POPULATION DYNAMICS OF THE ENTOMOPHILIC NEMATODE

ROMANOMERMIS CULICIVORAX

by

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TO MY PARENTS

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The major population parameters involved in the life-cycle of the entomophilic nematode Romanomermis culicivorax were estimated quantitatively by controlled laboratory experimentation using the mosquito Culex pipiens fatigans as the host. Parameters estimated included the rates of survival, immigration and emigration for, and between, each of the five stages in the nematode life-cycle. Emphasis was placed upon estimation of the rate of parasite transmission (infection) and the influences of the density and age of both infective stage and host were investigated, together with duration of exposure and the size of the infection arena.

The sex of individual nematodes was shown to be environmentally determined: dependent upon the parasite burden per host. A close study of the statistical distribution of the numbers of parasites within the host population, linked with the density-dependent sex determination of the nematode, showed that the parasite population would be regulated in a density-dependent manner.

A mathematical model, incorporating the parameters estimated in the laboratory,was used to predict the equilibrium level of infection in the host population. Predictions obtained from the model were compared with the results from long-term, free-running, experiments. The parasite was found to depress the equilibrium host population significantly.

Results from both experimental and theoretical investigations are discussed in relation to such host-parasite interactions in the field. The major factors which regulate the parasite population are also considered. Finally, given the density-dependent nature of the environmental sex determination, the statistical distribution of the numbers of parasites within the host population and the model predictions, the potential of the parasite as a biological control agent of mosquitoes is critically assessed.

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ENCLOSURE

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CHAPTER 1 : INTRODUCTION

1. INTRODUCTION

An understanding of the processes that determine the dynamics of animal (and plant) populations is of major importance. The reasons for the importance of this subject are many and varied, ranging, at one extreme, from the need to understand and predict population levels for harvesting, to the control of agricultural pests and agents of human disease.

Recent advances in understanding the dynamics of populations have largely stemmed from the use of mathematical techniques, supported by laboratory and field studies. One area where much progress has been made is in the study of insect populations, both because of the relative ease with which they may be studied and their economic and medical importance (see Varley, Gradwell & Hassell, 1973; Hassell, 1978).

There are several reasons why the understanding of the population ecology of parasites is less advanced than that of free-living organisms. The most important of these are the closeness of the physical association of the parasites and hosts (usually necessitating destructive sampling of the host in order to study the parasite), and the complexity of many parasite life-cycles (see Smyth, 1976).

Despite such problems, substantial advances have been made, again, by utilizing a mathematical approach in association with field and laboratory studies (see Anderson, 1982c). The basis of these advances, the use of modelling techniques, dates back to the early part of the century, to work upon malaria prevention (Ross, 1911). Expansion of this early research has taken several routes, a principal one being directed towards free-living organisms (Volterra, 1931; Lotka, 1932), culminating in the formulation of models of the dynamics of insect populations (Nicholson, 1933, 1954; Rogers, 1972; Hassell, Lawton & Beddington, 1976; Beddington, Hassell & Lawton, 1976; Hassell, Lawton & Beddington, 1977).

The theory of epidemiology and disease transmission also showed advances (Kermak & McKendrick, 1927), but without such an expansive data base, progress was slower. The introduction of stochastic components into models (Bartlett, 1960; Baily, 1975) was a major step, as was the incorporation of seasonality (Dietz, 1976).

Within the field of schistosomiasis, there is a body of literature that deals specifically with mathematical models of transmission (see Cohen, 1977; Fine & Lehman, 1977). Macdonald (1965) was one of the first to explore the whole life-cycle of the schistosome in terms of its transmission, producing the concept of a 'breakpoint', a transmission threshold below which the parasite population tends towards extinction (see also May, 1977b). Further work,derived from that of Macdonald,has shown the importance of the statistical distribution of the adult worms; particularly in respect of the breakpoint concept and the need of these dioecious parasites to form pairs (May, 1977c; Bradley & May, 1978). Studies have also explored the prevalence and dynamics of transmission in the intermediate snail host, showing factors such as severe parasite-induced host mortality and the survival and infectivity of miracidia to be important determinants of the prevalence of the parasite (Anderson & May, 1979; Anderson, Mercer, Wilson & Carter, 1982).

Investigations of the relationships between aspects of parasite ecology and the dynamics of parasite populations (and those of their hosts), has provided a major starting point for further recent work. Specifically, the effects of the statistical distribution of parasites within the host population and parasite-induced host mortality (Crofton, 1971a & b). This has formed the base of much expansion in conceptual terms, particularly related to parasite burdens, the lethal limit and parasite-induced host mortality (May, 1977a; Anderson & May, 1978; May & Anderson, 1978; Anderson, 1979 b & c).

With an ever expanding theoretical framework, experimental data supportive of theory became harder to find from within the literature. This resulted in specific experimental investigations linked to aspects of theory (Lanciani, 1975, 1979; Anderson & Lethbridge,

1975; Anderson & Michel, 1977). Investigation of complete or large parts of parasite life-cycles, tailored to further theoretical examination, was the next natural progression. As yet there are few such studies, but those conducted have considered the cestodes Caryophyllaeus laticeps (Anderson, 1974) and Hymenolepis diminuta (Keymer & Anderson, 1979; Keymer, 1980b, 1981, 1982), the digenean Transversotrema patialense (Anderson & Whitfield, 1975; Anderson, Whitfield & Mills, 1977; Whitfield, Anderson & Bundy, 1977; Anderson, Whitfield & Dobson, 1978; Anderson, Whitfield, Dobson & Keymer, 1978; Mills, Anderson & Whitfield, 1979; Mills, 1979, 1980a & b), the monogenean Gyrodactylus bullatarudis (Scott, 1982) and the protozoan Ichthyophthirius multifiliis (McCallum, 1982).

The basis of this thesis is to present the results of an experimental study on the population biology of the mermithid nematode Romanomermis culicivorax Ross and Smith. Quantitative estimates of the rate parameters that determine the flow through the parasite life-cycle were obtained and the dynamics investigated with the use of a simple, deterministic mathematical model. The model was formulated upon the framework described by Anderson & May (1978) and May & Anderson (1978).

Given the direct nature of the parasite life-cycle, it was attempted to examine the whole of the life-cycle, and, in addition, the effect upon the host population. The aim of the study was two-fold: (1) to investigate the ecology of the parasite, with particular emphasis upon those factors of major importance in the dynamics of the parasite population and (2) to better assess the potential of the parasite as a biological control agent of mosquitoes.

CHAPTER 2 : POPULATION DYNAMICS OF *R. CULICIVORAX*

2.1 BIOLOGY OF THE HOST

In any host-parasite association the host and its biology will clearly be of prime importance in the persistence and dynamics of the parasite population. Consideration must therefore be given to the role of the host and aspects of it's life-history that impinge upon the association, both directly and indirectly.

In the association which forms the subject of this thesis the host is one of a range of larval mosquitoes (Diptera: Culicidae), which may be parasitized by the nematode Romanomermis culicivorax (Nematoda: Mermithidae).

The parasite is known to penetrate other aquatic arthropods but development within such hosts has not as yet been demonstrated (Ignoffo, Biever, Johnson, Sanders, Chapman, Petersen and Woodard, 1973; Poinar, 1979). The description of host biology will therefore be restricted to mosquitoes. However, as R. culicivorax has been shown to utilize at least 58 species of mosquito larvae as hosts (Poinar, 1979), the initial account will cover mosquitoes in general.

The life-history of mosquitoes involves free-flying, dioecious adults and aquatic developmental stages (the eggs, larvae and pupae) which show a complete metamorphosis. Unembryonated eggs are laid in a suitable aquatic environment (usually the surface film of a body of still, freshwater) by the female. The eggs embryonate and hatch to give the first larval stage; there are then three additional larval stages (instars) with a moult between each. The larvae feed as browsers, predators and, or filter-feeders, depending upon instar and species. The fourth instar moults to the pupal stage, which is a buoyant, active (but non-feeding) stage. Adult mosquitoes emerge from the pupae on to the surface film of the water. The time required for this developmental series varies with temperature and species (Shelton, 1973). The adults feed; males utilizing principally sugars, the females utilizing sugars and blood obtained from a range of vertebrate hosts. Males are polygamous and females which have been mated and blood fed proceed to develop and lay eggs. Females can undergo many gonotrophic cycles and lay many batches of eggs throughout their life-span; even

those unable to obtain a blood meal may still be able to produce viable eggs, a process known as autogeny (Clements, 1963; Jones, 1968).

The species of mosquito used as the host will obviously affect the method of maintenance and possibly the protocol of some experiments. The biology of the species of mosquito used in this study will therefore be described in more detail.

Culex pipiens fatigans Wied. can develop over the temperature range 12°C. to 32°C. with optimum survival to the imago (adult) at 26°C. (Shelton, 1973). The approximate developmental times for the various stages, at 26°C., are shown in table 2.1.1.

Table 2.1.1

Development times for C. p. fatigans

Stage	Mean Development time (hours)	Source
Egg	37	Gómez, Rabinovich & Machado-Allison, 1977
1st Instar	20	Shelton, 1973
2nd Instar	51	Shelton, 1973
3rd Instar	27	Shelton, 1973
4th Instar	43	Shelton, 1973
Pupae	48	Shelton, 1973; Gómez et al, 1977

The eggs are laid in rafts on the surface film of water, particularly in and around dwellings. The approximate developmental time from the egg to imago is 9 to 10 days at 26°C. The generation time (from egg to egg) is longer and depends upon the speed with which a female can obtain a blood meal and produce eggs. The mean shortest time from emergence to egg laying reported by Gómez et al, (1977) was 22 days, with a mean generation time of 45 days. The minimum generation time can be as short as 16 to 20 days, as estimated from colony maintenance.

The reproductive potential of C. p. fatigans is high, with a mean rate of oviposition of 9.2 eggs per female per day under laboratory conditions (Gomez et al, 1977). Such a high reproductive potential is

required as these mosquitoes frequently inhabit temporary environments where mortalities may be excessively high due to desiccation. Many other mosquito species also suffer from high rates of mortality, even when developing in permanent or semi-permanent aquatic environments (Lakhani and Service, 1974; Service, 1977, 1982).

Mosquitoes show many characteristics typical of 'r' strategists in the 'r-K' spectrum of bionomic strategies, (see Southwood, 1981) which gives them the (typical) ability of pest species to utilize new or temporary habitats and to recover from catastrophic crashes in population levels. This makes mosquito 'control' difficult and eradication almost impossible, except in certain circumstances (e.g. where the mosquito population exists as a small, isolated ecological island).

