospiroides dubius, S. stroma possesses no free-living infective stage and infection of new hosts is dependent upon physical contact between infected and uninfected individuals. Reinfection may also take place during grooming. The minimum generation time for this parasite was found to be 12 days by Lewis (1968 a) and as the infective eggs of S. stroma are not very resistant to adverse environmental conditions, it is unlikely that the generation time could be extended much beyond this period.

Trematoda (Digenea):-

The life cycle of the dicrocoeliid digenean Corrigia vitta is still unknown but it is probably similar to other dicrocoeliid life cycles which involve two intermediate hosts. The first intermediate host is likely to be a terrestial snail or slug, the second an insect or insect larva. The ingestion of an infected second intermediate host by a vole would lead to the formation of the mature fluke, which inhabits the main pancreatic ducts. Further details of this life history are described by Lewis (1964).

The incidence and intensity of infection of Apodemus sylvaticus with helminth parasites

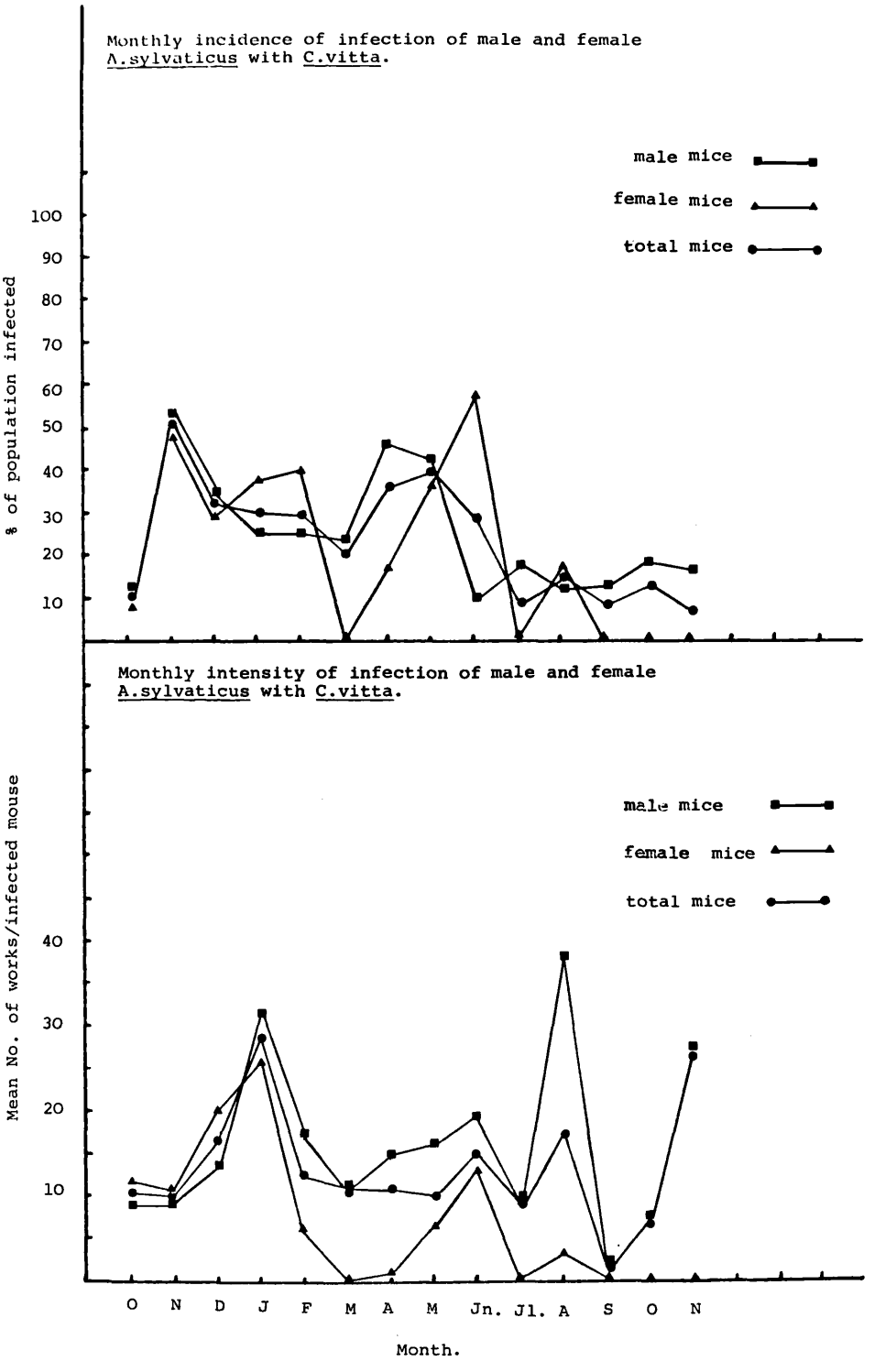
(1) Corrigia vitta

(a) Incidence of infection

Corrigia vitta occurred in every month of the sampling period, 23.27% of the total sample of Apodemus sylvaticus was infected. There was no significant difference in the incidence of C. vitta in male and female mice ($p > 0.05$), 24.14% of the males and 21.84% of the females being infected. The level of infection fluctuated from month to month, but there was no obvious seasonal variation (Figure 5a). There was a high incidence of C. vitta in November, 1968, but this result was unreliable as the sample size was very small. Infection levels were generally higher during April, May and June, dropping to a lower level in July which was maintained until the end of the sampling period in November, 1969. Out of 54 infected individuals, only four were juveniles, indicating that this age group is less susceptible than adults to infection with C. vitta.

(b) Intensity of infection

It can be seen from Figure 5b that the variation in intensity of infection of Apodemus sylvaticus with Corrigia vitta was seasonal, the largest worm burdens being recorded during the winter months at each end of the sampling period. During December, 1968, the mean number of C. vitta per

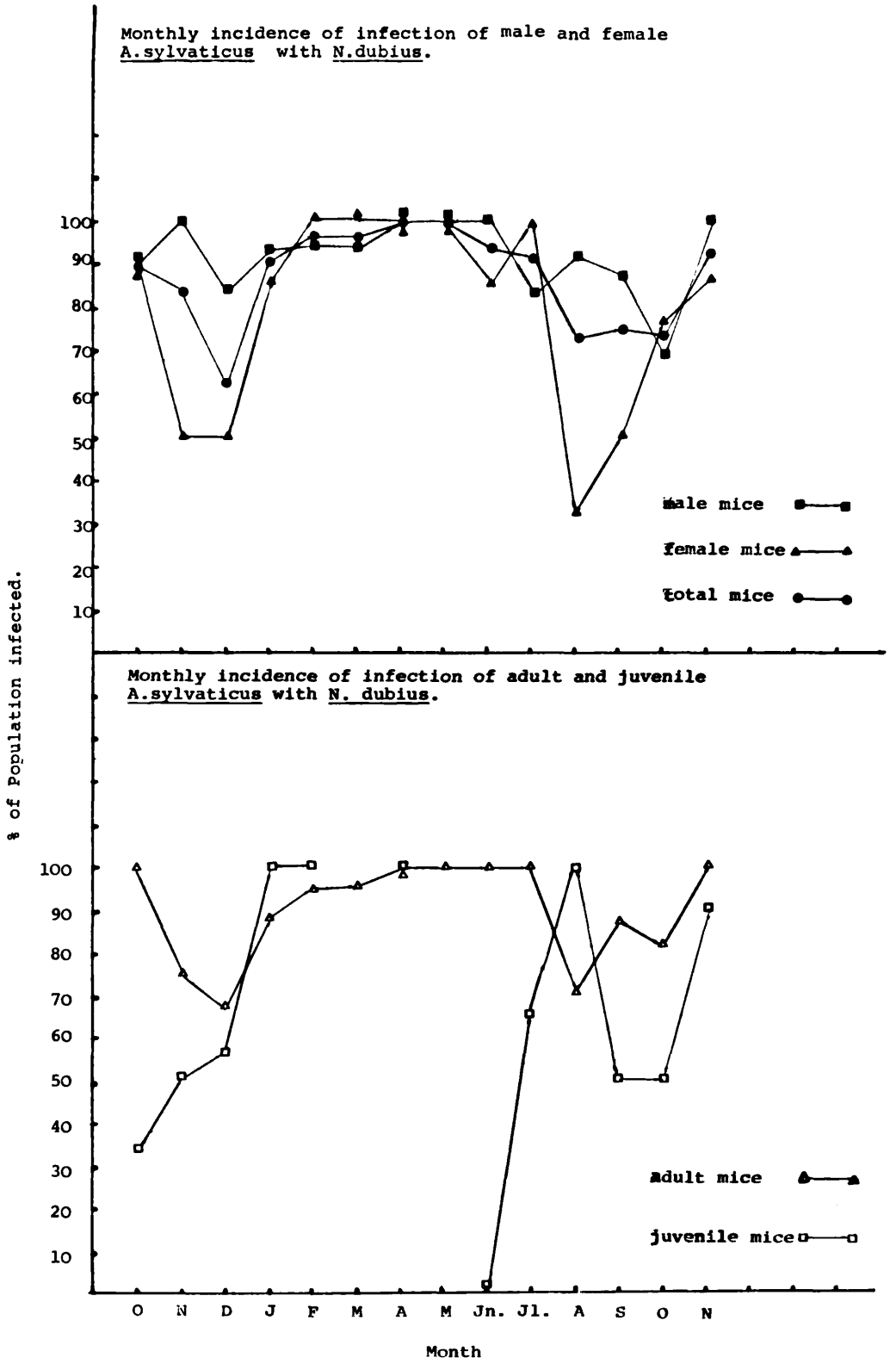

Monthly incidence of infection of male and female A.sylvaticus with C.vitta.
male mice
female mice
total mice
% of population infected
100
90
80
70
60
50
40
30
20
10
Monthly intensity of infection of male and female A.sylvaticus with C.vitta.
male mice
female mice
total mice
Mean No. of works/infected mouse
40
30
20
10
O N D J F M A M Jn. Jl. A S O N
Month.

infected host rose to reach a peak in January, 1969. The mean worm burden dropped in February and remained approximately the same in each month until August when it rose slightly. The intensity of infection was very low in September but rose to a peak in November. Although there was no significant difference in the mean worm burdens in male and female mice ($p > 0.05$), males generally harboured larger numbers of C. vitta. Adult mice carried significantly more worms than juvenile mice ($p < 0.05$).

(2) Nematospiroides dubius

(a) Incidence of infection

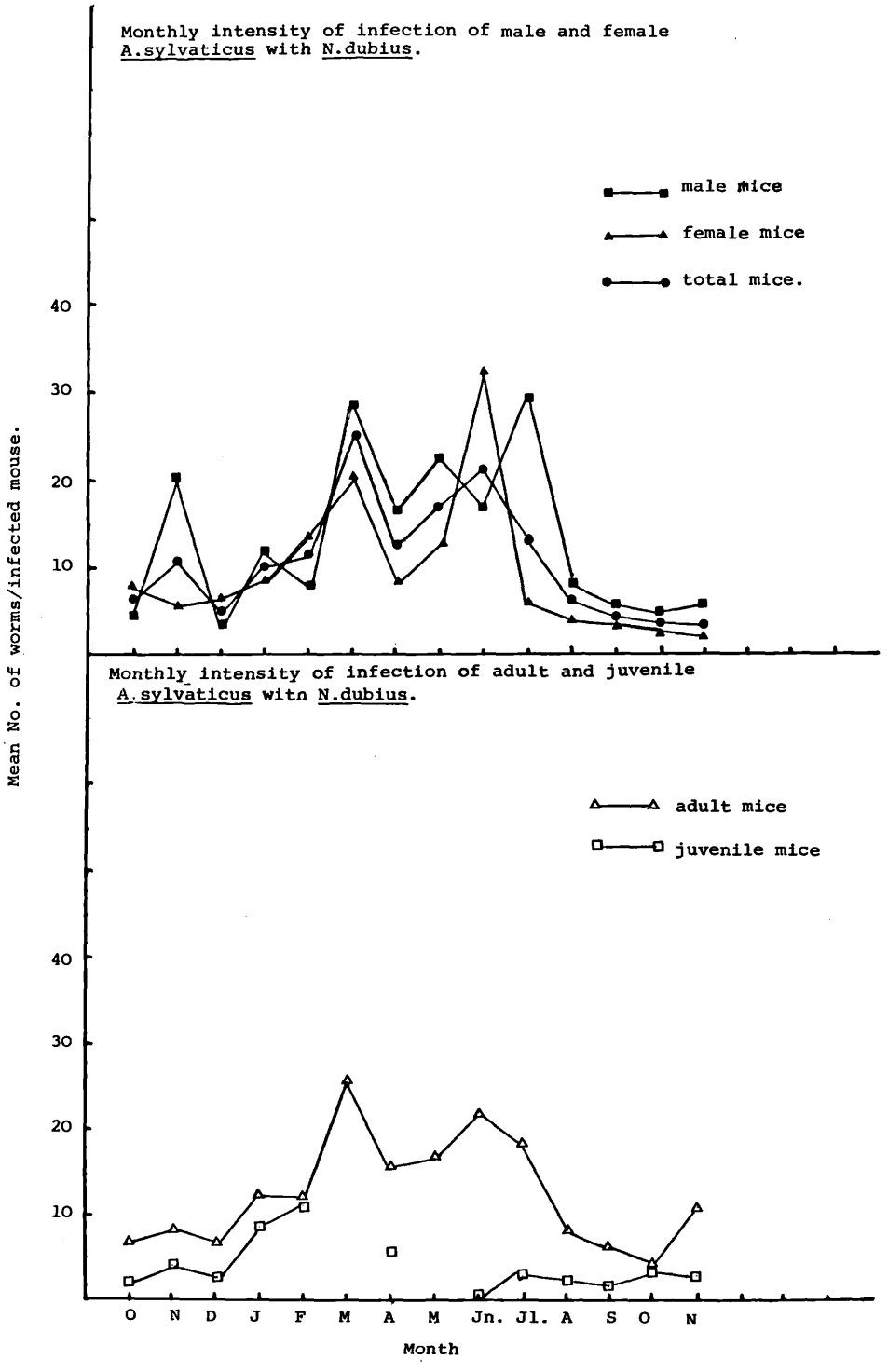
Nematospiroides dubius was the most commonly occurring parasite of Apodemus sylvaticus, 87% of the total sample being infected. There was evidence of a seasonal variation in incidence (Figure 6a). In October, 1968, 90% of both male and female mice were infected with N. dubius. Although the infection level dropped during November and December, it started to increase in January, 1969; the decrease in incidence of N. dubius was most pronounced in female mice. The level of infection remained high until July when it dropped, and remained low until October; again the decrease in incidence was more pronounced in female mice; in November, 1969, the number of mice infected increased again. A significantly greater proportion of female mice were infected with N. dubius ($p < 0.01$). The monthly incidence in adult and juvenile mice followed the same general trend, the highest incidence


Monthly incidence of infection of male and female A.sylvaticus with N.dubius.
% of Population infected.
100
90
80
70
60
50
40
30
20
10
Male mice
female mice
Total mice
Monthly incidence of infection of adult and juvenile A.sylvaticus with N. dubius.
100
90
80
70
60
50
40
30
20
10
Adult mice
juvenile mice
O N D J F M A M Jn. Jl. A S O N
Month

occurring during the spring and summer months (Figure 6b). The incidence was greater in adult than juvenile mice ($p < 0.001$), and in addition it was more variable in juveniles remaining almost constant in adults.

(b) Intensity of infection

The mean monthly worm burdens of Nematospiroides dubius are shown in Figure 7a. There was a seasonal variation in the intensity of infection with N. dubius which was similar to the variation in incidence of infection, higher worm burdens being recorded over the spring and summer months. During the winter months, October, 1968 to January, 1969, the intensity of infection was low, ranging from 6.07 worms/mouse in October to 9.26 worms/mouse in January. There was a high degree of infection in November - however, this result was unreliable as the sample size was very small. In February, the mean worm burden increased and a peak was reached in March; the monthly worm burdens decreased slightly in April and May and reached a second peak in June of 24 worms per infected mouse. In August, the worm burden dropped to 6 worms/mouse, and a similar intensity of infection was maintained until November, 1969. The mean number of Nematospiroides dubius in male mice was significantly greater than that in female mice ($p < 0.05$). The monthly intensity of infection of adult and juvenile mice is shown in Figure 7b; the worm burdens in adult mice were significantly

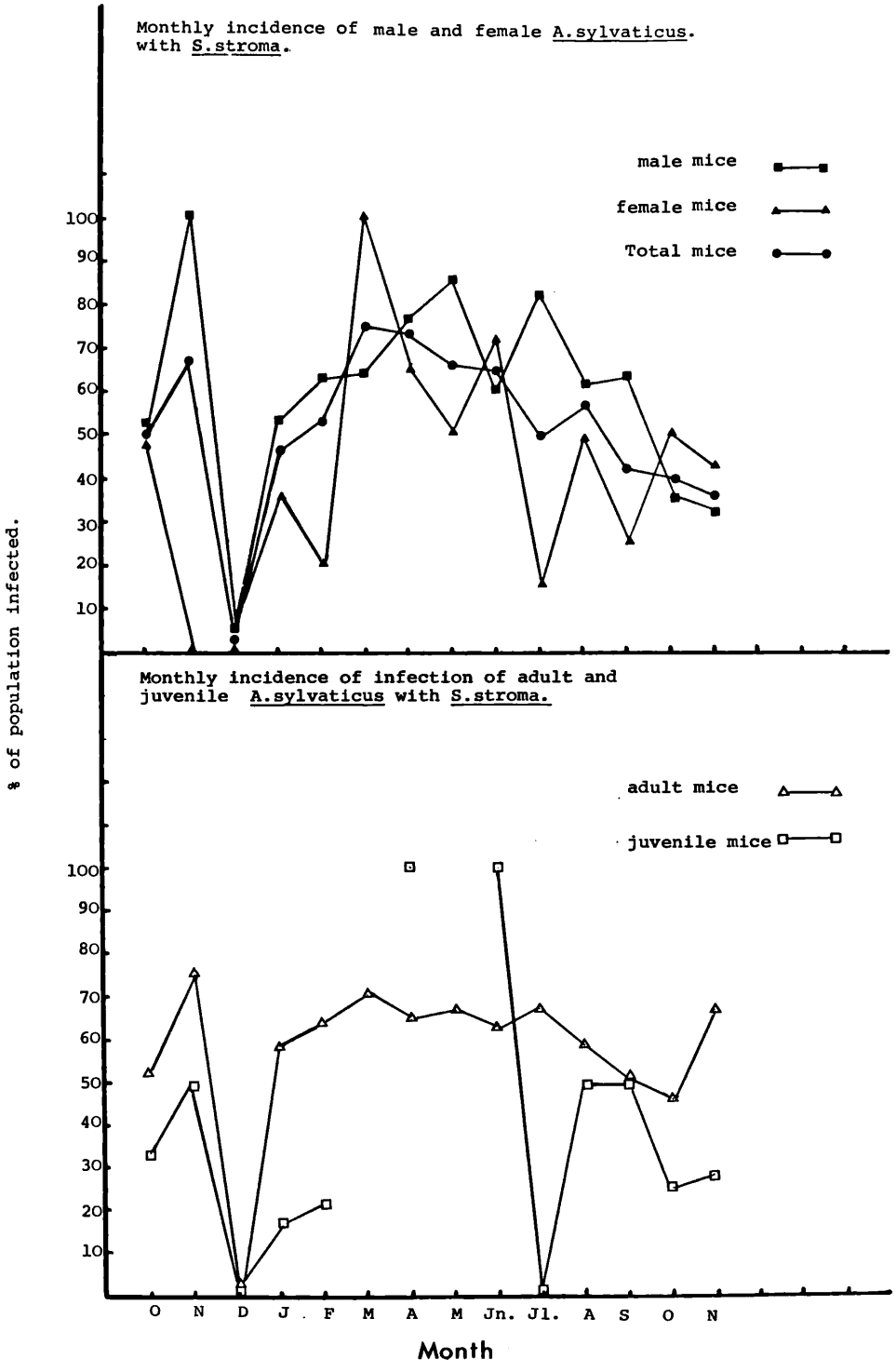

Monthly intensity of infection of male and female A.sylvaticus with N.dubius.
male mice
female mice
total mice.
40
30
20
10
Mean No. of worms/infected mouse.
Monthly intensity of infection of adult and juvenile A.sylvaticus with N.dubius.
adult mice
juvenile mice
40
30
20
10
O N D J F M A M Jn. Jl. A S O N
Month

greater than those in juvenile mice ($p < 0.001$). The adult worms showed a similar variation in intensity of infection as that shown by the total population, the highest worm burdens occurring during the period March to August. During the months December 1968 to February 1969, the worm burdens harboured by juvenile mice were similar to those harboured by the adults; in all other months they were much lower. There was no significant difference in the mean number of N. dubius harboured by pregnant and non-pregnant females ($p > 0.05$).

(3) Syphacia stroma

(a) Incidence of infection

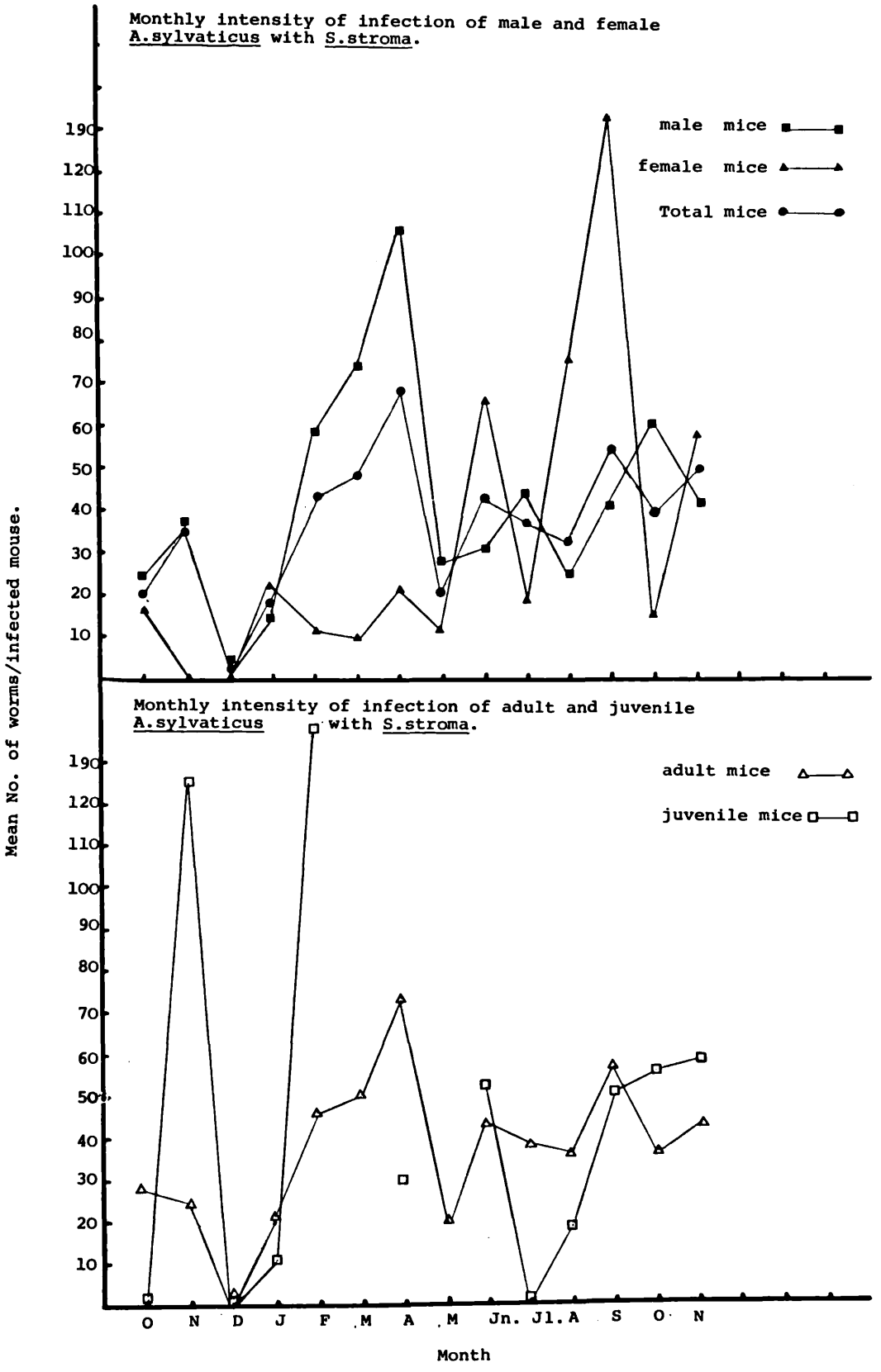
Syphacia stroma occurred in every month of the sampling period except December, 1968; 52% of the total sample was infected; 59.3% of the male and 40.2% of the female mice were infected. Figure 8a shows the monthly incidence of infection in males. females and the total population. There was seasonal variation in the incidence of S. stroma, higher levels of infection generally occurring during the spring and summer months. In October, 1968, 50% of the population was infected; the incidence of S. stroma rose in December, but this result was unreliable due to a small sample size. The infection levels were high from March to June ranging from 64.7% to 75%. However, they started to drop in July and continued to do so until September when 42.86% of the


Monthly incidence of male and female A.sylvaticus. with S.stroma.
male mice
female mice
Total mice
100
90
80
70
60
50
40
30
20
10
% of population infected.
Monthly incidence of infection of adult and juvenile A.sylvaticus with S.stroma.
adult mice
juvenile mice
O N D J F M A M Jn. Jl. A S O N
Month

population was infected; the incidence of infection remained at a low level until November, 1969. The incidence of infection was significantly greater in male than female mice ($p < 0.01$). Figure 8b shows the monthly incidence of S. stroma in adult and juvenile mice; 58.5% of the adults and 29.4% of the juveniles were infected, and the incidence was significantly higher in adult mice ($p < 0.001$). There was more variation in the incidence in juvenile mice; the infection level remained more or less constant in the adult mice, although it was slightly higher from March to July. Only a few juveniles were trapped during this period, but their infection level was high.

(b) Intensity of infection

The mean monthly worm burdens of Syphacia stroma are shown in Figure 9a, which indicates that no distinct seasonal variation in the intensity of infection occurred. However, two peaks of infection could be distinguished. A gradual build up in the number of S. stroma per infected host took place from January to April but in May the mean worm burden was lower; the number of worms per host increased during June and July to reach a second peak in September. The degree of infection was low in October 1968 and 1969 but had increased by November in both years. Male mice had their highest worm burdens in April, and females had their highest worm burdens in September; the latter sample consisted of


Monthly intensity of infection of male and female A.sylvaticus with S.stroma.
male mice
female mice
Total mice
Monthly intensity of infection of adult and juvenile A.sylvaticus with S.stroma.
adult mice
juvenile mice
Mean No. of worms/infected mouse.
190
120
110
100
90
80
70
60
50
40
30
20
10
O N D J F M A M Jn. Jl. A S O N
Month

a single heavily infected individual and this result was therefore not reliable. There was no significant difference in the mean worm burdens harboured by male and female mice ($p > 0.05$). There was great variation in the monthly intensity of infection in adult and juvenile mice (Figure 9b), the variation being more extreme in juvenile mice; However, there was no significant difference between the worm burdens in the two age groups ($p > 0.05$). Pregnant and non-pregnant female mice also harboured similar worm burdens.

The results of the multiple regression and correlation analyses and the regression equations are shown in Table 1.

The incidence and intensity of infection of Clethrionomys glareolus with helminth parasites

(1) Capillaria muris sylvatici

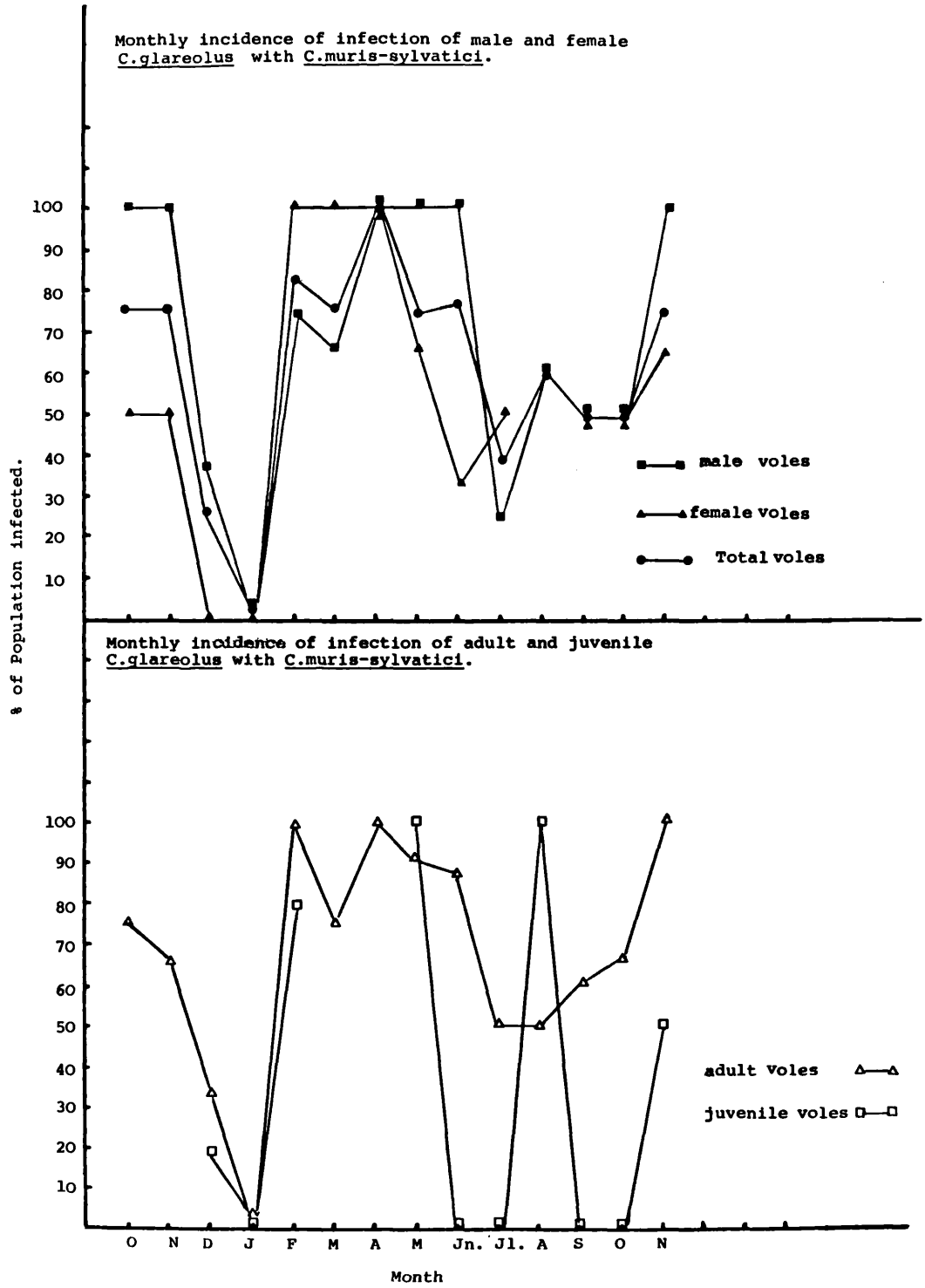
(a) Incidence of infection

Capillaria muris sylvatici was found in every month of the sampling period except January, 1969, 62.3% of the trapped population being infected. The monthly incidence of infection of male and female voles shows that there was a seasonal variation in the level of infection (Figure 10a). A lower percentage of the voles harboured C. muris sylvatici between February and June, 1969 than during the periods October, 1968 to January, 1969, and July and October, 1969. A significantly greater number of male voles than females

Results of the multiple regression and correlation analyses examining the relationship between the incidence and intensity of infection of A. sylvaticus with helminth parasites and selected environmental variables

	Multiple r^2	°C r (sig.)	R.H. r (sig.)	Rainfall r (sig.)	F (sig)	°C $\beta 1^2$	R.H. $\beta 2^2$	Rainfall $\beta 3^2$	°C B1	R.H. B2	Rainfall B3	Multiple regression equation
Incidence of C.vitta/ concurrent weather	0.387	-0.486	-0.158	0.026	2.105	0.436	0.173	0.000	-1.858	-1.033	-0.01	$Y = 129.9063 - 1.858X_1 - 1.033X_2 - 0.01X_3$
Incidence of C.vitta/ previous weather	0.326	-0.570	0.183	0.058	1.616	0.327	0.000	0.000	-1.62	-0.026	0.01	$Y = 42.165 - 1.62X_1 - 0.026X_2 + 0.01X_3$
Intensity of C.vitta/ concurrent weather	0.539	-0.408 *	0.278	0.686 ***	3.898 *	0.039	0.024	0.365	-0.311	0.246	0.154	$Y = -13.61 - 0.311X_1 + 0.246X_2 + 0.154X_3$
Intensity of C.vitta/ previous weather	0.263	-0.355	0.367	-0.125	1.192	0.061	0.148	0.076	-0.390	0.533	-0.058	$Y = -24.549 - 0.390X_1 + 0.533X_2 - 0.058X_3$
Incidence of N.dubius/ concurrent weather	0.152	-0.112	0.203	-0.239	0.598	0.000	0.100	0.124	-2.117	18.730	-31.533	$Y = -911.56 - 2.117X_1 + 18.730X_2 - 31.533X_3$
Incidence of N.dubius/ previous weather	0.317	-0.425	-0.193	0.186	1.553	0.304	0.174	0.017	-0.132	-0.088	0.004	$Y = 17.3 - 0.132X_1 - 0.088X_2 + 0.004X_3$
Intensity of N.dubius/ concurrent weather	0.495	-0.120	-0.584 *	-0.101	3.261	0.172	0.590	0.002	-0.616	-1.006	0.012	$Y = 102.112 - 0.616X_1 - 1.006X_2 + 0.012X_3$
Intensity of N.dubius/ previous weather	0.329	-0.400	-0.024	0.008	1.632	0.293	0.214	0.019	-0.808	-0.609	0.028	$Y = 69.808 - 0.808X_1 - 0.609X_2 + 0.028X_3$
Incidence of S.stroma/ concurrent weather	0.5275	-0.220	-0.582 *	-0.055	3.35 *	0.19	0.555	0.005	-1.250	-1.751	0.0325	$Y = 214.204 - 1.250X_1 - 1.751X_2 + 0.0325X_3$
Incidence of S.stroma/ previous weather	0.353	-0.441	-0.204	0.094	1.643	0.302	0.195	0.048	-1.448	-1.025	0.075	$Y = 152.935 - 1.448X_1 - 1.025X_2 + 0.075X_3$
Intensity of S.stroma/ concurrent weather	0.491	-0.170	-0.370	-0.566 *	2.933	0.143	0.114	0.291	-1.201	-0.896	-0.269	$Y = 142.147 - 1.201X_1 - 0.896X_2 - 0.269X_3$
Intensity of S.stroma/ previous weather	0.057	-0.027	0.022	-0.212	0.182	0.000	0.013	0.065	0.040	0.300	-0.010	$Y = 21.288 + 0.040X_1 + 0.300X_2 - 0.010X_3$

* $p < 0.05$
*** $p < 0.01$


Monthly incidence of infection of male and female C.glareolus with C.muris-sylvatici.
% of Population infected.
100
90
80
70
60
50
40
30
20
10
male voles
female voles
Total voles
Monthly incidence of infection of adult and juvenile C.glareolus with C.muris-sylvatici.
adult voles
juvenile voles
O N D J F M A M Jn. Jl. A S O N
Month

were infected with this parasite ($p < 0.05$). It can be seen from Figure 10b that the level of infection was greater in adult than juvenile voles, and the incidence was significantly higher in the adult voles ($p < 0.02$).

(b) Intensity of infection

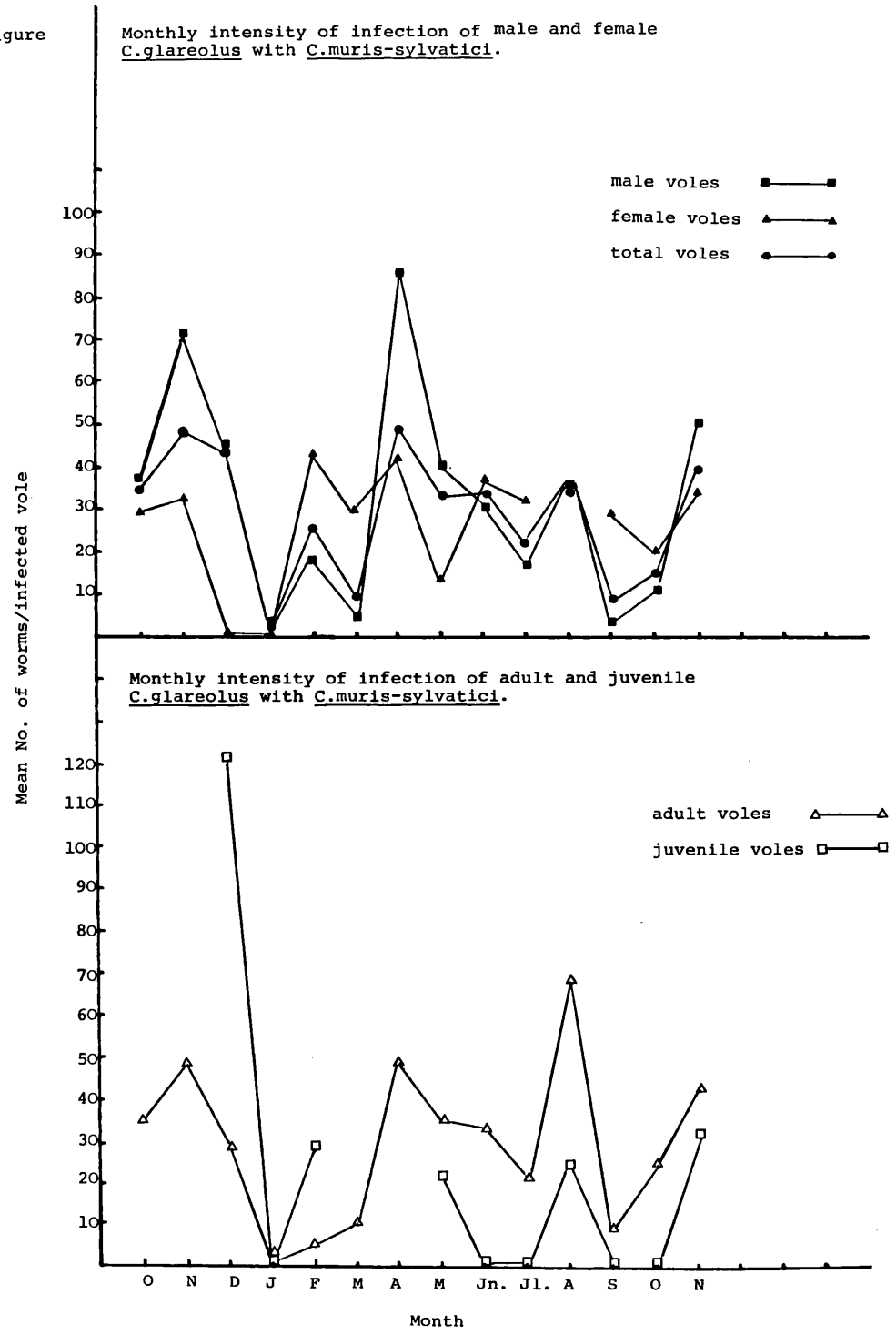
The mean monthly worm burdens of C. muris sylvatici showed considerable variation which did not appear to be seasonal, although some of the highest worm burdens were recorded during the summer months (Figure 11a). There was no significant difference in the mean number of worms harboured by male and female voles ($p > 0.05$) or adult and juvenile voles ($p > 0.05$), that is all the sex and age groups were equally susceptible to infection with C. muris sylvatici. The monthly worm burdens in adult and juvenile voles are shown in Figure 11b.