The ecology of the larvae of C. p. fatigans has already been briefly touched upon in terms of their development times. Further factors associated with both their ecology and behaviour likely to affect the host-parasite relationship include the need of the larvae for access to atmospheric oxygen for the purpose of respiration via the siphon (MacFie, 1917). This tends to produce a spatial clumping of the larvae at the top of the water column, their normal resting place. Despite this need to surface for breathing, C. p. fatigans larvae are denser than water and when not supported by the surface film or actively swimming, sink through the water column (Mellanby, 1958).

In addition to exhibiting aggregation at the top of the water column, mosquito larvae also show spatial clumping in their distribution over the habitat surface (Hocking, 1953; Wada, 1965; Service, 1968, 1977). This, associated with the high probability of infective stage aggregation, may have important consequences, both for the level of parasitism attained (depending upon how frequently the aggregations of host and infective stage coincide), and upon the stability of the host-parasite interaction (Crofton, 1971a, b; Anderson and May, 1978; Keymer and Anderson, 1979).

The larvae of the C. pipiens group of mosquitoes all tend to be positively phototactic and are attracted to any brighter than ambient

light source (Rudolfs and Lackey, 1929). This phenomenon has a clear implication for the lighting of infection arenas.

Disturbance, either by movement of the water or by shadowing, results in a combined active swimming-sinking response by the larvae in order to gain depth, a response used by the larvae in predator avoidance (Mellanby, 1958). Disturbance, assuming that it will interfere with or in some other way affect the infection process, must clearly be avoided in experimental infection regimes.

In non-tropical areas of the world, almost all exothermic organisms exhibit a seasonality in behaviour and abundance, the length of the season being directly linked to factors such as the environmental temperature and the day length (Uvarov, 1931; Ricklefs , 1973). Such seasonal effects also affect mosquito populations and impose upon the general population dynamics a cycle of seasonality which is inherent in the biology of the organism. Even in the tropics, usually viewed as an aseasonal environment, seasonality in mosquito abundance may be imposed by the occurrence of seasonal rainfall, e.g. monsoon areas.

The basic dynamics of the mosquito population are determined by inherent biological properties and seasonally imposed fluctuations. It is apparent that the effects of parasites, pathogens and predators will be super-imposed upon this basic condition to produce an infinitely complex inter-relationship between the various contributing factors.

2.2 BIOLOGY OF THE PARASITE

The biology of the entomophilic nematode R. culicivorax is characteristic of many mermithid nematodes. The life-cycle is direct and usually totally aquatic, with the host being a larval mosquito (figure 2.2.1).

Eggs, laid in the substrate of the aquatic environment, hatch to give infective preparasitic nematodes which directly penetrate larval mosquitoes and undergo a period of growth and development before emerging from the host as free-living postparasitic juveniles. The post-parasitic nematodes moult to become adults which mate; the females then proceed to develop and lay unembryonated eggs.

At ambient temperatures of around 25°C, the average generation time, i.e. the mean time taken to complete one life-cycle, is four weeks (Petersen, Chapman & Woodard, 1968). Embryonation of eggs proceeds in the manner described for R. communensis by Thornton and Brust (1979), giving rise to fully formed first stage juvenile, (J_1), nematodes within the egg membranes. The J_1 nematodes moult and hatch from the eggs as free-swimming infective J_2 (second stage) nematodes (Poinar & Otieno, 1974). The J_2 nematodes are frequently referred to as preparasites, a description indicating their position in the parasite life-cycle. The preparasitic nematodes are equipped with amphids, penetration glands and an anterior stylet (Nickle and Högger, 1974; Poinar, 1979) which are used in host location and penetration. Host infection is by direct penetration, the infective nematodes attach to and eventually pass through the integument of the larval mosquitoes, into the host haemocoele. Once within the haemocoele of a suitable host a period of diphasic growth and development is initiated.

Initial slow growth is followed by a very much more rapid phase. The change in the rate of development is probably associated with the second moult to the J_3 (third stage) nematode (Poinar and Otieno, 1974). Gordon, Bailey and Barber, (1974), stated that in the host Aedes aegypti, Reesimermis nielseni showed an 18 fold increase in length and a 16 fold increase in diameter during the 6-8 day developmental period, which was also diphasic. The parasitic phase in the R. culicivorax - C. p. fatigans

Figure 2.2.1 Diagrammatic representation of the life-cycle of Romanomermis culicivorax.

a Egg

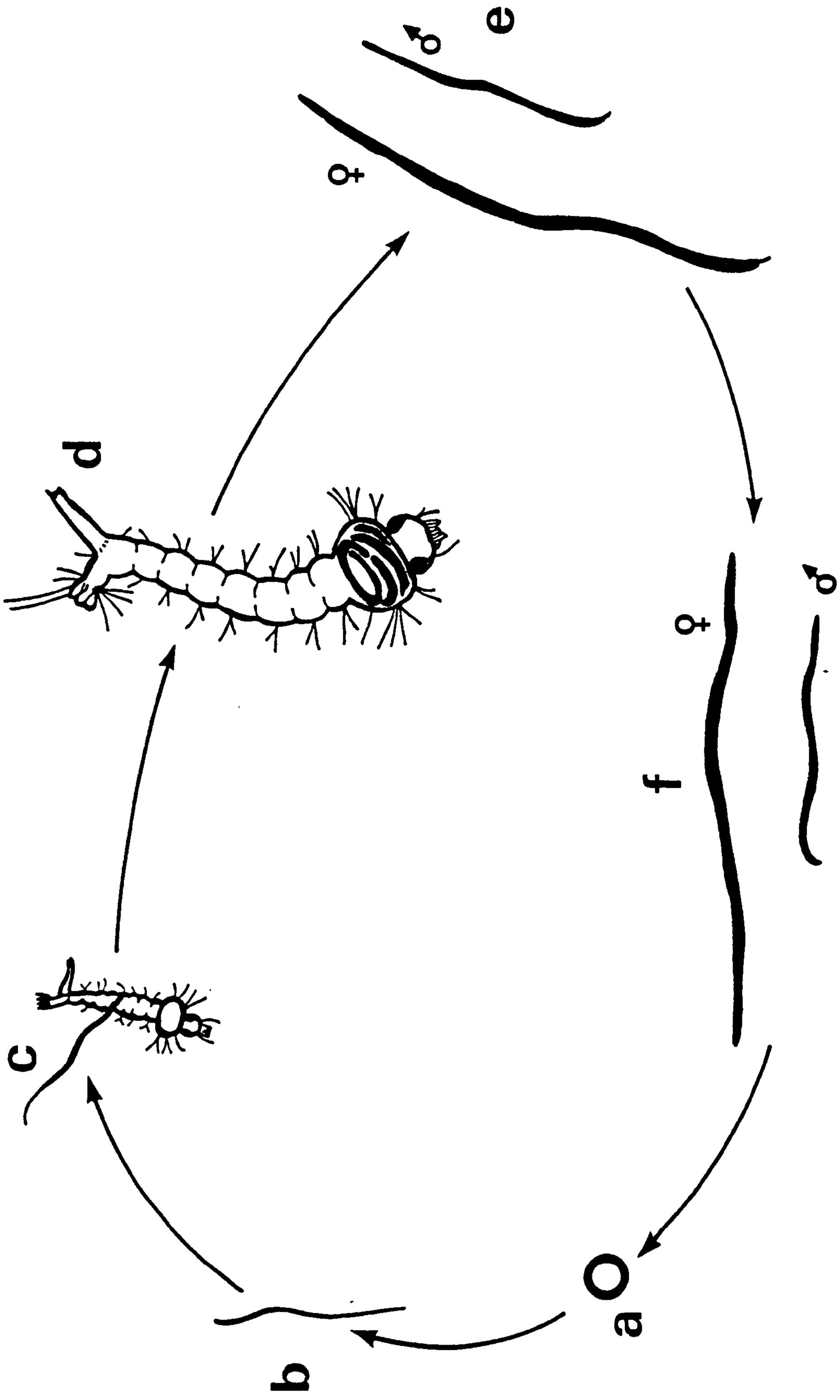
b Preparasitic (infective) nematode

c Penetration of the host

d Parasitic nematode within the host

e Male and female postparasitic juvenile nematodes

f Adult (sexually mature) male and female nematodes


e
♂
♀
♀
d
c
♂
♀
♀
a
b

association lasts from 6 to 11 days at 25°C. The length of this period depends upon the number of parasites per host (see section 2.5. (ii)).

Feeding occurs during the parasitic phase, and in the absence of a functional gut parasitic mermithids absorb nutrients across their body wall (Lee and Atkinson, 1976). Evidence for this mode of uptake is sparse, but, in the main, convincing. Rutherford and Webster (1974) demonstrated transcuticular uptake of glucose by Mermis nigrescens; Poinar and Hess (1977) showed that parasitic stages of R. culicivorax could take up ferritin particles through pores in their body wall membranes, and Riding (1970) showed the Tylenchid nematode Bradynema sp. to have a surface covered by microvilli, suggestive of an absorptive function.

The nutrients absorbed by mermithids are stored in the trophosome, chiefly in the form of lipids (Gordon, Finney, Condon and Rusted, 1979), which, in spatial terms is the most economic way of storing energy. The principle components of the stored food reserves and the effect that the absorption of these substances has upon the haemolymph of the host is discussed in detail by Rutherford and Webster, (1978), Gordon, Condon, Edgar and Babie (1978), Gordon et al. (1979) and Schmidt and Platzer, (1980a and b).

During the parasitic phase the nematode stores food that lasts for the remainder of its life-cycle; being used by the postparasitic juvenile forms and by the adults as an energy source.

Neither the postparasitic forms nor the adults have a functional gut and both are non-feeding (Nickle, 1972; Lee and Atkinson, 1976).

A somewhat unusual characteristic of this parasite is that the sex of the adult is determined environmentally, during the parasitic phase; the controlling variable is the number of parasites per host (Petersen, 1972). The same phenomenon has been reported for other mermithids (Parenti, 1962; Welch, 1965; Ezenwa and Carter, 1975; Petersen, 1977; Charnov and Bull, 1977) and a similar situation occurs in some plant parasitic nematodes (Ellenby, 1954; Triantaphyllou and

Hirschmann, 1973) and insect parasitoids (Charnov, Los-den Hartogh, Jones and van den Assem, 1981).The precise cause of this phenomenon in mermithids is unknown, but is presumed to be nutritional; other possibilities include accumulation of nematode secretions or waste products within the host and oxygen deficiency (Welch, 1965). In some associations the sex of the host affects the sex of the parasite (Strelkov, 1964 cited by Petersen, 1977). In mermithids the trend is towards a higher proportion of male nematodes as the parasite burden increases. (See section 2.6 for full discussion).