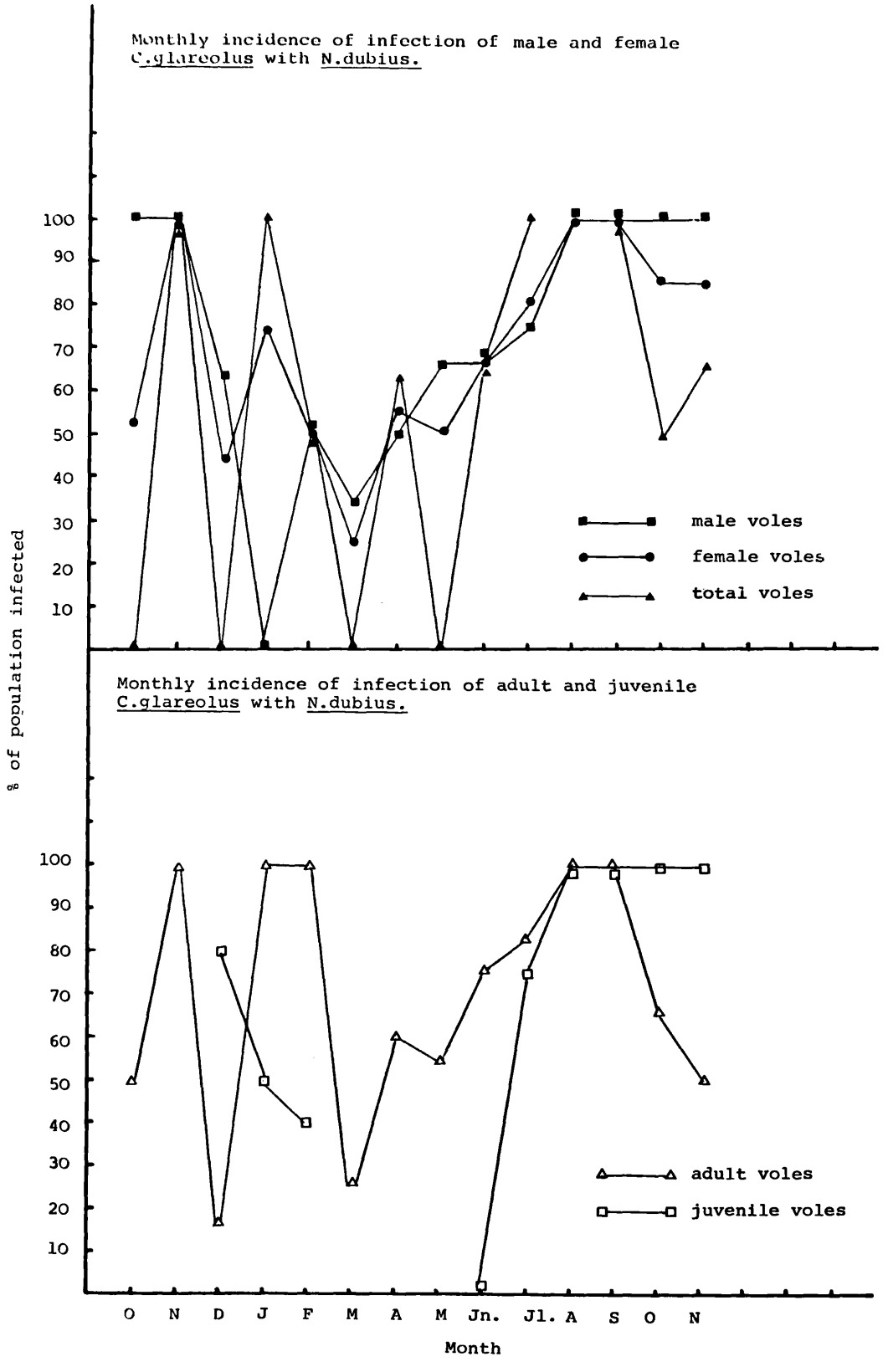
(2) Nematospiroides dubius

(a) Incidence of infection

Nematospiroides dubius occurred in every month of the sampling period, 67.3% of the voles being infected. The monthly incidence of infection of male and female voles is shown in Figure 12a; there was no significant difference in the percentage of each sex infected ($p > 0.05$). A distinct seasonal variation in incidence was observed, which was high during the period October, 1968 until January, 1969, and from August to the end of the sampling period in November, 1969.

Figure


Monthly intensity of infection of male and female C.glareolus with C.muris-sylvatici.
male voles
female voles
total voles
100
90
80
70
60
50
40
30
20
10
Monthly intensity of infection of adult and juvenile C.glareolus with C.muris-sylvatici.
adult voles
juvenile voles
120
110
100
90
80
70
60
50
40
30
20
10
Mean No. of worms/infected vole
O N D J F M A M Jn. Jl. A S O N
Month


Monthly incidence of infection of male and female C.glareolus with N.dubius.
% of population infected
100
90
80
70
60
50
40
30
20
10
male voles
female voles
total voles
Monthly incidence of infection of adult and juvenile C.glareolus with N.dubius.
adult voles
juvenile voles
O N D J F M A M Jn. Jl. A S O N
Month

The incidence was lower from February until July. A similar trend also occurred in adult and juvenile voles (Figure 12b) and there was no significant difference in the percentage of each age group infected ($p > 0.05$).

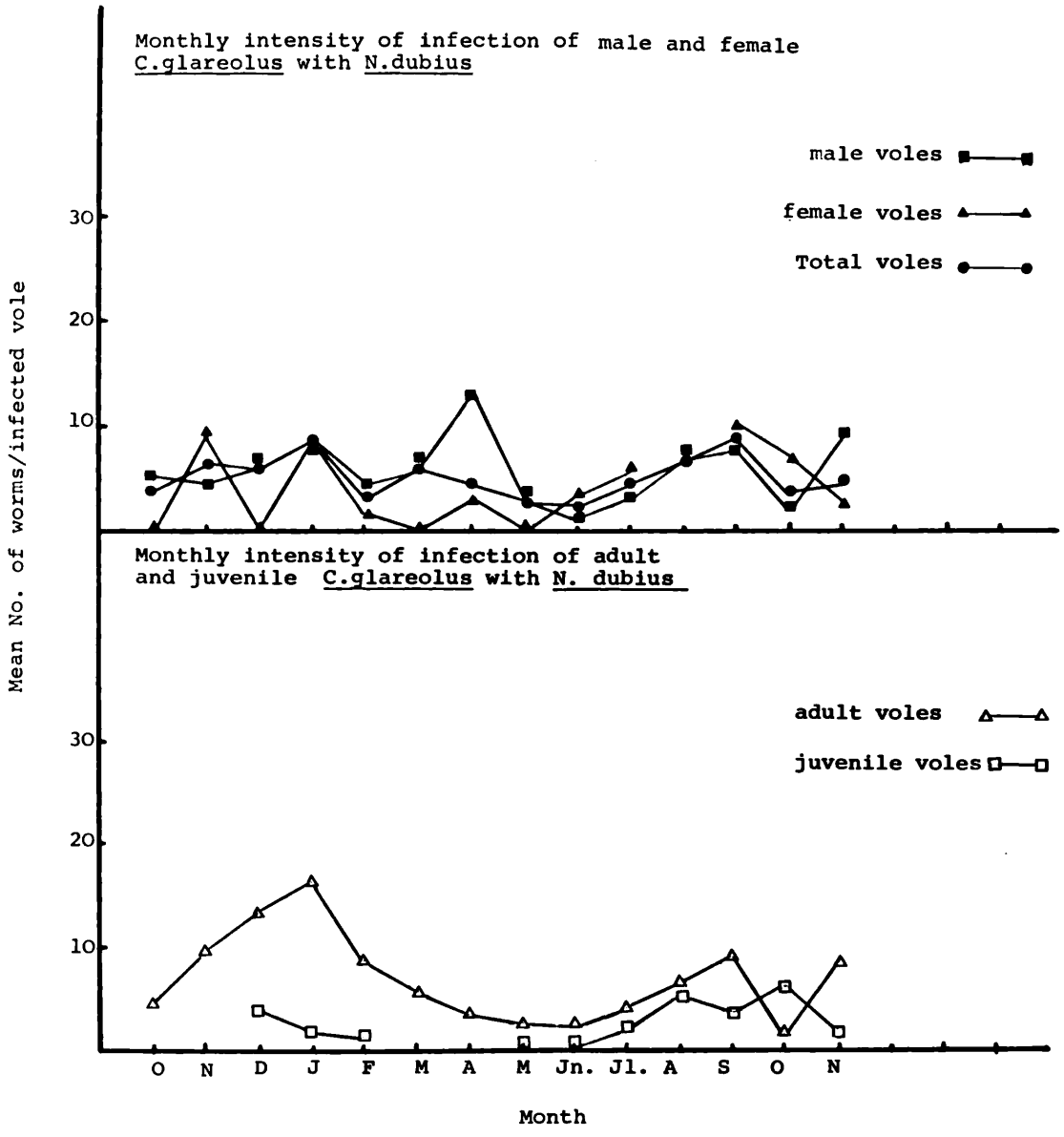
(b) Intensity of infection

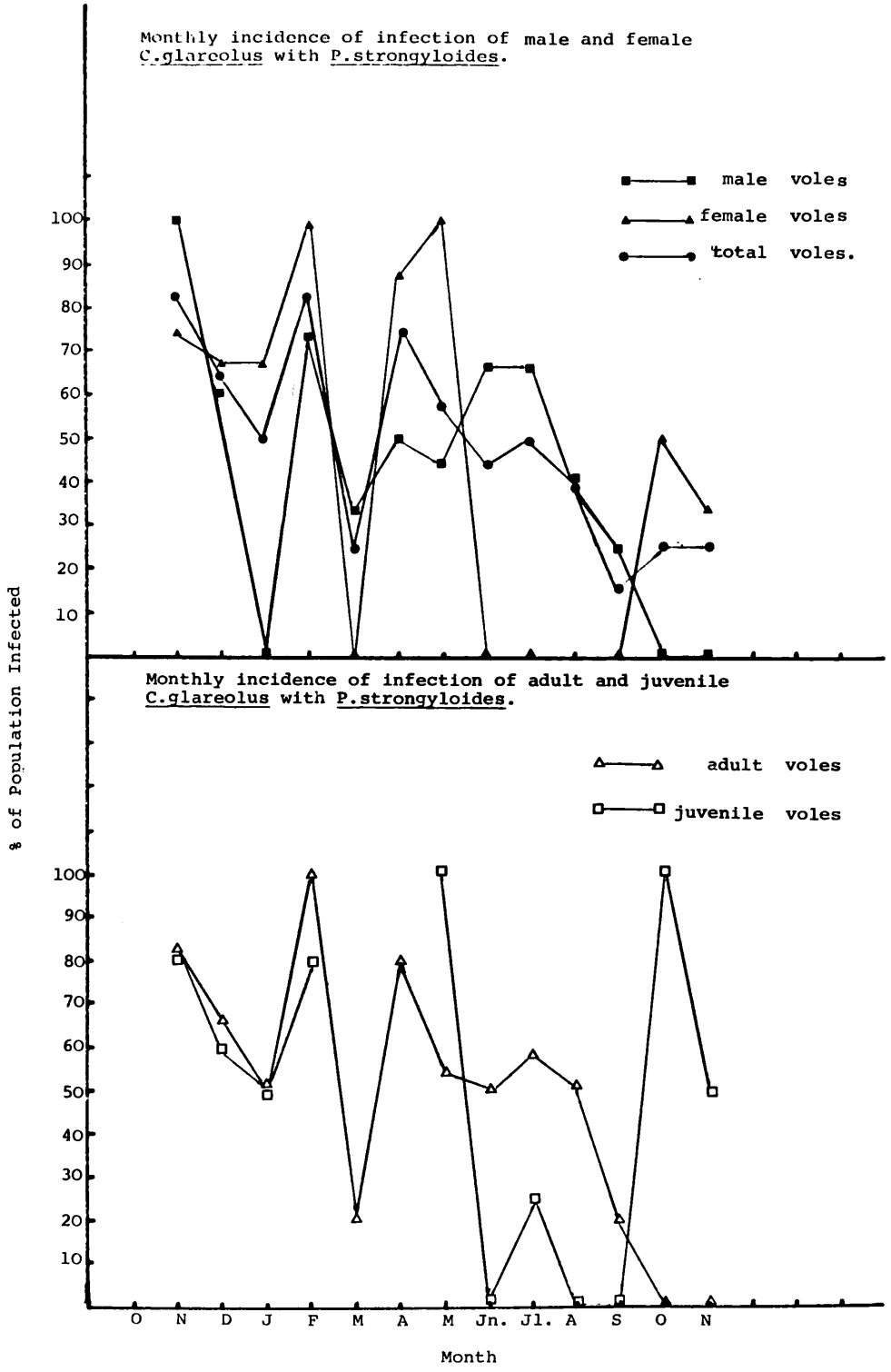
The mean monthly worm burdens of the total sample and male and female voles separately are shown in Figure 13a. There was no significant difference in the intensity of Nematospiroides dubius in male and female voles ($p > 0.05$). There was some evidence of a seasonal variation in intensity of infection, similar to that found for the incidence of infection, as lower mean worm burdens were recorded from February to July, 1969, than for the other months of the sampling period. This trend was also apparent in the monthly worm burdens recorded for adult and juvenile voles (Figure 13b). The worm burdens in adults were significantly larger than those in juvenile voles ($p < 0.02$).

(3) Pelodera strongyloides

(a) Incidence of infection

Pelodera strongyloides occurred in every month of the sampling period, 51.4% of the population being infected. The monthly incidence of infection in male and female voles is shown in Figure 14a, and there was no significant


Monthly intensity of infection of male and female C.glareolus with N.dubius
male voles
female voles
Total voles
30
20
10
Mean No. of worms/infected vole
Monthly intensity of infection of adult and juvenile C.glareolus with N. dubius
adult voles
juvenile voles
30
20
10
O N D J F M A M Jn. Jl. A S O N
Month


Monthly incidence of infection of male and female C.glareolus with P.strongyloides.
male voles
female voles
total voles.
100
90
80
70
60
50
40
30
20
10
Monthly incidence of infection of adult and juvenile C.glareolus with P.strongyloides.
adult voles
juvenile voles
% of Population Infected
O N D J F M A M Jn. Jl. A S O N
Month

difference in the incidence of either sex ($p > 0.05$). No seasonal variation in infection levels occurred although a smaller percentage of the population was infected during the latter half of the sampling period. Similar variation in monthly incidence occurred in adult and juvenile voles (Figure 14b), and there was no significant difference in the percentage of each age group infected ($p > 0.05$).

(b) Intensity of infection

The mean monthly worm burdens of Pelodera strongyloides show considerable variation, but again it was not seasonal (Figure 15a). However, the highest worm burdens generally occurred after rainfall had been low. There was no difference in the intensity of infection of P.strongyloides in male and female voles ($p > 0.05$), or adult and juvenile voles ($p > 0.05$) (Figure 15b).

The results of the multiple regression and correlation analyses and the regression equations are shown in Table 2.

Discussion

Apodemus sylvaticus

The variation in incidence of infection of Apodemus sylvaticus with Corrigia vitta was not seasonal, as was the variation in intensity of infection with this parasite. The overall seasonal variation in intensity of infection with C. vitta is a reflection of the type of food eaten throughout the year, as A. sylvaticus is omnivorous and its diet is known to vary according to the season (Ashby, 1967).

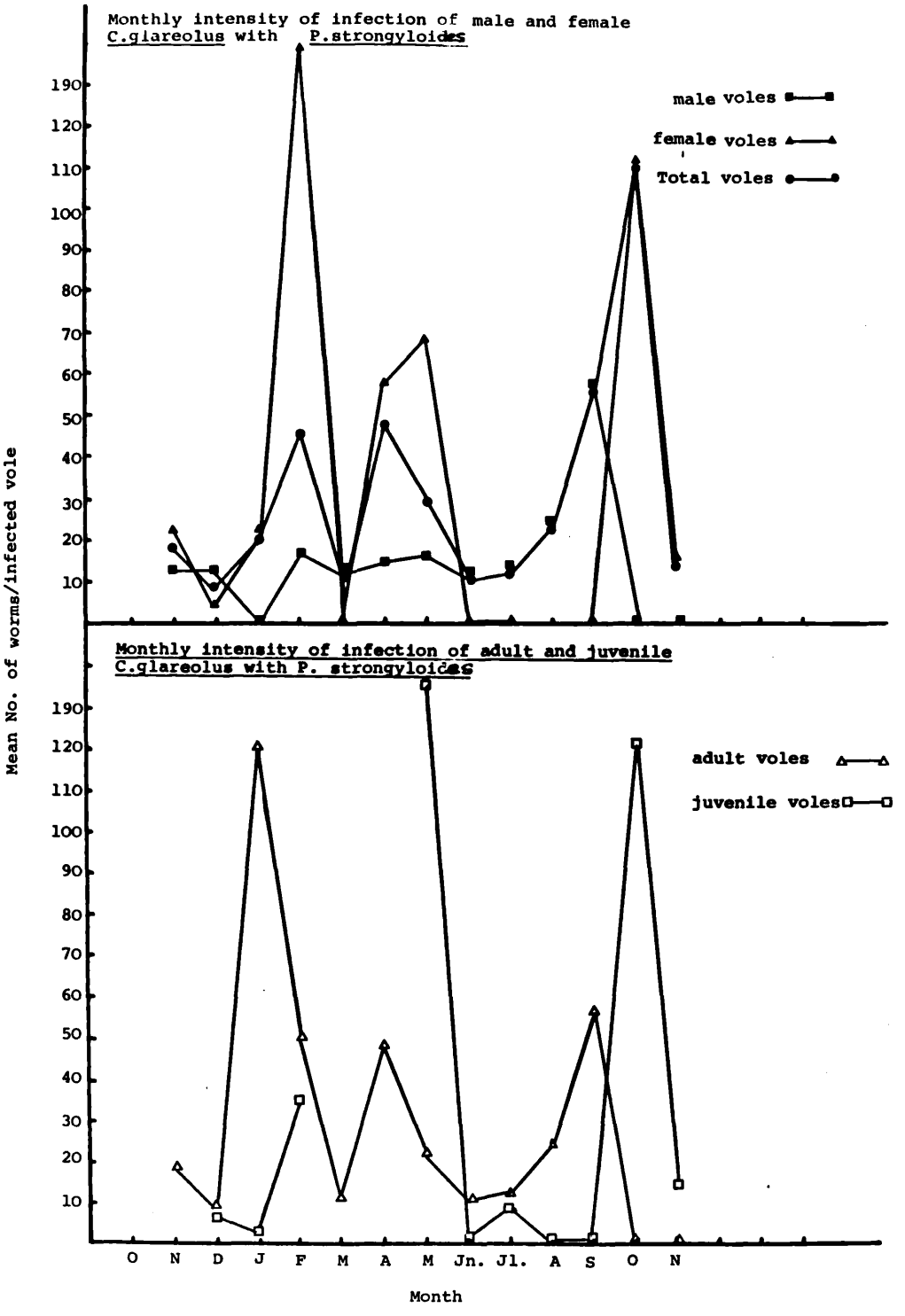

Monthly intensity of infection of male and female C.glareolus with P.strongyloides
male voles
female voles
Total voles
Monthly intensity of infection of adult and juvenile C.glareolus with P. strongyloides
adult voles
juvenile voles
Mean No. of worms/infected vole
190
120
110
100
90
80
70
60
50
40
30
20
10
O N D J F M A M Jn. Jl. A S O N
Month

TABLE 2

Results of the multiple regression and correlation analyses examining the relationship between the incidence and intensity of infection of C. glareolus with helminth parasites and selected environmental variables

	Multiple r^2	°C r (sig.)	R.H. r (sig.)	Rainfall r (sig.)	F (sig.)	°C β_1^2	R.H. β_2^2	Rainfall β_3^2	°C B1	R.H. B2	Rainfall B3	Multiple regression equation
Incidence of C.muris-sylvatici/ concurrent weather	0.286	-0.220	-0.365	-0.145	1.205	0.180	0.239	0.011	-1.744	-1.785	-0.070	$Y = 237.703 - 1.744X_1 - 1.785X_2 - 0.07X_2$
Incidence of C.muris-sylvatici/ previous weather	0.005	-0.018	-0.053	0.015	0.016	0.001	0.006	0.002	-0.174	-0.305	0.024	$Y = 88.115 - 0.174X_1 - 0.305X_2 + 0.024X_3$
Intensity of C.muris-sylvatici/ concurrent weather	0.266	-0.298	-0.022	0.408	1.054	0.083	0.064	0.171	-0.834	-0.651	0.199	$Y = 83.628 - 0.834X_1 - 0.651X_2 + 0.199X_3$
Intensity of C.muris-sylvatici/ previous weather	0.080	-0.203	0.232	0.066	0.261	0.028	0.036	0.000	-0.539	0.498	0.007	$Y = -5.363 - 0.539X_1 + 0.498X_2 + 0.007X_3$
Incidence of N.dubius/ concurrent weather	0.244	-0.164	0.487	0.234	1.076	0.003	0.231	0.006	0.022	0.179	0.006	$Y = -0.686 + 0.022X_1 + 0.179X_2 + 0.006X_3$
Incidence of N.dubius/ previous weather	0.432	0.650 **	-0.104	-0.006	2.559	0.473	0.014	0.000	3.243	0.500	-0.005	$Y = -7.505 + 3.243X_1 + 0.500X_2 - 0.005X_3$
Intensity of N.dubius/ concurrent weather	0.326	0.465	0.094	-0.177	1.615	0.329	0.140	0.019	2.686	1.546	-0.106	$Y = -3.232 + 2.686X_1 + 1.546X_2 - 0.106X_3$
Intensity of N.dubius/ previous weather	0.167	-0.008	0.284	0.367	0.669	0.006	0.041	0.090	0.032	0.076	0.017	$Y = 2.757 + 0.032X_1 + 0.076X_2 + 0.017X_3$
Incidence of P. strongyloides/ concurrent weather	0.212	-0.338	-0.109	0.103	0.808	0.245	0.132	0.003	-2.327	-1.550	0.046	$Y = 199.84 - 2.327X_1 - 1.550X_2 + 0.046X_3$
Incidence of P. strongyloides/ previous weather	0.263	-0.456	0.152	0.326	1.068	0.182	0.007	0.062	-2.026	-0.348	0.198	$Y = 88.704 - 2.026X_1 - 0.348X_2 + 0.198X_3$
Intensity of P. strongyloides/ concurrent weather	0.699	0.216	0.199	-0.685 ***	6.982 ***	0.046	0.311	0.626	1.352	3.184	-0.816	$Y = -203.695 + 1.352X_1 + 3.184X_2 - 0.816X_3$
Intensity of P. strongyloides/ previous weather	0.121	0.220	0.075	-0.212	0.415	0.065	0.057	0.050	1.622	1.338	-0.240	$Y = -83.295 + 1.622X_1 - 1.338X_2 + 0.24X_3$

** $p < 0.02$
*** $p < 0.01$

During the spring, summer and early autumn, there is a greater abundance of buds, seeds and green vegetation available to the mice and the proportion of animal food eaten decreases; during the winter months when less vegetable matter is available the mice rely more on animal matter for food, including insects that are thought to act as intermediate hosts for C. vitta. Such dietary variations could have accounted for the increase in intensity of infection with C. vitta during the winter months, and its decrease in intensity during spring and summer. Although differences in intensity of infection between male and female mice were not significant, the males generally harboured larger numbers of C. vitta; this was probably due to dietary differences known to exist between the two sexes, as male mice generally eat more animal material and less green plant material than female mice (Southern, 1964), thus increasing the males' chance of picking up an infected intermediate host.

Both the incidence and the intensity of infection with C. vitta were significantly lower in juvenile than adult mice, which may have been due to differences in the type and quantity of food taken by these two age groups. Young mice eat less animal and more vegetable food than adults (Southern, 1964) and they also forage less widely than the adults; both of these factors would decrease their chance of infection with C. vitta.

Very little of the variation in the incidence of infection with C. vitta was accounted for in terms of environmental variables for the previous or concurrent months, and in addition very little of the variation in intensity of infection could be accounted for by the previous month's weather. However, 53% of the variation in intensity of infection by C. vitta was accounted for in terms of rainfall, temperature and relative humidity for the concurrent month. The analysis of variance F ratio of 3.898 was significant at the 5% level, which indicated that the fit of the regression equation $Y = 83.63 - 0.311x_1 + 0.246x_2 + 0.154x_3$ was good. The gradation of importance of the environmental variables given by the β^2 values showed that rainfall and temperature were the most important environmental variables acting upon the intensity of infection. The correlation coefficient of rainfall and intensity of infection was 0.68, significant at 1% level and the correlation coefficient of temperature and intensity of infection was -0.408, significant at 5% level. Thus, increases in rainfall and decreases in temperature for the concurrent month were significantly related to the intensity of infection of A.sylvaticus with C.vitta. These environmental factors probably acted mainly upon the intermediate hosts of C. vitta. Increased rainfall and lowered temperature may have caused changes in the activity of these intermediate hosts causing

larger numbers to be available to A. sylvaticus as food.

It is therefore clear that seasonal variations in the intensity of infection of Apodemus sylvaticus with Corrigia vitta were due to a combination of environmental factors and behavioural differences of the various sex and age groups of the host. It is probable that variations in incidence of C. vitta were also due to differences in host behaviour.

Both the incidence and intensity of infection of Apodemus sylvaticus with Nematospiroides dubius were subject to similar seasonal variations; and there was a relationship between the two as high worm burdens occurred when a larger percentage of the population was infected. The number of N. dubius harboured by A. sylvaticus is directly related to the number of free-living infective stages encountered and ingested by the host during its foraging activity. Brown (1956b) observed that male mice have a wider home range than female mice, which may increase their chance of encountering infective N. dubius larvae giving rise to higher incidence in males. The larger worm burdens harboured by male mice may have been due to the fact that they generally eat more food than females, being more successful in intraspecific competition (Ashby, 1967).

The incidence of infection in both male and female mice was similar from February until July, which coincided with the onset of the breeding season, starting in February for

Apodemus sylvaticus. The female mice require more food when breeding starts in order to rear young successfully, and therefore foraged more widely and competed more effectively. This behavioural change could have accounted for the similarity in the occurrence of Nematospiroides dubius in males and females at this time of year. The drop in incidence of N. dubius in female mice during August and September may also have been due to some behavioural difference between the sexes, as the peak of the breeding season was over by September and the food requirements of the females may have been lower at this time.

Apodemus sylvaticus practises retraction (Ashby, 1967) which could account for the overall high incidence of infection with N. dubius, as constant reinfection would take place. The majority of the breeding of A. sylvaticus in 1969 occurred from March to August, the period when high worm burdens were observed. Brown (1956a) and Ashby (1967) showed that the activity of A. sylvaticus was strictly limited by light, and as the daylength increases during the spring and summer proportionally more time is spent by A. sylvaticus underground in runways and nests. Ashby also found that retraction was practised to a fairly large extent by A. sylvaticus during daylight hours in the laboratory and evidence that it occurred in the field was provided by Clemingsen (personal communication to Ashby). This

increased time spent in the nest, coupled with the habit of retraction in a community already infected with Nematospiroides dubius may have contributed to the increased worm burdens during the period March to August. Young mice do not practise refraction during the suckling period, and even when weaned they eat less food than the adults, and also exhibit a more limited foraging activity. This may explain the lower level and degree of infection of juvenile mice with N. dubius. However, during the months December, 1968 to February, 1969, both the incidence and intensity of N. dubius were similar in the two age groups. It has been shown that adults and juveniles are very difficult to distinguish during this period as the juveniles were becoming mature, and this therefore might explain the similarities in their infection levels.

Multiple regression analyses showed that very little of the variation in incidence of infection with Nematospiroides dubius was accounted for by the environmental variables for the concurrent or preceding month; also, the intensity of infection could not be explained by the previous month's weather. However, 49% of the variation in intensity of infection with N. dubius was accounted for by the concurrent environmental variables. The analysis of variance F ratio was 3.26, which is not significant indicating that the fit of the regression equation was poor. It is therefore not

possible to place great importance on this result. The correlation coefficient for relative humidity and intensity of infection with N. dubius was -0.58, significant at 5% level. The correlation was negative, i.e. a decrease in relative humidity was related to an increase in infection with N. dubius. The reason for this is not clear, although the third-stage larvae are known to be resistant to desiccation. The β^2 values showed that relative humidity was the most important and temperature the least important factor influencing the variation in intensity.

It therefore seems that behavioural differences between the various age and sex groups of Apodemus sylvaticus were most important in determining seasonal variation in incidence and intensity of infection with Nematospiroides dubius although environmental factors did affect the intensity of infection to some degree. However, N. dubius was found not to be as susceptible to the influence of environmental variables as was Corrigia vitta.

The feeding habits of Apodemus sylvaticus were not likely to affect either the incidence or intensity of infection with Syphacia stroma, as this parasite does not have an infective stage that may be ingested with food. The infective eggs are laid on the perianal fur and are most likely to be transferred by physical contact and ingested by the host during grooming, and the amount of physical contact

between individuals is related to the density of the population within the burrow system. The increased incidence of this parasite during the spring and summer months may have been due to the longer periods of time spent in the nest by A. sylvaticus in response to increased day-length at this time of year, because the chance of physical contact and subsequent infection was greater. The habit of refraction would not have affected either the incidence or intensity of infection with S. stroma as the infective eggs were rarely found in faecal pellets.

Although juvenile mice were less frequently infected with Syphacia stroma, they usually harboured very large numbers of worms. This may have been due to prolonged contact with a single infected female during suckling, leading to repeated infections.

The multiple regression and correlation analyses showed that an insignificant amount of the variation in incidence or intensity of infection could be accounted for in terms of the previous month's weather. This was to be expected as the previous month's weather would not affect a parasite whose infective stages only remain viable for several hours. The concurrent environmental variables accounted for 52% of the variation in incidence of Syphacia stroma and the F ratio was almost significant at the 5% level, indicating that the fit of the regression equation was quite good. The

correlation coefficient for relative humidity and incidence was -0.58, which was significant at 5% level, i.e. decreases in relative humidity were associated with an increase in the occurrence of S. stroma. Forty-nine per cent of the intensity of infection was accounted for by the concurrent environmental variables. However, little importance could be placed on this result as the F ratio was not significant, indicating that the fit of the regression equation was poor. However, the correlation coefficient between relative humidity and intensity of infection was -0.584, which was significant at the 5% level. Increases in both incidence and intensity of infection with S. stroma were related to decreases in relative humidity; the reason for this is not clear. As was found with Corrigia vitta and Nematospiroides dubius, variations in the incidence and intensity of infection with S. stroma are due to a combination of behavioural differences of the various age and sex groups of the host and external environmental variables.

Clethrionomys glareolus

As the seasonal variation in incidence of infection with Capillaria muris sylvatici could not be explained in terms of the environmental variables, it seems reasonable to assume that behavioural differences of the host population (over the year) were responsible for the increased incidence

of this parasite during the spring and summer months. The infective agent of C. muris-sylvatici is the embryonated egg, which develops in faecal pellets after they have left the host, infection taking place by ingestion of these eggs. It is likely that voles have an increased food requirement during the breeding season, which started in March in 1969, and would forage more widely to satisfy this need, thereby increasing their chance of encountering and ingesting the infective eggs.

The general level of infection with C. muris-sylvatici was high, and although Clethrionomys glareolus has not been shown to practise refraction, it is known to be active throughout 24 hours (Miller, 1955) which could increase their chances of infection with C. muris-sylvatici. The reasons for variations in intensity of infection are not known.

There was evidence of a seasonal variation in incidence of infection with Nematospiroides dubius, which was low during the spring and summer and higher during the autumn and winter, the opposite situation being found with Capillaria muris-sylvatici. The suggestion put forward that the increased foraging activity of Clethrionomys glareolus during the summer months increased their chance of picking up infective eggs of C. muris-sylvatici obviously does not increase their chances of incurring infective stages of N. dubius. The multiple regression and correlation analyses

showed that very little of the variation in incidence of infection with N. dubius could be accounted for in terms of the environmental variables of the concurrent month. However, 43% of the variation in incidence was accounted for by the previous month's environmental variables, although the analysis of variance F ratio was not significant indicating that the fit of the regression equation was poor. The correlation coefficient of temperature and incidence of infection was 0.65, significant at 2% level, i.e. increases in temperature in the previous month were associated with increased incidence of Nematospiroides dubius. As has already been discussed on page 16, the environmental variables may be expected to act chiefly on the infective stages of N. dubius; however, the relationship between temperature and incidence of this parasite is difficult to explain. It is possible that changes in environmental conditions affected the behaviour of Clethrionomys glareolus in such a way that they were less likely to encounter infective stages of N. dubius. This result is contrary to the situation in Apodemus sylvaticus where there was no correlation between any of the environmental variables and incidence of infection with N. dubius.

All the age and sex groups of voles were equally susceptible to infection with Nematospiroides dubius, whereas male and adult Apodemus sylvaticus were more suscept-

ible than the females and juveniles of this species. It is possible that there are less behavioural variations in the different groups of voles than in the corresponding groups of mice, which could have caused the above result.

The seasonal variation in intensity of infection of Clethrionomys glareolus with Nematospiroides dubius could not be accounted for in terms of the environmental variables, and was probably due to behavioural differences of the whole host population over the sampling period. The lower worm burdens recorded in juvenile voles were probably a reflection of their more limited foraging activity decreasing their chances of encountering the infective larvae of N. dubius. Both male and female C. glareolus carried similar worm burdens of N. dubius in contrast to female Apodemus sylvaticus which harboured significantly larger numbers of this parasite than the males. Juveniles of both host species harboured smaller worm burdens than adults. The seasonal variation in intensity of infection with N. dubius in C. glareolus was different to that found in A. sylvaticus as the highest worm burdens occurred during autumn and winter in voles and during the summer in mice. High worm burdens of N. dubius in C. glareolus coincided with high incidence of this parasite; a similar situation was also found in A. sylvaticus.

All the age and sex groups of voles were also found to

be equally susceptible to infection with Pelodera strongyloides, and once infected they harboured similar worm burdens. No significant part of the variation in incidence of P. strongyloides was accounted for by environmental variables; nor was the variation in intensity due to the previous month's weather. However, 69.9% of the variation in intensity of infection with P. strongyloides was accounted for by the concurrent months environmental data. The analysis of variance F ratio was 6.98, significant at 1% level, which indicated that the fit of the regression equation $Y = 203.69 + 1.352x_1 + 3.184x_2 - 0.816x_3$ was good. The β^2 values showed rainfall to be the most important factor and relative humidity the second most important factor in determining this variation in intensity. The correlation coefficient between rainfall and intensity of infection was -0.685, significant at 1% level, i.e. decreased rainfall resulted in an increased intensity of infection. This result adds support to the theory of Poinar (1965) that drought conditions in the soil stimulate the formation of "infective" larval forms of P. strongyloides which are then picked up on the feet of voles and transferred to the eyes during grooming. These worms pass out of the eyes back to the soil during damp favourable conditions.

It is clear that environmental variables were the cause of variations in the intensity of infection with P. strongy-

loides but the reasons for the variations in incidence are not known.

CHAPTER 2

COMPETITION BETWEEN NEMATOSPIROIDES DUBIUS AND SYPHACIA STROMA IN THE ALIMENTARY CANAL OF APODEMUS SYLVATICUS

Introduction

It was reported by Lewis (1964, 1966) that interspecific competition takes place between Nematospiroides dubius and Syphacia stroma along the alimentary canal of Apodemus sylvaticus, N. dubius having an antagonistic effect on S. stroma. This interaction was manifested as a difference in the distribution of S. stroma when it occurred alone and concurrently with N. dubius; the distribution of the latter was similar in both single species and concurrent infections. Lewis (1964) suggested that oxygen could be a limiting factor in the mouse gut for which these two nematodes were competing.

There have been several other reports of varying degrees of competition between parasitic helminths inhabiting the gastrointestinal tract of vertebrates. Cross (1934) noted that an antagonism existed between the cestode Proteocephalus exigus (La Rue, 1911) and an acanthocephalan of the genus Neoicanthorinchus when they occurred concurrently in ciscoes of Silver Lake, Wisconsin. High worm burdens of cestodes were associated with low acanthocephalan burdens; conversely, fish harbouring large numbers of acanthocephalans had a low

infection with tapeworms. He ascribed the phenomenon to a non-specific immunity as opposed to a crowding effect, as each species occupied a different region of the alimentary tract. Larsh and Donaldson (1944) showed that young white mice infected with Nippostrongylus muris (Yokogawa, 1920) just prior to infection with Hymenolepis nana var fraterna (Siebold, 1853) exhibited a marked resistance to infection by the tapeworm and harboured only half the number of cysticercoids observed in controls of the same age infected with the cestode only. Larsh and Donaldson were unable to offer any explanation for this phenomenon. A similar antagonism between Aspiculuris tetraptera and Trichuris muris (Schrank, 1788) was reported by Keeling (1961), in which the susceptibility of mice to infection by T. muris was lowered in the presence of A. tetraptera, but no distributional differences in single and concurrent infections were noted. Competition between the two species for essential nutrients was probably taking place or/the emanation of materials by one species proved deleterious to the other by interfering with its normal physiological processes. Holmes (1961) showed that the size, general body form and spatial distribution of Hymenolepis diminuta (Rudolph, 1819) and Moniliformis dubius (Meyer, 1932) in the rat, were affected in concurrent infections as compared to single species infections and that H. diminuta was affected to a greater degree than M. dubius. Holmes suggested that the worms may have been competing for carbo-

hydrates. A second case of antagonism between two species resulting in the alteration of the spatial distribution of each has been reported by Chappell (1969). In concurrent infections of Proteocephalus filicollis (Rudolphi, 1819) and Neoechinorhynchus rutili (Müeller, 1780) in the three spined stickleback Gasterosteus aculeatus L., the distribution of each species in the gut was significantly different from when these species occurred alone; this was thought to be due to competition for nutritional or spatial requirements, or both.

In view of the above results, the spatial distributions of Nematospiroides dubius and Syphacia stroma in both single and concurrent infections in naturally infected Apodemus sylvaticus have been analysed in order to ascertain whether an antagonistic effect similar to that described by Lewis existed. The mean worm burdens of both species in single and concurrent infections were calculated and compared statistically, to determine if the susceptibility of A. sylvaticus to infection by S. stroma was altered by the presence of N. dubius. In addition, the relationship between intensity of infection with N. dubius and intensity of infection with S. stroma in concurrent infections was analysed. These results should indicate the degree of competition that exists between these two parasitic nematodes.

Materials and Methods

The small intestines from stomach to caecum were removed from Apodemus sylvaticus and each was divided into ten equal sections. The position of each worm was noted, and the number of each species in every section of the gut was recorded.

Results

The distribution of Nematospiroides dubius and Syphacia stroma in single and concurrent infections is shown in Figure 16; the percentage of the total population in each section was plotted (Table 3). The distribution of N. dubius was different in single and concurrent infections as was that of S. stroma. A χ^2 test was performed on the proportion of each worm population in the anterior and posterior halves of the gut in single and concurrent infections. The hypothesis that the population of N. dubius was equally distributed in the anterior and posterior halves of the gut in both single and concurrent infections was tested; the same hypothesis was tested for S. stroma. The χ^2 for N. dubius was 8.96 ($p < 0.01 > 0.001$), the χ^2 for S. stroma was 73.55 ($p < 0.001$) (Table 4a). Both these results were highly significant thus disproving the null hypothesis, i.e. there was a greater proportion of both N. dubius and S. stroma in the anterior half of the gut in single than in concurrent infections. However, the χ^2 value was much larger for

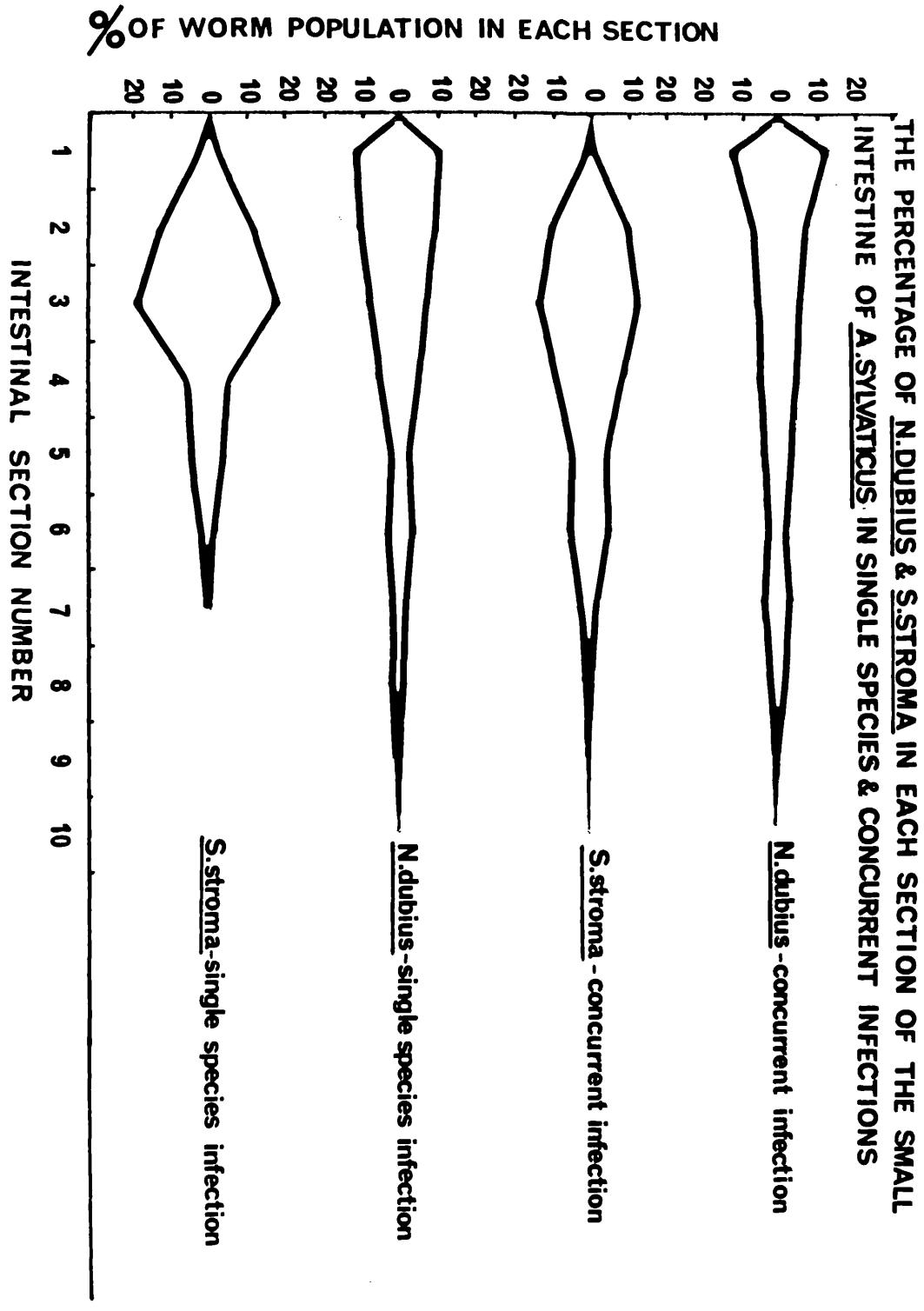

THE PERCENTAGE OF N.DUBIUS & S.STROMA IN EACH SECTION OF THE SMALL INTESTINE OF A.SYLVATICUS IN SINGLE SPECIES & CONCURRENT INFECTIONS
%OF WORM POPULATION IN EACH SECTION
20 10 0 10 20
N.dubius-concurrent infection
S.stroma-concurrent infection
N.dubius-single species infection
S.stroma-single species infection
1 2 3 4 5 6 7 8 9 10
INTESTINAL SECTION NUMBER

TABLE 3

The percentage of N. dubius and S. stroma populations in each section of the small intestine of A. sylvaticus in single species and concurrent infections

Section No.	N. dubius single species infection		N. dubius concurrent infection		S. stroma single species infection		S. stroma concurrent infection	
	Total no.of worms	% of popu-lation	Total no.of worms	% of popu-lation	Total no.of worms	% of popu-lation	Total no.of worms	% of popu-lation
1	333	23.63%	580	26.93%	52	6.03%	380	3.11%
2	292	20.72%	350	16.25%	122	25.78%	2603	21.32%
3	242	17.17%	2017	13.79%	319	37.04%	3167	25.95%
4	188	13.34%	236	10.96%	111	12.89%	2256	18.48%
5	92	6.52%	199	9.24%	91	10.56%	1426	11.68%
6	95	6.74%	160	7.43%	46	5.34%	1508	12.35%
7	83	5.89%	180	8.36%	20	2.32%	560	4.58%
8	74	5.25%	119	5.52%	-	-	238	1.95%
9	9	0.63%	30	1.39%	-	-	51	0.41%
10	1	0.07%	2	0.09%	-	-	15	0.12%
TOTALS	1409	100%	2153	100%	861	100%	12204	100%

TABLE 4a

The numbers of N. dubius and S. stroma occurring in the anterior and posterior regions of the small intestine of A. sylvaticus in single species and concurrent infections

N. dubius

	Anterior half of S.I.	Posterior half of S. I.	Totals
Single species infections	1147	262	2153
Concurrent infections	1162	491	1409
Totals	2809	753	3562

$\chi^2 = 8.96, p > 0.001 < 0.01$

S. stroma

	Anterior half of S. I.	Posterior half of S. I.	Totals
Single species infections	795	66	861
Concurrent infections	9832	2372	12204
Totals	1062	2438	13065

$\chi^2 = 73.55, p < 0.001$

S. stroma which indicated that the effect of concurrent infection was greater on S. stroma than N. dubius.

Nematospiroides dubius was equally widely distributed in both types of infections, and worms were found in all ten sections of the small intestine. Syphacia stroma was more widely distributed in the presence of N. dubius, worms being found in all ten sections in concurrent infections but only in the first seven sections in single species infections.

The mean worm burdens of N. dubius and S. stroma in single and concurrent infections are shown in Table 4b. There was no significant difference in the mean number of N. dubius or S. stroma in either type of infection, i.e. the susceptibility of Apodemus sylvaticus to infection with S. stroma was not affected by the presence of N. dubius.

The correlation coefficient between the number of N. dubius and the number of S. stroma that occurred in concurrent infections was 0.042, which was not significant, i.e. there was no relationship between intensity of infection of either species.

Discussion

The alteration of the distribution of Nematospiroides dubius and Syphacia stroma in the gut of Apodemus sylvaticus would seem to be due to a competitive interaction between the two species. It appears that these worms were competing for some unknown factor which was more abundant in the

TABLE 4b

A comparison of the mean worm burdens of *N. dubius* and *S. stroma* in *A. sylvaticus* in single species and concurrent infections

	Mean worm burden	't'
N.dubius-single species infections	8.86	$1.8462(\infty d.f.)p>0.05$
N.dubius-concurrent infections	11.93	
S.stroma-single species infections	56.94	$0.6978(\infty d.f.)p>0.05$
S.stroma-concurrent infections	39.80	

anterior than in the posterior half of the small intestine. As a result of this competition, a greater proportion of the S. stroma population were forced into the posterior region of the intestine which was a less favourable environment. The N. dubius population was also shifted to a more posterior position, but the shift was not so pronounced, i.e. N. dubius competed more successfully than S. stroma which resulted in the partial exclusion of the latter from the anterior region of the intestine.

There was considerable overlap of the distributions of the two species in concurrent infections; this contrasts with the situation found between Hymenolepis diminuta and Moniliformis dubius in the rat, where very little overlap of the distributions of the two species was found in concurrent infections (Holmes, 1961), as the distribution of each species "shifted" in the opposite direction. This indicated that the degree of competition between N. dubius and S.stroma was not as great as that found between H. diminuta and M. dubius.

The lack of correlation between the numbers of Nematospiroides dubius and Syphacia stroma in concurrent infections differs from the findings of Paperna (1964). He showed that when the monogenean Dactylogyrus vastator (Nybelin, 1924) was present on the gills of the carp, Cyprinus carpio, in large numbers, few of the closely related

species Dactylogyrus extensus (Müeller and Van Cleave, 1932) were present. There was a high degree of competition between these two species in which D. vastator was the most successful, as its presence caused histological changes in the carp gills, rendering the environment unsuitable for D. extensus.

Although the presence of Nematospiroides dubius affected the distribution of Syphacia stroma, the presence of one species did not alter the hosts' susceptibility to infection with the other. Chappell (1969) found a similar situation between Proteocephalus filicollis and Neoechinorhynchus rutili in naturally infected sticklebacks, as there was no significant difference in the worm burdens of either species in single and concurrent infections, despite a marked difference in the spatial distribution in the two types of infection.

The above results indicated that although a definite competitive interaction existed between Nematospiroides dubius and Syphacia stroma in Apodemus sylvaticus, it did not operate so strongly as the other examples of competition cited, as it did not result in the total exclusion of either species from any one region in the small intestine. All the results were obtained from naturally infected A.sylvaticus and therefore no previous knowledge of the time or duration of the infections, or of the chronological order of infection with each species was known. Since it is possible that

these factors could have affected the degree of competition, no definite conclusions may be drawn.

A series of experiments was therefore planned in which mice of known age were to be infected with standardized doses of Nematospiroides dubius and Syphacia stroma, and the resulting worm burdens and their spatial distribution were to be examined. The number of each species administered was to be varied in some experiments, in order to study the effect of the variation of intensity of infection on the competitive interaction; the chronological order of infection would also have been varied in order to study its effect. Control experiments were to be performed in which the worm burdens and their distribution in single species infections subjected to similar variations described above, were to be studied. However, it was not possible to obtain infective S. stroma eggs from gravid females in naturally infected hosts in order to initiate infections in laboratory mice. Considerable difficulty has been experienced by other workers attempting to initiate laboratory infections of oxyurid nematodes (Philpot, 1954), although Lewis (1964) did successfully carry out a small number of S. stroma infections from wild to laboratory mice. Attempts to infect laboratory mice by association with naturally infected Apodemus sylvaticus were unsuccessful in the present work. Eggs removed from gravid female S. stroma

and incubated in Tyrodes saline at 10°C, 15°C, 20°C and 35°C, for varying periods of time never became infective. As it was not possible to obtain controlled infections with this oxyurid nematode, the above series of experiments were discontinued for obvious reasons. The distribution of N. dubius in single species infections has, however, been studied in detail, and this topic has been dealt with in Chapter 3.

It is difficult to ascertain what limiting factors Nematospiroides dubius and Syphacia stroma were competing for. Although it is not known precisely what these two species feed upon, it is probable that the supply of nutrients such as carbohydrates and fats in the host intestine would be greater than the requirements of the heaviest worm burdens (Read, 1951). Competition for mucosal space may also be involved in this interaction, but it has been shown by Lewis (1964) that pH was not a limiting factor. It is known that oxygen is present in the intestine of rats and furthermore that higher concentrations exist in the anterior regions of the gut (Rogers, 1949a); a similar situation is likely to occur in the mouse intestine. It is therefore possible that N. dubius and S. stroma may have been competing for the more abundant supply of oxygen in the anterior sections of the small intestine of Apodemus sylvaticus; however, the situation

is probably very complex involving several other limiting factors.

CHAPTER 3

THE DISTRIBUTION OF NEMATOSPIROIDES DUBIUS IN THE SMALL INTESTINE OF THE LABORATORY MOUSE

Introduction

The distribution of Nematospiroides dubius within the mouse intestine has been described by several authors, including Bawden (1969) and Panter (1969). They showed that the adult worms always take up a position in the anterior part of the duodenum. Dobson (1960) also reported that the relative length of the intestine infected on the fifth day was greater than that infected on the tenth day, i.e. the adult worms migrate to a more anterior position in the small intestine than that occupied by the pre-adult stages. Dobson put forward the theory that this anterior migration was stimulated by the secretion of bile into the duodenum and that the worms tended to move to a position that was anterior to the entry of the bile duct. Bawden suggested that the anterior migration may also be influenced by other conditions such as the supply of dietary nutrients, ph or O_2 tensions beyond the first few cm. of the small intestine.

An experiment was therefore designed to determine the positions taken up by Nematospiroides dubius in the small

intestine of the laboratory mouse throughout the course of infection from the ingestion of the free-living infective third stage larvae to the formation of the sexually mature adult worms. Mice with infections of 21 and 42 days' duration were also examined, in order to see if the infection had altered after these periods. The results enabled the degree of aggregation of the worms to be quantified. Knowledge of the changes in distribution of the parasite was also useful to interpret changes that occurred in the respiration throughout its life cycle. Both male and female mice were used in this experiment, in order to determine whether the distribution was in any way affected by host sex.

Materials and Methods

110 mice were used in this study, 10 being autopsied on each of the 11 days of the experiment. Male and female mice were infected with a standard dose of 70 infective *Nematospiroides dubius* larvae as described in general methods. Five mice of each sex were autopsied on the fourth day after infection, which is when the worms are clearly visible in the gut mucosa. Thereafter, 5 male and 5 female mice were autopsied daily until the eleventh day after infection when mature worms are present in the intestinal lumen and the life cycle has been completed. Mice were also autopsied at arbitrarily chosen periods of 2, 3 and 6 weeks after infection in order to see if the distribution of the worms

had changed.

Mice were killed by cervical dislocation, the small intestine from the stomach to the caecum was removed and the surrounding mesenteries and fat dissected away. The small intestine was laid on a board with a graduated scale and divided into twenty equal sections. Each individual section was slit open under Tyrodes saline and examined under a dissecting microscope, and the number, sex and position of the worms were noted. Worms which spread over two different sections were counted as being in that section in which the head was situated or embedded.

Initially, this experiment was to have been repeated using mice infected with Syphacia stroma and Syphacia stroma and Nematospiroides dubius together, as a competitive interaction is known to occur between these two nematode species in the natural host Apodemus sylvaticus, which is manifested as a difference in distribution of the worms when they occur singly and together. This experiment was not feasible as Syphacia stroma proved to be impossible to culture, as previously mentioned.

The results of these experiments were analysed using Morisita's (1959) indices of dispersion, which have the great advantage that they are relatively independent of the type of distribution, the number of samples and the size of the mean (Southwood, 1966). The index is given by the

formula

$$I\delta' = N \frac{\sum_{i=1}^{N} n_i(n_i - 1)}{\sum x (\sum x - 1)}$$

where N = total samples, n_i = nos. in the i^{th} sample, and $\sum x$ = the sum of the number of individuals found in all the samples. When the distribution is random, the index will give a value of unity; when the distribution is contagious, the index will be greater than one, and when the distribution is regular, less than one. The significance of the departure from a random distribution shown by the index may be tested by comparing Fo with the value of F in tables, where $N_1 = N - 1$ and $N_2 = \infty$

$$F_o = \frac{I\delta(\sum x - 1) + N - \sum x}{N - 1}$$

Results

The daily mean worm burdens of Nematospiroides dubius in male and female mice are shown in Figure 17. There was no significant difference between the mean number of N. dubius found in male and female mice on any one day (Table 5). The mean daily worm burdens were more variable in male than in female mice, worms in the latter remaining almost constant from day 7 onwards; these results also indicate that the dosage received by the mice was not unacceptably variable.

An example of the distribution of Nematospiroides dubius in each sex of mouse throughout the course of

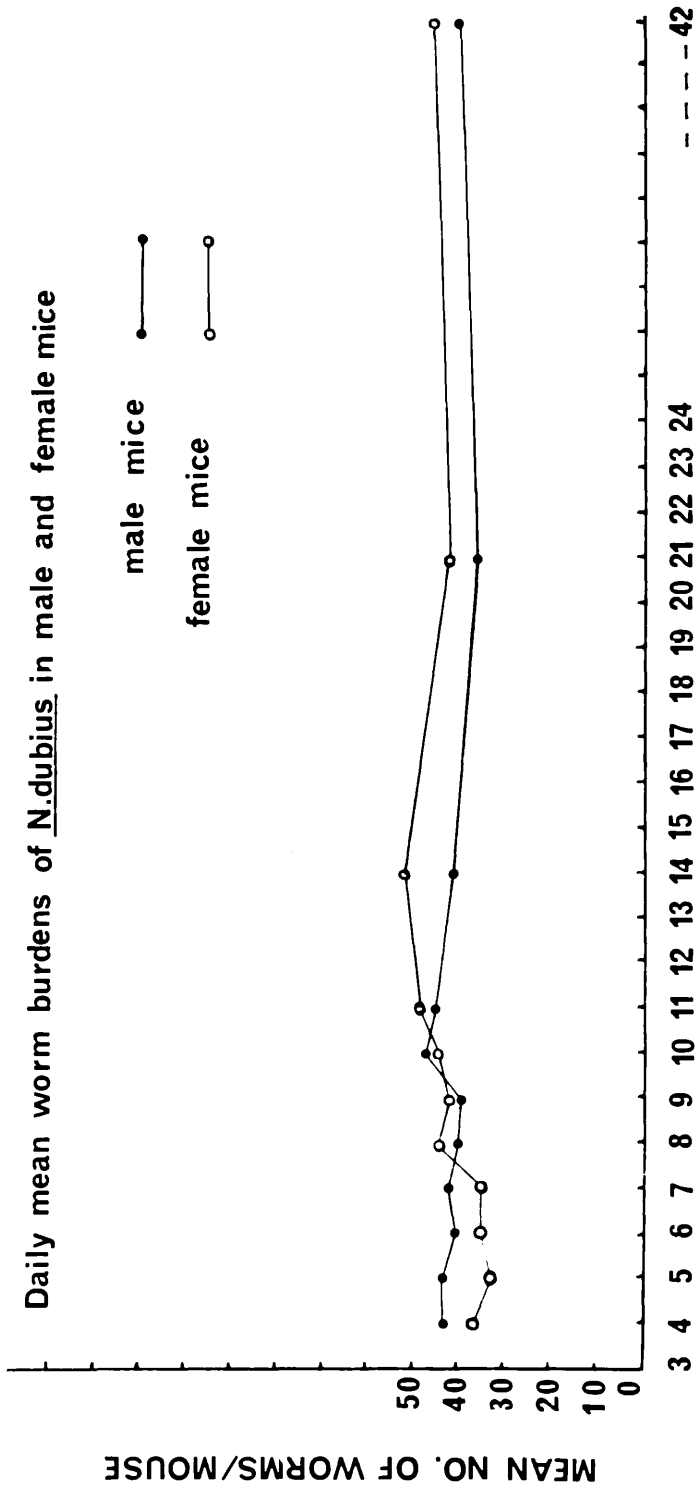

Daily mean worm burdens of N.dubius in male and female mice
male mice
female mice
MEAN NO. OF WORMS/MOUSE
0
10
20
30
40
50
3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
TIME IN DAYS FROM INFECTION
- - - - 42

TABLE 5

A comparison of the daily mean worm burdens of N. dubius in male and female laboratory mice

Days after start of infection	Mean worm burden in male mice (S.D.)	Mean worm burden in female mice (S.D.)	't'
4	37.2 ± 15.193	44.2 ± 7.63	0.8863(9d.f.)p > 0.05
5	33.8 ± 12.48	44.0 ± 9.43	1.4581(9d.f.)p > 0.05
6	35.2 ± 12.03	40.4 ± 8.35	0.7940(9d.f.)p > 0.05
7	35.2 ± 11.21	42.4 ± 6.11	1.2611(9d.f.)p > 0.05
8	44.2 ± 8.53	41.0 ± 18.64	0.3490(9d.f.)p > 0.05
9	41.2 ± 14.07	40.4 ± 7.4	0.1124(9d.f.)p > 0.05
10	44.6 ± 7.64	46.04± 17.51	0.1639(9d.f.)p > 0.05
11	48.0 ± 3.94	46.6 ± 10.45	0.2802(9d.f.)p > 0.05
14	52.4 ± 6.8	42.2 ± 14.75	1.4038(9d.f.)p > 0.05
21	42.6 ± 10.13	36.2 ± 10.75	1.2062(9d.f.)p > 0.05
42	45.6 ± 11.76	39.6 ± 3.36	1.0969(9d.f.)p > 0.05

infection is shown in Figure 18. It is clear that from the beginning of the infection, N. dubius always took up a position in the anterior half of the small intestine. During the initial period of the infection, the worms occupied up to 50% of the small intestine. As the infection progressed, the number of sections in which worms were found decreased, and by the eighth day they were only found in the anterior two or three sections of the intestine. However, the mode of the distribution was in approximately the same region throughout the infection, i.e. the distribution changed in extent but not position in the intestine.

Indices of aggregation ($I\delta_T$) were calculated daily for the worm population with respect to the host intestine for both sexes of mice; indices of aggregation ($I\delta_A$) of the worms within the population with respect to each other were also calculated each day throughout the course of infection (Table 6).

The degree of aggregation of the parasite population gradually increased with relation to the small intestine up to day 8, after which time it remained more or less constant. The degree of aggregation increased the most between days 7 and 8, which was the time in the life cycle when worms left the cysts in the intestinal wall and moved into the intestinal lumen; once there they had the freedom to migrate anteriorly, which occurred on day 7 for both male and female

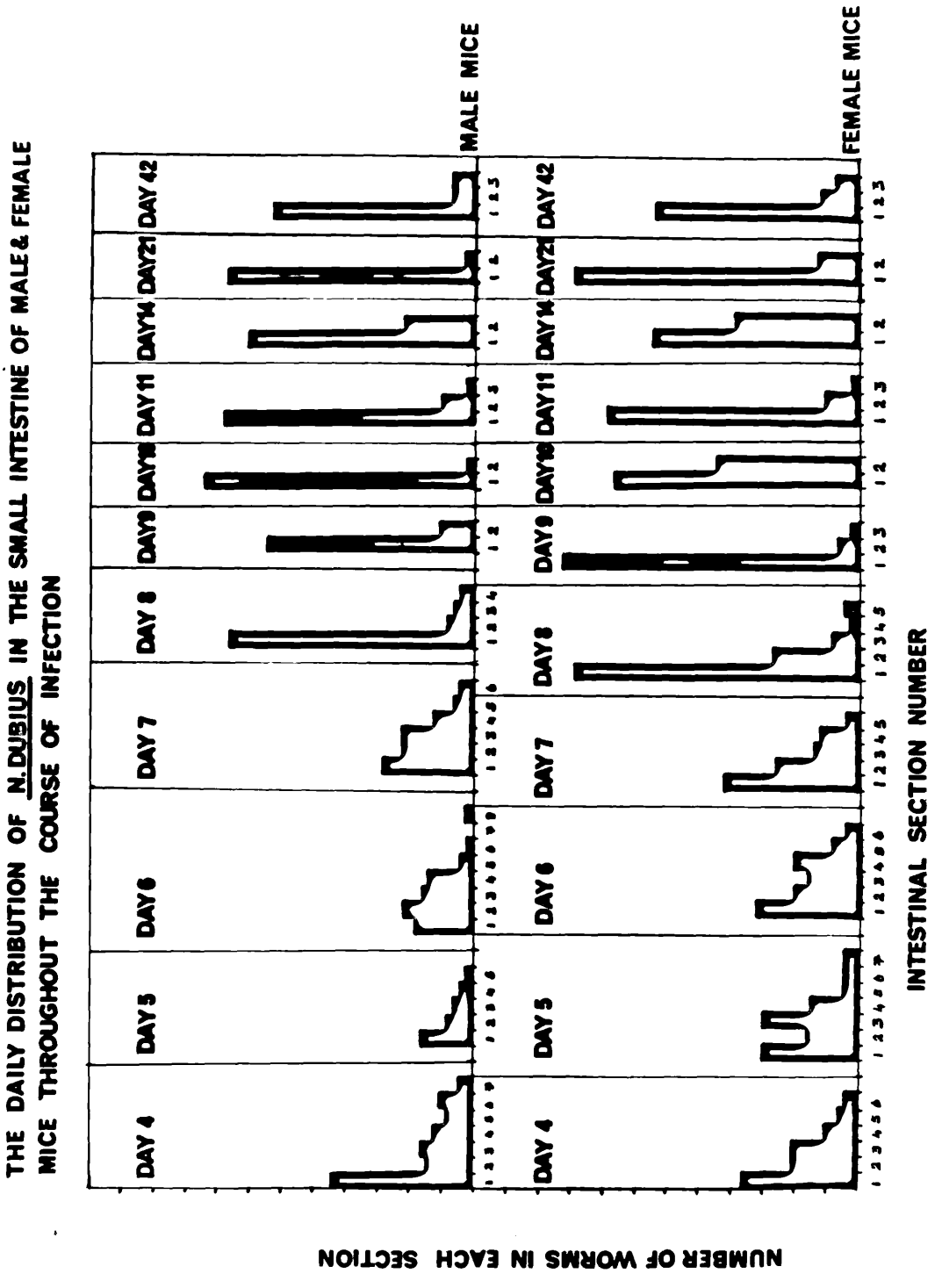

THE DAILY DISTRIBUTION OF N.DUBIUS IN THE SMALL INTESTINE OF MALE & FEMALE MICE THROUGHOUT THE COURSE OF INFECTION
DAY 4
DAY 5
DAY 6
DAY 7
DAY 8
DAY 9
DAY 10
DAY 11
DAY 14
DAY 21
DAY 42
MALE MICE
FEMALE MICE
NUMBER OF WORMS IN EACH SECTION
INTESTINAL SECTION NUMBER

TABLE 6

The indices of aggregation for populations of N. dubius with respect to the host intestine ($I\delta_T$) and with respect to each other ($I\delta_A$)

Indices were calculated each day throughout the course of infection

Male Mice

DAY	$I\delta_T$	F	Significance level	$I\delta_A$	F	Significance level	Dist.
4	4.44	5.71	0.1%	1.33	3.15	1%	C
	3.27	3.08	0.1%	.98	.93	N.S.	R
	4.36	10.37	0.1%	1.53	5.65	0.1%	C
	3.49	5.33	0.1%	1.22	2.23	5%	C
	4.06	9.39	0.1%	1.42	5.27	0.1%	C
5	6.06	9.52	0.1%	1.82	6.24	0.1%	C
	4.97	4.55	0.1%	1.24	2.03	N.S.	R
	5.87	9.20	0.1%	1.47	4.74	0.1%	C
	5.12	7.72	0.1%	1.28	3.17	1%	C
	5.47	3.24	0.1%	1.92	8.26	0.1%	C
6	6.81	4.98	0.1%	1.70	3.29	5%	C
	6.15	11.31	0.1%	2.46	8.93	0.1%	C
	3.81	6.66	0.1%	1.33	3.10	1%	C
	3.67	6.47	0.1%	1.28	2.17	5%	C
	2.88	5.24	0.1%	1.29	2.58	1%	C
7	5.0	6.05	0.1%	1.25	2.5	5%	C
	4.07	8.43	0.1%	1.23	3.03	1%	C
	5.54	11.27	0.1%	1.66	6.69	0.1%	C
	5.78	10.3	0.1%	1.16	3.07	5%	C
	6.93	7.55	0.1%	1.39	3.70	5%	C
8	6.5	17.49	0.1%	1.95	11.82	0.1%	C
	13.51	25.37	0.1%	2.70	22.0	0.1%	C
	6.74	9.95	0.1%	2.02	5.74	0.1%	C
	7.56	14.11	0.1%	1.51	7.48	0.1%	C
	13.17	30.47	0.1%	2.63	26.06	0.1%	C
9	7.43	17.58	0.1%	1.11	3.82	5%	C
	10.76	14.37	0.1%	1.08	2.30	N.S.	R
	8.51	25.12	0.1%	2.55	19.56	0.1%	C
	20.0	31.0	0.1%	1.0	0	N.S.	R
	15.20	27.9	0.1%	1.52	19.7	0.1%	C

TABLE 6 (continued)

DAY	$I\delta_T$	F	Significance level	$I\delta_A$	F	Significance level	Dist.
10	9.88	24.84	0.1%	1.98	17.59	0.1%	C
	20.0	43.0	0.1%	2.0	38.6	0.1%	C
	5.56	11.32	0.1%	1.11	2.26	N.S.	R
	8.60	13.80	0.1%	1.72	8.68	0.1%	C
	16.99	43.07	0.1%	2.55	39.71	0.1%	C
11	15.17	33.2	0.1%	2.28	29.08	0.1%	C
	5.60	12.39	0.1%	1.12	2.89	5%	C
	5.22	11.66	0.1%	1.31	4.66	0.1%	C
	6.66	13.81	0.1%	1.33	5.86	0.1%	C
	8.65	22.34	0.1%	1.73	13.90	0.1%	C
14	12.56	28.88	0.1%	1.26	72.52	0.1%	C
	6.01	16.04	0.1%	1.20	4.85	1%	C
	6.33	16.87	0.1%	1.27	5.96	0.1%	C
	11.12	23.9	0.1%	1.11	5.82	5%	C
	8.80	23.91	0.1%	1.76	15.17	0.1%	C
21	9.78	19.94	0.1%	.98	.09	N.S.	R
	20.0	34.0	0.1%	1.0	0	N.S.	R
	13.83	25.98	0.1%	1.38	15.16	0.1%	C
	10.07	19.14	0.1%	1.01	1.25	N.S.	R
	9.02	25.91	0.1%	1.80	16.814	0.1%	C
42	15.42	48.06	0.1%	1.54	17.81	.1%	C
	11.62	27.23	0.1%	1.74	18.47	.1%	C
	12.31	24.21	0.1%	1.23	10.00	.1%	C
	8.47	12.80	0.1%	1.32	5.34	1%	C
	9.41	20.92	0.1%	1.88	14.23	.1%	C
				Female Mice			
4	6.4	13.73	.1%	1.92	8.87	0.1%	C
	4.56	9.8	.1%	1.37	4.45	0.1%	C
	4.65	7.92	.1%	1.16	2.47	5%	C
	4.12	9.71	.1%	1.44	4.91	0.1%	C
	7.17	12.37	.1%	2.15	14.44	0.1%	C
5	7.16	18.82	.1%	2.50	8.17	0.1%	C
	4.84	7.87	.1%	0.96	0.78	N.S.	R
	4.11	9.18	.1%	1.44	4.65	0.1%	C
	4.77	7.75	.1%	1.43	8.14	0.1%	C
	3.90	7.4	.1%	1.36	3.55	1%	C

TABLE 6 (continued)

DAY	Id_T	F	Significance level	Id_A	F	Significance level	Dist.
6	4.68	6.42	0.1%	1.17	2.19	N.S.	R
	2.55	4.76	0.1%	1.28	2.41	1%	C
	4.17	7.01	0.1%	1.46	3.77	0.1%	C
	4.00	8.74	0.1%	1.20	2.96	5%	C
	5.61	10.23	0.1%	1.40	4.83	0.1%	C
7	5.53	12.44	0.1%	1.38	5.58	0.1%	C
	6.02	11.82	0.1%	1.80	7.60	0.1%	C
	9.08	16.73	0.1%	1.36	7.68	0.1%	C
	4.10	6.55	0.1%	1.23	2.57	5%	C
	5.23	11.46	0.1%	1.83	7.51	0.1%	C
8	7.18	4.90	0.1%	1.08	1.46	N.S.	R
	10.23	31.60	0.1%	2.5	25.52	0.1%	C
	8.09	14.07	0.1%	1.62	11.83	0.1%	C
	7.39	15.81	0.1%	1.48	8.02	0.1%	C
	7.16	15.91	0.1%	1.43	7.62	0.1%	C
9	10.05	16.72	0.1%	2.01	12.12	0.1%	C
	9.85	20.1	0.1%	1.48	10.79	0.1%	C
	9.24	19.65	0.1%	1.39	9.29	0.1%	C
	8.39	13.05	0.1%	2.10	9.5	0.1%	C
	16.95	42.13	0.1%	2.54	38.78	0.1%	C
10	15.29	18.29	0.1%	2.29	15.88	0.1%	C
	10.55	30.67	0.1%	1.06	4.27	5%	C
	13.98	20.81	0.1%	2.10	16.9	0.1%	C
	9.25	26.17	0.1%	1.39	12.22	0.1%	C
	6.63	17.60	0.1%	1.66	10.20	0.1%	C
11	8.71	20.06	0.1%	1.74	12.61	0.1%	C
	6.64	16.73	0.1%	1.0	0.89	N.S.	R
	15.7	27.3	0.1%	2.35	24.03	0.1%	C
	8.26	14.75	0.1%	1.24	5.3	1%	C
	7.21	19.96	0.1%	1.44	9.55	0.1%	C
14	11.53	22.1	0.1%	1.73	14.85	0.1%	C
	7.8	18.31	0.1%	1.60	10.39	0.1%	C
	8.9	23.5	0.1%	1.34	10.06	0.1%	C
	10.46	25.9	0.1%	0.88	4.53	5%	C
	10.98	9.93	0.1%	1.65	6.500	1%	C
21	9.37	18.61	0.1%	1.40	8.60	0.1%	C
	13.98	24.23	0.1%	2.10	19.66	0.1%	C
	15.28	24.30	0.1%	3.06	22.25	0.1%	C
	8.67	21.20	0.1%	1.73	12.24	0.1%	C
	13.51	14.82	0.1%	2.03	11.77	0.1%	C

TABLE 6 (continued)

DAY	Id_T	F	Significance level	Id_A	F	Significance level	Dist.
42	10.98	13.39	0.1%	1.10	4.44	5%	C
	10.99	19.93	0.1%	1.10	4.57	5%	C
	10.55	9.55	0.1%	1.58	14.62	0.1%	C
	13.10	27.11	0.1%	1.31	13.71	0.1%	C
	12.81	27.[illegible]	0.1%	1.28	13.09	0.1%	C

worms. The worms were all highly aggregated in relation to the intestine from the beginning of the infection; the Fo values indicated that the indices of aggregation were all significant at the 0.1% level. The indices of aggregation of the worms with respect to each other were more variable within each age group. On day 4, some of the worms in male mice tended to be distributed randomly with respect to each other. Occasionally a population of worms was found at a later stage of the infection which were also randomly distributed; however, in the majority of cases the worms were significantly aggregated with respect to each other. After the tenth day of infection when all the worms were sexually mature, only a single population of worms was not aggregated.

Discussion

The mean worm burdens in both male and female mice remained more or less constant throughout the course of infection, but it is clear that the positions of the worms altered as the infection progressed, with an increased degree of aggregation in relation to the intestine. Furthermore, the worms themselves are clumped with respect to each other.

A similar situation was reported for Nippostrongylus brasiliensis in the small intestine of the rat (Alphey, 1970), the worms being aggregated both with respect to each other and to the intestine. Alphey suggested that the latter

aggregation was in response to some unknown factor acting over a relatively large area of the intestine, probably im the form of a secretion into the lumen,which may diffuse setting up a gradient along the gut to which the worms may respond, thus giving rise to their characteristic distribution.

It was shown by Rogers (1949a) that appreciable O_2 tensions exist close to the mucosa in the rat intestine, such tensions decreasing as the distance from the pylorus of the stomach increased. It is possible that this gradient of O_2 tension is an important factor governing the orientation of intestinal parasites within the small intestine. The juvenile Nematospiroides dubius which emerged into the lumen of the intestine on the seventh day of infection were still growing and also needed energy for egg and sperm production and copulation. It has been shown in Chapter 6 that these worms consume appreciable quantities of oxygen when it is available, and it is possible that they migrate to a more anterior position in the intestine where higher concentrations of O_2 exist,to help fulfil their energy requirement.

The aggregation of Nematospiroides dubius with respect to each other has also been reported for Nippostrongylus brasiliensis by Alphey. He suggested that intrinsic behaviour patterns were responsible for this, one of which

is sexual attraction. Both Lee (1969) and Alphey (1971) have demonstrated thigmokinesis in N. brasiliensis; Lee suggested that this would be advantageous to the worms in maintaining their position between the villi on the intestinal mucosa.

Experiments on the oxygen consumption of Nematospiroides dubius have indicated that the clumping of worms may have a sparing effect on their oxygen consumption, as the oxygen uptake of individuals decreased when they were crowded together. This could be of economic value to a nematode which inhabits an environment where the oxygen tension fluctuates. This fluctuation is mainly due to the release of food from the stomach into the intestine at irregular intervals and the consequent variation in digestive activity, O_2 tensions tending to be lower during high digestive activity. One can speculate that the degree of clumping of individual worms may alter (i.e. increase) as food passes through the intestine.