The nematodes emerge from their hosts once their development is complete. Emergence is almost always from a larval mosquito; infection generally prevents the pupation of the host. The parasite creates a hole in the integument of the host through which it escapes into the external environment. This rupturing of the host cuticle results in the rapid loss of haemolymph and the death of the host (Petersen, Chapman and Woodard, 1968; Nickle, 1974). In other mermithid-host associations the emergence of the parasite does not necessarily result in the death of the host (Petersen, Chapman and Woodard, 1967; Poinar and Petersen, 1978).

The newly emerged J_3 nematodes are identifiable as male and female by the difference in the distance between the posterior end of the trophosome and the base of the caudal appendage and by the obvious genetalia of the female (Tsai and Grundmann, 1969; Ross and Smith, 1976). The postparasitic juvenile nematodes are distinguishable from the adults by the presence of a distinct caudal appendage, which is lost during the final moults (Tsai and Grundmann, 1969; Ross and Smith, 1976).

The juvenile postparasitic R. culicivorax burrow into the substrate of the aquatic environment, where, after a developmental period (see section 2.7 (ii)) a double moult takes place (Poinar and Otieno, 1974), resulting in sexually mature male and female nematodes. The adult nematodes are sexually active as soon as they are free of their J_3 and J_4 cuticles; mating even occurs before the female has completely moulted. The males are polygamous (see section 2.7 (iv)), the number of females with which they copulate being dependent upon the number

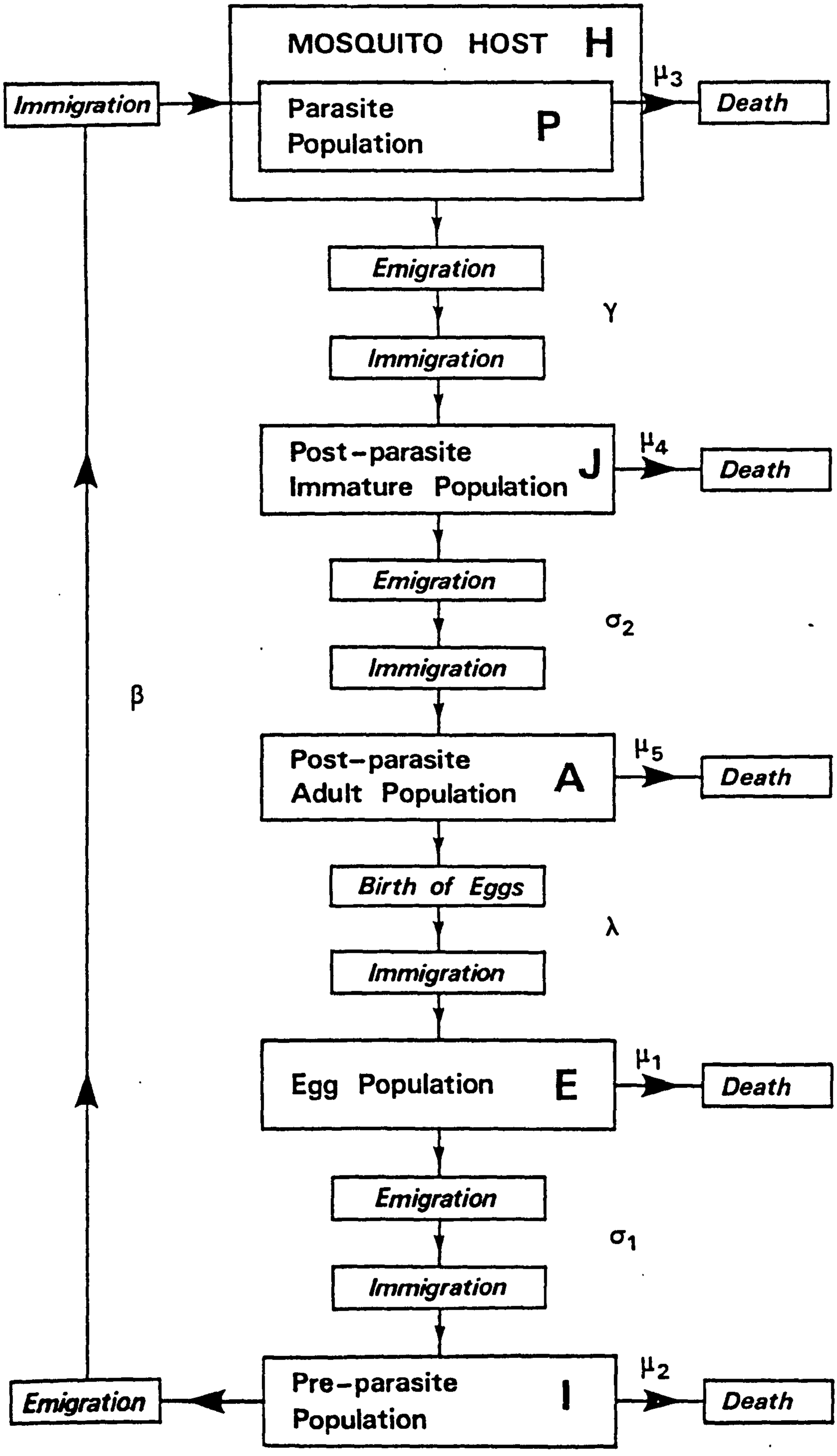
of females available and the length of time males and females are in proximity. After a brief developmental delay females initiate egg laying, producing between 1,388 and 4,431 eggs (mean 2,480) over about a three week period (Petersen, 1975). The eggs are covered with a sticky surface coat and adhere to anything they contact. Once females have completed egg laying their food reserves are depleted and they die.

The life cycle of the parasite is shown in figure (2.2.2) as a flow chart; this indicates the various sub-populations and also illustrates the direction of flow of individual organisms between the various populations of distinct developmental stages. Figure (2.2.2) also defines the population rate parameters which determine the size of the populations.

Figure 2.2.2 Representation of the life-cycle of R. culicivorax as a flow-chart.

Symbol	Definition
H	Population density of the host at time t
P	Population density of the parasitic nematodes at time t
J	Population density of postparasitic juvenile nematodes at time t
A	Population density of adult nematodes at time t
E	Population density of the nematode eggs at time t
I	Population density of the preparasitic (infective) nematodes at time t
μ_1	Instantaneous rate of nematode egg mortality/egg/unit time
μ_2	Instantaneous rate of preparasitic nematode mortality/preparasite/unit time
μ_3	Instantaneous rate of parasitic nematode mortality/parasite/unit time
μ_4	Instantaneous rate of postparasitic juvenile nematode mortality/juvenile/ unit time
μ_5	Instantaneous rate of adult nematode mortality/adult/unit time
σ_1	Instantaneous rate of nematode egg hatch /egg/unit time
β	Instantaneous rate of infection/host /preparasite/unit volume/unit time
γ	Instantaneous rate of parasitic nematode emergence from the host/parasite/unit time
σ_2	Instantaneous rate of moulting of juvenile nematodes (maturation)/juvenile/unit time
λ	Birth rate of nematode eggs/female/unit time

Other symbols used are defined in the text where appropriate. Redefinitions of symbols, in the light of changing attitude, also occur in the text.


MOSQUITO HOST H
Parasite Population P
Immigration
Death
μ_3
Emigration
Immigration
Post-parasite Immature Population J
μ_4
Death
Emigration
Immigration
Post-parasite Adult Population A
μ_5
Death
Birth of Eggs
Immigration
Egg Population E
μ_1
Death
Emigration
Immigration
Pre-parasite Population I
μ_2
Death
Emigration
γ
σ_2
β
λ
σ_1

2.3 MATERIALS AND METHODS

2.3 (i) Host and Parasite Species

The host species used in this study was Culex pipiens fatigans (=quinquefasciatus), supplied by Mr. R. Page and Dr. G. B. White of the Entomology Department, the London School of Hygiene and Tropical Medicine. The strain of the nematode Romanomermis culicivorax was supplied by Dr. J. J. Petersen formally of the U.S.D.A. Louisiana, U.S.A.

2.3 (ii) Maintenance of the Host

The mosquitoes were maintained in cubic, net cages (side length 45 cm.) in a constant temperature room at 25°C. and 70% RH. They were given 10% sucrose solution on cotton wool ad libitum. Blood meals were provided, by presenting laboratory mice twice weekly, for a period of 20 - 60 minutes. The mice were previously anaesthetized with "Sagatal" (pentobarbitone sodium) (May and Baker Ltd.).

Egg rafts were collected by placing 500 ml crystalizing dishes half full of water into the cages overnight. The egg rafts were removed and placed into plastic sandwich boxes of various sizes containing about 3 cm depth of aged tapwater.

Mosquito larvae hatched from the egg rafts were fed breadcrumbs made from dry household brown bread passed through a 600 μm sieve. The water was changed and more food provided on a daily basis. Pupae were removed as they formed and were placed in containers of water in empty mosquito cages, into which the adults emerged.

2.3 (iii) Maintenance of the Parasite

The procedure described by Petersen and Willis (1972) was followed with slight alterations as necessary. For parasite maintenance, stock jars were prepared using wide-mouth glass jars of approximately 160 ml volume with plastic caps. Into each jar was placed about 2 cm depth of sterile (autoclaved) gravel with a particle size of between 1 and 2 mm. In addition to the gravel approximately 100 ml of fish tank water was

placed into each jar, giving a water depth above the gravel of about 2 cm. Jars thus prepared were set aside for a period of at least three weeks, as the nematodes would not burrow into recently sterilized gravel.

Postparasitic nematodes were placed into prepared culture jars at the rate of about 60-100 females and 100-200 males per jar. After three weeks the surplus water was drained off, leaving viable eggs ready to hatch in the moist sand. Eggs remained viable for up to 6 months when kept at 25°C. The eggs were induced to hatch by flooding with dechlorinated (aged) tap water. Hatching was allowed to continue for 2-4 hours before decanting the liquid containing the free-swimming infective stages. By removing the surplus water, restoppering and storing the nematode cultures for three to six weeks, it was possible to obtain one or two further hatchings from each culture.

Twenty-four hour old (1st instar) mosquito larvae were exposed to newly hatched infective nematodes for up to 24 hours at a ratio of approximately ten nematodes to one mosquito (10:1). The infections were carried out in a plastic sandwich box containing between 1000 and 10,000 mosquito larvae in 0.5 to 1.0 litre of water. Infected hosts were maintained in the manner described above (section 2.3 (iii)) for uninfected larvae. After 6-8 days, exposed but uninfected hosts pupated and were removed and destroyed, this was to obviate any possibility of the mosquito stock developing resistance to the parasite (Petersen, 1978a). Infected hosts died as a result of the emergence of postparasitic nematodes on days 6-11 post infection. Newly emerged nematodes were washed several times in distilled water to remove all detritus and were then sexed according to the criteria described by Tsai and Grundmann (1969). These 'cleaned' nematodes were then placed into the culture vessels as described above.

All utensils used in handling the nematodes were kept scrupulously clean to avoid infection of the cultures with fungal pathogens, which can cause severe problems in maintaining this nematode (Dhillon & Platzer 1978; Platzer & Stirling 1978; Stirling & Platzer 1978). Despite these precautions, however, some cultures were lost due to fungal infection.

2.3 (iv) Volumetric Estimation of Preparasite Density

To provide defined and constant conditions for infecting hosts it was necessary to place each host with a known number of infective stages per arena. Preliminary trials indicated that the density per unit volume of preparasites required to give a reasonable degree of infection was greater than could be practically counted by hand. For this reason a method of volumetric estimation of nematode density was employed. Variable volumetric pipettes, finnpipettes (Jencons Ltd., U.K.) accurate to ± 1% were used throughout the study.

A culture of eggs was flooded and the eggs allowed to hatch for two hours. The suspension of infective nematodes was decanted and thoroughly mixed. From this mixture a number of 0.2 ml aliquots were removed and placed onto petri-dishes. A 0.2 ml sample volume was chosen as this was the largest volume in which nematodes could be counted quickly and accurately. The samples were scanned using a dissecting microscope and the number of infective nematodes in each 0.2 ml. aliquot was recorded. The mean and variance of the samples were calculated. If the variance-to-mean ratio was less than or equal to unity, the distribution of the preparasites in the suspension was assumed to be random or random tending towards regularity (Elliot, 1971). Thus, each aliquot could be considered as being a representative sample and the mean value obtained, an accurate estimate of the nematode density (per 0.2 ml). If the variance-to-mean ratio was greater than unity the suspension was remixed and resampled.. Having obtained an estimate of the mean number of nematodes per 0.2 ml in the suspension, the number of nematodes in an aliquot of a given volume could be calculated. With this information it was possible to prepare a range of nematode densities in the infection arena by adjusting the volume of the nematode suspension used. This method facilitated the rapid replication of the infection experiments. The suspension was remixed frequently during the removal of material to maintain the distribution of the nematodes as random as practically possible.

The accuracy of this volumetric technique was assessed for three initial suspension densities, one high, one medium and one low density. A number of 0.2 ml aliquots were taken as described above and the mean

and variance of the samples were calculated. In each case the variance-to-mean ratio was less than unity and application of the χ^2 statistic (Elliot 1971) indicated that the nematode distributions were all random (table A1, appendix I). An aliquot size was then chosen and the expected number of nematodes for that volume was determined. Aliquots of the chosen volume were obtained and the numbers of nematodes in each aliquot counted. Using this data the mean, variance-to-mean ratio and 95% confidence limits about the mean were determined. This procedure was repeated several times and in each case the expected number of nematodes fell between the upper and lower 95% confidence limits at the sample mean. This suggested that, when averaged out over a number of samples, this volumetric technique of delivering infective nematodes was of sufficient accuracy to be used as an experimental procedure.

2.4 INFECTION OF THE HOST MOSQUITO LARVA

Infection of larval mosquitoes by R. culicivorax results from the direct penetration of infective, preparasitic nematodes through the host cuticle (Petersen, Chapman and Woodard, 1968). This section describes the experimental investigation of some of the factors, both biotic and abiotic, that influence the dynamics of parasite transmission.

For a parasitic species to maintain itself within a host population, it must obviously be able to effect transmission between hosts at a rate that will replace parasite mortalities, either natural or host induced. In a direct life-cycle parasite such as R. culicivorax where there is only one host involved in the parasite life-cycle, there is only one period within that cycle when transmission occurs. For indirect life-cycle parasites there may be two, three or four different hosts within the life-cycle and a similar number of periods of transmission. The factors which influence the rate of transmission will be of major importance in determining the equilibrium level of the parasite population. (Anderson, 1980).

There are many different modes of transmission dependent upon the precise form of the host-parasite relationship. Transmission may occur by the passive ingestion of eggs or other transmission stages, e.g. the eggs of Ascaris spp., and larvae of Ostertagia spp. The mode of infection may require the direct penetration of the host by an active infective stage e.g. schistosome miracidia penetrating the snail host and the larvae of Ancylostoma spp. penetrating mammals. Other methods of transmission include the use of a predator-prey link, where the infective stage may be sought out and consumed as a food item in itself (e.g. the eggs of Hymenolepis diminuta by the beetle intermediate host) or, where the infective stage occurs within the body of an intermediate host, infection occurring when the definitive host consumes the intermediate host as a prey item (e.g. cysticercoids of taeniid cestodes). The dynamics of infection will clearly be influenced by the mode of transmission. Rate determining factors frequently occur during this stage in the parasite life-cycle, especially when satiation effects occur in predator-prey interactions (Keymer and Anderson, 1979) and handling time or interference mechanisms occur in insect

parasitoid-host associations (Holling, 1965; Hassell, Lawton and Beddington, 1976 and 1977).

Available data relating to the infection dynamics of mermithid nematodes is largely unstandardized, previous investigations having used several experimental designs for assessing the success of infection. Petersen (1975) and many other workers have recorded the percentage of exposed hosts found to be infected, either by squashing or dissecting the hosts. This method may be used at any time during the parasitic phase, and can provide information relating to parasite establishment and also survival in the hosts. This method of assessing transmission does however have one major drawback, in that the percentage of hosts infected is dependent upon the frequency distribution and the degree of dispersion of the parasites within the host population. For a given mean or total number of parasites within a host population there will be a greater percentage of hosts infected when the parasite distribution is regular or random than when it is overdispersed (aggregated).

Galloway and Brust (1976) isolated individual hosts and maintained them until emergence of the nematodes. This method results in the loss of information relating to parasite establishment and survival but gives data on the proportion of surviving hosts that are infected, the parasite burden, the distribution of the parasites within the host population and information relating to the sex ratio of the nematodes. The method adopted to assess parasite transmission in this study was to dissect hosts exposed to infection as soon as possible after exposure and always within twenty-four hours; the number of newly established parasites being recorded for each host. This provided data on the proportion of hosts infected, the mean parasite burden per host and the distribution of parasites within the host cohort.

In this section the results of experiments designed to show the effect of various biotic and abiotic factors upon the dynamics of transmission will be presented and discussed. Those factors examined include infective stage age, density and survival; host density, host age, the duration of exposure and changes in arena size.

2.4 (i) Preparasite Survival

The dynamics of parasite transmission may be affected by many factors. The survival of the infective stages is one of the more important of these factors. Survival, however, should not be confused with infectivity, for although it would be expected that infectivity and the rate of mortality both vary with time, they need not vary at the same rate [see section 2.4 (iii)].

The average life-expectancy of the preparasitic nematodes will be a direct determinant of their density (at any period in time) within the environment; the longer the life expectancy the higher the density. This is an example of the well known ecological relationship between standing crop (the density of infective stages) and the rate of turnover (the rate of mortality). Increased infective stage density leads directly to increased parasite burdens and assuming that there is no loss of infectivity with age, the increase, as shown in section 2.4 (ii), is likely to be one of direct proportionality.

Petersen (1975) measured preparasite survival of R. culicivorax by estimating the proportion of actively swimming preparasites remaining at successive periods of time; maximum longevity was recorded as 120 hours at 24 - 27°C. This estimate of survival ignored the possibility of nematodes being alive but unable to swim actively in the water column. For this reason the survival characteristics of preparasitic R. culicivorax were examined in the following manner. Preparasitic nematodes, less than one hour old, were placed individually into plastic arenas containing 0.3 ml of filtered water obtained from an established aquarium (approximately neutral pH). For each experiment a cohort of one hundred preparasites was used, each nematode being examined at regular intervals. Death was defined as the failure to respond to three touches with a dissecting needle. To reduce errors each "dead" individual was rechecked at the two successive sampling periods. The experiments were carried out at constant temperature and in the absence of light. The water was partially replaced at every examination to prevent a build-up in salinity due to evaporation; salinity having been demonstrated to be injurous to preparasitic nematodes (Brown & Platzer, 1978).

Survival experiments were conducted at six temperatures, from 10°C to 35°C at five degree intervals. The proportions of preparasitic

nematodes surviving to time, t, at each temperature are displayed in figure (2.4.1 a-f).

From the proportion of nematodes surviving to time t, the instantaneous death rate (/nematode/unit time) may be estimated by using the method described by Anderson and Whitfield (1975), which assumes that the instantaneous death rate($\mu_{(t)}$) is constant over very small intervals of time. Thus,

$$\mu_{(t+0.5)} = [\ln P_{(t+1)} - \ln P_{(t)}]/[t_{(+1)} - t] \qquad (2.4.1)$$

where $P_{(t)}$ is the proportion of preparasites surviving to time t. The estimates of the rates of the instantaneous preparasite mortality thus obtained are shown in figure (2.4.2). In each case the instantaneous death rate, $\mu_{(t)}$, increases exponentially with preparasite age, the relationship being best described by a simple exponential model of the form,

$$\mu_{(t)} = a+\exp(bt). \qquad (2.4.2)$$

where the constants a and b may be estimated by a linear regression of $\ln \mu_{(t)}$ upon t. For each temperature this model yields a good statistical fit to the data (Figure 2.4.2).

Using the values of the constants a and b obtained from equation (2.4.2), predicted values of the proportion of preparasites surviving to time t may be calculated using the age-dependent survival model of Anderson and Whitfield (1975), where

$$P_{(t)} = \exp\left[\frac{a}{b}[1-\exp(bt)]\right] \qquad (2.4.3)$$

Predicted values of $P_{(t)}$ are plotted for each temperature in figure (2.4.1). To give some idea of the goodness of fit of this age-dependent survival model to the data, the 95% confidence limits about the model predictions are also plotted in figure (2.4.1) using the method described by Elliot (1971). The variances (S^2), about the model predictions, necessary to obtain the 95% confidence limits, were calculated from the

Figure 2.4.1 Preparasitic (infective) nematode survival at six temperatures.

The points represent observed values of the proportion of preparasites surviving to time t. The solid lines are the predictions of an age-dependent survival model (equation 2.4.3) and the dashed lines indicate the 95% confidence limits about the model predictions (see text).