A red pigment is present in the walls of Nematospiroides dubius which is thought to be similar to the red pigment found in the walls of Nippostrongylus brasiliensis. This latter pigment is known to be a haemoglobin with a low loading tension (Davenport, 1949 ; Rogers, 1949c) which probably acts as an oxygen store. If a similar O_2 store were present in Nematospiroides dubius, it would release O_2

to the worms during times of O_2 lack; this could occur when O_2 tensions are lowered in the intestine due to digestive activity, and may also be related in some way to the changes which occur in the respiration rate under crowded conditions.

The parasitic third and fourth stage larvae of Nematospiroides dubius, which do not need to contact other worms for sexual reproduction, are found encysted individually in the submucosal layer of the duodenum. This area is richly supplied with blood vessels and histological sections have shown that the larvae invariably take up a position close to a blood vessel (Figure 19). The structure of the larval "cyst", formed by the larvae is difficult to determine, as no definite boundaries can be seen. Red blood corpuscles were often observed surrounding the "encysted" larvae and occasionally were packed inside the intestines of some of the worms. Liu (1965) in a study of the pathology of Nematospiroides dubius in mice also reported haemorrhages in the small intestine with larvae in the centre of them. The larvae stages would thus seem to have a plentiful supply of O_2 from the blood which they should be fully capable of exploiting as they, like the adult stages of N. dubius, consume oxygen when it is available to them (Chapter 6). Whether or not the larvae obtain nutritional value from the blood cells or whether they simply utilise them as a source

FIGURE 19

a - encysted larva

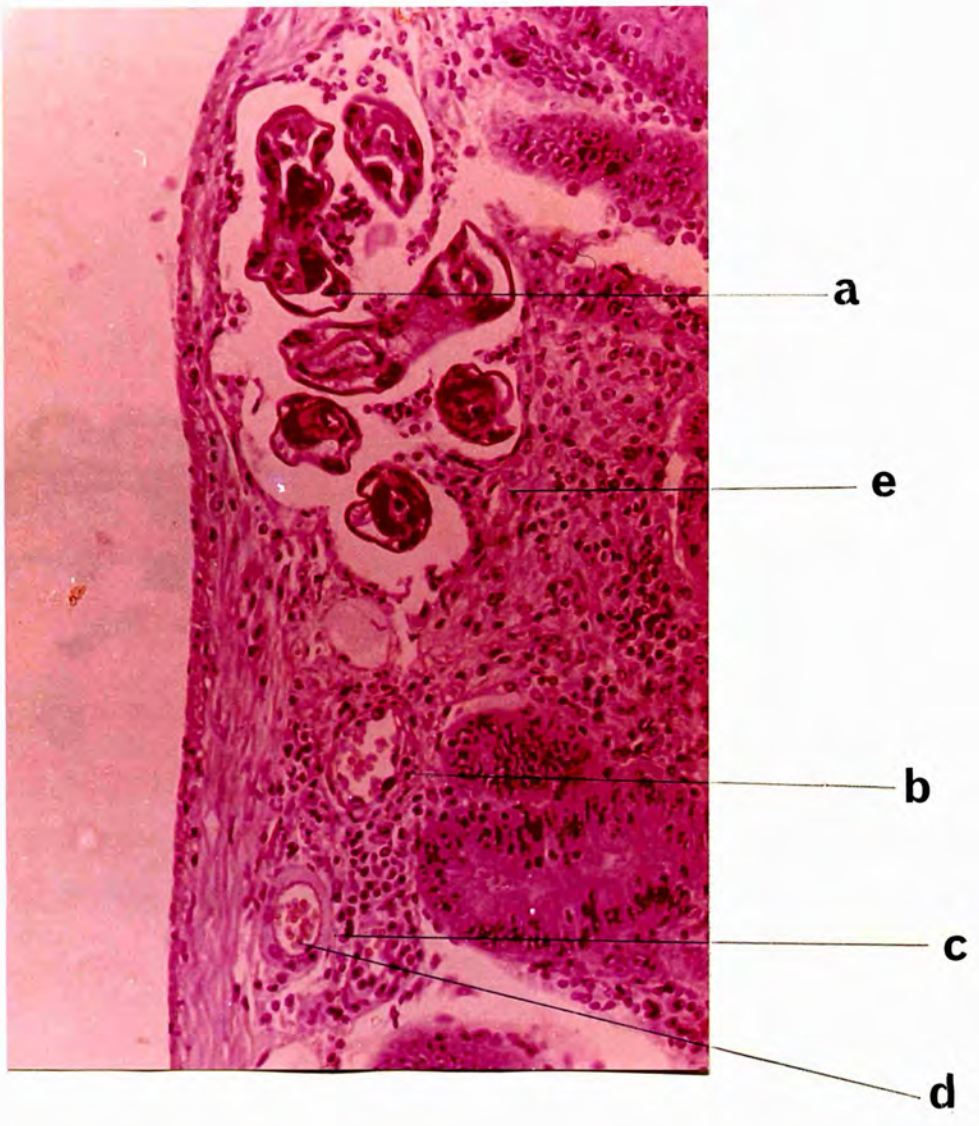
b - host venous blood vessel

c - host arterial blood vessel

d - red blood corpuscles

e - mucosal cells of host small intestine

THE POSITION OF AN ENCYSTED PARASITIC LARVA OF N.DUBIUS IN RELATION TO THE HOST BLOOD SUPPLY



of O_2 is not known. Crowding of third and fourth stage larvae in respiration experiments did not lead to a pronounced decrease in individual respiration rates, as was found with adult worms. This indicates that they obtained sufficient O_2 for their needs in the positions they took up in the gut, and thus have less need of this "sparing effect". The above results suggest that oxygen is an important factor in determining the distribution of both larval and adult stages of Nematospiroides dubius.

The distribution of both larval and adult Nematospiroides dubius was similar in both male and female mice; in addition, there was no difference in the worm burdens harboured by male and female mice. The result was unexpected as female mice have been shown to harbour significantly lower burdens of N. dubius than male mice (Dobson, 1961; Bawden, 1969). Newton, Weinstein and Sawyer (1962) reported that there was no difference in yields of N. dubius from male and female germ free mice, but that in control experiments, using mice of the same genetic stock maintained outside the germ free system, infections in males averaged two to three times those found in females. Thus, the sex difference would appear to be linked to the presence of an intestinal flora. The mice used in these experiments were a Specific Pathogen Free Strain; however, they were not maintained under germ free conditions. Populations of male Apodemus sylvaticus

harboured larger worm burdens than the females, and the reason for such a difference in susceptibility to infection with *N. dubius* of laboratory and field mice is consequently difficult to explain.

CHAPTER 4

THE LIFE CYCLE OF NEMATOSPIROIDES DUBIUS

Introduction

Nematospiroides dubius is a Trichostrongylid nematode, belonging to the family Heligmosomatidae. It has a direct life cycle involving a free-living phase and a parasitic phase. Infective ensheathed third-stage larvae enter the host via the mouth and after a period in the intestinal mucosa, take up a position in the lumen of the duodenum when adult. N. dubius parasitizes a variety of small mammals and in the present study has been found in Apodemus sylvaticus and Clethrionomys glareolus.

There have been several published accounts of the life cycle of Nematospiroides dubius, few of which agree about the moulting times and duration of the different larval stages. The discrepancies in the duration of the free-living larval stages were mainly due to differences in culture temperature used by the various authors.

The first account of the life cycle of this parasite was published by Spurlock (1943), in which the moulting times of the free-living stages were not given, but it was stated that infective stages were produced by the fourth to sixth day after eggs were placed in culture. Spurlock indicated that the first parasitic moult occurred in the lumen of the

intestine before the worms entered the mucosa, and that the final moult occurred while the worms were encysted in the mucosa, before returning to the intestinal lumen.
Ehrenford (1945) produced a more detailed study in which he maintained his cultures between 23°C and 28°C. At this temperature the eggs hatched 26 hrs after they passed from the host, and the first ecdysis was initiated approximately 48 hours after hatching. Ehrenford gave no indication of the time of the second moult, merely stating that the larvae required four to six days to become infective. He observed two parasitic moults within the host, the first occurring 48 hours after infection, the second 6 - 8 days after infection, after which time the worms returned to the intestinal lumen. In 1956, Fahmy made a study of this life cycle with particular reference to the free-living stages, which he cultured at three different temperatures, 22°C, 28°C and 40°C. At 22°C hatching occurred 23 - 24 hours after the eggs passed from the host; at 28°C, hatching occurred 19½ hours after the eggs left the host; at 40°C the eggs did not develop larvae. The L1 moulted to the L2 24 hours after hatching at 26°C, and moulted to give rise to the ensheathed L3 68 - 80 hours after hatching. These larvae were found to be capable of initiating an infection in laboratory mice, but Fahmy did not investigate the duration times of the parasitic instars.

A fourth account of the life cycle was produced by Dobson (1960). He described three parasitic moults, the first occurring 48 hours after infection, the second 96 hours after infection, and the third some time before the sixth day after infection. He maintained that the infective larvae were ensheathed in the old cuticle of the first larval stage instead of the second larval stage. All authors agreed that the life cycle takes approximately 15 days from egg to egg.

It was therefore necessary to study this life cycle in some detail at a selected culture temperature, as accurate knowledge of the time of infectivity, and the duration of each larval stage was necessary for further studies on the growth and metabolism of Nematospiroides dubius.

Materials and Methods

(a) Development of free-living larvae

Cultures were set up as described above. Mouse faeces used for culturing were collected over 30 minute periods, in order that the larvae produced would be of as uniform an age as possible. However, this did not account for any age difference which may have been caused by periodicity of egg laying by female worms within the host. All cultures were maintained at 20°C ± 0.1°C. Eggs and larvae were removed from culture at two-hourly intervals, temporarily mounted on a slide in a drop of distilled water, and examined under a microprojector x 20 magnification. Any changes in the

cuticle, particularly at the tail end of the worm where the first evidence of ecdysis can be seen, were noted.

(b) Development of the parasitic stages

S.P.F. ASH/CS1 mice aged 6 - 8 weeks were infected with a standard dose of infective larvae as described in general methods. Mice were autopsied at six-hourly intervals and the small intestine removed and placed in Tyrodes' saline. The position of worms in the mucosa or lumen of the gut was noted before they were removed. Temporary mounts were made of the worms and they were examined under a microprojector. In some cases, worms could only be seen in the mucosa by squashing it between two glass plates and examining under a microscope.

Results

(a) Free-living stages

The eggs which passed out of the host were at the 8 - 16 cell stage; approximately 12 hours later the eggs had reached the many celled blastula stage, and the gastrula stage was reached 18 hours after the eggs had left the host; this stage exhibited slight twitching movements. By 28 hrs the eggs contained fully developed larvae which performed vigorous movements within the egg shell. (Figures 20. a-d). The eggs hatched 36 - 37 hours after passing from the host giving rise to the first larval stage. Twenty-eight to twenty-nine hours after hatching the L1's were observed to

FIGURE 20

a 64 cell stage

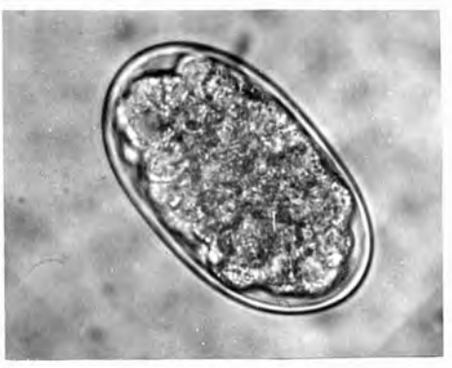
b blastula stage

c gastrula stage

d infective larva

STAGES IN THE DEVELOPMENT OF THE EGG OF N.DUBIUS, AND THE INFECTIVE THIRD STAGE LARVA.

a



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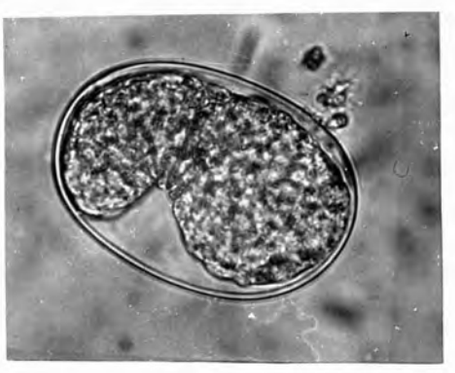

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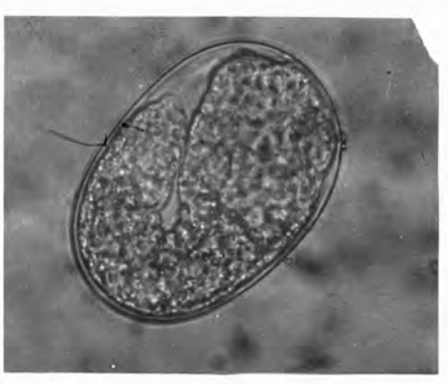
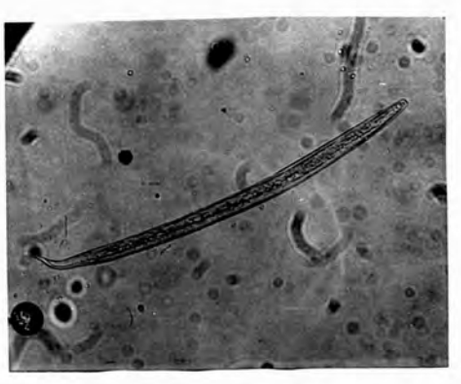


d



be starting to moult; 46 - 47 hours after hatching the second larval stages underwent a partial moult to form the ensheathed third stage larvae which are active but non-feeding. The first two larval stages feed on bacteria in the faeces (Lee, 1965). The infective larvae were administered orally to laboratory mice, as a standard dose.

(b) Parasitic stages

A small number of third-stage larvae could be detected in the intestinal lumen 18 hours after infection, and could be seen to be in the process of losing their protective sheaths. No larvae were found in the intestinal lumen 24 hours after infection, indicating that they had all penetrated the mucosa by this time. Worms could be seen in the mucosa 30 hours after infection; they were larger than the infective L3 and had less pointed tails (Figure 20e). Larvae removed 48 hours after infection were beginning to show a differentiation in the tail region; the tails of the male larvae developed a bulge at one side, whereas those of the female larvae remained more pointed. This difference was much clearer 70 hours after infection. Between 70 and 80 hours, the cuticle at the posterior end of the worms could be seen to be separating from the underlying tissues, and the worms moulted to the L4 stage between 90 and 96 hours. The tail end of the female L4 had a definite spike which was clearly distinguishable from that shown by the male L4's (Figures 20 f and g).

FIGURE 20

e larva extracted 30 hrs after infection

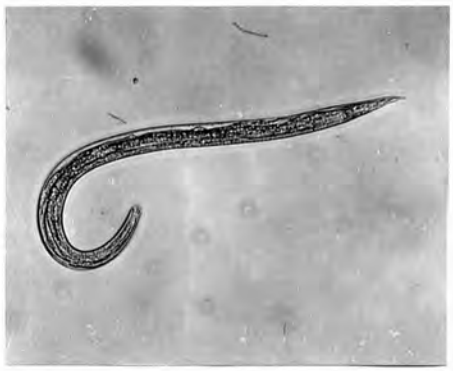
f tail end of male L4

g tail end of female L4

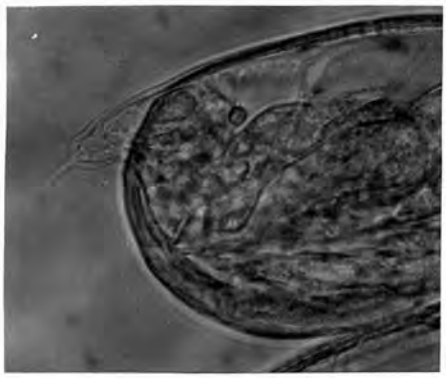
h tail end of male L4 showing developing bursal rays

i tail end of male L5 showing released bursal rays

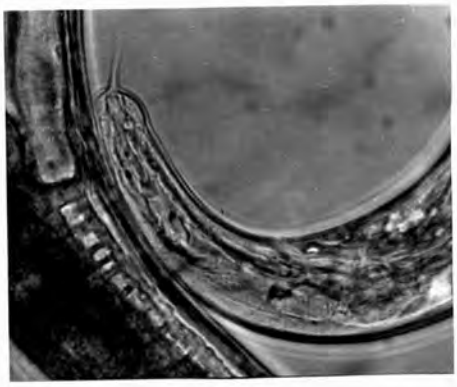
e



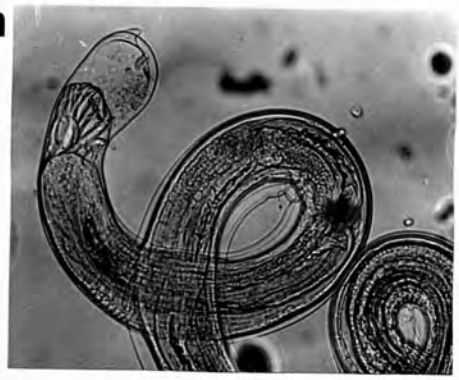
f



g



h



i

More pronounced changes were visible at the posterior end of the male worms; the rays of the copulatory bursa could be seen developing within the L4 cuticle (Figure 20h). By 138 hours, the old cuticle could be seen separating away from the new one in both male and female worms and the second parasitic moult occurred between 144 and 166 hours after infection. The posterior end of the L5 female was similar to the L4 but the (copulatory) bursal rays of the male were now spread out (Figure 20i). By 191 hours, most of the juvenile worms had passed from the mucosa into the intestinal lumen to take up their adult position. Developing eggs could be detected within the female worms by 200 hours, and by 240 hours the females contained large numbers of eggs, and male and female worms were observed in copulo. The first eggs were detected in the host faeces on the tenth day of infection, so the life cycle can be seen to take 14 days from egg to egg in the strain of mouse used and when a culture temperature of 20°C is used. A summary of the duration times of the different stages is given in Table 7.

TABLE 7

DURATION TIMES OF THE EGG AND LARVAL STAGES OF N. DUBIUS WHEN CULTURED AT 20°C

	STAGE	DURATION TIME
free-living stages 20°C	Egg	36 - 37 hours
	L1	28 - 29 hours
	L2	18 - 19 hours
	L3	indefinitely
parasitic stages 37°C	L3	90 - 96 hours
	L4	48 - 76 hours
	L5	until death

CHAPTER 5

THE GROWTH OF NEMATOSPIROIDES DUBIUS

Introduction

The growth of Nematospiroides dubius has been studied throughout its entire life cycle; the primary purpose of this experiment being to determine the type of growth curve exhibited by this worm during its free-living and parasitic phases. This was necessary for later work in which the metabolism/size relationship was established for each stage in the life cycle.

The life cycle of nematodes involves four moults; the onset of each moult is commonly associated with a period of inactivity known as lethargus, during which no growth occurs (Lee, 1965). Several workers have demonstrated that a sequence of growth, lethargus and ecdysis occurs throughout the life cycle of these animals. This has been shown in the animal parasites Haemonchus contortus (Cobb, 1898) by Veglia (1915) and Cooperia curticei (Ransom, 1907), by Sommerville (1960); both these species belong to the superfamily Trichostrongylidae, which also includes Nematospiroides dubius.

Most previous work on the growth curves of nematodes, both free-living and parasitic, have taken changes in body

length as a measure of growth. This method gives rise to several errors. The length of a nematode may vary due to the amount of food in the gut (Townsley et al., 1963), the time of defaecation (Mapes, 1965), or the osmotic pressure of the external medium (Lee, 1960a; Anya, 1966).

Also an increase in size of the internal organs such as ovaries may not be accompanied by increases in body length (Blake, 1962). Weight is a more reliable measure of growth.

Weiser and Kanwisher (1960) plotted a growth curve for the marine nematode Enoplus communis using fresh weights calculated from volume and specific gravity measurements. However, they pointed out that it is probable that changes in water content take place in connection with the moulting process giving rise to additional errors.

Both fresh and dry weights were obtained for the free-living stages of Nematospiroides dubius and this work showed that the dry/fresh weight ratio was not constant for different instars and it was therefore decided to use dry weight as the best measure of growth. The growth curves plotted were based on these dry weight measurements, the main advantage being that the chief source of error was experimental. This method does not include errors inherent in calculated weights and is more independent of the physiological state of the animal; however, it does involve the

collection of large numbers of accurately aged worms.

The growth rates of N. dubius were studied in both male and female laboratory mice. Both sexes of host were used as it was shown by Dobson (1960) that there is stunting of growth of N. dubius in female mice which is linked with a sex resistance, which also manifests itself as a reduction of the worm burden. As there was no evidence of reduced worm burdens in female mice of the strain employed (Chapter 3) it was of interest to observe if there was any resistance mechanism affecting growth.

Materials and Methods

Free-living stages of Nematospiroides dubius were extracted from cultures and parasitic stages were removed from mice as described in general methods. Measurements of growth for both free-living and parasitic stages were made at 12 or 24 hourly intervals. All worms used were freed from debris and washed a minimum of three times in distilled water. No damaged worms were used.

The first free-living larvae used, which hatched 36 hours after the eggs were placed in culture, were called day 2 larvae.

(a) Measurement of Fresh Weights of Free-Living Larvae

The fresh weights were calculated using the formula of Andrassy (1956): $G = \frac{a^2 \cdot x\ b}{100,000}$ in which G = fresh weight in micrograms (µg), a = the greatest body width in

microns (μ), and b = body length in microns (μ), 16 = the empirical key number. Andrassy derived his formula by first obtaining the volume and specific gravity of nematodes. He obtained the former by dividing the body into three geometrical figures and calculating their cubic content, and the latter by suspending worms in liquids of known specific gravity. He found that regardless of species and body size all the nematodes he tested had very similar specific gravities. From detailed calculations, Andrassy derived his empirical key number of 16, which could be used in a simplified equation to calculate fresh weight. When compared to the more detailed method, the simplified method showed at most an error of ± 5%.

Washed larvae were mounted on a slide in a drop of distilled water and gently heated on a hot plate until they relaxed and died. A temporary mount was made of the larvae and their outlines were drawn on graph paper using a microprojector; for each age group a minimum of 22 larvae were drawn. The length and greatest body width were measured using a calibrated map-measuring wheel; the measurements were substituted in the above formula.

The standard deviation for drawing was obtained by drawing the same worm twenty times and measuring each one and was ± 19.25 μ; the standard deviation for measuring was obtained by measuring a single worm drawing twenty times and

was $\pm$ 1.257 μ.

(b) Measurement of Dry Weight of Free-Living larvae

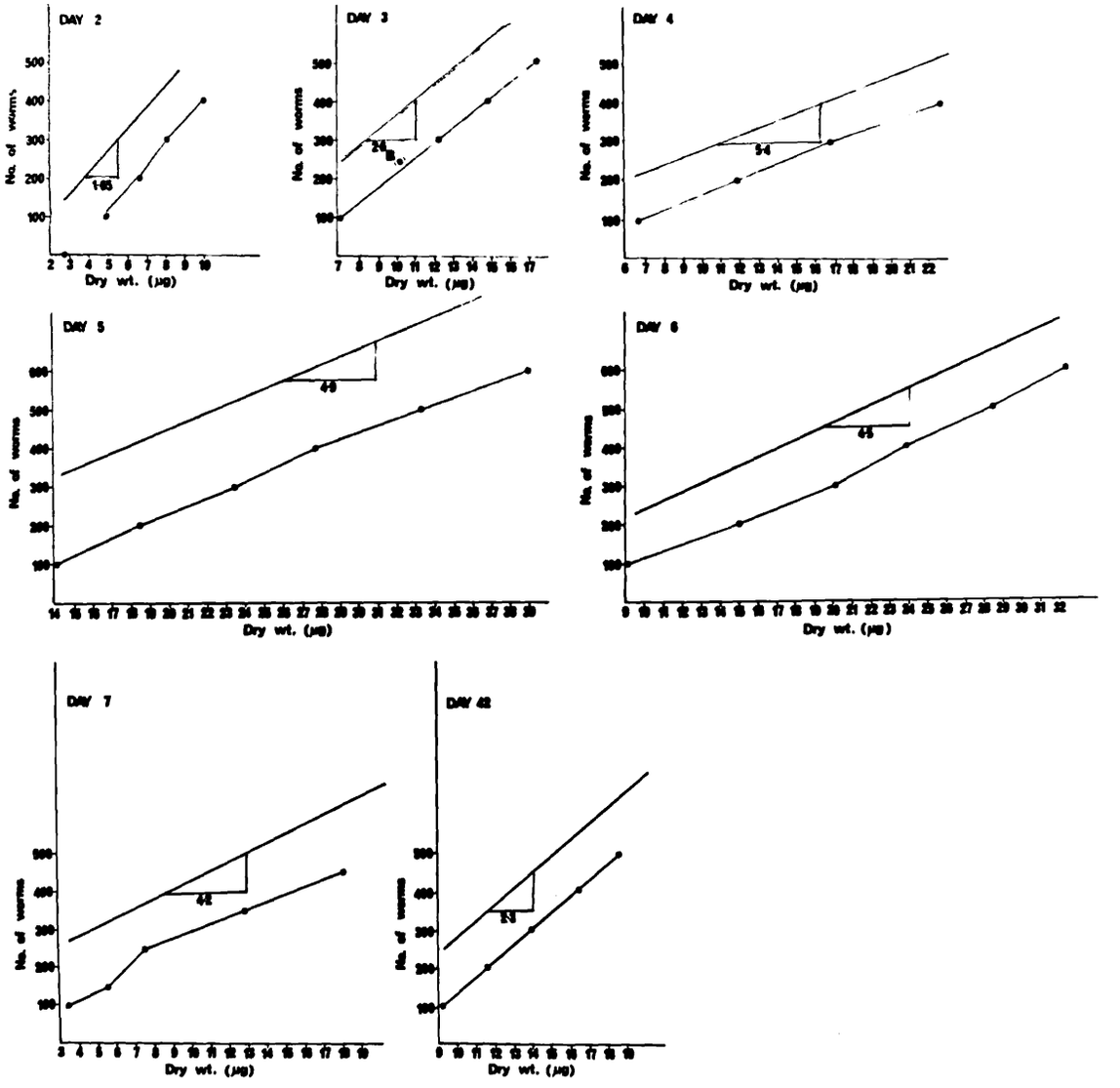
Dry weights of free-living larvae were determined using the cumulative method of Doohan and Rainbow (1971). Samples of 100 washed larvae were counted on to a cavity slide and dried in a desiccator over calcium chloride at room temperature for a minimum of 48 hours. A sample of 100 larvae was transferred to the balance pan of a Cahn Gram Electrobalance (10^{-7}g; sensitivity, 0.2 μg.; accuracy 0.05% of the scale range). A watch glass of silica gel was placed in the weighing chamber to prevent hygroscopic uptake of water by the dried nematodes. After weighing a second sample of 100 larvae was added to the balance pan and weighed. The cumulated number of worms was plotted against their cumulated weight, which mostly took the form of a straight line, from which it was possible to read off the average weight of 100 individuals, and calculate the mean weight of one worm. The straightness of the line was a check upon possible loss of worms during transfer to the pan. The point B in Figure 21, Day 3, represents a probable increase in moisture content of the sample.

(c) Measurement of Dry Weight of Parasitic Stages

Infected mice were killed by cervical dislocation and the small intestine was removed and opened under Tyrodes saline.

FIGURE 21

THE CUMULATED DRY WEIGHTS OF AGED FREE-LIVING LARVAE OF N. DUBIUS.



Encysted third and fourth stage larvae were teased out of the submucosal region of the small intestine using fine Tungsten dissecting needles. Debris was removed, the worms washed three times in distilled water and placed on cavity slides in a desiccator at room temperature for a minimum of 72 hours. Very small worms were dried and weighed in batches of five; all other worms were treated singly. Growth curves were obtained by plotting weight against age.

The youngest parasitic stages obtained from infected mice were 48 hours old. The infective larvae do not penetrate the intestinal mucosa until approximately 18 hours after infection. Although they may occasionally be seen moving in the submucosal layers using a H. P. binocular microscope 36 hours after infection; it is not possible to remove them without damage until they are at least 46 hours old.

(d) Fat staining

Aged free-living larvae were stained for fats using Oil Red O (Lee , 1960b).

(e) Growth curves

Growth curves were obtained by plotting weight against age. In order to obtain a quantitative measure of growth the various curves were converted into straight lines by plotting on arithlog paper as described by Brody (1945). It was then possible to calculate daily growth rates using

the formula:-

$$g = \frac{\ln W_2 - \ln W_1}{t_2 - t_1}$$

where W_1 and W_2 are the weights at the times t_1 and t_2. The straight lines obtained by converting the curves enable W_1 and W_2 to be read directly from the graph. A conversion factor of0.4342 was used to convert the natural logarithms to log base^{10}. The formula thus becomes:-

$$\frac{\text{Log } W_2 - \text{Log } W_1}{t_2 - t_1 (0.4342)}$$

The differences in g values for a single sex of worm in different sexes of hosts were not tested directly. Instead the regression coefficients calculated from the converted growth curves were compared.

Results

(a) Fresh weights - free-living larvae

The individual measurements of drawings and the calculated weights are shown in Appendix 1. Larvae were weighed at 24 hourly intervals from the second day of culture. An average weight was obtained for each age group based on a minimum of 18 different calculated weights (Table 8a). During the first two days of their life, the free-living larvae increased in weight. There are two larval stages during this period,both of which feed upon bacteria in the host faeces. By the fourth day from the

TABLE 8a

The average fresh weights of the free-living larval stages of N. dubius

Larval Stage	Age in days from start of culture	Fresh weight µg (S.D.)	No. of worms measured
1	2	0.1567 ± 0.0205	32
2	3	0.2236 ± 0.0444	22
3	4	0.2204 ± 0.0346	26
3	5	0.2104 ± 0.0282	29
3	6	0.2081 ± 0.0250	20
3	7	0.1979 ± 0.0219	40

TABLE 8b

The average dry weights of the free-living larval stages of N. dubius

Larval Stage	Age in days from start of culture	Dry weight µg.	No. of samples weighed	No. of worms in each sample
1	2	0.016	5	100
2	3	0.026	5	100
3	4	0.054	4	100
3	5	0.049	6	100
3	6	0.045	6	100
3	7	0.042	5	100
3	42	0.023	5	100

start of culture, the larvae have undergone the second incomplete moult to form the ensheathed infective third stage larva, which is non-feeding but active. After this point in time the larvae began to show a decrease in fresh weight which continued for the following three days.

(b) Dry weights - free-living larvae

The dry weight for each age group was based on a minimum of 500 larvae, weighed in batches of 100. The graphs from which the weights were calculated are shown in Figure 21 and the weights are shown in Table 8b. As was shown with fresh weights there was an increase in weight for the first two feeding larval stages extracted on the second and third days of culture. The larvae undergo the second moult and become infective approximately 84 hours after the eggs are placed in culture at 20°C, so it is not clear how closely weight loss coincided with the onset of infectivity. However, by day 5 a weight loss was recorded which continued up to day 7 and beyond.

(c) Fat content of free-living larvae

The staining of free-living larvae for the presence of fats revealed a build up in fat in the bodies of the larvae during the first two days of their life when they were feeding. Although the method was not quantitative, it indicated that the amount of fat in the body decreased between days 4 and 8. There was a very marked decrease in the amount of

fat present in 42 day old larvae (Figure 22).

(d) The growth curves of free-living larvae

The growth curves of the free-living larvae in terms of both dry and fresh weight are shown in Figure 23 and it is clear that the same general trend in growth was shown by both measures of growth. However, the dry weight/fresh weight percentage was by no means constant (Table 9). For the first two larval stages, the dry weight was only approximately 10% of the fresh weight; for the third larval stage it ranged between 24.5% and 21.2% over the four days it was measured. These differences could be due to errors in either method but were more likely to have been due to changes in body water content during the moulting process.

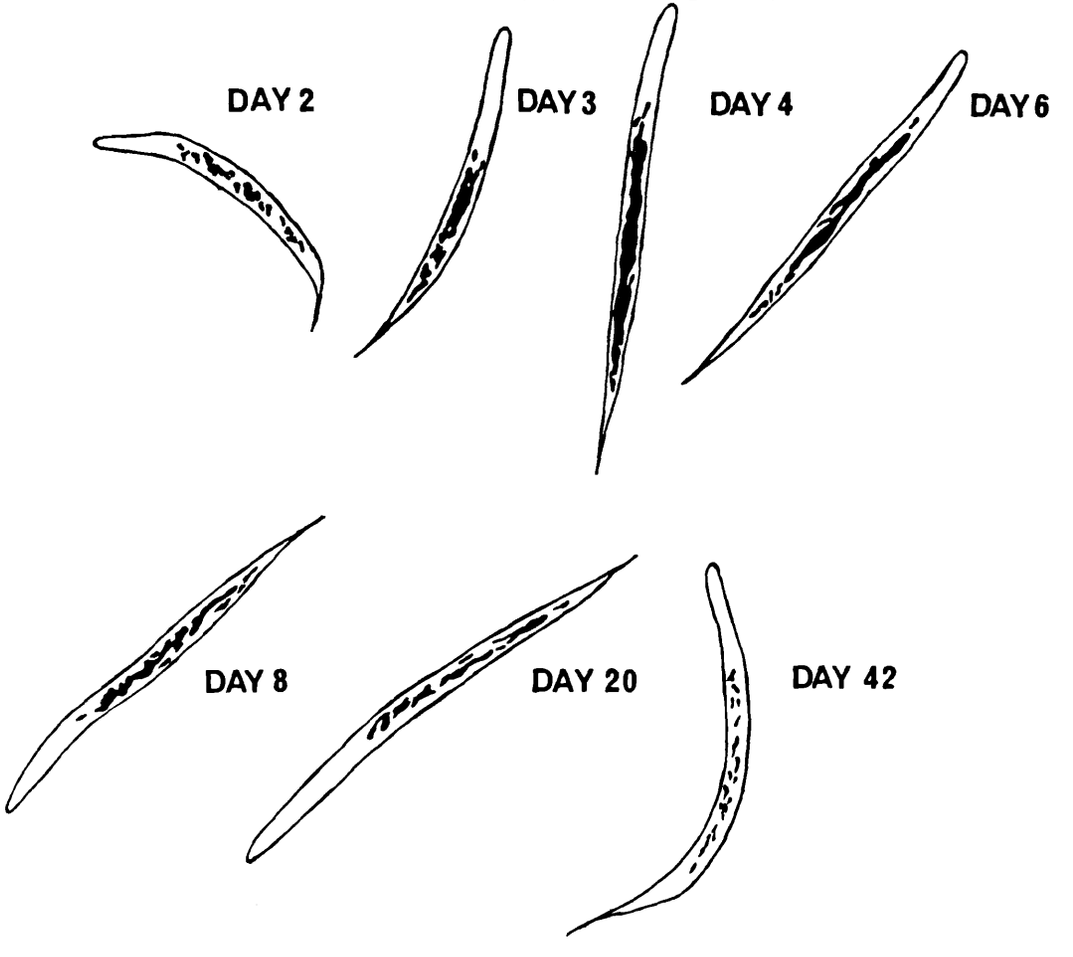
The daily loss of dry weight between days 5 and 7 was 0.0013 µg. per day, and that between days 7 and 42 was 0.0006 µg./day. That is to say the rate of daily weight loss decreased. However, previous to day 7 all larvae were maintained in culture at 20°C and after this they were stored at 4°C, so this result could possibly have been due to a temperature effect.

(e) Dry weights - Parasitic stages

Worms were weighed from the second to the eleventh day of infection at 24 hourly intervals and thereafter on Days 15, 18 and 42. Differences in structure of the tail region of the worms were apparent on Day 2, and enabled the sexes

FIGURE 22

THE FAT CONTENT OF AGED FREE-LIVING LARVAE OF N.DUBIUS, DEMONSTRATED BY STAINING WITH OIL RED O.



2-6 DAY OLD LARVAE STORED AT 20°C

8-42 DAY OLD LARVAE STORED AT 4°C

FIGURE 23

Growth curves of the free-living larvae of N.dubius based on dry and fresh weight

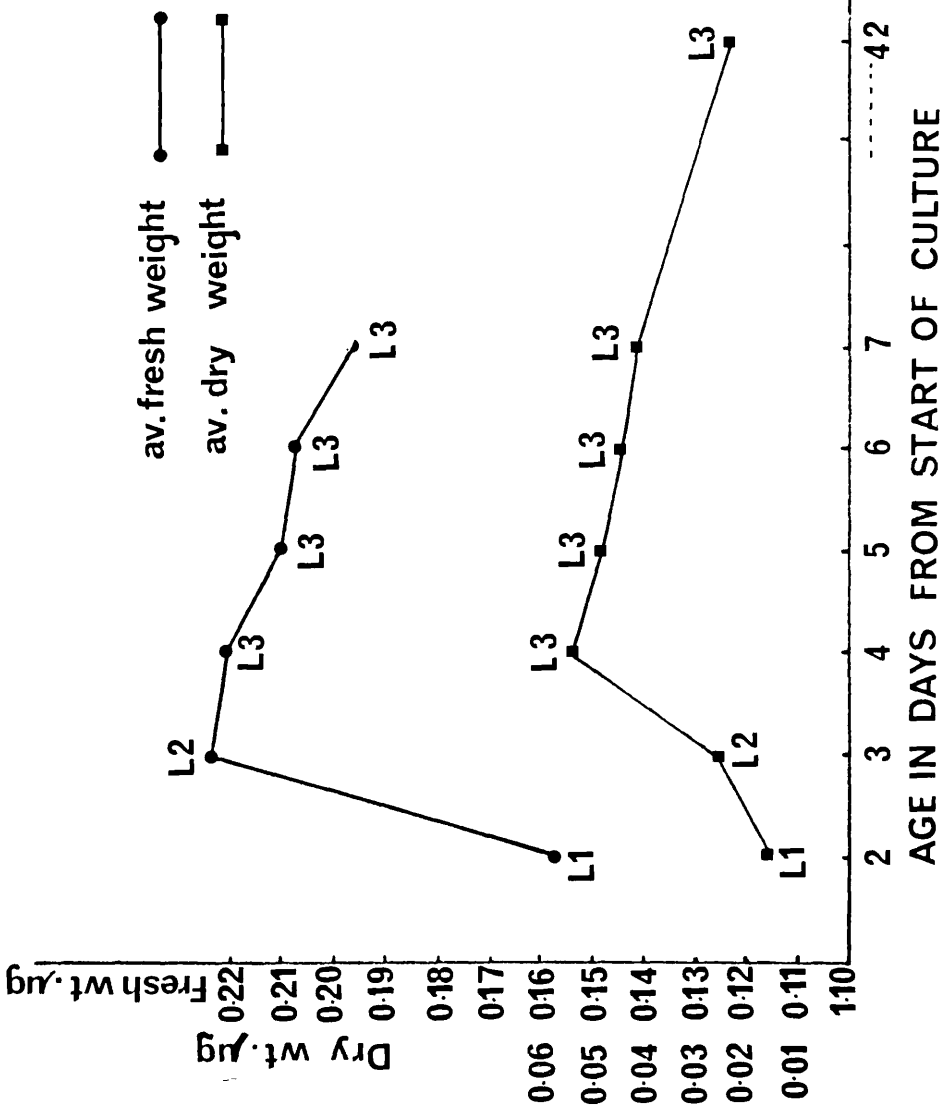


TABLE 9

The dry weights of aged free-living larval stages of N. dubius expressed as a percentage of the fresh weight

Larval Stage	Day of extraction from culture	Dry wt./Fresh wt. percentage
1	2	10.2%
2	3	11.63%
3	4	24.5%
3	5	23.29%
3	6	21.54%
3	7	21.22%

to be distinguished and treated separately; the individual weights are shown in Appendix 2.