At each temperature the values of the parameters a and b, of the model, are given.

a. 10°C a = 0.001367 b = 0.007163

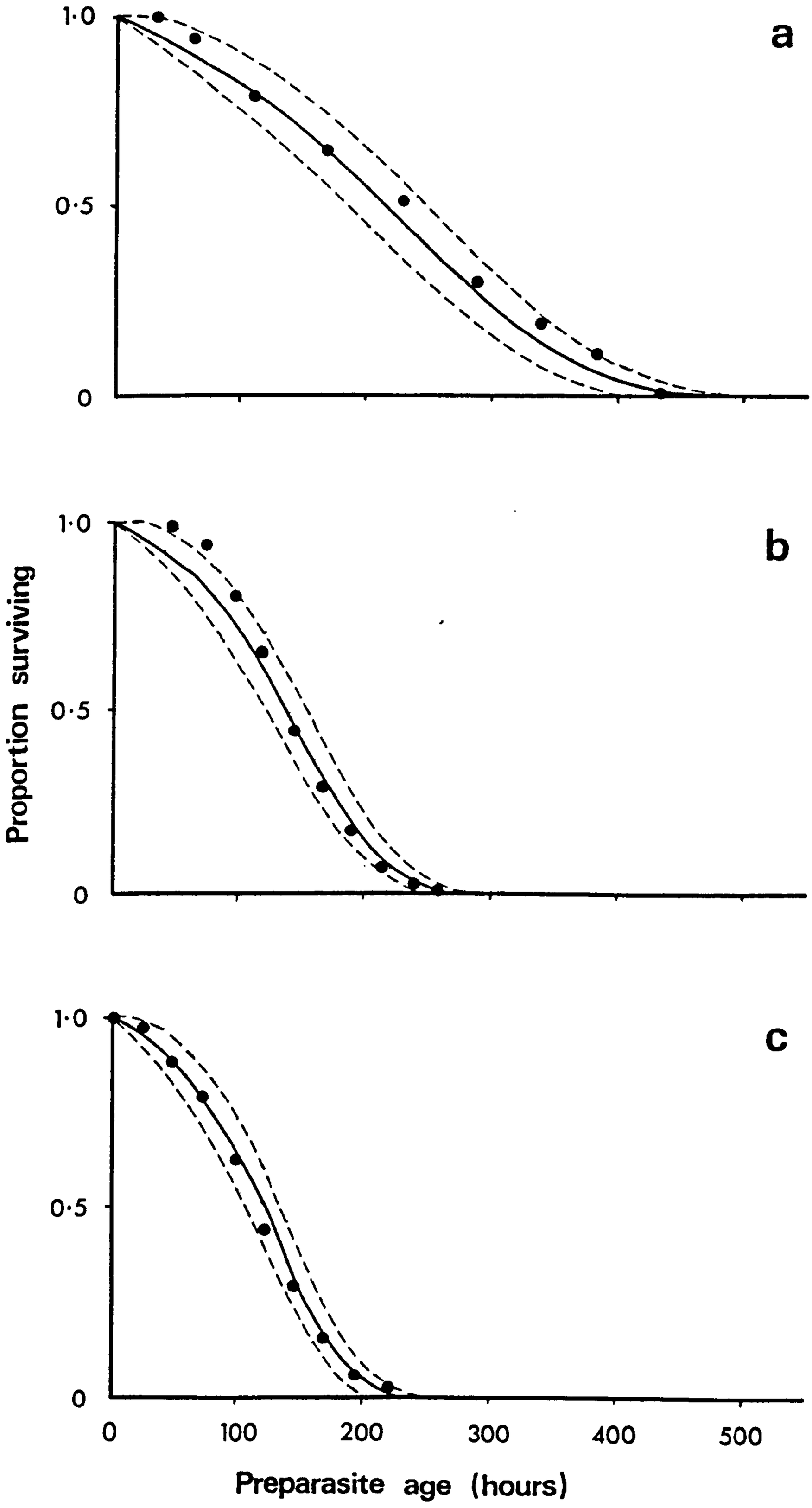
b. 15°C a = 0.001474 b = 0.015290

c. 20°C a = 0.001647 b = 0.017659

d. 25°C a = 0.000201 b = 0.048383

e. 30°C a = 0.002137 b = 0.048139

f. 35°C a = 0.005076 b = 0.094714


1·0
0·5
0
a
1·0
0·5
0
b
Proportion surviving
1·0
0·5
0
c
0
100
200
300
400
500
Preparasite age (hours)

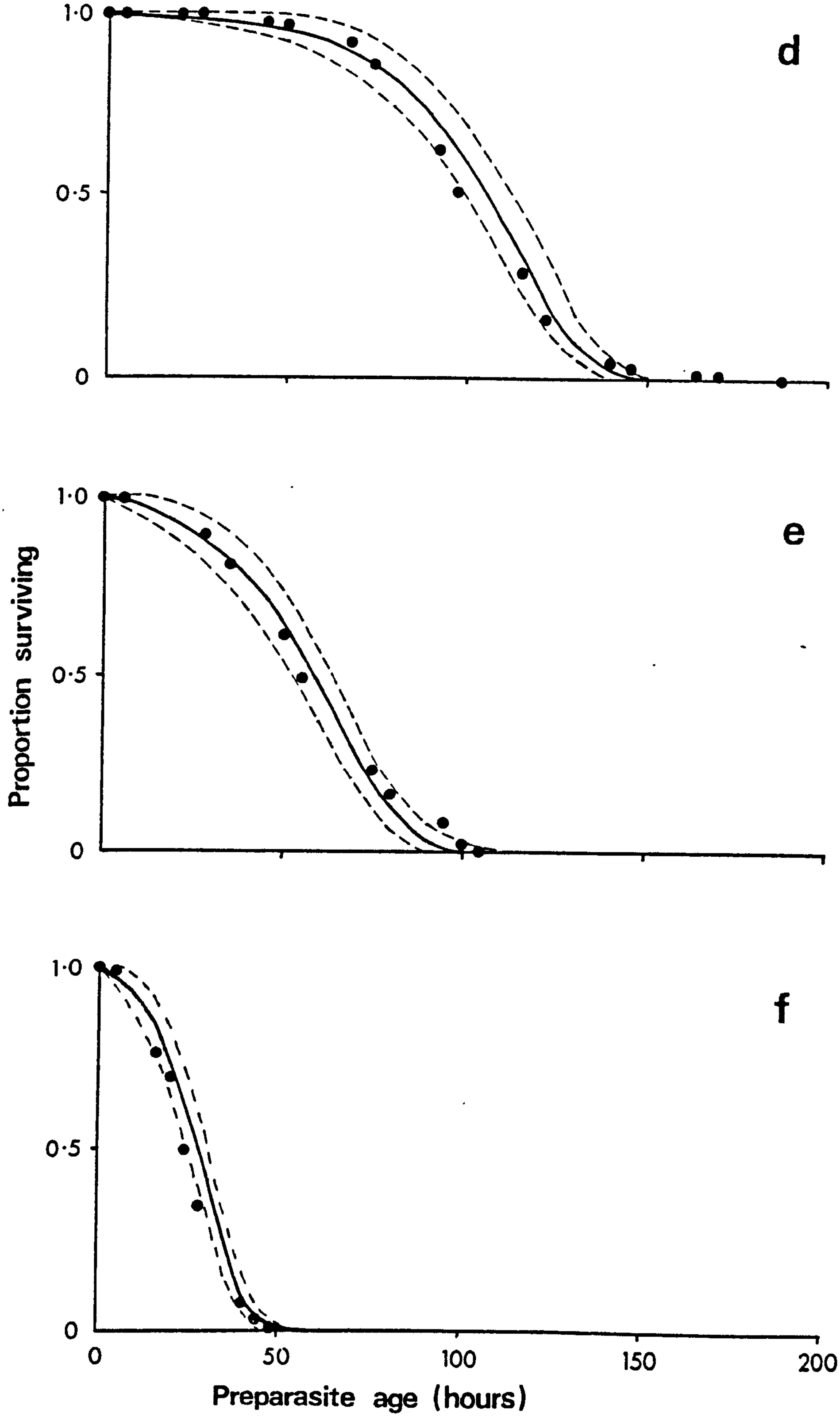
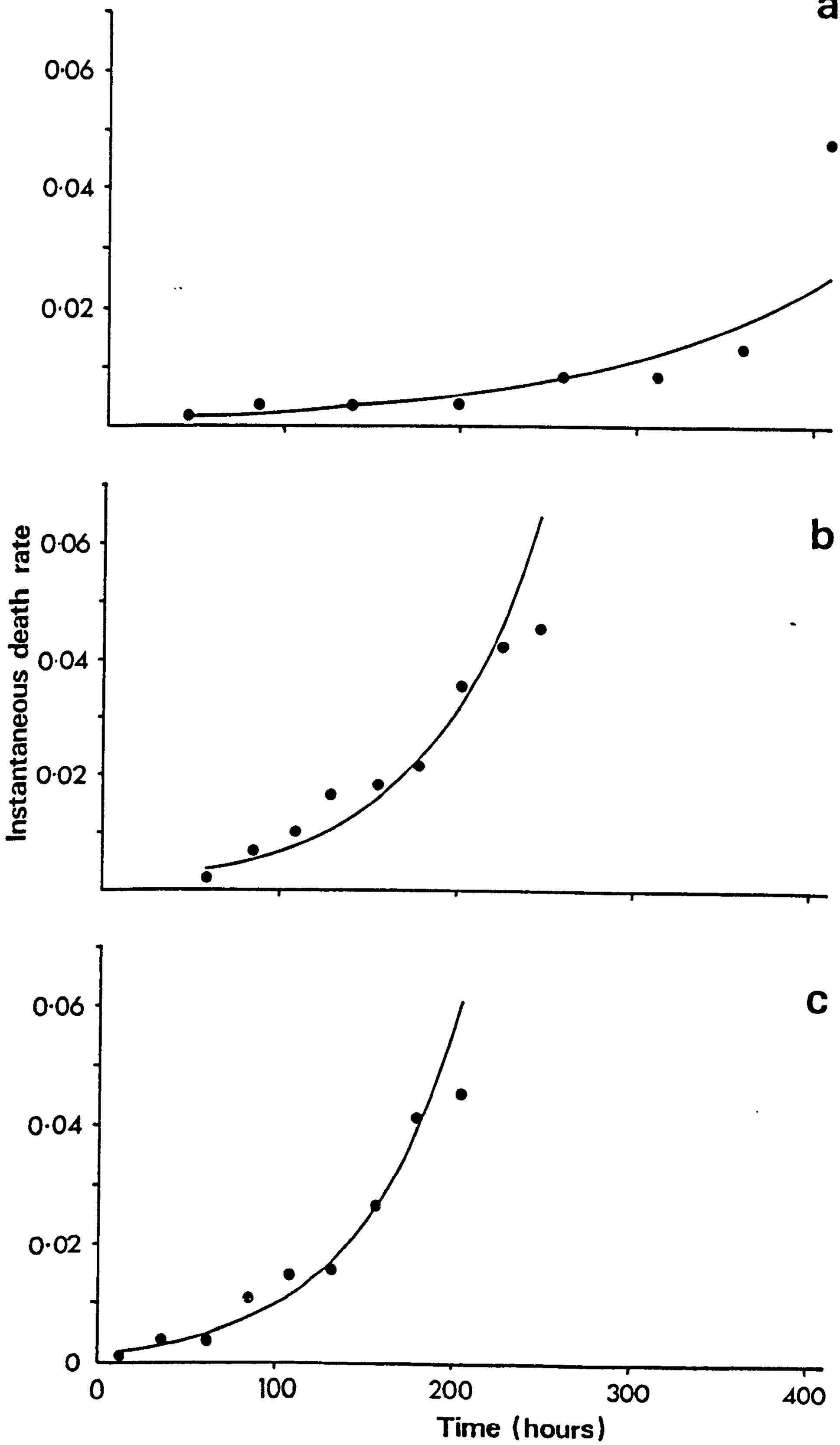
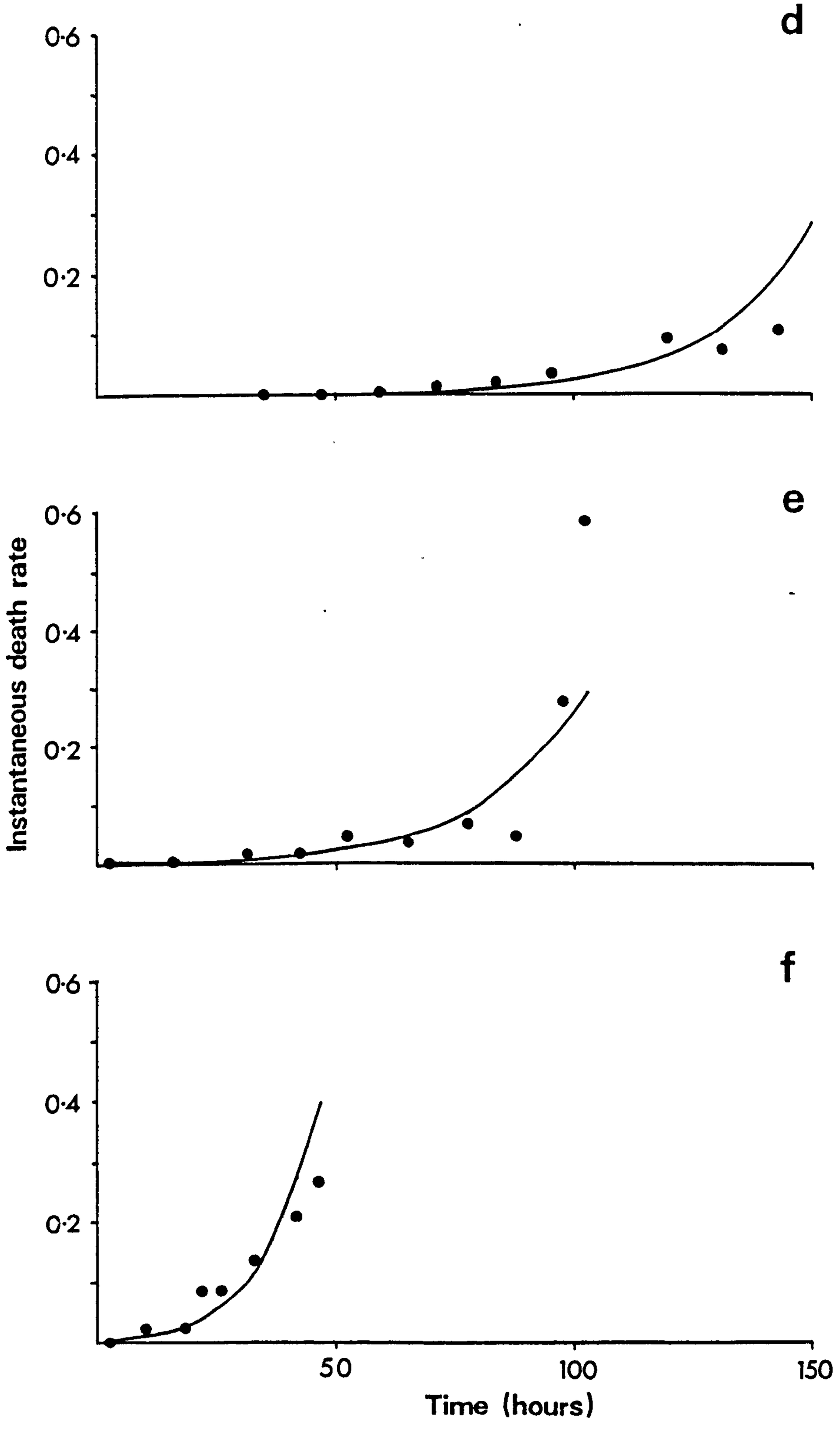

1·0
0·5
0
d
1·0
0·5
0
e
Proportion surviving
1·0
0·5
0
f
0
50
100
150
200
Preparasite age (hours)

Figure 2.4.2 The relationship between the age of preparasitic nematodes and the instantaneous rate of preparasite mortality, $\mu_{(t)}$, (per preparasite per hour).

The points are values of $\mu_{(t)}$ obtained from equation (2.4.1). The solid lines are given by a least-squares best-fit model (equation 2.4.2). The r^2 values of the fit of the model to the data are given at each of the six temperatures.

a	10°C	$r^2 = 0.8679$
b	15°C	$r^2 = 0.8692$
c	20°C	$r^2 = 0.9436$
d	25°C	$r^2 = 0.9295$
e	30°C	$r^2 = 0.9173$
f	35°C	$r^2 = 0.8775$


a
b
c
0·06
0·04
0·02
0
Instantaneous death rate
0
100
200
300
400
Time (hours)


d
0·6
0·4
0·2
e
Instantaneous death rate
0·6
0·4
0·2
f
0·6
0·4
0·2
50
100
150
Time (hours)

equation,

$$S^2 = \exp\left[\frac{a}{b}\left[1-\exp(bt)\right]\right]\left[1-\exp\left[\frac{a}{b}\left(1-\exp(bt)\right)\right]\right] \qquad (2.4.4)$$

(from Anderson and Whitfield, 1975).

The goodness of fit of the model to the data highlights the age-dependent nature of the survival of preparasites (figure 2.4.1) as does the exponential increase in the estimated instantaneous rate of preparasite mortality with time (figure 2.4.2).

Age-dependent survival characteristics are of some importance in the population dynamics of organisms (Hirsch, 1980). They are, however, frequently omitted from mathematical models of population processes because of the technical difficulties inherent in encapsulating age structure into non-linear models. Often, however, a constant rate may be used to capture the essential features of population change. For example, in the case of age-dependent survival, the inverse of the average life expectancy may often provide a good estimate of an average constant death rate over all age classes.

In this study, to facilitate the construction of a population model of the nematode (chapter 4), it was assumed that the instantaneous rate of preparasite mortality was constant. An estimate of this mortality rate may be obtained by taking the inverse of the mean expected life span, by calculating the integral of the predicted survival curve, (see Pollard, 1973),

$$1/\mu_2 = \int_{t=0}^{t_{(max)}} \exp\left[\frac{a}{b}\left[1-\exp(bt)\right]\right] dt \qquad (2.4.5)$$

where $\frac{1}{\mu_2}$ is the mean expected life span, μ_2 is the instantaneous rate of preparasite mortality (/preparasite/hour) and t_{max} is the maximum preparasite survival under the given conditions. Estimates of life expectancy at each temperature were calculated, (figure 2.4.3) and values of μ_2 for use in chapter 4 were also estimated. (table 2.4.1).

Figure 2.4.3 The influence of temperature upon preparasite life expectancy.

The points are values of the mean expected life-span of preparasites at six temperatures, estimated using equation (2.4.5). The solid line is a least-squares best-fit linear regression ($a = 260.08$, $b = -6.7517$, $r^2 = 0.9426$).