(f) Growth curves

The growth curves for male and female worms in male and female hosts are shown in Figures 24a-d they were all typically sigmoid. All groups of worms showed an increase in weight with age, which also corresponded with an increase in development. There was, however, a variation with weight within each age category - this is discussed in a later section. It can be seen that the curves of the female worms differed from those of the males, the former attaining a larger final size in an equivalent period of time. However, the curves of the same sex of worm in different sexes of host are essentially the same.

(g) Converted growth curves

The converted growth curves for both free-living and parasitic worms are shown in Figures 25 a - d. It can be seen that the growth of all the categories of parasitic worms falls into three separate phases. (Table 10).

The instantaneous growth rate for the free-living larvae for the first three days until infectivity was reached was 0.6244, i.e. on each day the body weight increased by 62.44% of its weight on the previous day. The instantaneous growth rate (g) for phase one for female worms was 0.4627 in female mice and 0.5279 in male mice. The g value for phase two was 0.1018 in female mice and

FIGURE 24a

The growth curve of male parasitic stages of N.dubius in male mice based on dry weight

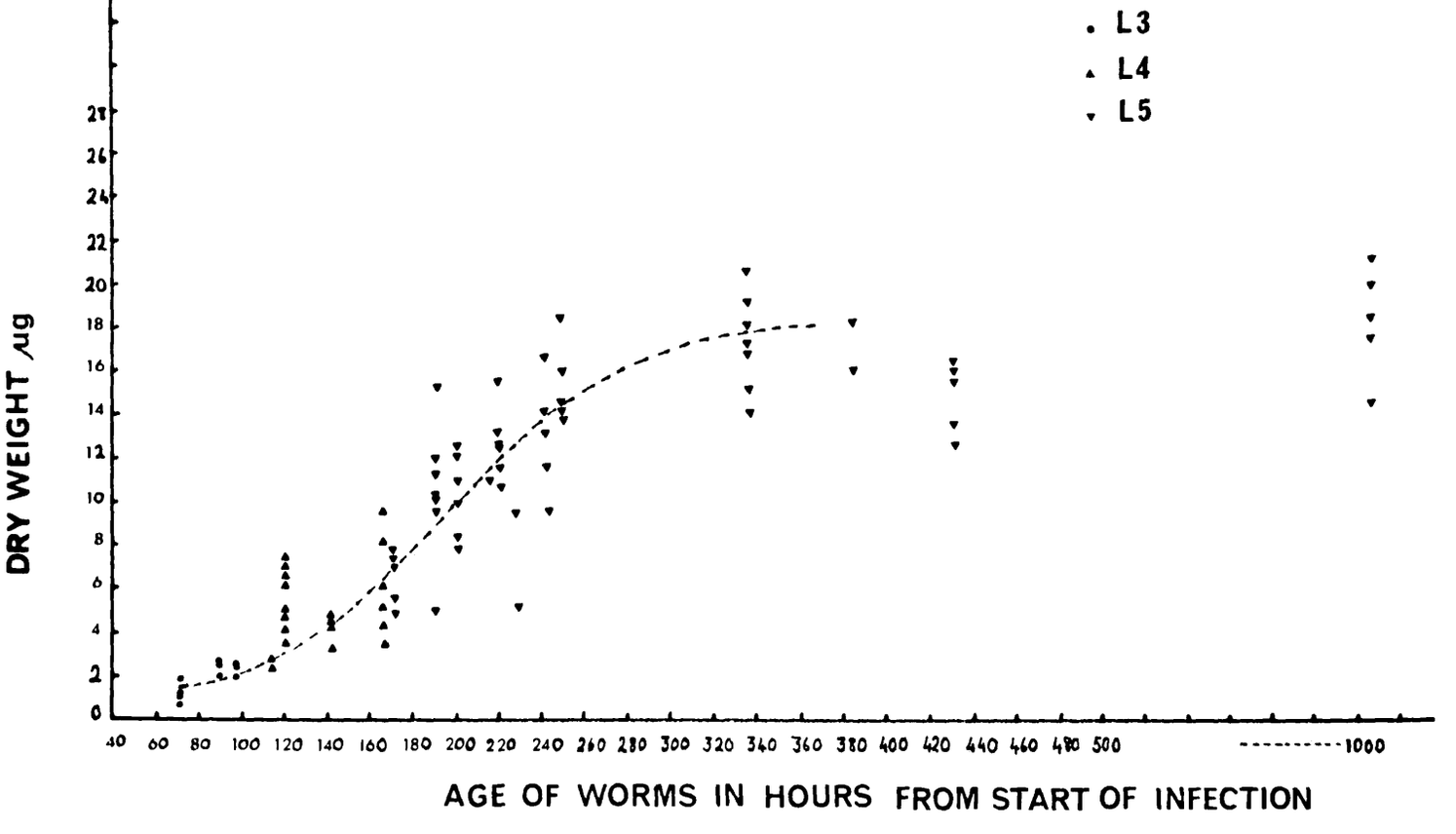


FIGURE 24b

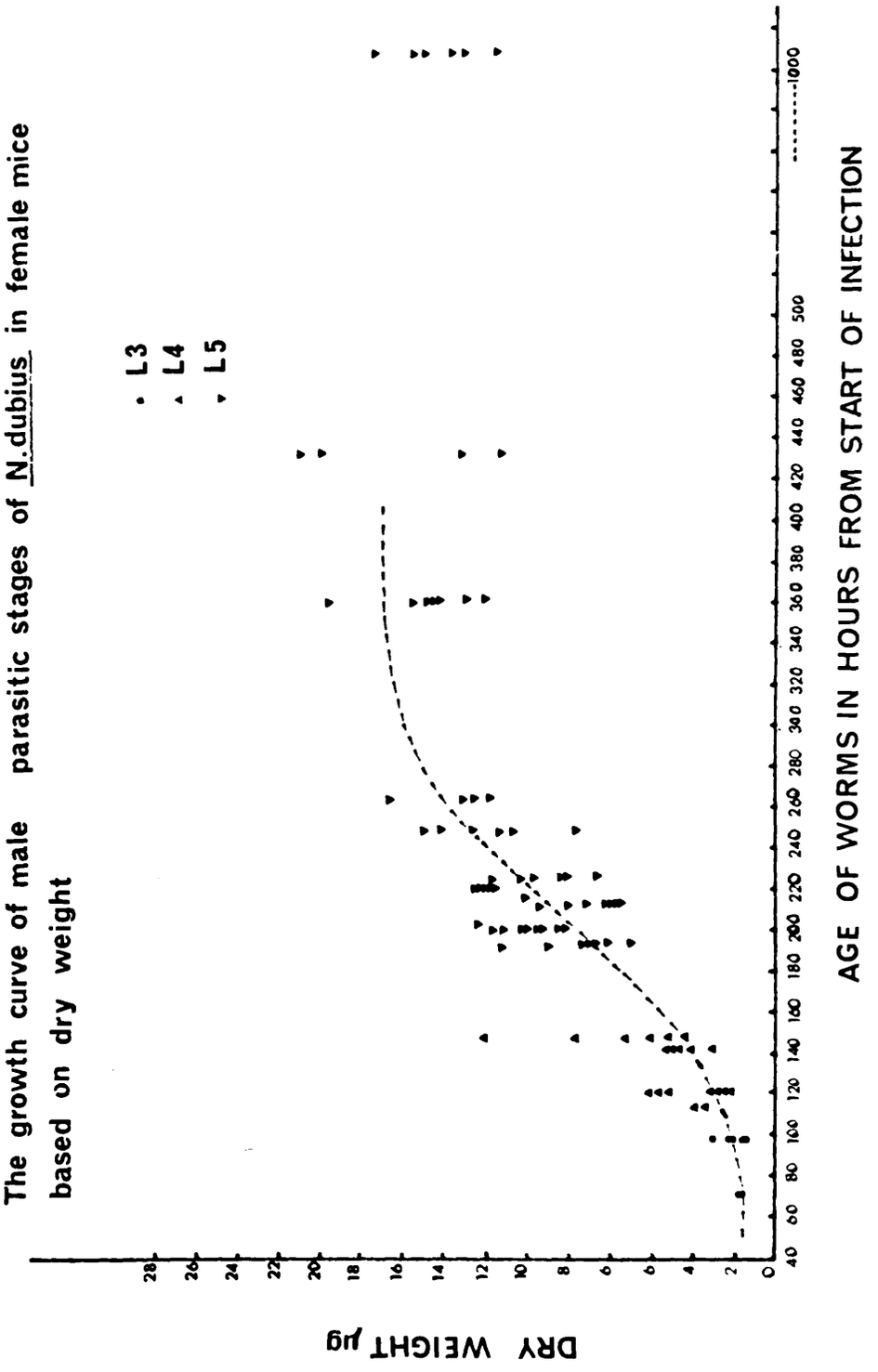

The growth curve of male parasitic stages of N.dubius in female mice
based on dry weight
L3
L4
L5
DRY WEIGHT µg
AGE OF WORMS IN HOURS FROM START OF INFECTION

FIGURE 24c

The growth curve of female parasitic stages of N.dubius in female mice based on dry weight

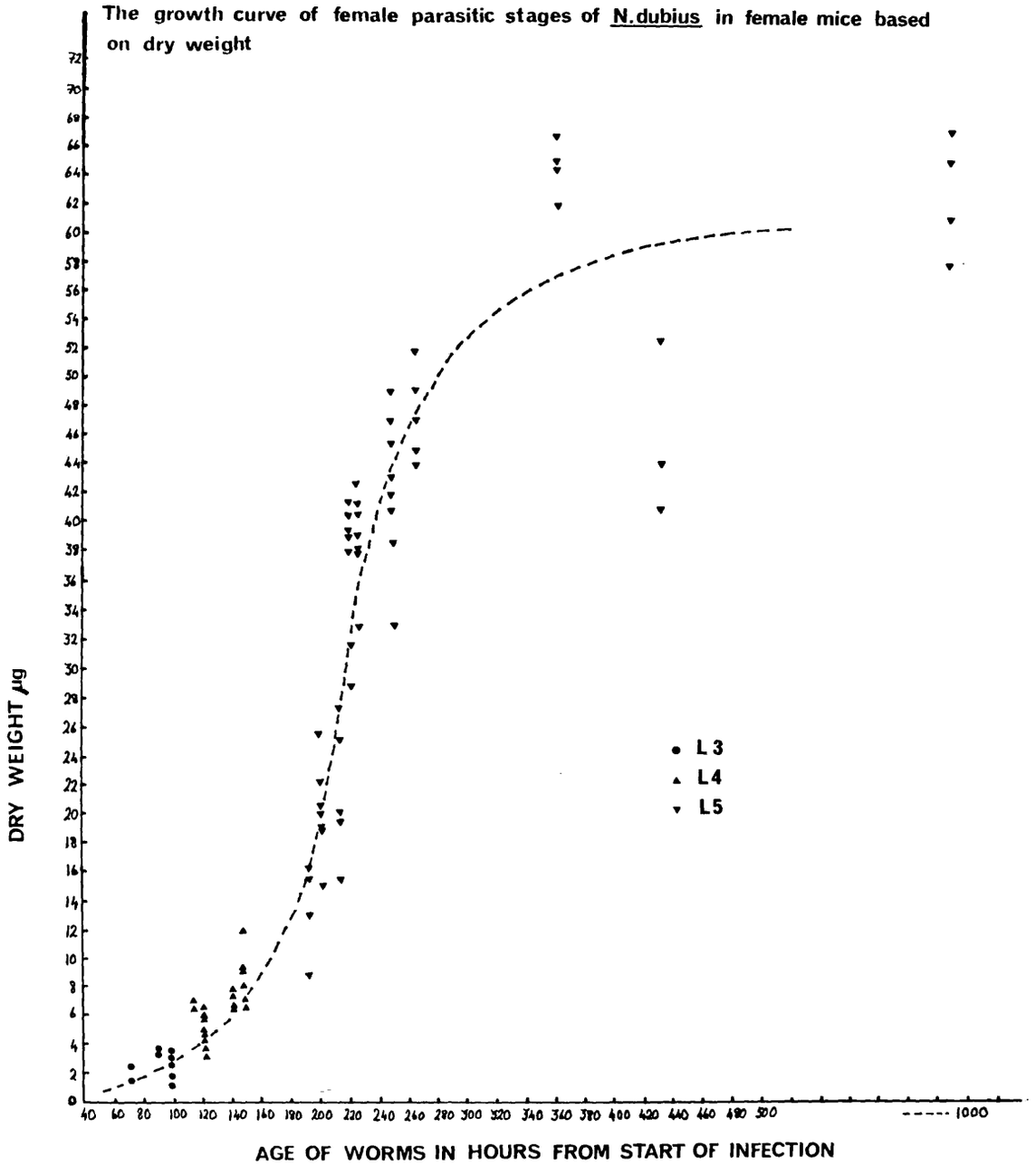


FIGURE 24d

The growth curve of female parasitic stages of N.dubius in male mice based on dry weight

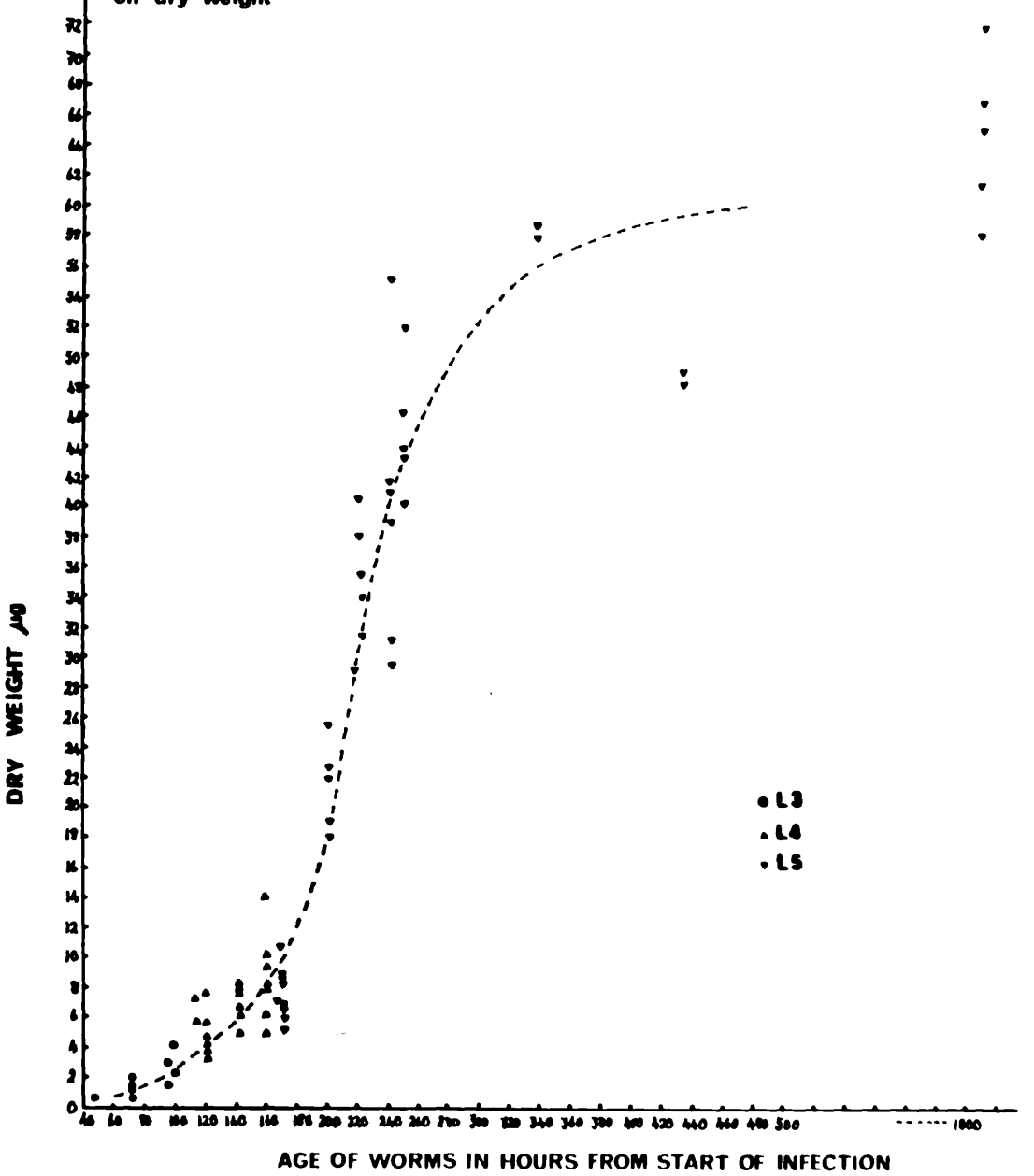


FIGURE 25a

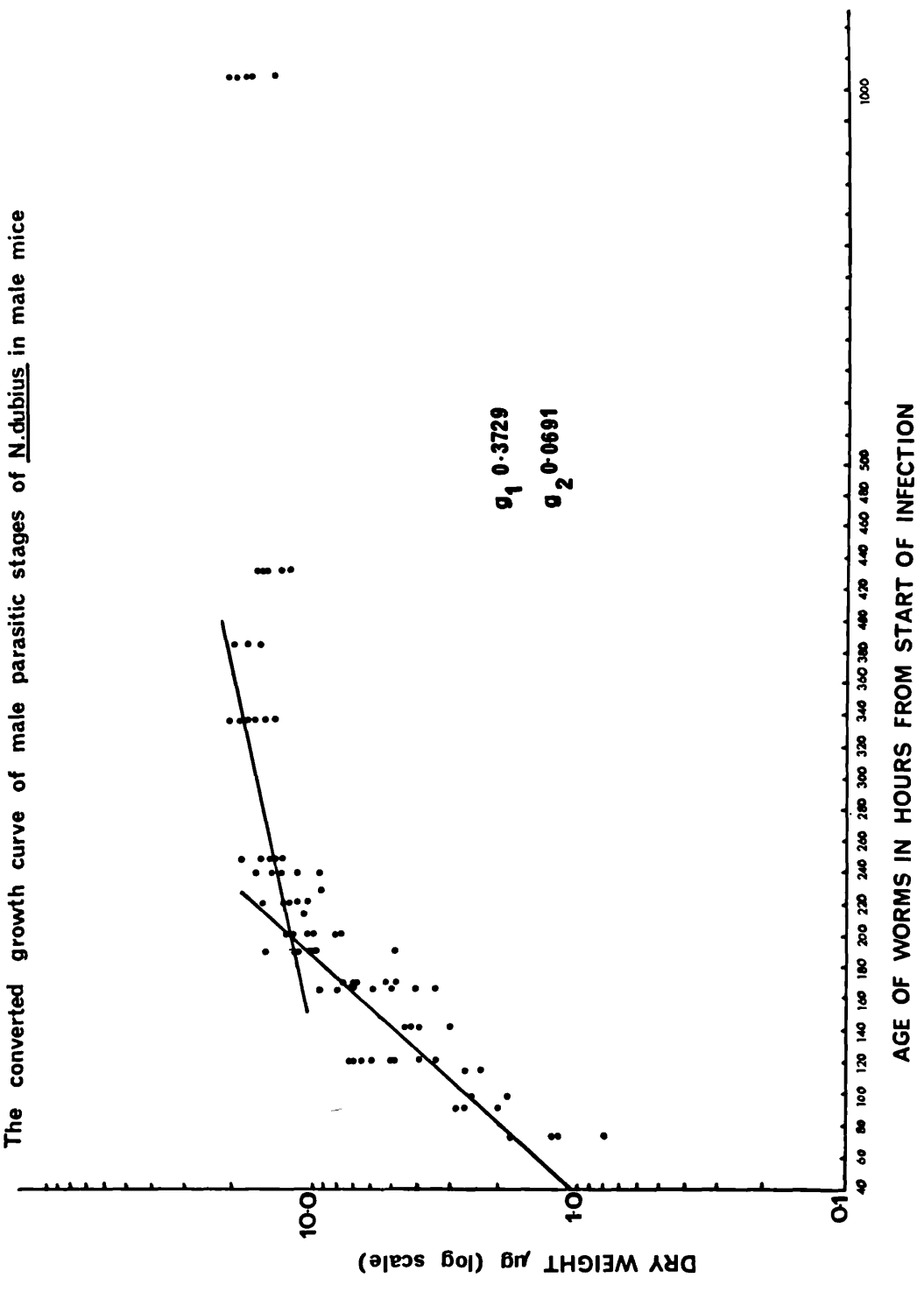

The converted growth curve of male parasitic stages of N.dubius in male mice
g_1 0·3729
g_2 0·0691
DRY WEIGHT µg (log scale)
100
10
01
40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380 400 420 440 460 480 500
1000
AGE OF WORMS IN HOURS FROM START OF INFECTION

FIGURE 25b

The converted growth curve of male parasitic stages of N.dubius in female mice

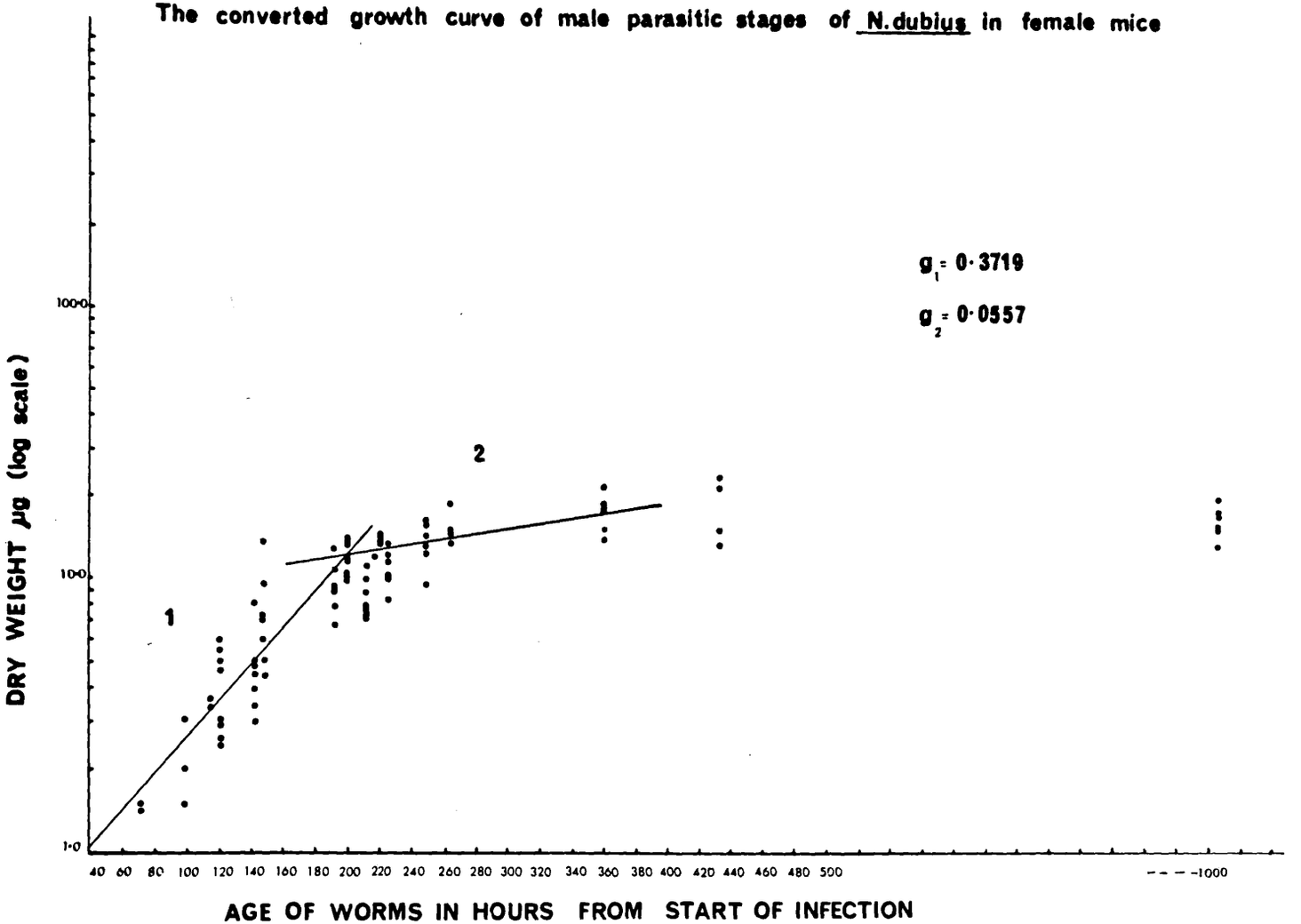


FIGURE 25c

The converted growth curve of female parasitic stages of N.dubius in female mice

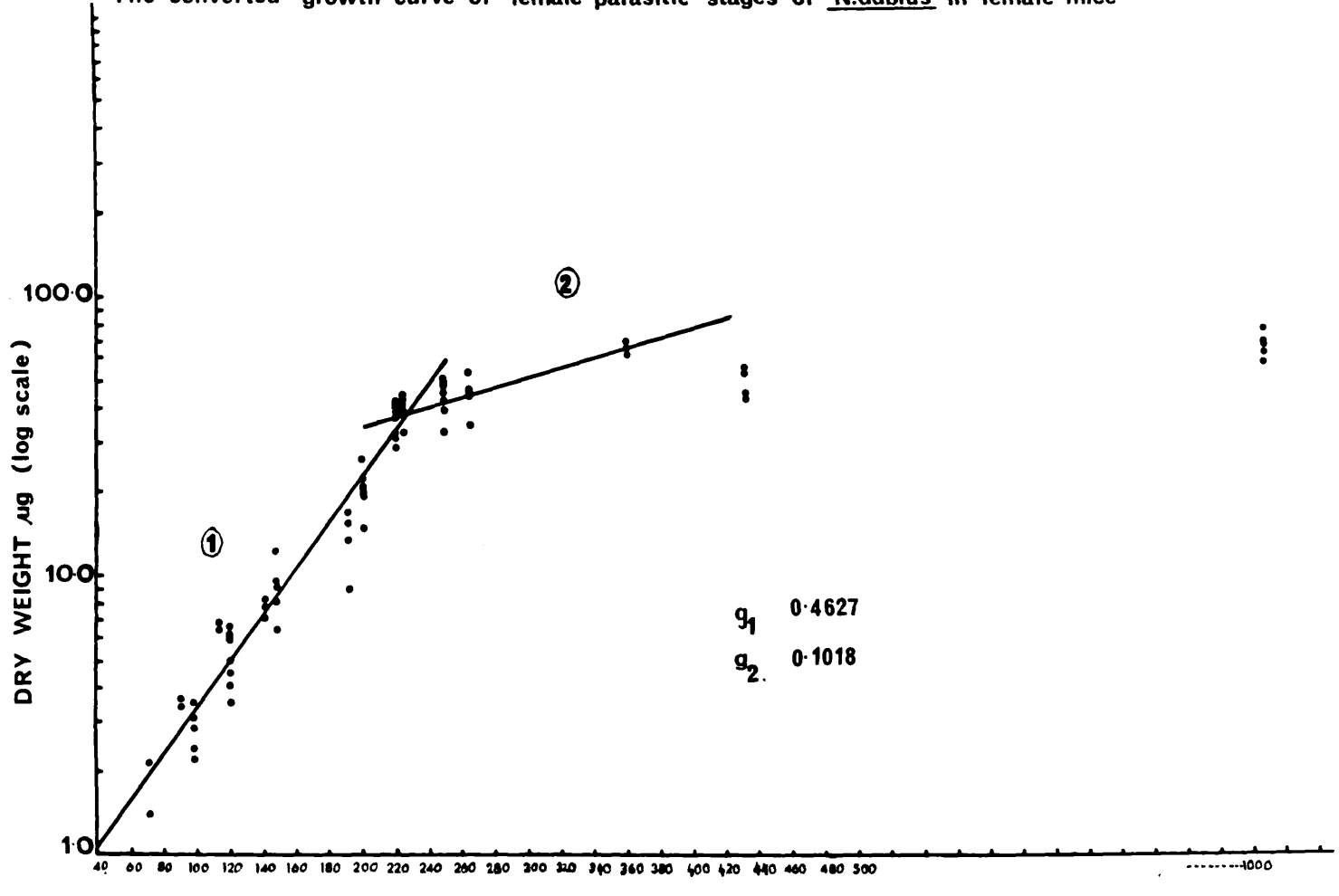


FIGURE 25d

The converted growth curve of female parasitic stages of N.dubius in male mice

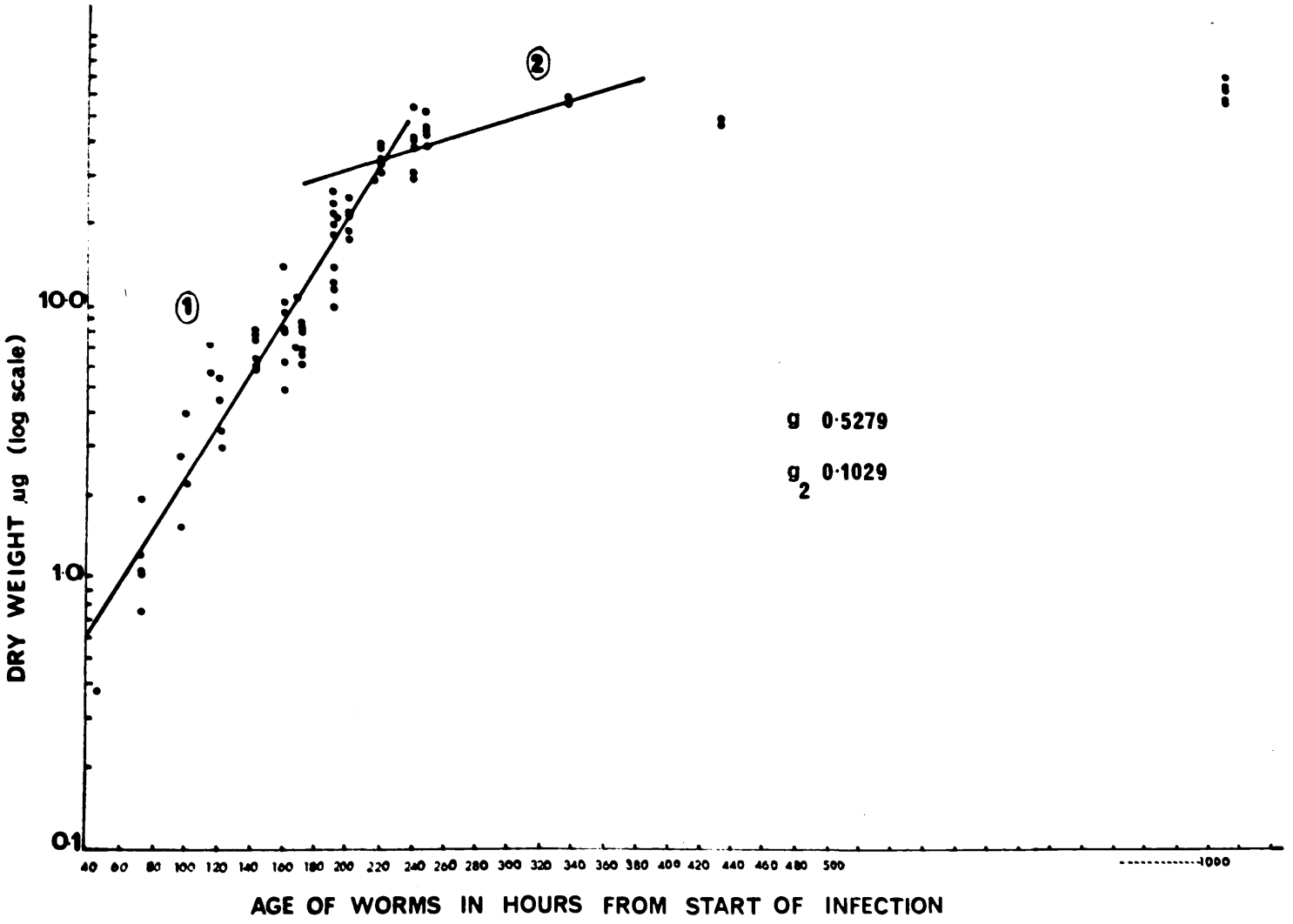


TABLE 10

The instantaneous growth rates (g), calculated for 24 hourly periods, of male and female parasitic stages of N. dubius in male and female mice

Worm Sex	Host Sex	g-phase (1)	g-phase (2)
Female	Female	0.4627	0.1018
Female	Male	0.5279	0.1029
Male	Female	0.3719	0.0557
Male	Male	0.3729	0.0691

Free-living larvae. (Days 2-4) g = 0.6244

t tests showed that there was no significant difference between the paired results - indicated in brackets

0.1029 in male mice. There was no significant difference in the growth rates in either sex of host. The same was also true for male worms, the g values for phase one being 0.3719 and 0.3729 in male and female mice respectively and those of phase two being 0.0557 and 0.0691 in male and female mice. There does not therefore seem to be any sex resistance that manifests itself as an alteration in growth rate of this parasite in the ASH/CS1 strain of mice used.

Discussion

It would seem that the increase in weight exhibited by the first two larval stages was due to two factors, the first being body growth and the second the laying up of fat reserves, as it has been shown that the fat content of the body increased at this time. When the larvae ceased feeding at infectivity they started to utilise these food reserves which was immediately reflected by a loss in both fresh and dry weight. The rate of loss in dry weight decreased after day 7; this may be of adaptive significance to an animal which is totally reliant upon stored food reserves, and has been discussed in Chapter 7 in relation to respiration.

The free living larvae exhibited a higher daily growth rate than any of the parasitic stages. This was probably related to the short time available to the first two larval stages in which to feed and build up food reserves which may

have to last them indefinitely once they become infective.

The first growth phase of the parasitic stages represented a period of intense growth which lasted until approximately 200 - 220 hours after infection. During this period the last two moults of the life cycle are completed. The first parasitic mould occurs 80 - 90 hours after infection and the second between 144 and 146 hours after infection. The growth rate appeared to be constant throughout this period of the life cycle in both male and female worms. Between 164 and 192 hours (7 - 8 days) after infection the young fifth stage larvae start to migrate back into the lumen of the intestine, from the mucosa where they developed.

Approximately 200 - 220 hours after infection, the growth rate changed and continued at a slower rate until approximately 360 hours (15 days). That is after the worms returned to the lumen they continued to grow although the majority of body growth had already been achieved. It is between 200 and 220 hours that the first eggs can be seen in the uteri of the female worms and ovarian development has been in progress for some time. This is probably also the time of onset of sperm production in male worms. The worms can be seen mating on Day 10 and eggs are laid by the females from this day onwards. The second growth phase lasted until Day 15 which is probably the time of maximum

egg and sperm production.

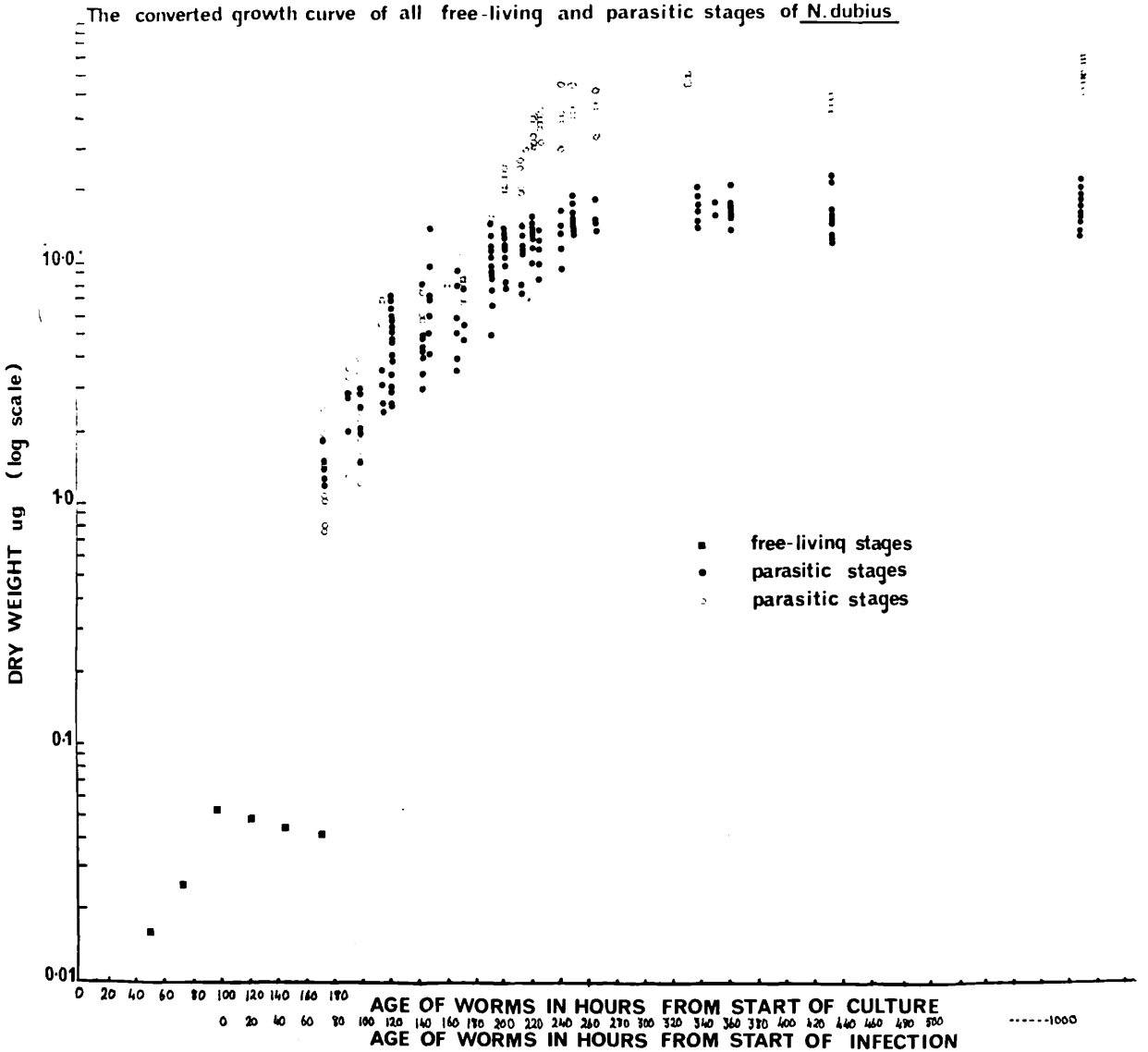
The third growth phase appeared as a levelling out of the curve, and there was no appreciable difference in the weight of worms weighed on days 15 and 42. This phase represented no body growth but constant reproductive growth, i. e. the egg and sperm production were more or less constant during this phase. There was a slight drop in the weight of worms on Day 18 in all groups of worms but the significance of this is not clear.