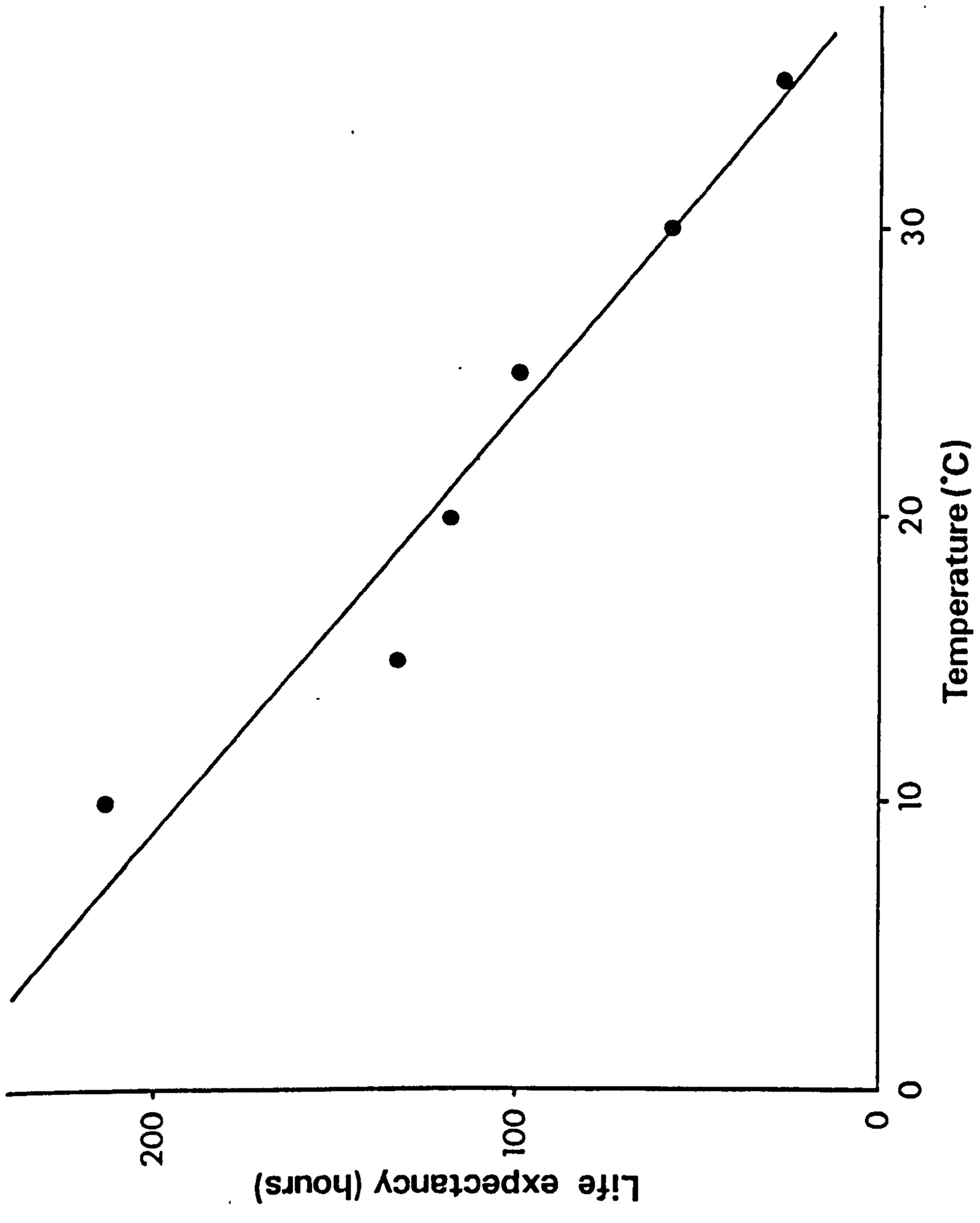

Life expectancy (hours)
0
100
200
0
10
20
30
Temperature (°C)

Table 2.4.1

Mean expected life-spans and mean instantaneous rates of mortality of preparasitic nematodes over a range of temperatures.

Temperature (°C)	μ_2	$\frac{1}{\mu_2}$
10	0.1126	8.88
15	0.1795	5.57
20	0.2047	4.89
25	0.2355	4.25
30	0.4281	2.34
35	0.8969	1.12

where μ_2 is the instantaneous rate of infective stage mortality (per preparasite per day) and $\frac{1}{\mu_2}$ is the mean life-expectancy in days.

The linear decrease in life expectancy with increasing temperature is most probably a reflection of the increasing use of finite reserves at the higher temperatures, linked to the temperature dependent increase in enzyme reaction speed. A similar relationship is found between life expectancy and temperature in the miracidia of Schistosoma mansoni (Anderson, Mercer, Wilson and Carter, 1982) and Fasciola hepatica (Wilson, Smith and Thomas, 1982).

2.4 (ii) Preparasite Density

Many investigations have shown that the density of infective stages within the environment directly affects the level of infection attained by potential hosts. For example Anderson (1978a) presented data showing direct proportionality between the initial density of infective stages and the mean parasite burden per host for cercariae of Transversotrema patialense infecting the definitive host Brachydanio rerio and for the miracidia of Echinostoma lindoense when infecting the intermediate host Biomphalaria glabrata. This situation is frequently the case, although at very high infective stage densities interference between the infective stages may occur or host behaviour may alter. Such effects tend to cause a decrease in the rate of infection, and result in a non-linear relationship between the degree of infection of hosts and infective stage density.

If the degree of infection is measured as the percentage or proportion of exposed hosts which become infected, then, as discussed in section 2.4, the relationship between the percentage infected and the infective stage density is dependent upon the statistical distribution of parasite numbers per host. Data relating the percentage of hosts infected to infective stage density classically show a non-linear rise, such that the percentage infection reaches a plateau as infective stage density increases (see Anderson, 1978a). This is as a consequence of the chance nature of the infection process where some hosts may be subject to multiple infections.

Published data, describing the relationship between infective stage density and the degree of infection for R. culicivorax, almost exclusively record the percentage of exposed hosts that become infected as the index of infectivity (Petersen and Willis, 1970; Petersen, 1973 a and b; Mitchell, Chen and Chapman, 1974; Galloway and Brust, 1977; Levy and Miller, 1977; Kurihara, 1979). Estimation of the rate of infection, defined per host per infective stage per unit time per unit volume, from such data is difficult in the absence of a knowledge of the degree of dispersion of parasite numbers per host.

With the object of developing a mathematical model to explore the dynamics of the parasite population, it was necessary to obtain estimates of all the relevant rate parameters. In this context, estimates of the transmission rate are especially important. A series of experiments were therefore designed to enable an estimate of the rate of infection to be made.

The experimental protocol consisted of exposing a single, first instar, 16 to 24 hour-old host, to a known density of preparasites in 3 ml of water at 25°C with a pH of approximately 7.0. Two durations of exposure were used in order to obtain two independent estimates; two and four hours. Infective stage density was varied over the range of 6 preparasites per arena to 90 per arena (i.e. 2/ml to 30/ml). The nematode densities in the infection arenas were prepared as described in section 2.3 (iv), with the exception of the lowest density (6 nematodes/arena) where absolute counts were made using a pasteur pipette. Between seventy-one (71) and two hundred and sixteen (216) hosts were exposed at each density of infective stages for each exposure duration (2 and 4 hours). After the assigned period of exposure had elapsed, the hosts were removed from the arenas, rinsed to remove any accidentally transferred preparasites and dissected. The number of parasites found within each host was recorded. The mean parasite burden per host was calculated for each exposure density and each exposure time, with the values plotted against the initial density of infective stages (figure 2.4.4 a and b). These data show, that under the experimental conditions employed, a relationship of direct proportionality exists between mean parasite burden and infective stage density.

The rate of parasite acquisition is dependent upon the relationship between the number of infections per host per unit time and the density of the infective stages (Anderson, 1978a). The change in the number of parasites (P) within the host population (H) through time, which reflects the rate of parasite acquisition, may be described thus,

$$dP_{(t)}/dt = \beta H I_{(t)} \qquad (2.4.6)$$

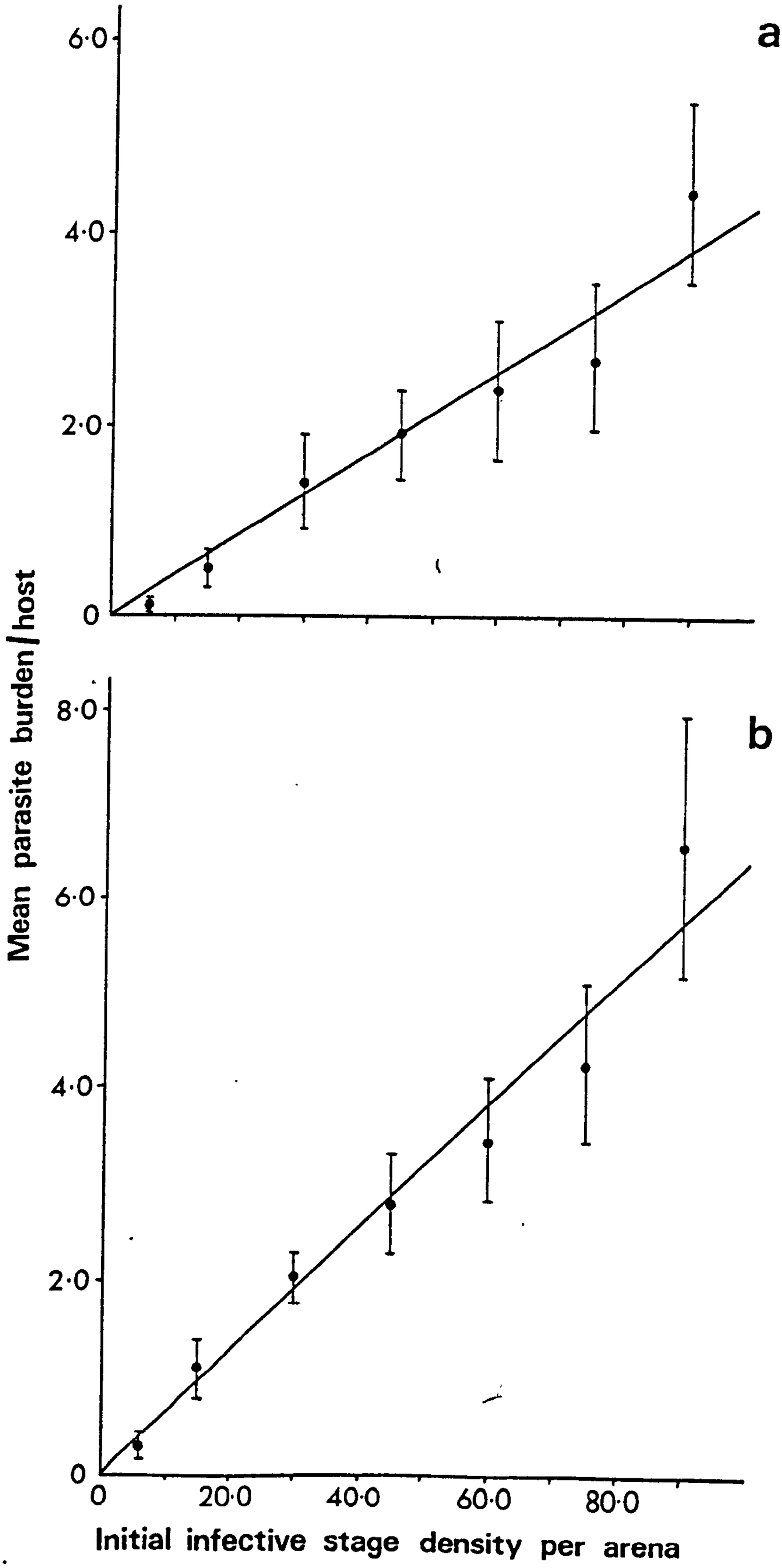
where $P_{(t)}$ is the number of parasites at time t, $I_{(t)}$ is the density

Figure 2.4.4 The relationship between the initial infective stage density and the resultant mean parasite burden per host.

The points are observed data (± 95% confidence limits) and the solid lines are linear regressions constrained to pass through the origin for exposure durations of:

a 2 hours
b 4 hours

The arena volume was 3 ml and the host density was one per arena.


a
b
Mean parasite burden/host
6·0
4·0
2·0
0
8·0
6·0
4·0
2·0
0
0
20·0
40·0
60·0
80·0
Initial infective stage density per arena

of infective stages at time t, H is the density of hosts and the constant β is the coefficient of transmission defined per infective stage per host per volume per unit time.