The overall converted growth curve for the entire life cycle is shown in Figure 26. It is clear that the growth of the free-living larvae until they reached infectivity was of the same order of magnitude as that exhibited by the worms once they became parasitic. The instantaneous growth rate for the first two larval stages (which are free-living) was similar to the growth rates of the last two larval stages (which are parasitic).

Sommerville (1960) in his study on the growth of Cooperia curticei in lambs did not detect any growth during the first 48 hours after infection. Although no Nematospiroides dubius were weighed before 48 hours after infection in the present study, it is clear from Figure 26 that growth must occur during this phase for the larvae to make up the weight between that at infection and that recorded 48 hours after ingestion.

FIGURE 26

The converted growth curve of all free-living and parasitic stages of N. dubius



Although periods of lethargus where no growth occurred were detected during the life cycle of Cooperia curticei by Sommerville, no lethargus was detected in the growth of Strongyloides ratti by Wertheim and Lengy (1965). However, S. ratti reaches maturity within 74 hours of entry into the host, whereas C. curticei takes 14 days to reach the same stage, and as the authors pointed out it would have been possible to miss periods of lethargus during such a short developmental period. Wertheim (1970) using increases in length and breadth as a measure of growth detected periods of lethargus prior to the two parasitic moults of Strongyloides venezuelensis. However, she suggested that cryptic growth must have gone on during the lethargus as at the moment of emergence from the old cuticle, the larvae were longer than at the previous stage. When growth was measured in terms of dry weight in the present study, no lethargus period in the growth was detectable. This would suggest that the idea of non-growth during the period of non-activity known as lethargus is incorrect, and has arisen as a result of using an unsuitable measure of growth to demonstrate it.

CHAPTER 6

THE RESPIRATION OF NEMATOSPIROIDES DUBIUS

Introduction and Historical Review

In this experiment the oxygen uptake of Nematospiroides dubius has been studied throughout its whole life cycle. The only published account of the aerobic respiration of a nematode for all stages of the life cycle is that of Weiser and Kangwisher (1960) who worked on Enoplus communis, which is a free-living marine nematode. Considerable research has been carried out on the respiration of animal parasitic nematodes; however, most workers have concentrated on the infective larval stages or the mature adult worms.

Many investigations have been made on the effect of physical factors such as pH, osmotic pressure and temperature, on the oxygen consumption of parasites (von Brand, 1943; Wilson, 1965; Barrett, 1969b). The effect of different partial pressures of oxygen and carbon dioxide has also been studied (von Brand and Simpson, 1945; Rogers, 1949b; Bair 1953). The latter conditions have been of great interest in relation to nematodes inhabiting the gastro-intestinal tract of vertebrates, which has traditionally been regarded as a predominantly anaerobic environment. The nematodes found in this habitat fall broadly into two categories. Those in the first category are mainly members of the

Ascaridoidea and are large worms (approximately 20 - 30 cm. in length) typified by Ascaris lumbricoides; those in the second category belong mainly to the Trichostrongyloidea and are much smaller worms (approximately 1 - 2 mm. in length). The relative importance of aerobic and anaerobic respiration to these two categories of worms seems to differ. In 1901, Weinland showed that Ascaris survived equally well under anaerobic or aerobic conditions in vitro. In vivo, it occupies a position in the central lumen of the host intestine where oxygen tensions are known to be very low (Read, 1950). Laser (1944) found that adult Ascaris suis were capable of taking up oxygen, but that the O_2 consumption was dependent upon the oxygen tension, negligible amounts being consumed at the O_2 tensions found in the physiological environment of this parasite. High O_2 tensions were found to be toxic to the worms because of a deficiency of catalase. Bueding and Charms (1952) were unable to detect cytochrome c or cytochrome oxidase activity in the muscle or female reproductive system of Ascaris. In 1969, Saz pointed out that the above observations lead to the conclusion that Ascaris is predominantly anaerobic. However, Smith (1969) maintains that it is essentially an aerobe.

The second category of intestinal nematodes is typified by the rat parasite Nippostrongylus brasiliensis. Rogers (1962) and Saz (1969) have summarised the available information on the respiratory metabolism of this parasite and have formulated several general conclusions. N. brasiliensis is capable of consuming O_2 at appreciable rates (Rogers, 1948); however, as was found with Ascaris, the rate of O_2 consumption varied with the oxygen tension (Rogers, 1949b). However, unlike Ascaris, N. brasiliensis inhabits the paramucosal region of the intestinal lumen where appreciable oxygen tensions have been shown to exist. Rogers (1949a) recorded oxygen tensions as high as 30.2 mmHg in the rat intestine close to the mucosa, 12 cm. away from the stomach. These oxygen tensions recorded in anaesthetized animals were probably lower than those occurring in normal animals as Campbell (1925) showed that certain anaesthetics lower the pressure of O_2 in animal tissues. A gradient in O_2 tension across the intestinal lumen has been shown to occur in domestic ducks (Crompton, Shrimpton and Silver, 1965), the highest tensions occurring close to the mucosa. A similar situation probably occurs in the intestine of all vertebrates and is due to the diffusion of O_2 from the richly vascularised wall of the intestine towards the central lumen (McIver et al., 1926). The rate of this diffusion is related to the ratio of the surface area of

the intestinal mucosa to the volume enclosed, and high ratios such as are found in rat and mouse guts lead to high rates of diffusion. It therefore seems clear that appreciable amounts of oxygen may be available to N. brasiliensis in vivo and Rogers (1949b) calculated that this parasite could probably approach its maximum in vitro rate of respiration in vivo.

Nippostrongylus brasiliensis possesses haemoglobin in its cuticle and body walls which gives the worm a brick red colour. Davenport (1949) showed that this haemoglobin has a high affinity for oxygen. It can thus extract oxygen from the host tissues but the oxygen tension in the tissue of the parasite would need to be extremely low before the oxyhaemoglobin would give up the oxygen (Lee, 1965). The oxyhaemoglobin of N. brasiliensis becomes deoxygenated under anaerobic conditions and reoxygenation occurs when air is re-admitted, indicating that it has a respiratory function (Davenport, 1949). It is possible that it may function as an oxygen store releasing it when oxygen pressure is very low. In addition, the haemoglobin may also function in the transfer of oxygen from the cuticle to the body fluid (Scholander, 1960; Lee, 1965).

Nematospiroides dubius also contains a bright red pigment in its body wall and it is thought that this is also a haemoglobin (Lewis - personal communication). Even if the haemoglobin of N. brasiliensis is not actively used for oxygen transport, Rogers (1962) calculated that the surface/volume ratio of this worm is sufficient for O_2 to be supplied to the internal tissues by diffusion alone. This was not found to be true for Ascaris. Even though theoretically N. brasiliensis could respire aerobically in vivo, Rogers questioned the ability of such parasites to obtain energy useful for their economy in vivo from oxygen consumed.

Roberts and Fairbairn (1965) reported that adult N. brasiliensis survived in vitro in air for 8 days, and in addition that it survived equally well in atmospheres containing 1% oxygen in nitrogen. Under anaerobic conditions either N_2 or N_2 - CO_2, motility was lost after 6 hours, the reaction being irreversible after 24 hours, thus showing that O_2 is necessary for the survival of this parasite. They also found that endogenous carbohydrates were utilised twice as rapidly anaerobically as aerobically. Saz (1969) interpreted this result as evidence of the existence of a Pasteur effect, indicating that some energy is derived from aerobic metabolism.

Nematospiroides dubius used in the present study is

closely related to Nippostrongylus brasiliensis and has a similar body form and size, and occupies a similar position in the host intestine. Therefore it is likely that similar amounts of oxygen will be available to this worm in vivo as are available to N. brasiliensis.

The ability of the different stages to withstand oxygen lack has been briefly studied, as well as their aerobic respiration. However, this was only a preliminary study and great emphasis cannot be placed on the results.

A short experiment on the effect of crowding of various stages on their respiration rate has been carried out, and the results related to the distribution of the worms within the host intestine.

Materials and Methods

Free-living and parasitic N. dubius were obtained as described in general methods. All worms used for respiratory measurements were freed from debris and washed three times in medium. Dechlorinated water was used as a medium for the free-living stages and Tyrodes saline was used for the parasitic stages.

Respiration measurements were made at 24 hourly intervals using Stoppered Cartesian Divers (Zeuthen, 1950; Klekowski, 1971). All experiments were carried out at 20°C. From 14-30 free-living nematodes of the same age were used in each diver, and a mean individual respiration rate was calculated.

Although the age of these worms from the start of culture was known, absolute certainty as to the homogeneity of each sample was not possible, as there was no knowledge of the time intervals involved in the egg laying of female worms within the host. Parasitic worms were removed from the host and placed in divers at 20°C; no acclimation period was allowed. Individual respiration rates were obtained for the larger, older worms, whereas mean individual rates were obtained for the smaller worms, two or three being used in each diver. In all experiments, control divers were run containing a sample of the final washing medium in order to detect any bacterial respiration. In all cases, the gas phase was air.

(a)
General Theory of the Cartesian Diver

The cartesian diver is a constant volume changing pressure system. It is essentially a container enclosing a gas bubble connected to a surrounding fluid medium in such a way that any changes in pressure in the medium are transferred on to the gas space in the diver. The bubble in a calibrated diver enables it to float at equilibrium. Increases or decreases in pressure cause the gas bubble to contract or expand and the diver correspondingly sinks or floats in the medium. A charged diver contains an animal in medium, a gas bubble and some alkali. The alkali is of the same composition as the flotation medium which is $\frac{N}{10}$

NaOH. Oxygen used by the animal is removed from the gas bubble and the CO_2 released back into the gas bubble is absorbed by the alkali, resulting in a net decrease in the volume of the gas bubble. An increase in pressure will then be needed to return the diver to equilibrium by changing the gas bubble to its original volume. These changes in gas volume may be measured manometrically and the amount of O_2 consumed can be calculated.

Theoretically, this method may be used to measure oxygen consumptions of 1×10^{-4} µl./hour.

(b)
General Apparatus

The general apparatus is shown in Figure 27. The temperature of the water bath was maintained at 20°C ± 0.01°C, using a Beckmann RU 8 cooling coil and two 180 watt heaters of variable resistance. The temperature control was tested over an 8 hour period using a Beckmann constant recording Quartz thermometer, and was found to be constant within 0.01°C. The flotation vessels were filled with $\frac{N}{10}$ NaOH.

(c)
Manufacture of divers

Divers were made using fine pyrex capillary; ideally the ratio of the internal to the external diameter of the glass used should be 0.9. Divers of gas volume 1.0717 µl. to 3.431 µl. were used.

Straight sided divers were used for free-living stages;

FIGURE 27

a - flotation vessels

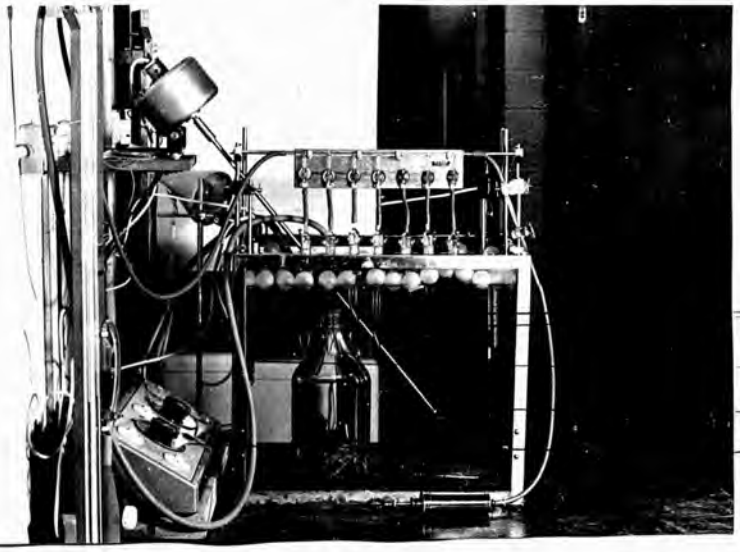
b - heating element

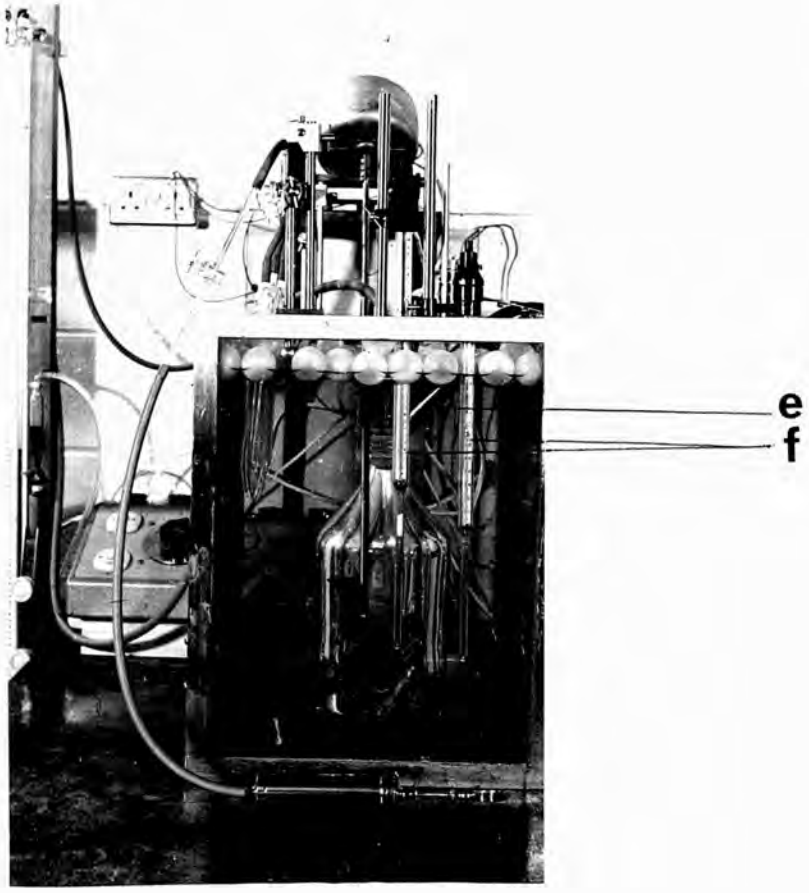
c - stirrer

d - control box

e - cooling coil

f - contact thermometers


a
b
c
d


e
f

divers with bulbs were used for the parasitic stages (Figure 28). All divers were cleaned with concentrated H_2SO_4 and washed three times with distilled water before use.

(d) Loading

Nematodes were introduced into the diver head using slow uncalibrated braking pipettes.

(e) Calculation of O_2 consumption

The pressure changes recorded on the manometer were plotted against time and the slope of the line fitted using a perspex rule marked with parallel lines. The average pressure difference per hour was read off and substituted in the following formula derived from Boyle's Law

$$\Delta VO_2 = \frac{Vg. \Delta P}{Po} \cdot \frac{273}{T}$$

ΔVO_2 = volume of O_2 consumed in µl/individual unit time at S.T.P.

Vg = volume of gas phase µl.

ΔP = pressure change in mm./unit time

Po = normal pressure for system 10,000 mm. Brodie's fluid)

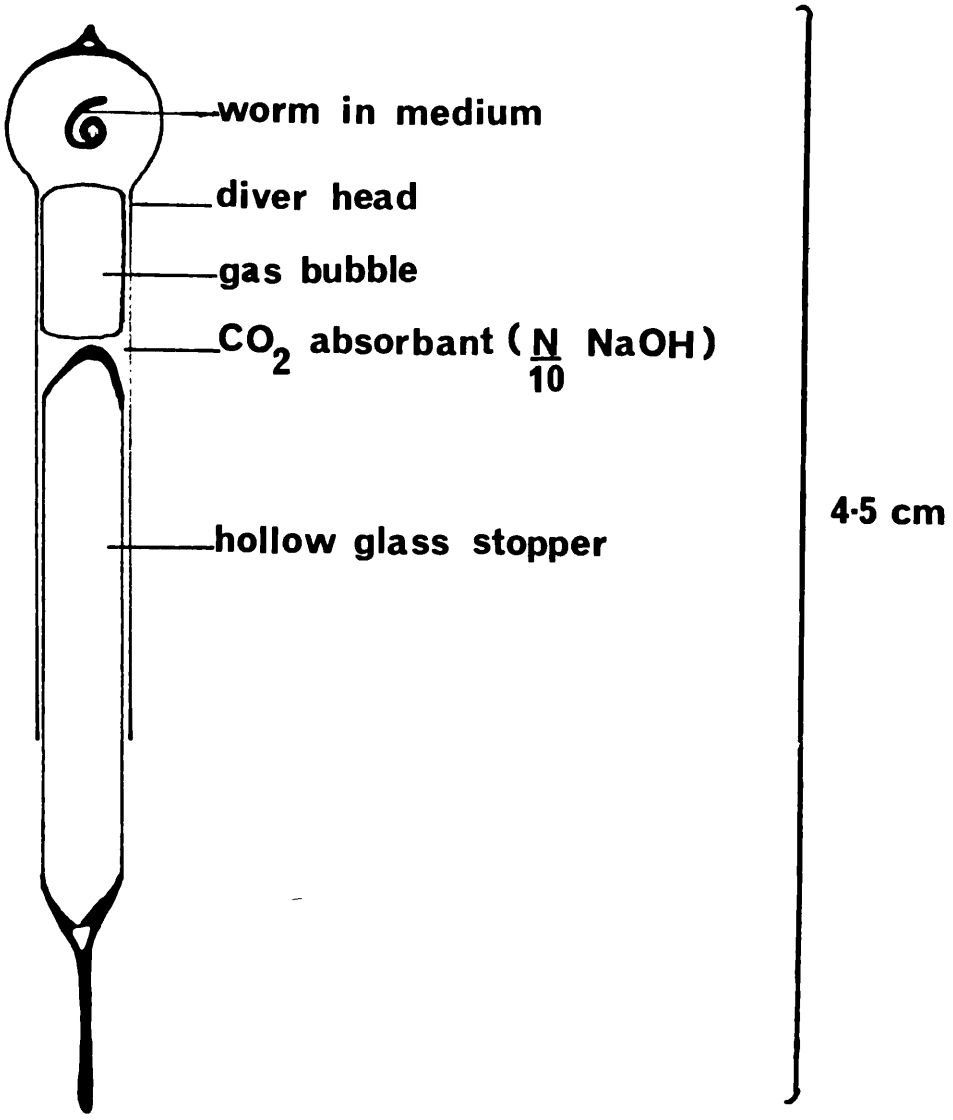
T = Temperature of system $(273 + t)^{\circ}C$.

It is only possible to accurately measure the length of air in a braking pipette (in order to load the diver with a standard air bubble) to the nearest 0.25 mm. This therefore introduces an error into the Vg which would be ± 1.2% for a diver with a calculated Vg of 3.4228 µl., and ± 4.02% for a diver with a calculated Vg of 1.0717 µl.

It is only possible to measure the manometric pressure

FIGURE 28

ZEUTHEN'S STOPPERED CARTESIAN DIVER FOR MEASURING OXYGEN CONSUMPTION



to within 0.5 mm, which introduces an error into the final line drawn of pressure change against time. The error will be greatest on a line with a shallow slope. On a slope of 10, derived from a line plotted on 7 points, the error in P would be ± 1.4%. In the case of a steep slope of 300 derived from a line plotted on 5 points, the error in P would be ± 0.66%. The two slopes were the most extreme cases found in the present study.

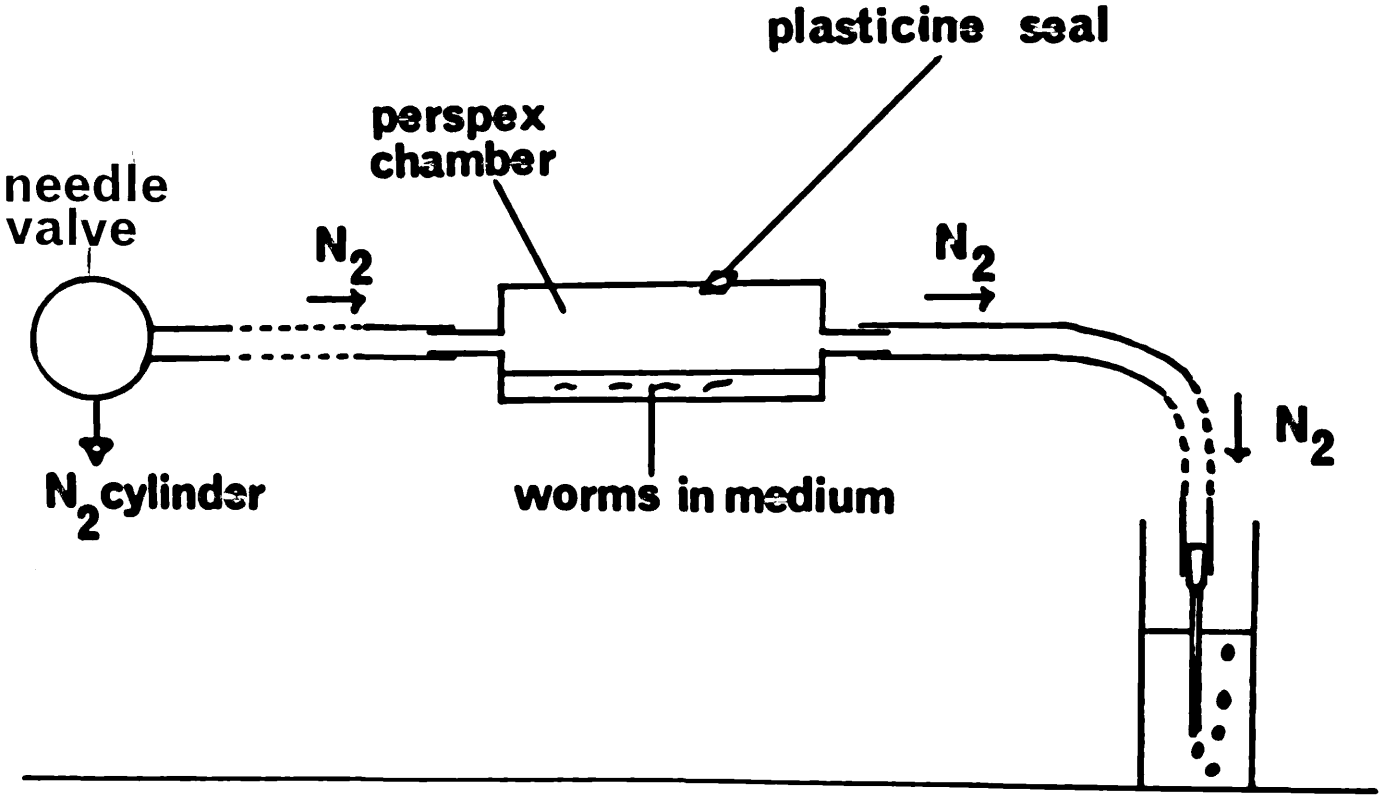
As the calculation of rate of O_2 consumption involves the multiplication of Vg and Δ P, the greatest error possible was ± 5.62%, and the smallest possible error was ± 0.792%. However, the smallest divers were rarely used, and the error involved in the measurement of O_2 consumption was rarely more than ± 2.5%.

(f)
Survival under anaerobic conditions

Worms were placed in medium in the chamber shown in Figure 29, and subjected to a constant flow of oxygen free nitrogen. Controls were run of worms in a similar chamber with a constant air flow passing through it. The chambers were mounted on the stage of a binocular microscope and the worms were examined at 30 minute intervals to ascertain whether or not they were moving. Worms which did not move, even when stimulated by shaking of the chamber, were considered dead if they did not recover when placed in an atmosphere of air.

FIGURE 29

Apparatus for the observation of worms maintained in an atmosphere of O_2-free N_2



Results

(a) Free-living stages

The first batch of larvae used for respiration measurements were extracted on the second day of culture, and were first stage larvae. Respiration rates were measured of larvae removed from culture every 24 hours until day 9. Oxygen was used as the gas phase. This was justified as the larvae in culture had access to air. In nature, the larvae develop in faecal pellets which are distributed over the ground. In the outer layers of the pellet O_2 would be plentiful, and even if O_2 was in short supply in the centre of the pellet, it would be possible for larvae to migrate to the outer layers if necessary.

The daily respiration rates are shown in Table 11. It can be seen that the average individual respiration rate increased during the first two days of the larvae's life (Figure 30). The mean respiration rate for first stage larvae (extracted on day 2 of culture) was 0.2970 μl.x 10^{-3}/hr. hr, the mean rate for second stage larvae (extracted on day 3 of culture) rose to 0.3324 μl.x 10^{-3}/hr. The respiration rate started to decrease on the fourth day of culture, which was when the second stage larvae moulted to form the infective stage, and continued to do so until the eighth day. No oxygen consumption could be measured for larvae older than this using Stoppered Divers; however, it is very probable

TABLE 11

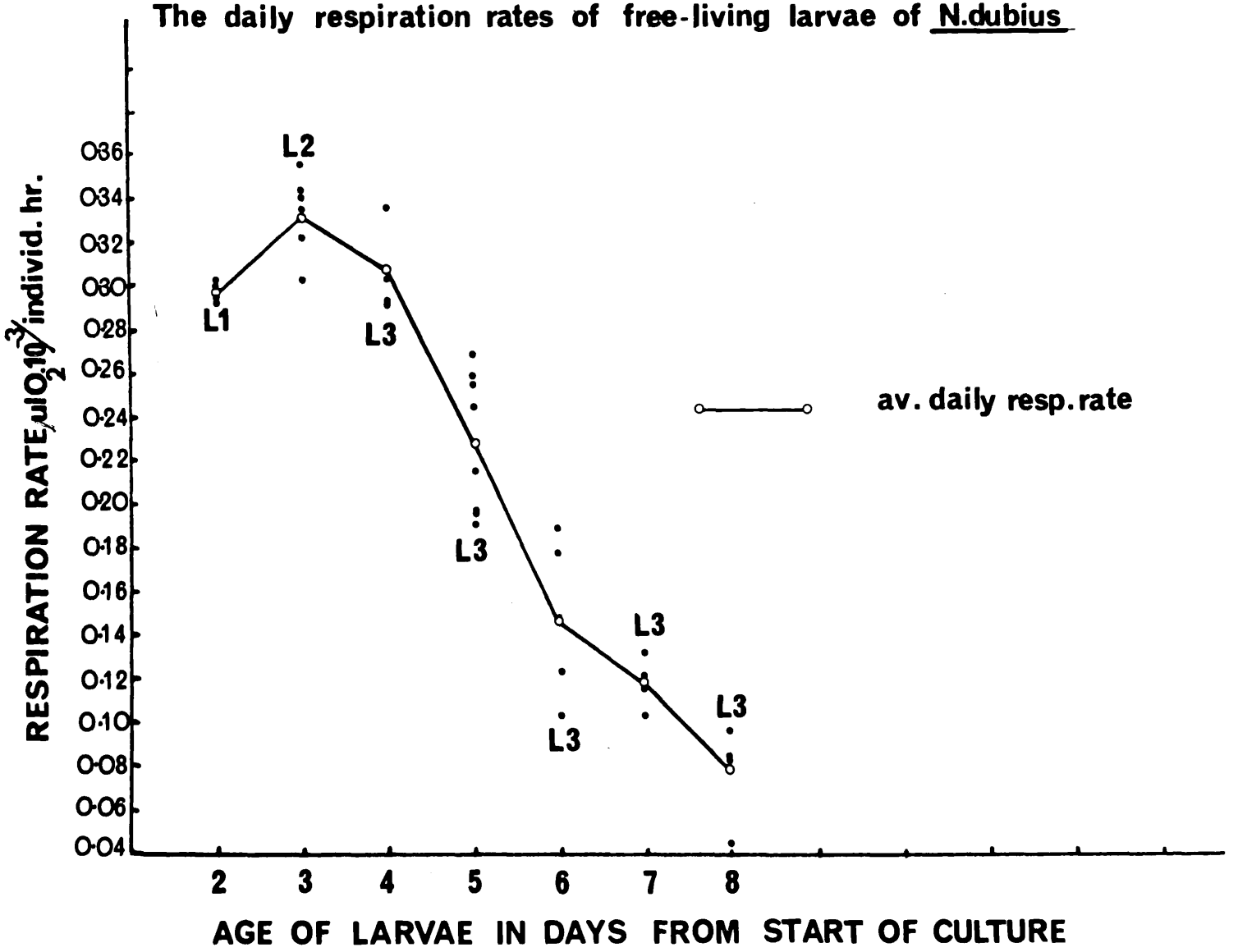
The Daily Respiration Rates of Free-Living Larval Stages of N. dubius

(each respiration rate is an average individual rate)

Age in days from start of culture	Larval stage	Respiration rate $ul O_2 . 10^{-3}$/individual hr.
2	LI	0.3014 0.2990 0.2957 0.2920
3	LII	0.3412 0.3307 0.3033 0.3564 0.3403 0.3228
4	LIII	0.2908 0.2919 0.3366 0.3047
5	LIII	0.2446 0.2150 0.2535 0.1948 0.2687 0.1945 0.2591 0.1924
6	LIII	0.1890 0.1224 0.1488 0.1794 0.1014 0.1441
7	LIII	0.1211 0.1036 0.1177 0.1307
8	LIII	0.0986 0.0455 0.0850 0.0838

FIGURE 30

The daily respiration rates of free-living larvae of N.dubius



that oxygen consumption could have been recorded using Ampulla divers (sensitivity 1 x 10^{-6} µl. O_2/hr.).

Larvae were not always consistently active when in the divers. Their behaviour varied from lying still on the meniscus of the air bubble to swimming actively in the medium in the diver head. All larvae exhibited both types of behaviour during a respiration run; however, no change in respiration rate was recorded whether they were active or resting. This may be accounted for to some extent by the fact that motility increases the metabolic rate over that of the basal metabolism at a lesser rate the smaller the organism (Zeuthen, 1947).

All three free-living larval stages were highly susceptible to anaerobic conditions, as they became immobile within 30 minutes exposure to oxygen free nitrogen (Table 14). This effect was irreversible if the larvae were not exposed to air within four hours. Oxygen is therefore necessary for the survival of all three free-living stages of Nematospiroides dubius.

(b) Parasitic stages

Parasitic larvae and adults were removed from the host at 24 hourly intervals for respiration measurements. The first worms used were removed 48 hours after infection. As males and females could be distinguished at this stage of their development, they have been treated separately.

Air was used as the gas phase in the divers as it has already been established by Rogers (1949a) that oxygen is available to worms inhabiting the paramucosal lumen of the small intestine.

Histological examination showed that the "encysted" L3 and L4 stages usually occupy a position in the sub-mucosa adjacent to a blood vessel, and in some cases the worms were surrounded by individual red blood corpuscles (Figure 19). It is probable that worms in this position obtain oxygen from the host blood supply by diffusion.

The experimental temperature used was 20°C although the worms normally live at the host's temperature of 37°C. It is therefore likely that the in vitro respiration rates recorded may have been low compared to in vivo rates, as the oxygen consumption of some parasites has been shown to increase with increasing temperature (von Brand, 1960b).

The daily rates of oxygen consumption of male and female parasitic stages of Nematospiroides dubius are shown in Figures 31a and b, Tables 12 and 13. It can be seen that the O_2 consumption per individual increased with age for both male and female worms (increase in age is synonymous with an increase in development until day 15(360 hrs). The pattern that emerged when individual respiration rate was plotted against age was similar to that obtained when weight was plotted against age, i.e. a sigmoid curve. The rate of

FIGURE 31a

Daily respiration rates of the male parasitic larvae and adult N.dubius

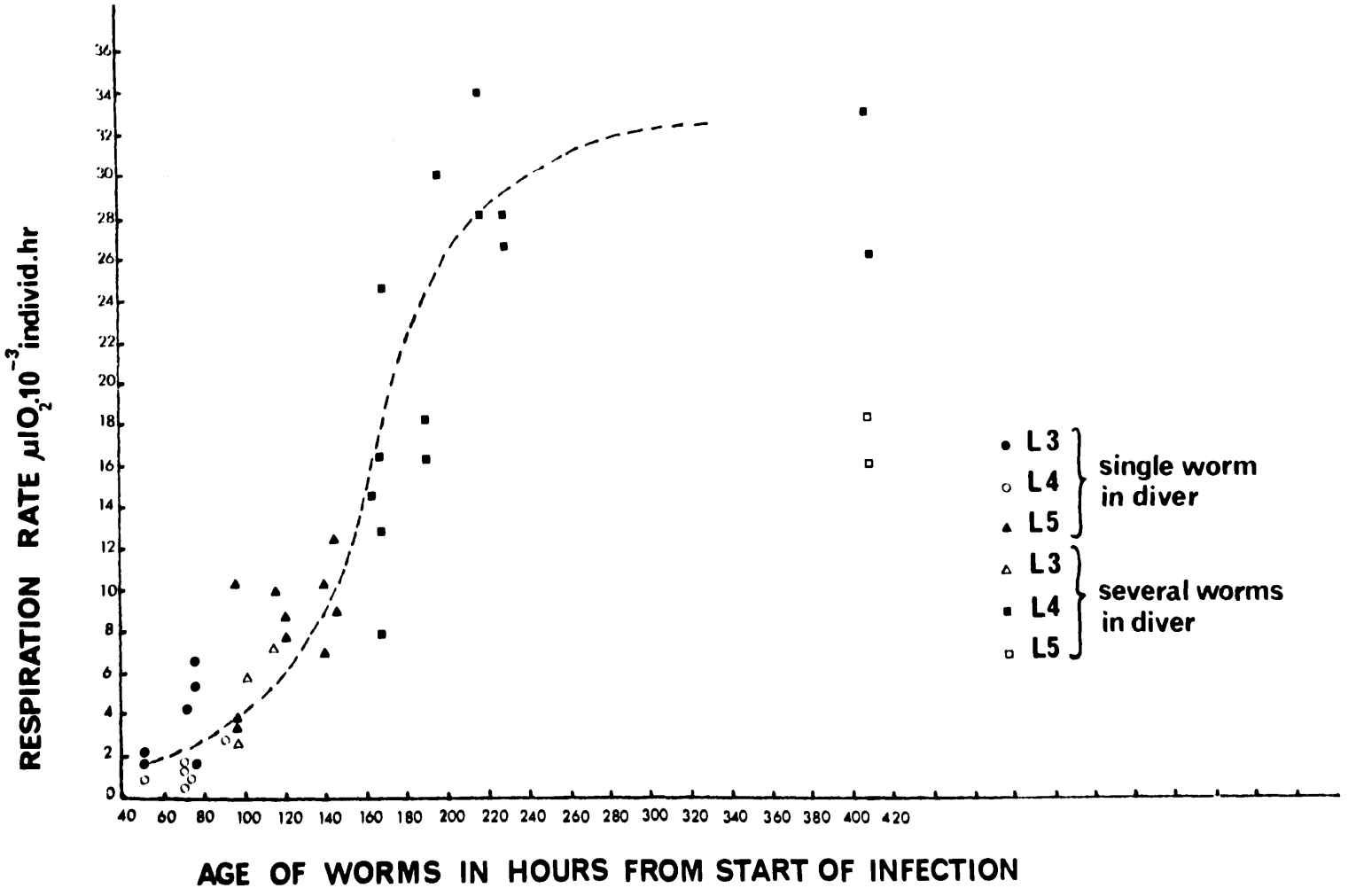


FIGURE 31b

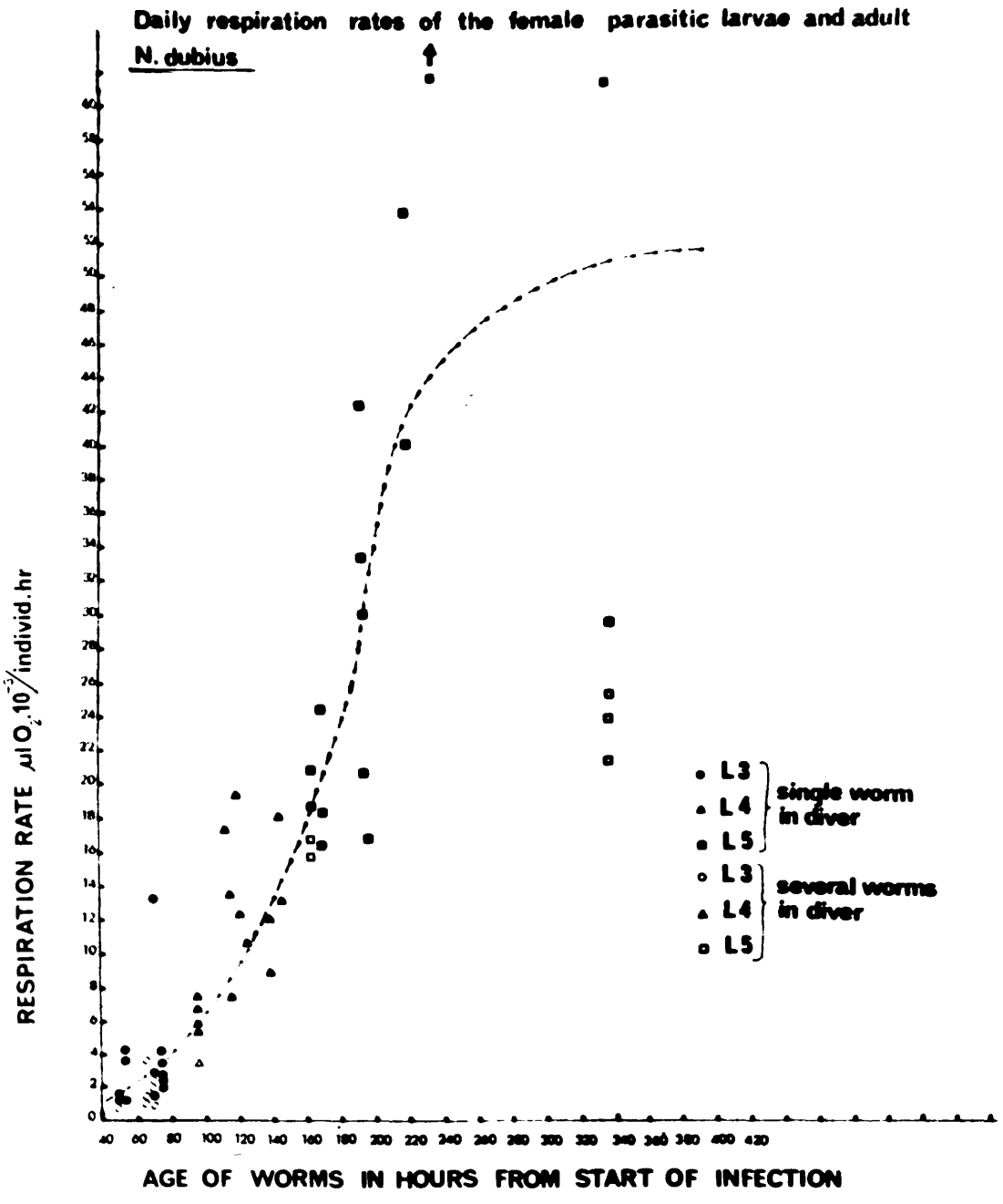

Daily respiration rates of the female parasitic larvae and adult
N. dubius
RESPIRATION RATE µl O2.10-3/individ.hr
AGE OF WORMS IN HOURS FROM START OF INFECTION
L3
L4
L5
single worm in diver
L3
L4
L5
several worms in diver

TABLE 12

Individual respiration rates of male parasitic stages of N. dubius extracted from male mice

Larval Stage	Age in Hours from start of infection	No. of worms in diver	Total respiration rate $\mu l O_2 . 10^{-3}$/hr.	Individual respiration rate $\mu l O_2 . 10^{-3}$/individ.hr
3	49	3	2.5039	0.8346
3	50	1	-	1.1619
3	50	1	-	1.9216
3	70	2	2.3233	1.1617
3	71	1	-	4.1341
3	72	4	3.3386	0.8346
3	74	1	-	1.5920
3	74	1	-	5.0999
3	74	1	-	6.40267
3	96	1	-	10.0389
3	96	1	-	3.66
3	96	1	-	3.505
3	96	3	10.0542	3.3514
3	96	4	10.0116	2.5029
4	100	3	17.1527	5.7175
4	114	4	28.3403	7.0851
4	116	1	-	9.9456
4	120	1	-	8.7626
4	120	1	-	5.5909
4	139	1	-	10.264
4	139	1	-	6.828
4	144	1	-	12.2951
4	146	1	-	8.8728
5	163	1	-	14.3280
5	164	1	-	11.87
5	164	1	-	25.4814
5	168	1	-	7.9065
5	168	1	-	12.5908
5	168	1	-	16.2445
5	168	1	-	24.7511
5	191	1	-	16.6360
5	191	1	-	18.2550
5	196	1	-	30.0665

TABLE 12 (continued)

Larval Stage	Age in Hours from start of infection	No. of worms in diver	Total respiration rate $\mu l O_2 . 10^{-3}/hr.$	Individual respiration rate $\mu l O_2 . 10^{-3}/individ.hr$
5	216	1	-	28.9410
5	216	1	-	34.3561
5	228	1	-	26.69
5	228	1	-	28.0759
5	408	1	-	23.617
5	408	1	-	26.193
5	408	4	73.0486	18.2622
5	408	3	48.2351	16.0783

TABLE 13

Individual respiration rates of female parasitic stages of N. dubius extracted from male mice

Larval Stage	Age in Hours from start of infection	No. of worms in diver	Total respiration rate $\mu l O_2 . 10^{-3}$/hr.	Individual respiration rate $\mu l O_2 . 10^{-3}$/individ.hr
3	49	4	3.9427	0.9857
3	50	1	-	1.5175
3	50	1	-	1.3089
3	53	1	-	3.6299
3	53	1	-	4.2450
3	53	1	-	1.2345
3	66	2	7.1724	3.5860
3	66	2	2.2418	1.1209
3	66	2	2.4690	1.2345
3	70	1	-	13.2025
3	71	1	-	2.6179
3	71	1	-	1.5175
3	72	4	8.5829	2.1457
3	72	4	7.8883	1.9721
3	73	1	-	2.7567
3	73	1	-	2.2500
3	73	1	-	2.0728
3	74	1	-	3.3017
3	74	1	-	4.0196
3	96	1	-	7.2841
3	96	1	-	6.6809
3	96	1	-	5.3067
3	96	1	-	5.7145
3	96	3	10.0825	3.3608
4	114	1	-	17.254
4	114	4	21.6051	5.4013
4	116	1	-	7.4026
4	116	1	-	13.5321
4	120	1	-	19.3535
4	120	1	-	12.1911
4	122	1	-	10.5362
4	139	1	-	12.0536
4	139	1	-	7.9638
4	144	1	-	13.1131
4	144	1	-	18.2104

TABLE 13 (continued)

Larval Stage	Age in Hours from start of infection	No. of worms in diver	Total respiration rate $\mu l O_2 . 10^{-3}/hr.$	Individual respiration rate $\mu l O_2 . 10^{-3}/individ.hr$
4	146	1	-	14.9914
4	146	1	-	10.1469
5	163	1	-	18.7103
5	163	1	-	20.7170
5	164	2	33.5383	16.7692
5	164	2	31.8551	15.9275
5	168	1	-	18.3349
5	168	1	-	16.4507
5	168	1	-	24.6690
5	191	1	-	30.3031
5	191	1	-	33.5495
5	191	1	-	21.4921
5	194	1	-	42.4191
5	196	1	-	16.1591
5	216	1	-	54.1316
5	216	1	-	40.1056
5	228	1	-	71.7390
5	336	1	-	61.41
5	336	1	-	29.26
5	336	3	63.7400	21.25
5	336	2	50.48	25.24
5	336	3	72.67	24.22

oxygen consumption increased exponentially between 48 and 240 hours of age which is the period during which the two parasitic moults occur and then levelled off as the worms reached maturity; this occurred with both male and female worms. The female worms attained a greater maximum rate of oxygen consumption per individual than the male worms as was to be expected as they grew to a larger size.

There was a wide variation in the oxygen consumption of individual worms within each age category; this was probably linked with the similar variation in individual weights found within each age category. The variation in size may also have been due to differences in physiological age of the infective larvae, although every effort was made to ensure that they were of as uniform an age as possible.

It can be seen from Figures 31a and 31b that there was a tendency for the respiration rates of adult worms to be lower when more than one worm was present in a diver, i.e. when they were subjected to crowded conditions. However, there were insufficient points to test this observation statistically. It seems that this reduced respiration may be of economic value to the parasite and has been discussed in Chapter 3, in relation to the distribution of the worms *in vivo*. The survival times of adult *Nematospiroides dubius* in different gas phases are shown in Table 14.

Both adult male and female *Nematospiroides dubius* were capable of surviving in air for 5 days when incubated at

TABLE 14

Survival times of various stages of N. dubius in atmospheres of air and O_2 free N_2

Stage	Temp.	Gas phase	Survival time
L1	20°C.	Air	∞
		O_2 free N_2	4 hrs
L2	20°C.	Air	∞
		O_2 free N_2	4 hrs
L3	20°C.	Air	∞
		O_2 free N_2	4 hrs
Adult Male and Female	20°C.	Air	4 days
	37°C.	Air	5 days
	20°C.	O_2 free N_2	18 hrs.

∞ Indicates survival time unknown but probably until food reserves are all utilized.

37°C in Tyrodes saline which contained glucose. They remained motile and bright red in colour throughout this period and the females laid eggs. When incubated for longer periods the worms became paler, they lost the power of movement and eventually died. However, the worms were not maintained under sterile conditions and it is therefore difficult to state precisely the cause of death, although it was not likely to be due to the presence of O_2. Adult worms of both sexes incubated in air at 20°C remained healthy and the females continued to lay eggs for four days. When they were incubated in O_2 free N_2 at 20°C, they lost motility within 18 hours and did not recover when air was reintroduced; the worms were therefore assumed to be dead. No eggs were laid by female worms in the O_2 free atmosphere.

Discussion

An increase in the respiration rate of the first two free-living larval stages of a parasitic nematode does not seem to have been recorded previously. The increase in respiration occurred at the time that the Nematospiroides dubius larvae were growing and laying up fat reserves. An increased respiration was therefore needed at this time to provide the necessary energy.

The decrease in respiration of larvae removed on the fourth day of culture coincided with the formation of the infective L3 and the associated cessation of feeding. As

a decrease in body weight was also recorded at this time, the larvae would appear to have been utilising their stored food reserves.

There have been many other reports of decreasing respiration rates of infective larvae with increasing age, as was found with third stage larvae of Nematospiroides dubius. In 1948, Rogers reported that the QO_2 of infective Nippostrongylus brasiliensis larvae decreased rapidly as they aged; he recorded an O_2 consumption until the twelfth day after infectivity had been reached. The oxygen consumption of Necator americanus (Fernando, 1963), Cooperia curticei (Eckert, 1967), and Strongyloides ratti (Barrett, 1969a) have also been shown to decrease with age. Fernando also noted that the rate of decrease was related not only to the length of time the larvae were stored but also to the temperature at which they were maintained. He related this phenomenon to a concurrent decrease in the food reserves of the larvae, which occurred at a faster rate at higher temperatures. Evidence for this was presented by Payne (1922, 1923) and Giovannola (1936) who showed by staining techniques that the number of fat granules decreased during storage. The fat staining of N. dubius during the present study suggests that this species also utilises its endogenous fat reserves during the period of decreased respiration.

Rogers (1962) suggested that the lowered respiration

rate of the infective larvae is probably an adaptation leading to the conservation of reserve materials allowing the infective larvae to survive longer, thus increasing their chances of encountering a host.

The loss of motility of free-living larvae in O_2 free N_2, coupled with the fact that oxygen is necessary for the release of energy from fat which is their main food reserve, suggests that their metabolism is predominantly if not completely aerobic. In addition, an anaerobic metabolism would be very wasteful for an animal whose chief priority is to survive as long as possible.

In 1953, Bair found similar low rates of O_2 consumption for the infective larvae of several small horse strongyles; in addition, he found that changes in PO_2 did not alter this basic rate, i.e. there was no critical oxygen tension. He suggested that this would be an advantageous adaptation for an animal which will eventually inhabit an atmosphere of low oxygen tension, for example the gastro-intestinal tract of a vertebrate, and could allow oxygen consumption to continue at these relatively low oxygen tensions. Thus, the decreased O_2 consumption of third stage Nematospiroides dubius could be of adaptive advantage for its survival within the host as well as outside it.

It is clear that all the parasitic stages of Nematospiroides dubius, in common with all animal parasitic

nematodes tested, are capable of consuming appreciable amounts of oxygen at atmospheric pressure. Whether or not similar amounts of O_2 if taken up *in vivo* could be utilised is discussed in Chapter 7.

It was not possible to study the effect of different O_2 tensions on the respiration rates of *Nematospiroides dubius* and thus to observe how *in vitro* rates might compare with those to be expected at the lower tensions *in vivo*. However, studies by Rogers (1949b) and Roberts and Fairbairn (1965) on the closely related species *Nippostrongylus brasiliensis* provide some indication of what may occur. The respiration rate of adult *N. brasiliensis* was found to decrease rapidly below a certain O_2 tension, above which the O_2 consumption remained more or less constant. This tension, known as the critical oxygen tension, was found by both workers to lie between 20 and 30 mmHg, and oxygen tensions of this order were reported in the rat intestine by Rogers (1949a). It is therefore possible that this parasite would be able to achieve its maximum *in vitro* rate *in vivo*, that is to say the respiratory rates would be similar in both situations. It is therefore also possible that the respiration rates obtained *in vitro* for the parasitic stages of *N. dubius* may approximate to those that occur *in vivo* at lower O_2 tensions.

The ability of adult *Nematospiroides dubius* to survive

in an oxygen free atmosphere could be due to the red pigment present in the body wall. If this was a haemoglobin with a low loading tension, as suggested on p.121, it could possibly have provided the worms with sufficient oxygen to survive for some time, death only occurring when this store was completely depleted. Roberts and Fairbairn (1965) reported that adult Nippostrongylus brasiliensis did not feed when incubated in glucose saline, and it is possible that N. dubius does not either. If this were true, the different survival times in aerobic and anaerobic conditions recorded in this work may only indicate that starving N. dubius utilise their food reserves more efficiently and slowly in aerobic conditions, and not that O_2 is essential for their survival in vitro. However, a study of the growth/metabolism relationship has indicated that oxygen is essential for its metabolism (Chapter 7).

CHAPTER 7

THE SIZE/METABOLISM RELATIONSHIP OF NEMATOSPIROIDES DUBIUS

Introduction

It is clear from the preceding two Chapters that the respiration of Nematospiroides dubius throughout its life cycle is closely linked with its growth. The question has been posed as to whether the aerobic respiration of parasites gives rise to energy which is useful in their economy in vivo (Rogers, 1962). In the present study, the O_2 consumption of the parasitic stages was measured in vitro; however, the weight data was obtained from worms that grew in vivo. The establishment of a meaningful relationship between these two factors would in some measure answer the above question and this was the main purpose of this experiment. The second purpose of the experiment was to provide knowledge of the size/metabolism relationship for both the free-living and parasitic stages of the life cycle of an animal parasitic nematode, as very little information is available about this relationship in nematodes. A useful method of establishing the relationship is to plot the logarithm of oxygen uptake per specimen against the logarithm of weight per specimen (Zeuthen, 1953). The relationship between body size and metabolism is given by the equation $Y = aX^b$ (Brody, 1945), where Y represents metabolic rate, X represents body size and a is a constant showing the value of Y when X = 1. The

exponent b is the constant which indicates the proportion of increase in Y with respect to X. When the b value is 1.00 the increase in metabolism parallels the increase in weight; when b is 0.67 the increase in metabolism is proportional to two-thirds power of body weight, i.e. it is directly proportional to the body surface (Sarrus and Rameux, 1839). In 1953, Zeuthen compared the metabolism and body size relationship in organisms ranging from bacteria to large poikilothermic and homiothermic animals and found the b values for all these animals to be between 0.67 and 1.

Materials and Methods

(a) Free-living stages

It was not possible to measure the respiration and dry weight of individual free-living larvae as they are too small; an average respiration rate and dry weight was therefore obtained for each age group. Measurements were made on larvae extracted from cultures at 24 hourly intervals, starting on the second day of culture and continuing until the seventh day.

(b) Parasitic stages

The respiration rate of individual worms, removed from the mouse intestine at approximately 24 hourly intervals, were measured. Each worm was removed from the diver at the end of the respiration run, washed, dried and weighed.

Results

(a) Free-living stages

The respiration rates of aged larvae and their corresponding dry weights are shown in Table 15. The double logarithmic plot is shown in Figure 32 and the graph can be divided into three parts. Part 1 represents the period of the life cycle involving the first two free-living larval stages which feed and grow and build up fat reserves. There were insufficient points to calculate a significant regression for this line. Part 2 of the graph represents the time between days 3 and 4 of culture when the second stage larvae moult to form the infective third-stage larvae; this appears to be a transitional period within the free-living phase of the life-cycle of N. dubius. Part 3 of the graph represents the period when the larvae have just become infective and ceased to feed. The slope of the line is negative with respect to time and has a very high calculated b value of 4.1831, with a correlation coefficient of 0.9218, which was significant at 0.1% level. The b value falls well out of the range calculated by Zeuthen (1953).

(b) Parasitic stages

The individual dry weights and respiration rates are shown in Table 16. The logarithmic plots for male and female worms are shown in Figures 33a and 33b. The relationship between weight and respiration for both sexes of

TABLE 15

The dry weight/respiration data for free-living larval stages of N. dubius

Larval Stage	X	Log X	Y	Log Y
1	0.016	-1.7959	0.3014	-0.5228
	0.016	-1.7959	0.2990	-0.5243
	0.016	-1.7959	0.2957	-0.5292
	0.016	-1.7959	0.2920	-0.5346
2	0.026	-1.5850	0.3412	-0.4669
	0.026	-1.5850	0.3307	-0.4806
	0.026	-1.5850	0.3033	-0.5182
	0.026	-1.5850	0.3564	-0.4481
	0.026	-1.5850	0.3403	-0.4681
	0.026	-1.5850	0.3228	-0.4910
3	0.049	-1.3098	0.2446	-0.6091
	0.049	-1.3098	0.2150	-0.6676
	0.049	-1.3098	0.2535	-0.5960
	0.049	-1.3098	0.1948	-0.7104
	0.049	-1.3098	0.2687	-0.5708
	0.049	-1.3098	0.1945	-0.7111
	0.049	-1.3098	0.2591	-0.5865
	0.049	-1.3098	0.1920	-0.7167
3	0.045	-1.3468	0.1890	-0.7235
	0.045	-1.3468	0.1224	-0.9122
	0.045	-1.3468	0.1448	-0.8393
	0.045	-1.3468	0.1794	-0.7461
	0.045	-1.3468	0.1014	-0.9940
	0.045	-1.3468	0.1441	-0.8413
3	0.042	-1.3768	0.1211	-0.9169
	0.042	-1.3768	0.1036	-0.9846
	0.042	-1.3768	0.1177	-0.9292
	0.042	-1.3768	0.1307	-0.8841
	0.054	-1.2676	0.2908	-0.5372
	0.054	-1.2676	0.2919	-0.5363
	0.054	-1.2676	0.3366	-0.4729
	0.054	-1.2676	0.3047	-0.5161

X = dry weight (µg)

Y = respiration rate ($\mu l O_2 . 10^{-3}$/individ. hr.)

FIGURE 32

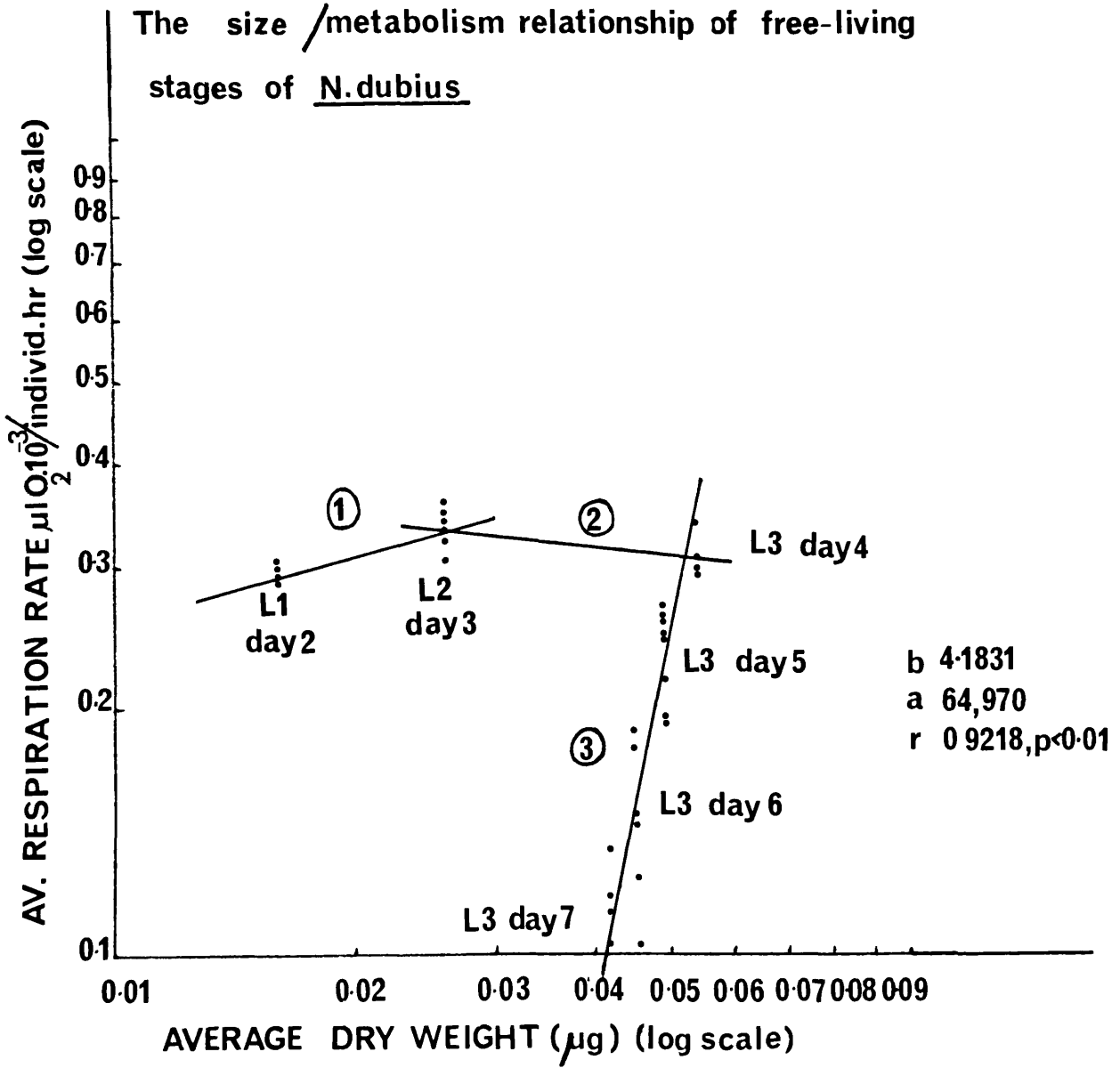

The size /metabolism relationship of free-living stages of N.dubius
AV. RESPIRATION RATE µl O2.10-3/indivd.hr (log scale)
0·9
0·8
0·7
0·6
0·5
0·4
0·3
0·2
0·1
0·01
0·02
0·03
0·04
0·05
0·06
0·07
0·08
0·09
AVERAGE DRY WEIGHT (µg) (log scale)
①
②
③
L1 day 2
L2 day 3
L3 day 4
L3 day 5
L3 day 6
L3 day 7
b 4·1831
a 64,970
r 0 9218, p<0·01

TABLE 16

The dry weight/respiration data for male and female parasitic stages of N. dubius

Male Mice

Larval Stage	X	Log X	Y	Log Y
3	0.8	-0.1969	5.01	0.6698
3	1.2	0.0792	6.4026	0.8063
4	2.0	0.3010	3.66	0.5635
4	3.0	0.4771	9.9456	0.9977
4	3.0	0.4771	7.926	0.8996
4	4.0	0.6021	7.5909	0.8802
4	5.0	0.6990	8.763	0.9426
5	5.0	0.6990	12.59	1.1000
5	5.9	0.7709	12.2951	1.0899
5	7.0	0.8451	16.245	1.2106
5	7.2	0.8573	24.751	1.3936
5	9.0	0.9542	28.076	1.4483
5	9.0	0.9542	25.4814	1.4062
5	9.2	0.9638	30.067	1.4786
5	9.5	0.9777	26.69	1.4264
5	10.00	1.0000	14.328	1.559
5	10.1	1.0043	19.193	1.2830
5	11.0	1.0414	21.492	1.3322
5	11.0	1.0414	34.356	1.5359
5	12.0	1.0792	28.941	1.4615
5	16.0	1.2041	33.617	1.5264
5	18.0	1.2553	26.193	1.4181

Female Mice

3	0.75	-0.1249	3.5861	0.5546
3	0.75	-0.1249	1.2090	0.0821
3	1.2	0.0792	3.301	0.5186
4	1.0	0.0043	5.3060	0.7245
4	2.5	0.3979	5.7140	0.7569
4	4.0	0.6021	13.5320	1.1313
4	5.0	0.6990	18.2104	1.2603
4	5.5	0.7404	12.191	1.0859
4	6.0	0.7782	7.4026	0.8694
4	6.5	0.8129	19.354	1.2867
4	7.0	0.8451	14.99	1.1758
4	7.9	0.8976	10.146	1.0060
4	8.9	0.7494	13.113	1.1176
5	7.1	0.8513	18.335	1.2632
5	7.5	0.8751	16.6507	1.2300
5	9.8	0.9912	24.66	1.3920
5	10.0	1.0000	21.906	1.3404
5	11.5	1.0607	18.7103	1.2720
5	11.9	1.0755	20.717	1.3162
5	14.0	1.1461	30.3031	1.4814
5	15.0	1.1761	33.55	1.5256
5	15.8	1.1987	21.492	1.3322
5	22.1	1.3444	16.159	1.2081
5	26.0	1.4150	54.137	1.7334
5	29.1	1.4639	40.106	1.6031
5	32.8	1.5159	71.739	1.8557
5	58.0	1.7634	61.41	1.7883
5	58.5	1.7672	29.26	1.4663

X = dry weight (μg)

Y = respiration rate ($\mu l O_2 . 10^{-3}$/individ. hr.)

FIGURE 33a

The size/metabolism relationship of male parasitic stages of N.dubius

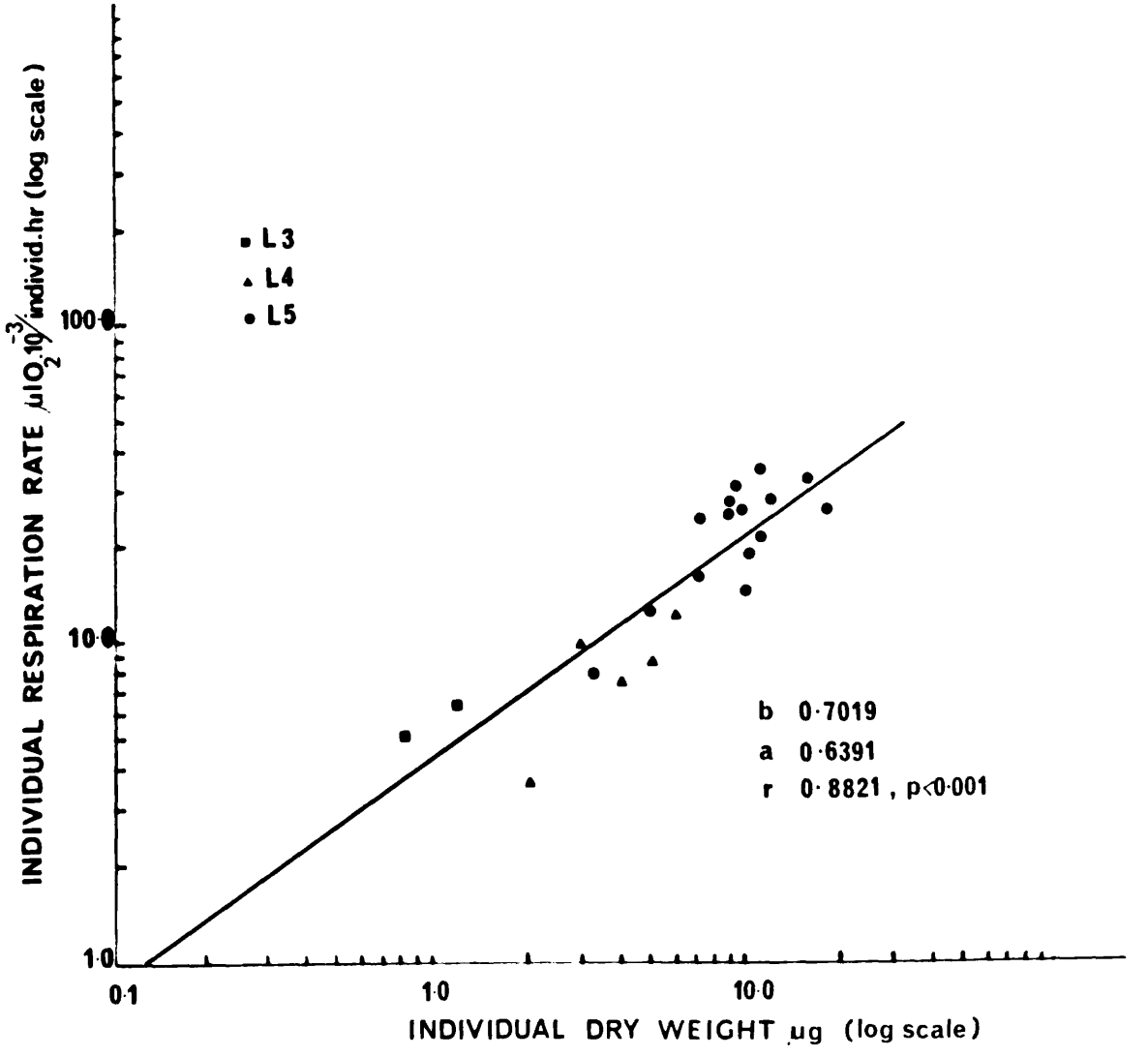
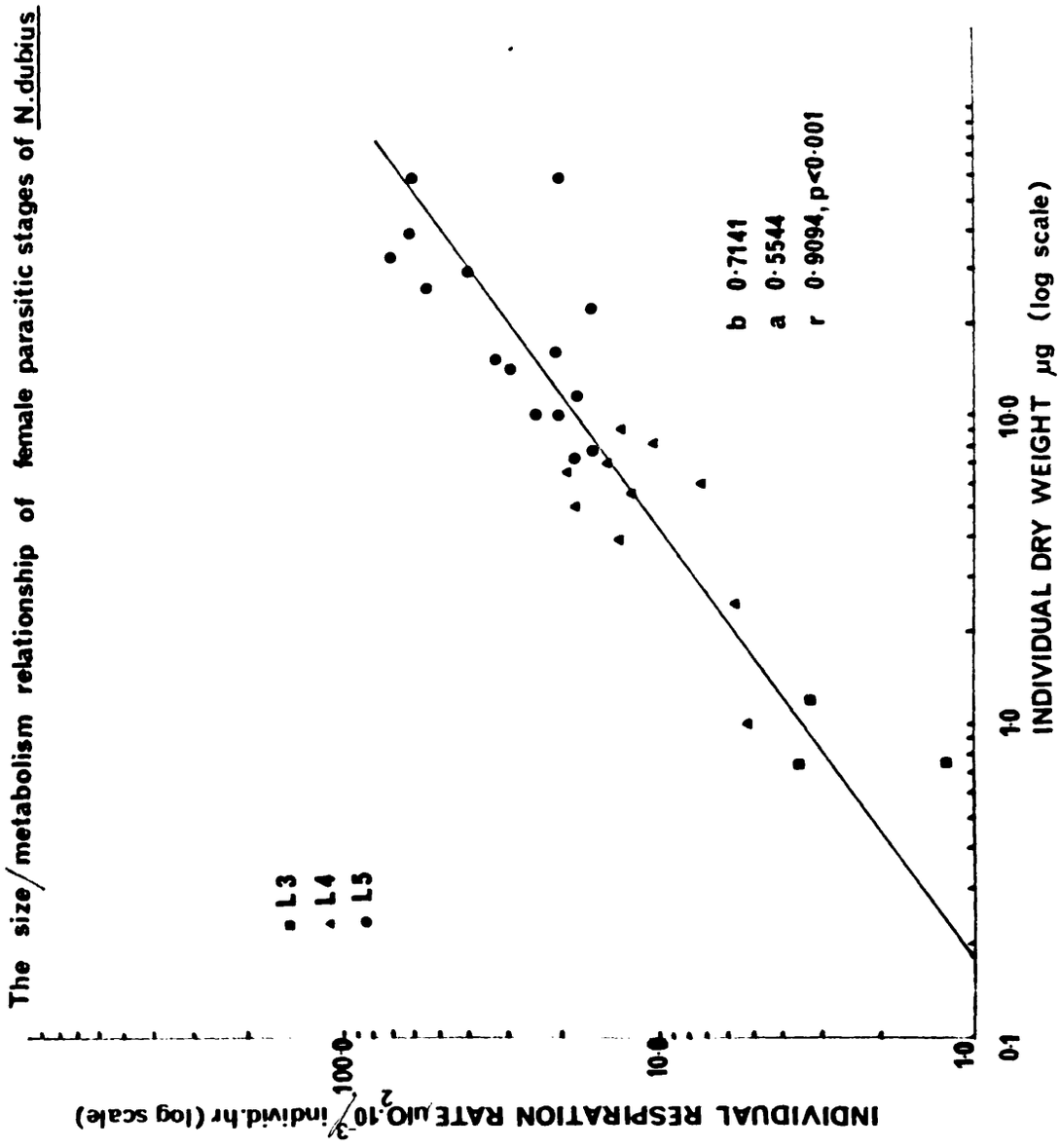


FIGURE 33b


The size/metabolism relationship of female parasitic stages of N.dubius
L3
L4
L5
b 0·7141
a 0·5544
r 0·9094, p<0·001
INDIVIDUAL RESPIRATION RATE $\mu lO_2.10^{-3}$/indiv.hr (log scale)
INDIVIDUAL DRY WEIGHT µg (log scale)
100·0
10·0
1·0
0·1
1·0
10·0

worms is expressed in the following equations

$$Y = 4.356\ X^{0.7019} \text{ for male worms}$$

$$Y = 3.584\ X^{0.7141} \text{ for female worms.}$$

The b values of 0.7019 and 0.7141 obtained for male and female parasitic stages of Nematospiroides dubius respectively, fall well within the normal range of b values calculated for poikilothermic animals (Zeuthen, 1947 and 1953; Hemmingsen, 1960). There was no significant difference in the b values for either sex of worm ($p > 0.05$).

The straight line relationship obtained when body weight was plotted against respiration on a double logarithmic scale represented a constant percentage increase in respiration for constant percentage increase in body size. Although the individual respiration rates of larger animals were higher than those of small animals, the fact that the b values were less than 1 indicates that metabolic intensity was lowered with increasing body size. This is a well established fact for most other animal species.

There was great variation in both size and respiration rate within an instar or age group of parasitic stages of Nematospiroides dubius (Chapters 5 and 6). However, this variation largely disappeared when the size/metabolism relationship was considered and instar does not seem to be important in this context.

Discussion

The b value for the infective free-living larvae of Nematospiroides dubius was very high, lying well out of the range of b values calculated by Zeuthen. Its main significance seems to be that it represents a decelleration of the metabolism of the worms from the higher level exhibited by the feeding and growing first two larval stages to a new lower level, the result being that the O_2 consumption per individual is drastically reduced. This is probably an adaptation which allows the stored fat, upon which the infective larvae are dependent, to be utilised as slowly as possible. The daily rate of weight loss slows down considerably after the seventh day of culture (Chapter 5, p.149) supporting this theory of a generally slower metabolism after a transitional period of change. It is extremely important that an animal, which may have to survive for several months in a viable state before being eaten by a suitable host, should be able to eke out its stored energy reserves for as long a time as possible.

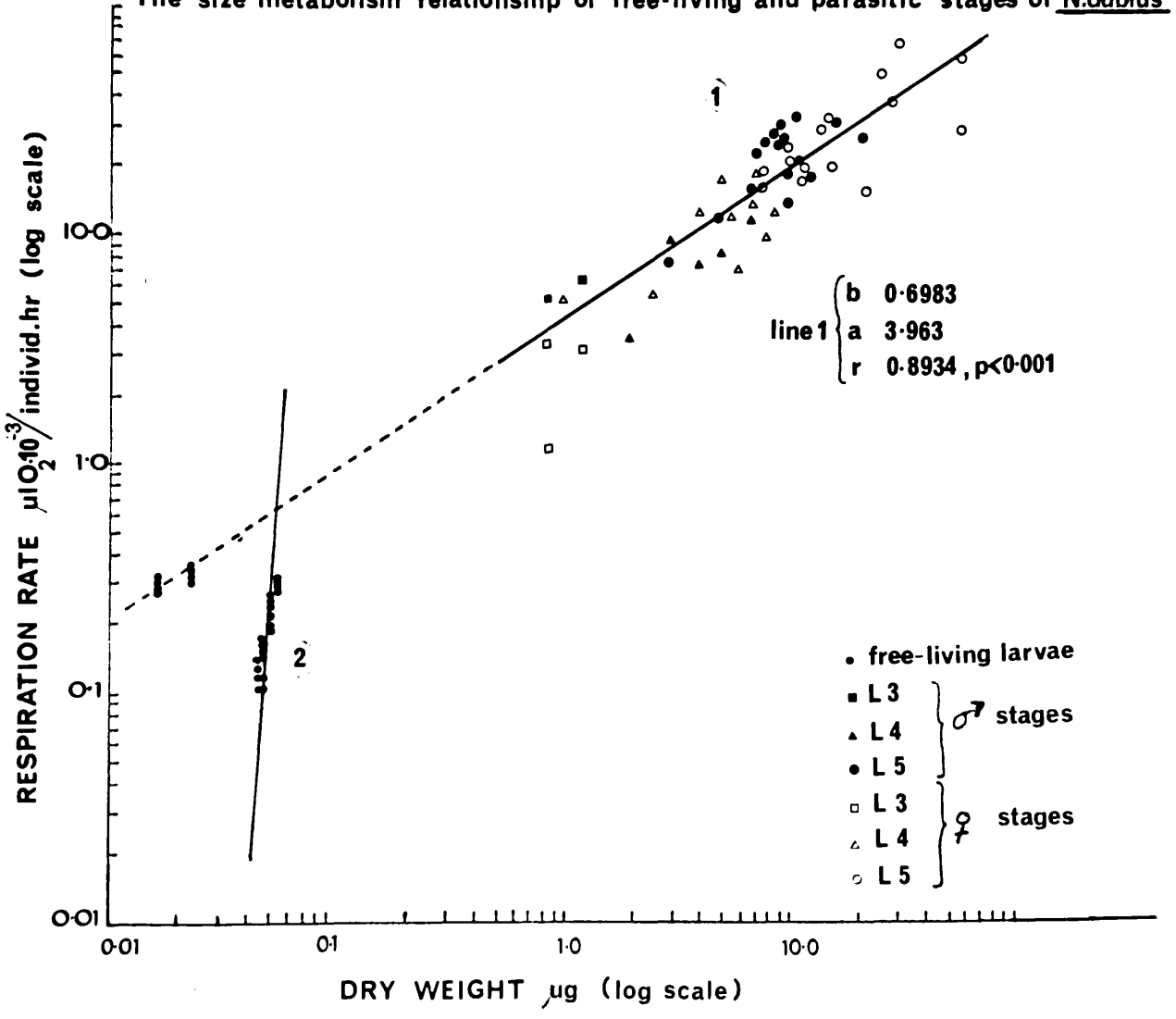
In 1949, Overgaard-Nielsen produced oxygen consumption/weight data for a wide range of soil-dwelling nematodes, and a double logarithmic plot of this interspecific data was produced by von Brand (1960a); the calculated b value of the line obtained was 0.87. The b values calculated by Zeuthen (1947 and 1953) and von Brand (1960a)were mainly for animals

known to have a predominantly aerobic metabolism. However, the b values for male and female parasitic N. dubius fall well within the range of these values. That is the relationship between size (data obtained in vivo) and the respiration (data obtained in vitro) of N. dubius was essentially the same as that for aerobic animals. This indicates that similar quantities of oxygen to those consumed in vitro would also need to be utilised in vivo to provide the energy necessary for this growth to be achieved.

Figure 34 shows the size/metabolism relationship throughout the whole life cycle of Nematospiroides dubius. The fitted regression line for male and female parasitic stages when considered together has been drawn (line 1). It is obvious that when this line is extrapolated back it passes very close to the position on the graph of the first two larval stages. Although there was insufficient data for these two stages for a significant regression to be calculated, it appears that a more or less constant relationship would exist between size and metabolism for both free-living and parasitic stages of N. dubius, provided the larvae were ingested immediately they became infective (i.e. between days 3 and 4 of culture). If the larvae are not ingested, the size/metabolism relationship is drastically altered as indicated by line 2.

FIGURE 34

The size metabolism relationship of free-living and parasitic stages of N.dubius



GENERAL DISCUSSION

It has been suggested by Lewis (1968) that changes in incidence of infection of small mammals with helminths vary with the changing population of hosts and the availability of infective stages throughout the year. He also suggested that the infective stages varied in response to seasonal climatic conditions, leading to a general decrease in the number of infective eggs or larvae during the winter months and an increase during the summer, and in addition, that the numbers of intermediate hosts of parasites would also be subject to similar variation.

The present survey has shown that most of the variation in incidence of infection of *Apodemus sylvaticus* and *Clethrionomys glareolus* with helminth parasites was due to changes in the age and sex ratio of the host population and the associated behavioural changes. Differences in density of the host population were probably also important in determining variations in both the level and degree of infection, but no measure of population density was feasible during the present work. In general, the infection levels of the various parasites studied were similar to those found by Lewis (1968a and b) in *A. sylvaticus* and *C. glareolus* trapped in North Wales.

It has been shown that, contrary to expectation, the incidence of infection with any of the five species studied

was rarely affected by weather factors, either of the previous or concurrent month. An exception was Syphacia stroma, as 52% of the variation in incidence of infection with this parasite in Apodemus sylvaticus was accounted for by the concurrent weather, and there was a negative correlation with relative humidity. This result was surprising as the infective eggs of S. stroma, which are passed to new hosts by physical contact, would seem to be less likely to be influenced by external environmental conditions than any of the infective stages of the other parasites studied, which have a free-living stage away from the host. The negative correlation with relative humidity was also difficult to explain as decreases in relative humidity would be expected to desiccate eggs on the host fur thereby lowering their viability,which would be reflected as a decrease, rather than an increase in incidence.

The incidence of infection of Clethrionomys glareolus with Nematospiroides dubius was negatively correlated with the temperature of the preceding month. This may have been due to increases in temperature causing desiccation of the infective third stage larvae, although they have been shown to be fairly resistant to this effect (Dobson, 1960). There was no correlation between temperature and incidence of infection of Apodemus sylvaticus with N. dubius; this was possibly because A. sylvaticus practises refraction and eats

faeces containing larvae before they have had time to become desiccated by high temperatures. C. glareolus is not known to practise refraction and this is reflected in the lower level and degree of infection with this parasite, i.e. it had less chance of becoming infected, and also of picking up large numbers of N. dubius than A. sylvaticus.

The intensity of infection of small mammals with helminth parasites was affected by environmental conditions to a greater degree than the incidence. A significant proportion of the variation in intensity of infection of Apodemus sylvaticus with Corrigia vitta was due to concurrent environmental variables. The intensity of infection with C. vitta was correlated with rainfall and negatively correlated with temperature, and this was thought to be due to changes in the availability of infected intermediate hosts under different weather conditions.

The intensity of infection of Apodemus sylvaticus with Nematospiroides dubius was negatively correlated with concurrent relative humidity, and the intensity of infection with S. stroma was negatively correlated with rainfall, but the reasons for these relationships are not clear. The preceding month's weather did not affect the degree of infection with any of these parasites.

The intensity of infection of Clethrionomys glareolus with helminth parasites could not be accounted for in terms

of environmental variables, except in the case of Pelodera strongyloides. Almost 70% of the variation in intensity of infection with this nematode was due to variations in concurrent weather and there was a highly significant negative correlation with rainfall. This result provides additional evidence for the theory that drought conditions stimulate the production of "infective" larvae (Poinar, 1965).

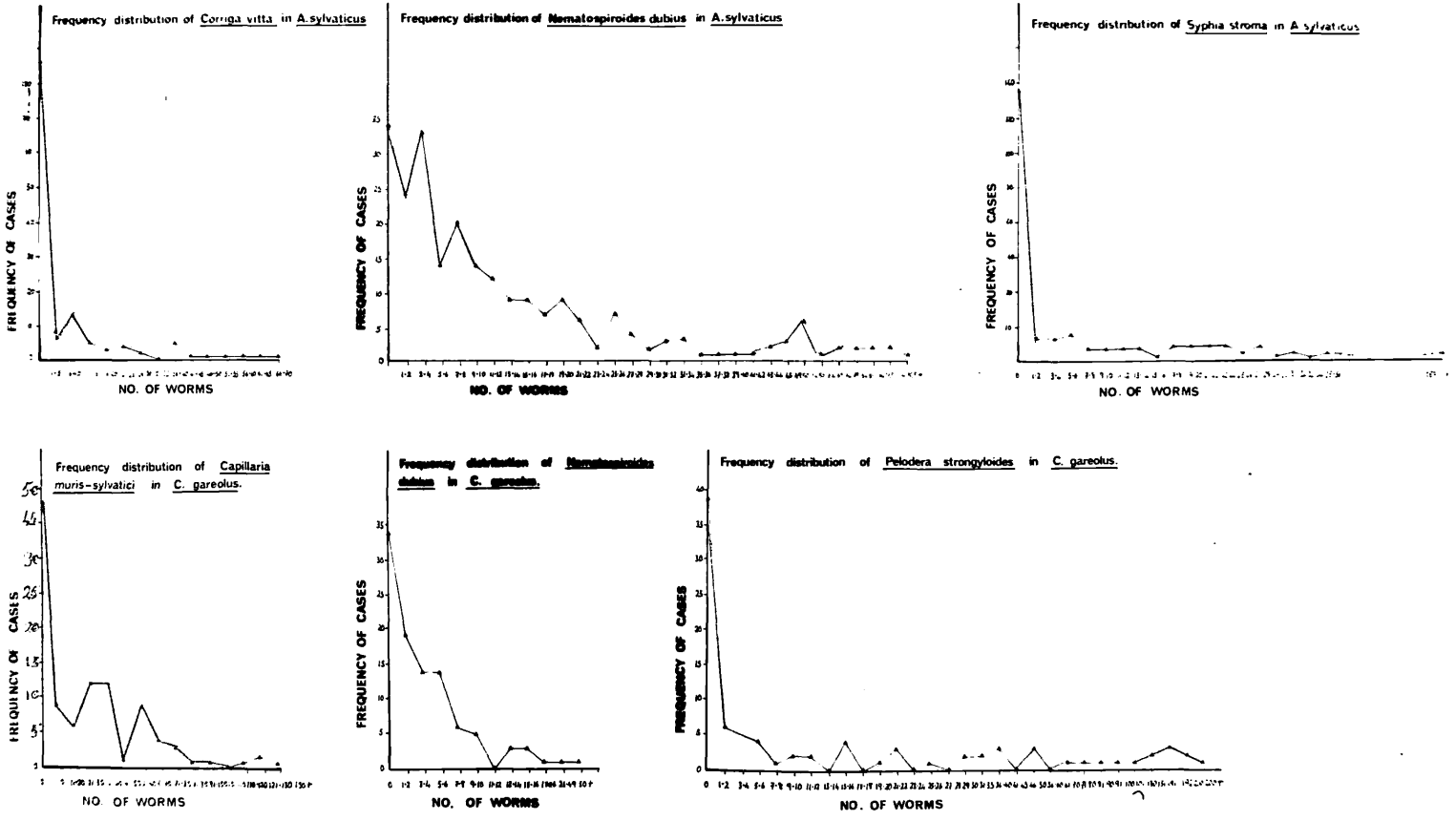
The relationship between incidence and intensity is a complex one and various workers have suggested that a parallel variation exists between these two factors. Hu (1931) in studies on Dicrofilaria immitis in the mosquito vector found that high intensities were associated with high incidences of infection. Bertram (1949) also showed that the intensity of infection of Litomosoides carinii in the mite Liponyssus bacoti fed on infected cotton rats, was associated with the incidence of infection but that the relationship was not a simple one. More recently, Williams and Harris (1962) have shown that the highest incidence of infection of Gulls with various cestode species was associated with the highest intensity of infection and that the lowest incidence was associated with low intensity. However, Lewis (1968a) found no such relationship between the numbers of individual parasites and the incidence of infection of the helminth parasites of small mammals of North Wales, and a similar situation was observed during the present work.

A correlation analysis was performed on the monthly incidence and intensity of infection of the individual parasites studied and in all cases except one the correlation coefficients were not significant, showing that there was no relationship between incidence and intensity of infection. The exception was Nematospiroides dubius in Apodemus sylvaticus, as the correlation coefficient was 0.6172, significant at 5% level, i.e. high incidence was related to high intensity and low incidence to low intensity. This may also be due to refraction practised by A. sylvaticus and the development of infective larvae of N. dubius in the faecal pellets. Repeated ingestion of infective stages would lead to high worm burdens with associated high egg output in the host faeces, thus facilitating the production of large numbers of infective larvae for reingestion.

The frequency distributions of the individual parasites in their naturally infected hosts were plotted and a skewed distribution curve resulted in all cases (Figure 35). This type of distribution appears to be a general phenomenon of parasitic infections as similar results were obtained by Li and Hsu (1951) who plotted the frequency distributions of twenty individual parasitic species in their naturally infected hosts, showing that in most cases the distributions were well represented by the type Ij Pearsonian Frequency curve, although their range, incidence and intensity of

FIGURE 35

THE FREQUENCY DISTRIBUTIONS OF PARASITIC HELMINTHS IN THEIR NATURALLY INFECTED HOSTS



infection were different. Lewis (1968a)also found that the frequency distributions of Nematospiroides dubius and Syphacia stroma in Apodemus sylvaticus were of the same general shape.

A competitive interaction took place between Nematospiroides dubius and Syphacia stroma when they occurred concurrently in the alimentary canal of Apodemus sylvaticus. This competition resulted in the partial exclusion of S.stroma from the more favourable habitat in the anterior portion of the gut, but there was no significant reduction in the worm burden of either species when they occurred concurrently compared to single species infections. These results indicated that there was not a "strong" interaction between these two nematode species. It was not possible to verify the theory of Lewis (1964) that the limiting factor in this competitive interaction was oxygen, as S. stroma was unculturable, and therefore its possible oxygen requirement could not be assessed. However, populations of N. dubius in experimentally infected mice were shown to be highly aggregated in relation to the anterior portion of the small intestine. This indicated that oxygen is an important resource to this worm as the aggregation was thought to be in response to an oxygen gradient known to exist in the small intestine, the highest tensions being found in the most anterior region. The individual N. dubius were also

shown to be highly aggregated with respect to each other. This observation, linked with the fact that the crowding of these worms decreased their individual rate of O_2 consumption, suggested that the response may be of economic importance to the parasites, reducing their need for a resource that is intermittently present in limited quantities, due to the passage of food through the gut. If S. stroma had been shown to have a similar oxygen requirement to N. dubius, it would have been possible for oxygen to have been a limiting factor for which they competed.

Nematospiroides dubius was shown to have a high daily growth rate both in the free-living and parasitic stages of the life cycle. The free-living larvae grew from the time of hatching until infectivity was reached three days later (at 20°C), when the larvae started to lose weight; this coincided with a decreased respiration rate. The slowing down of the metabolic rate, which has been reported many times for free-living infective nematode larvae, appears to be of adaptive significance, enabling the worms to survive as long as possible on their stored food reserves.

There was no significant difference in the growth rate of male parasitic stages of Nematospiroides dubius in male or female mice, and the same was also true of the female parasitic stages.

The relationship between size and metabolism of the

free-living infective third stage larvae of Nematospiroides dubius indicated that the level of their metabolism decreased from the higher level exhibited by the feeding and growing first two larval stages, to a new lower level. This was probably an adaptation leading to the conservation of stored food reserves.

The growth/metabolism relationship of male and female parasitic stages of Nematospiroides dubius was very similar, and there was no significant difference in the b values obtained from the double logarithmic plots. These b values were comparable to those which have been obtained for free-living aerobic animals (Zeuthen, 1947, 1953; Hemmingsen, 1960). The fact that the size/metabolism relationship was so similar to that found in aerobic organisms indicates that an aerobic metabolism may also be important to N. dubius.

In order to achieve its high daily growth rates, Nematospiroides dubius requires a large amount of energy. The relevant question is could it obtain enough energy from anaerobic respiration to achieve this growth or is it more likely to use aerobic respiration if oxygen is available. Parasites are usually surrounded by a plentiful food supply, and may therefore have sufficient substrate for anaerobic respiration to supply the energy required for growth. Oxygen is probably available to N. dubius at least part of the time in vivo; it is most likely that it would use

aerobic respiration whenever possible to fulfil its energy requirements. It was pointed out by Bryant (1971) that parasites, like most animals, are metabolic opportunists, and will utilise different resources when they become available; their type of metabolism whether aerobic or anaerobic is fitted to the prevailing environment. Bryant reasoned that there were probably varying degrees of dependence on both aerobic or anaerobic metabolism and that the two processes are not mutually exclusive.

Although it is unlikely that Nematospiroides dubius relies totally on an aerobic metabolism, it is probable that aerobic respiration is of considerable importance in its life in vivo.

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APPENDIX 1a

The measurements of drawings of free-living N. dubius larvae extracted on Day 2 of culture and their calculated fresh weight

A Greatest body width μ	B Body length μ	Fresh weight μg. ($A^2B/16 \times 10^5$)	Mean Fresh Weight μg.	S.D.	S.E.
26.25	437.50	0.18841	0.1567	0.0205	0.0036
26.25	420.00	0.18087			
24.50	430.50	0.16150			
22.75	420.00	0.13586			
22.75	416.50	0.13472			
24.50	428.75	0.16084			
26.25	448.00	0.19293			
22.75	434.00	0.14038			
26.25	430.50	0.18540			
24.50	455.00	0.17069			
24.50	451.50	0.16938			
22.75	406.00	0.13133			
22.75	399.00	0.12906			
24.50	430.50	0.16150			
22.75	411.25	0.13302			
24.50	423.50	0.15887			
24.50	430.50	0.16150			
22.75	427.00	0.13812			
24.50	448.00	0.16807			
22.75	421.75	0.13642			
22.75	430.50	0.13925			
24.50	427.00	0.16019			
22.75	451.50	0.14604			
24.50	455.00	0.17069			
24.50	441.00	0.16544			
26.25	462.00	0.19896			
24.50	451.50	0.16938			
24.50	476.00	0.17857			
22.75	406.00	0.13133			
24.50	393.75	0.14771			
22.75	406.00	0.13133			
22.75	423.50	0.13699			

APPENDIX 1b

The measurements of drawings of free-living N. Dubius larvae extracted on Day 3 of culture and their calculated fresh weights

A Greatest body width μ	B Body length μ	Fresh weight μg. ($A^2B/16 \times 10^5$)	Mean Fresh Weight μg.	S.D.	S.E.
24.50	486.50	0.1825	0.2236	.04440	.00946
24.50	472.50	0.1772			
24.50	488.50	0.1832			
26.50	530.25	0.2283			
31.50	553.00	0.3429			
28.00	490.00	0.2400			
26.25	563.50	0.2426			
26.25	560.00	0.2411			
26.25	560.00	0.2411			
26.25	574.00	0.2472			
26.25	539.00	0.2321			
24.50	486.50	0.1825			
24.50	483.00	0.1812			
24.50	556.50	0.2087			
24.50	500.50	0.1877			
26.25	532.00	0.2291			
26.25	500.50	0.2155			
29.75	574.00	0.3175			
24.50	462.00	0.1733			
24.50	465.50	0.1746			
26.25	567.00	0.2441			
28.00	504.00	0.2469			

Appendix 1c

The measurements of drawings of free-living N. dubius larvae extracted on Day 4 of culture and their calculated fresh weights

A Greatest Body width u	B Body length u	Fresh weight ug. ($A^2B/16 \times 10^5$)	Mean Fresh Weight ug.	S.D.	S.E.
26.25	493.90	0.2127	0.2204	0.0346	0.0067
24.50	528.50	0.1982			
24.50	533.75	0.2002			
28.00	533.00	0.2611			
24.50	523.25	0.1963			
26.25	525.00	0.2260			
26.25	507.50	0.2185			
26.25	549.50	0.2366			
26.25	540.75	0.2328			
28.00	546.00	0.2675			
24.50	504.00	0.1890			
24.50	504.00	0.1890			
26.25	528.00	0.2273			
22.75	479.50	0.1551			
26.25	490.00	0.2110			
26.25	511.00	0.2200			
26.25	518.00	0.2230			
28.00	535.50	0.2623			
24.50	483.00	0.1812			
28.00	553.00	0.2709			
24.50	472.50	0.1772			
22.75	528.50	0.1709			
28.00	532.00	0.2606			
28.00	560.00	0.2743			
24.50	528.50	0.1982			
28.00	553.00	0.2709			

APPENDIX 1d

The measurements of drawings of free-living N. dubius larvae extracted on Day 5 of culture and their calculated fresh weights

A Greatest body width µ	B Body length µ	Fresh weight µg. ($A^2B/16 \times 10^5$)	Mean Fresh Weight µg.	S.D.	S.E.
26.25	514.50	0.2215	0.2104	0.0282	0.0052
24.50	535.50	0.2208			
22.75	542.50	0.1754			
24.50	539.00	0.2022			
22.75	532.00	0.1720			
28.00	528.50	0.2589			
26.25	532.00	0.2291			
22.75	548.00	0.1772			
24.50	539.00	0.2022			
24.50	528.50	0.1982			
28.00	539.00	0.2641			
22.75	535.50	0.1732			
26.25	539.00	0.2321			
24.50	532.00	0.1995			
24.50	535.50	0.2008			
24.50	521.50	0.1956			
24.50	514.50	0.1930			
26.25	528.50	0.2276			
28.00	588.00	0.2881			
26.25	553.00	0.2381			
26.25	532.00	0.2291			
24.50	539.00	0.2022			
24.50	546.00	0.2048			
24.50	532.00	0.1995			
24.50	549.50	0.2061			
26.25	549.50	0.2366			
24.50	532.00	0.1995			
22.75	539.00	0.1743			
24.50	532.00	0.1995			

APPENDIX 1e

The measurements of drawings of free-living N. dubius larvae extracted on Day 6 of culture and their calculated fresh weights

A Greatest body width µ	B Body length µ	Fresh weight µg. ($A^2B/16 \times 10^5$)	Mean Fresh Weight µg.	S.D.	S.E.
24.50	528.50	0.1982	0.2081	0.0250	0.0056
24.50	535.50	0.2008			
24.50	493.50	0.1851			
22.75	521.50	0.1686			
26.25	518.00	0.2230			
26.25	504.00	0.2170			
24.50	528.50	0.1982			
26.25	518.00	0.2230			
24.50	539.00	0.2022			
24.50	546.50	0.2050			
24.50	528.50	0.1982			
24.50	581.00	0.2179			
24.50	539.00	0.2022			
24.50	518.00	0.1943			
22.75	560.00	0.1811			
26.25	528.50	0.2276			
28.00	528.50	0.2589			
28.00	544.25	0.2666			
22.75	532.00	0.1720			
26.25	514.50	0.2215			

APPENDIX 1f

The measurements of drawings of free-living N. dubius larvae extracted on Day 7 of culture and their calculated fresh weights

A Greatest body width µ	B Body length µ	Fresh weight µg. ($A^2B/16 \times 10^5$)	Mean Fresh Weight µg.	S.D.	S.E.
26.25	532.00	0.2291	0.1979	0.0219	0.0034
22.75	532.00	0.1720			
26.25	504.00	0.2170			
22.75	518.00	0.1675			
24.50	521.50	0.1956			
22.75	535.00	0.1730			
22.75	535.00	0.1730			
24.50	549.50	0.2061			
24.50	532.00	0.1995			
24.50	574.00	0.2153			
26.25	504.00	0.2170			
24.50	518.00	0.1943			
24.50	518.00	0.1943			
24.50	518.00	0.1943			
24.50	532.00	0.1995			
24.50	532.00	0.1995			
22.75	540.75	0.1749			
22.75	560.00	0.1811			
24.50	542.50	0.2035			
24.50	535.50	0.2008			
22.75	493.50	0.1596			
22.75	563.50	0.1822			
22.75	539.00	0.1743			
22.75	514.50	0.1664			
22.75	586.25	0.1896			
24.50	540.75	0.2028			
26.25	539.00	0.2321			
24.50	518.00	0.1943			
24.50	574.00	0.2153			
26.25	514.50	2215			
24.50	528.50	0.1982			
24.50	553.00	0.2074			
26.25	490.00	0.2110			
24.50	521.50	0.1956			
26.25	519.75	0.2238			
24.50	535.50	0.2008			
22.75	483.00	0.1562			
24.50	553.00	0.2074			
24.50	542.50	0.2035			
28.00	546.00	0.2675			

APPENDIX 2a

The individual dry weights of aged male parasitic stages of N. dubius extracted from male mice

Larval Stage	Age	Wt(ug)	Log Wt.
3	72	1.24	0.0934
	72	1.78	0.2504
	72	0.8	-0.0969
	72	1.2	0.0792
3	90	2.84	0.4533
	90	2.0	0.3010
	90	2.68	0.4281
3	98	2.5	0.3979
	98	1.9	0.2788
4	114	2.38	0.3766
	114	2.62	0.4183
4	120	5.0	0.6990
	120	7.0	0.8451
	120	4.9	0.6902
	120	4.0	0.6021
	120	3.5	0.5441
	120	6.0	0.7782
	120	3.5	0.5441
	120	6.0	0.8195
	120	6.6	0.7782
	120	7.2	0.8573
	120	4.0	0.6021
4	142	4.5	0.6532
	142	3.0	0.4771
	142	4.0	0.6021
	142	4.3	0.6335
4	166	5.1	0.7076
	166	4.1	0.6128
	166	6.0	0.7782
	166	6.0	0.7782
	166	3.5	0.5441
	166	9.5	0.9771
	166	8.0	0.9031
	166	6.0	0.7782

Appendix 2a (continued)

Larval Stage	Age	Wt.(ug)	Log Wt.
5	168	7.2	0.8573
	168	7.0	0.8451
5	170	7.2	0.8573
	170	7.2	0.8573
	170	5.5	0.7404
	170	4.8	0.6812
	170	7.0	0.8451
	170	7.8	0.8921
5	190	9.5	0.9777
	190	10.1	1.0043
	190	5.0	0.6990
	190	9.9	0.9956
	190	11.1	1.0453
	190	11.8	1.0719
	190	10.0	1.0000
	190	15.0	1.1761
	190	5.0	0.6990
5	200	10.5	1.0212
	200	7.9	0.8976
	200	12.0	1.0792
	200	10.0	1.0000
	200	8.2	0.9138
	200	12.5	1.0969
5	216	11.0	1.0414
5	220	15.5	1.1903
	220	11.5	1.0607
	220	13.0	1.1139
	220	10.5	1.0212
	220	12.5	1.0961
5	240	13.1	1.1173
	240	16.5	1.2175
	240	11.6	1.0645
	240	14.1	1.1492
	240	9.5	0.9777

Appendix 2a (continued)

Larval Stage	Age	Wt(μg)	Log. Wt.
5	248	16.0	1.2041
	248	18.5	1.2672
	248	13.6	1.1335
	248	14.5	1.1614
	248	14.0	1.1461
5	336	19.0	1.2788
	336	17.3	1.2380
	336	19.0	1.2788
	336	15.0	1.1761
	336	14.0	1.1461
	336	20.5	1.3118
	336	19.0	1.2788
	336	18.0	1.2553
	336	16.8	1.2253
5	384	18.0	1.2553
	384	16.0	1.2041
5	432	15.5	1.1903
	432	15.8	1.1987
	432	13.5	1.1303
	432	16.2	1.2095
	432	12.5	1.0969
5	1008	17.6	1.2455
	1008	20.0	1.3010
	1008	18.5	1.2672
	1008	21.0	1.3222
	1008	14.5	1.1614

APPENDIX 2b

The individual dry weights of aged male parasitic stages of N. dubius extracted from female mice

Larval Stage	Age in hours from start of infection	Dry weight µg.	Log dry weight
3	71	1.4	0.1461
	71	1.45	0.1614
3	98	3.0	0.4771
	98	2.0	0.3010
	98	2.0	0.3010
	98	1.5	0.1761
	98	1.5	0.1761
	98	1.5	0.1761
4	114	3.68	0.5658
	114	3.42	0.5340
4	120	3.0	0.4771
	120	5.5	0.7404
	120	2.5	0.3979
	120	2.6	0.4150
	120	6.0	0.7782
	120	2.9	0.4642
	120	5.0	0.6990
	120	4.6	0.6628
	120	2.5	0.3979
4	142	4.8	0.6812
	142	8.2	0.9138
	142	3.0	0.4771
	142	5.0	0.6990
	142	4.5	0.6532
	142	4.0	0.6021
	142	3.4	0.5315
4	147	13.9	1.1430
	147	6.0	0.7782
	147	9.5	0.9777
	147	5.0	0.6990
	147	7.0	0.8451
	147	7.2	0.8573
	147	4.4	0.6435
5	191	10.9	1.0374
	191	9.0	0.9542
	191	13.0	1.1139
	191	8.0	0.9031
	191	8.9	0.9494
	191	8.0	0.9031
	191	9.1	0.9590
	191	6.9	0.8388

Appendix 2b (continued)

Larval Stage	Age in hours from start of infection	Dry weight ug.	Log dry weight
5	200	12.0	1.0792
	200	13.5	1.1303
	200	13.0	1.1139
	200	11.1	1.0453
	200	10.5	1.0212
	200	11.9	1.0755
	200	13.0	1.1139
	200	11.2	1.0492
	200	14.0	1.1461
	200	9.9	0.9946
5	212	7.5	0.8751
	212	9.0	0.9542
	212	11.1	1.0453
	212	8.1	0.9085
	212	7.8	0.8921
	212	7.3	0.8633
	212	10.0	1.0000
	212	10.0	1.0000
	212	8.0	0.9031
5	216	12.0	1.0792
5	220	14.0	1.1461
	220	13.3	1.1239
	220	14.2	1.1523
	220	13.9	1.1430
	220	13.8	1.1399
	220	14.0	1.1461
5	224	12.1	1.0828
	224	10.1	1.0043
	224	13.5	1.1303
	224	10.0	1.0000
	224	11.7	1.0682
	224	8.6	0.9345
5	248	16.8	1.2253
	248	9.5	0.9777
	248	14.5	1.1614
	248	13.0	1.1139
	248	16.0	1.2041
	248	16.0	1.2041
	248	12.8	1.1072
5	264	18.5	1.2672
	264	14.5	1.1614
	264	13.7	1.1367
	264	15.0	1.1761
	264	18.5	1.2672

Appendix 2b (continued)

Larval Stage	Age in hours from start of infection	Dry weight ug.	Log dry weight
5	360	16.1	1.2068
	360	21.5	1.3324
	360	15.0	1.1761
	360	16.5	1.2175
	360	17.5	1.2430
	360	16.7	1.2227
	360	14.0	1.1461
	360	15.0	1.1761
	360	16.5	1.2175
5	432	23.8	1.3766
	432	22.0	1.3424
	432	13.2	1.1206
	432	15.2	1.1818
5	1008	15.1	1.1790
	1008	13.5	1.1303
	1008	19.5	1.2900
	1008	17.0	1.2304
	1008	17.3	1.2380
	1008	15.5	1.1903

APPENDIX 2c

The individual dry weights of aged female parasitic stages of N. dubius extracted from female mice

Larval Stage	Age	Wt.(µg)	Log Wt.
3	71	2.1	0.3222
	71	1.4	0.1461
3	90	3.37	0.5776
	90	3.6	0.5563
3	98	3.0	0.4771
	98	1.8	0.2553
	98	1.2	0.0792
	98	3.5	0.5441
	98	2.4	0.3802
	98	3.0	0.4771
4	114	6.6	0.8195
	114	6.8	0.8325
4	120	4.5	0.6532
	120	5.9	0.7709
	120	6.0	0.7782
	120	4.9	0.6902
	120	6.5	0.8129
	120	6.1	0.7853
	120	4.5	0.6532
	120	6.0	0.7782
	120	4.0	0.6021
	120	3.5	0.5441
4	141	8.0	0.9031
	141	7.0	0.8451
	141	7.8	0.8921
	141	7.0	0.8451
	141	7.0	0.8451
	141	7.0	0.8451
	141	7.0	0.8451
4	147	11.9	1.0
	147	7.0	0.8451
	147	7.0	0.8451
	147	9.0	0.9542
	147	9.3	0.9685
	147	8.0	0.9031
	147	6.5	0.8129

Appendix 2c (continued)

Larval Stage	Age	Wt.(ug)	Log Wt.
5	191	15.5	1.1903
	191	16.2	1.2095
	191	9.0	0.9542
	191	13.1	1.1173
5	200	22.0	1.3421
	200	19.1	1.2810
	200	20.0	1.3010
	200	18.9	1.2765
	200	20.5	1.3118
	200	25.5	1.5502
	200	20.5	1.3118
	200	15.0	1.761
5	212	19.5	1.2900
	212	27	1.4314
	212	20.2	1.3054
	212	25.0	1.3979
	212	15.5	1.1903
5	220	40.5	1.6075
	220	39.1	1.5922
	220	31.5	1.4983
	220	38.0	1.5798
	220	39.0	1.5911
	220	28.5	1.4548
	220	41.5	1.6180
	220	36.0	1.5563
	220	40.5	1.6075
5	224	40.9	1.6117
	224	42.5	1.6284
	224	40.5	1.6075
	224	39.0	1.5911
	224	38.2	1.5809
	224	38.2	1.5809
	224	37.9	1.5786
	224	32.9	1.5051

Appendix 2c (continued)

Larval Stage	Age	Wt (mg)	Log Wt.
5	248	41.8	1.6212
	248	43.1	1.6345
	248	45.5	1.6580
	248	42.0	1.6232
	248	38.6	1.5866
	248	49.0	1.6902
	248	47.0	1.6776
	248	32.6	1.5132
5	264	33.5	1.5250
	264	45	1.6532
	264	44	1.6435
	264	51.9	1.7152
	264	47.1	1.6730
5	360	65.1	1.8136
	360	66.5	1.8228
	360	64.5	1.8096
5	432	41.1	1.6138
	432	44.0	1.6435
	432	44.0	1.6435
	432	44.0	1.6435
	432	52.5	1.7202
5	1008	57.9	1.7627
	1008	67.0	1.8261
	1008	74.5	1.8722
	1008	65.0	1.8129
	1008	61.5	1.7889

APPENDIX 2d

The individual dry weights of aged female parasitic stages of N. dubius extracted from male mice

Larval Stage	Age in hours from start of infection	Dry weight ug.	Log dry weight
3	46	0.38	-0.4202
3	72	1.01	0.0004
	72	1.04	0.0017
	72	0.7667	-0.1148
	72	1.96	0.2923
	72	1.01	0.0004
	72	1.2	0.0792
3	96	2.8	0.4472
	96	2.8	0.1761
3	98	2.2	0.3424
	98	4.0	0.6021
4	114	5.64	0.7513
	114	7.12	0.5514
4	120	5.5	0.7404
	120	3.5	0.5441
	120	4.5	0.6532
	120	3.5	0.5441
	120	3.0	0.4771
	120	7.5	0.6021
	120	4.0	0.8751
4	142	6.5	0.8129
	142	5.8	0.7634
	142	6.0	0.7782
	142	6.5	0.8129
	142	7.5	0.8751
	142	7.9	0.8976
	142	8.0	0.9031
	142	6.5	0.8129
4	160	10.1	1.0043
	160	8.1	0.9085
	160	9.5	0.9777
	160	14.0	1.1461
	160	6.2	0.7924
	160	6.2	0.7924
	160	8.0	0.9031
	160	5.0	0.6990

Appendix 2d (continued)

Larval Stage	Age in hours from start of infection	Dry weight µg.	Log dry weight
5	170	7.0	0.8451
	170	6.2	0.7924
	170	8.6	0.9345
	170	7.0	0.8451
	170	6.6	0.8195
	170	8.5	0.9294
	170	8.0	0.9031
5	168	7.1	0.8513
	168	10.6	1.0253
5	190	22.9	1.3598
	190	20.8	1.3181
	190	10.0	1.0000
	190	11.5	1.0607
	190	26.5	1.4232
	190	12.2	1.0804
	190	24.5	1.3892
	190	14.0	1.1461
	190	18.5	1.2672
	190	20.5	1.3118
5	192	22.1	1.3444
5	200	25.5	1.4065
	200	18.0	1.2553
	200	22.0	1.3424
	200	19.0	1.2833
	200	22.5	1.3522
5	216	29.1	1.4639
5	220	31.5	1.4793
	220	38.0	1.5798
	220	34.0	1.5315
	220	34.0	1.5315
	220	40.5	1.6075
	220	35.5	1.5502
5	240	38.9	1.5899
	240	40.9	1.6117
	240	55.0	1.7404
	240	41.4	1.6170
	240	29.5	1.4698
	240	31.0	1.4914

Appendix 2d (continued)

Larval Stage	Age in hours from start of infection	Dry weight µg.	Log Dry weight
5	248	43.8	1.6415
	248	51.9	1.7152
	248	46.0	1.6628
	248	40.0	1.6021
	248	43.2	1.6355
5	336	58.0	1.7672
	336	58.5	1.7634
5	432	48.8	1.6884
	432	48.2	1.6830
5	1008	57.9	1.7627
	1008	67.0	1.8261
	1008	74.5	1.8722
	1008	65.0	1.8129
	1008	61.5	1.7889

APPENDIX 3

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Determination of Dry Weights of Small Aschelminthes (< 0.1 µg)

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A method is described here by which the individual dry weight may be determined for Rotifera and Nematoda of less than 0.1 μg dry weight. Fresh weights of rotifers and nematodes have previously been obtained either by the weighing of scale models of known density and volume or by approximating the animal shape to geometrical figures from which weight can be calculated (Andrássy, 1956; Overgaard-Nielsen, 1949; Neuwerck, 1963). Dry weights for groups of nematodes (> 10 μg), using a relatively coarse balance, were obtained by Wieser (1960), and Fernando weighed groups of 100000 larvae of *Necator americanus* to the nearest 0.01 mg on a semi-micro balance (Fernando, 1963). No previous attempts have been described for rotifer dry weights although Depoorter and Magis report that 12000 washed eggs of *Brachionus leydigi*, dried at 80° C, weighed 630 μg (Depoortere and Magis, 1967). By cumulating relatively small groups of dried animals, we have been able to detect differences in dry weight of 0.005 μg/individual.

The rotifers used were *Keratella quadrata* (O.F. Muller) of mean length 141.7 ± 14 μ. The nematodes were the free-living stages of a mammalian parasite, *Nematosporoides dubius* (Bayliss) divided into 24-hour age groups, the first batch being hatched 48 hours after the eggs were passed from the host animal and placed in culture, the last being seven days old; the length range was $393 \rightarrow 581$ μ.

Homogeneous samples of approximately 50 rotifers were accurately counted, briefly washed in distilled water and dried on cavity slides in a $CaCl_2$ desiccator at room temperature for at least 48 hours (Lovegrove, 1966). After this period a sample was transferred with tungsten needles or fine hairs to the scale pan of a Cahn Gram Electrobalance (10^{-7} g;

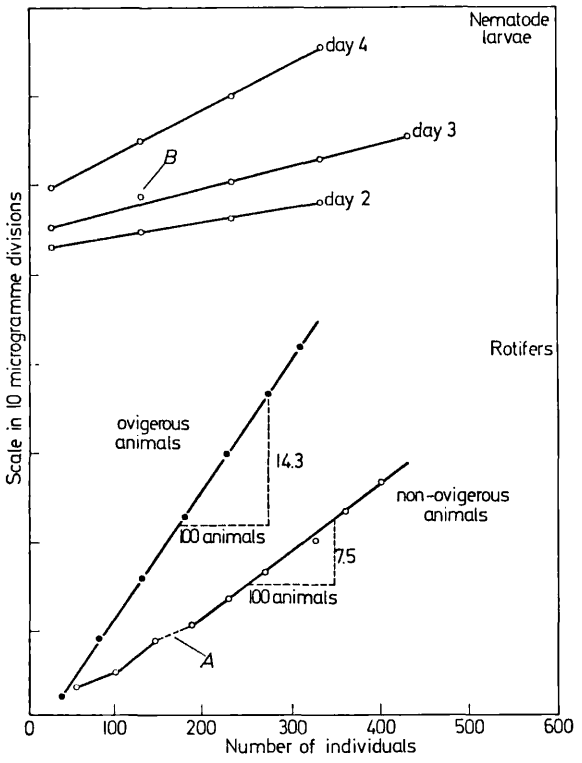

Fig. 1. The dry weight of *Keratella quadrata* and larval *Nematosporoides dubius* from cumulated samples of 50 or 100 individuals. *A* Known loss of animals from scale pan. *B* Probable increase in moisture content of sample

sensitivity, 0.2 μg; accuracy, 0.05% of the scale range), all damaged animals being discarded. Weighings were within the 1 mg range of the balance scale and each weighing was delayed for a short period to allow moisture absorbed during transfer to be removed by silica gel in the balance chamber. After the first reading, the balance was not returned to zero but a second sample was added to the loaded scale pan and the new weight recorded. This process was continued until up to 400 rotifers had been accumulated and weighed. The method gives equally satisfactory results after only four or five sets of cumulated weighings.

Fig. 1 shows the linear relationship between ovigerous or non-ovigerous rotifer numbers and their dry weights; since weights are cumulated an absolute scale of weight is not necessary and therefore the vertical axis is simply marked in 10 μg divisions. From the line fitted

by eye, the weight of 100 individuals was read off and the weight of one calculated. The table presents the mean weight of one individual *Keratella*, without eggs and carrying one egg. Five replicate weighings of a single sample containing approximately 90 animals gave a mean sample weight and standard error of 6.62 ± 0.07 µg. The cumulation of samples provides an automatic check on loss of animals during transfer to the scale pan or possible gain in moisture content during the weighing (see Fig. 1). A regression could be calculated (and has been for other results) but it was not considered necessary for this data.

The nematodes were treated in the same way as the rotifers, using samples of 100 larvae and cumulating up to 600 individuals. However, their number could be accurately determined only before drying and not immediately prior to weighing since they tend to stick together so that those lost during transfer to the pan could not be counted. The mean individual dry weights obtained are presented in the Table. This demon-

Table. *Mean dry body weight of* Keratella quadrata (Rotifera) *and larval* Nematosporoides dubius (Nematoda)

Keratella quadrata (Muller)		*Nematosporoides dubius* (Bayliss)	
Reproductive state	Mean individual weight (µg)	Age of larvae in days	Mean individual weight (µg)
Ovigerous ♀	0.143	day 2	0.016
Non-ovigerous ♀	0.075	day 3	0.026
Egg (by subtraction)	0.068	day 4	0.054

strates the usefulness of the technique for distinguishing different dry weights of various developmental stages of a very small species such as this nematode, from which a growth curve in terms of dry weight rather than calculated wet weight can be derived. Processes involving changes in hydration render wet weight estimates unreliable for comparisons of metabolism of different growth stages of a species or between different groups of species. Accurate dry weights are essential for this purpose.

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