The rate of change in $I_{(t)}$ through time is,

$$dI_{(t)}/dt = - \beta H I_{(t)} - \mu_2 I_{(t)} \qquad (2.4.7)$$

where μ_2 is the mean instantaneous rate of preparasite mortality (section 2.4(i)). Clearly the rate of change of $I_{(t)}$ will be a function of the rate of parasite acquisition (βHI) and the natural death rate of the infective stages. The major assumption in this model is that the net rate of infection is directly proportional to the density of hosts (H) multiplied by the density of infective stages. Equation (2.4.6) has the solution

$$P_{(t)} = \frac{\beta H I_o}{(\beta H+\mu_2)} \left[1-\exp(-(\beta H+\mu_2)t) \right] \qquad (2.4.8)$$

where I_o is the density of infective stages at t=0, (i.e. the initial density of infective stages). The mean parasite burden per host at time t, $(M_{(t)})$ is therefore

$$M_{(t)} = P_{(t)}/H = \frac{\beta I_o}{(\beta H+\mu_2)} \left[1-\exp(-(\beta H+\mu_2)t) \right] \qquad (2.4.9)$$

As the time of exposure, t, becomes large, equation (2.4.9) predicts an increase in $M_{(t)}$ which asymptotically approaches the value

$$M_{(t\to\infty)} = \beta I_o/(\beta H+\mu_2). \qquad (2.4.10)$$

If the duration of exposure is kept constant, then the relationship between $M_{(t)}$ and I_o (figure 2.4.4) will be one of direct proportionality with slope

$$\beta \frac{[1-\exp(-(\beta H + \mu_2)t]}{(\beta H + \mu_2)} \quad .$$

For the durations of exposure used to generate figure (2.4.4), the death rate of preparasites is negligable (figure 2.4.2 d) and may thus be eliminated from equations (2.4.7) and (2.4.9). Under these circumstances the model takes the form

$$dI_{(t)}/dt = -\beta H I_{(t)} \qquad (2.4.11)$$

with the solution

$$M_{(t)} = \frac{I_o}{H} [1-\exp(-\beta H t)] \qquad (2.4.12)$$

The slope (b) of the lines in figure (2.4.4 a and b) is given by

$$b = \frac{[1-\exp(-\beta H t)]}{H} \qquad (2.4.13)$$

Given an estimate of b from the experimental data, (figure 2.4.4), equation (2.4.13) may be used to estimate the rate of infection β, where

$$\beta = -\ln[1-bH]/Ht \qquad (2.4.14)$$

Estimates of β thus obtained for the two and four hour exposure periods

from figure (2.4.4a and b) are very similar; at 0.0220 per host per infective stage per 3 ml water per hour and 0.0166 per host per infective stage per 3 ml water per hour respectively. The average value of β is 0.0193 per host per infective stage per 3 ml water per hour.

The transmission coefficient or rate of infection, β, may be interpreted as the product of two components; namely the inverse of the average duration between infective stage-host contacts and the probability that such a contact leads to a successful infection. Individual estimation of either of these two components is invariably difficult for host-parasite interactions.

It should be noted that the relationship of direct proportionality between infective stage density and mean parasite burden per host breaks down under certain conditions. For example, when interference occurs between the infective stages, as seen in certain insect parasitoids (Hassell, 1971), and also when transmission is via a predator-prey link, where non-linear effects are generated by predator-satiation (Keymer and Anderson, 1979).

In addition to enabling estimation of the transmission coefficient, the series of experiments described above also provide information relating to the distribution of parasites within populations of exposed hosts and how the distribution varies with changing experimental conditions. One set of results, those of the four hour exposure experiments, will be further examined to show, in detail, the range of information contained within a frequency distribution and particularly, to highlight some of the problems associated with using the percentage of exposed hosts infected as a measure of infectivity.

The distribution of the parasites has many effects upon the dynamics of the parasite population, especially in relation to both the development of the sex of the adult nematodes (section 2.6) and to the degree of parasite induced host mortality (section 2.5).

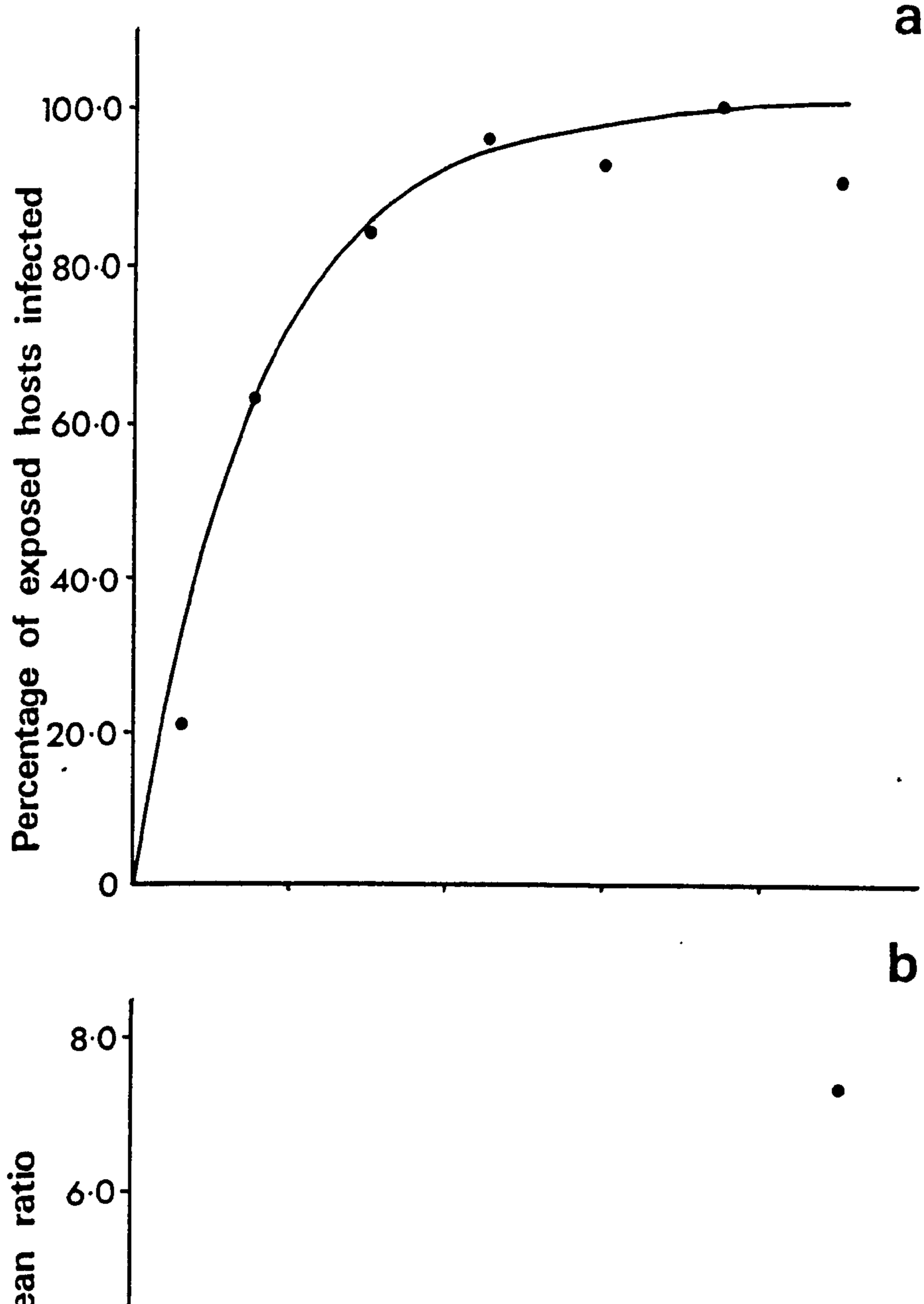
The line in figure (2.4.5a) denotes the percentage of hosts infected (Pcent) predicted by the Poisson distribution,

Figure 2.4.5 The relationship between the initial infective stage density and characteristics of the distribution of parasites amongst the hosts.

a Percentage of hosts infected. The points are observed data and the line is the prediction of equation (2.4.15) which assumes a Poisson parasite distribution amongst the hosts.

b The variance-to-mean ratio of the number of parasites per host. The dashed line represents the variance-to-mean ratio for a Poisson distribution.

The arena volume was 3 ml, the exposure duration was four hours and the host density was one per arena.


a
100·0
80·0
60·0
40·0
20·0
0
Percentage of exposed hosts infected
b
8·0
6·0
4·0
2·0
0
Variance-to-mean ratio
0
20·0
40·0
60·0
80·0
Initial infective stage density per arena

$$Pcent = (1-exp(-m)) \times 100 \qquad (2.4.15)$$

where m is the mean worm burden per host.

The values of m used were obtained from the best fit linear regression of y on x in figure (2.4.4 b). The data points are the observed percentage of hosts which became infected during the series of experiments that generated figure (2.4.4 b).

The predicted line, which assumes a Poisson distribution of parasites within the hosts, shows a good fit to the data, suggesting that the distribution of parasites within the host population at each density of infective stages is approximately random. However, it is relatively easy to show that this assumption is in error. An examination of the variance-to-mean ratios of parasites per host, an indicator of the degree of dispersion of the parasites within the host population (Pielou, 1969; Elliot, 1971; Southwood, 1978), shows that as the density of infective stages increased there was a corresponding increase in the degree of overdispersion, well beyond that compatible with the assumption of a random distribution, where the variance equals the mean (figure 2.4.5 b). In addition the majority of the observed frequency distributions do not show a statistically significant fit to the Poisson distribution (figure 2.4.6), whereas the negative binomial model, which describes overdispersed patterns, provides a significant fit to the majority of the observed frequency distributions. It is obvious from this, that, although some of the observed distributions are random, not all are and the apparently good fit of the predictions of equation (2.4.15) (figure 2.4.5a) to the data is misleading. For a fixed total number of parasites acquired by a host population, the percentage infection will vary according to the degree of dispersion. For this reason it is preferable to use the mean worm burden per host as the index of infectivity, because the mean worm burden is independant of the degree of dispersion, whether underdispersed, randomly dispersed or overdispersed.

The degree of overdispersion presented in figure (2.4.5b) and (2.4.6) is that immediately post-infection and would be expected to change through time if there is any density-dependent parasite induced host mortality (section 2.5), or, if the host was capable of mounting an immune response against the parasite.

Figure 2.4.6 The frequency distribution of parasites within the host population: the influence of infective stage density.

The dashed lines are fitted Poisson distributions and the solid lines are fitted negative binomial distributions.

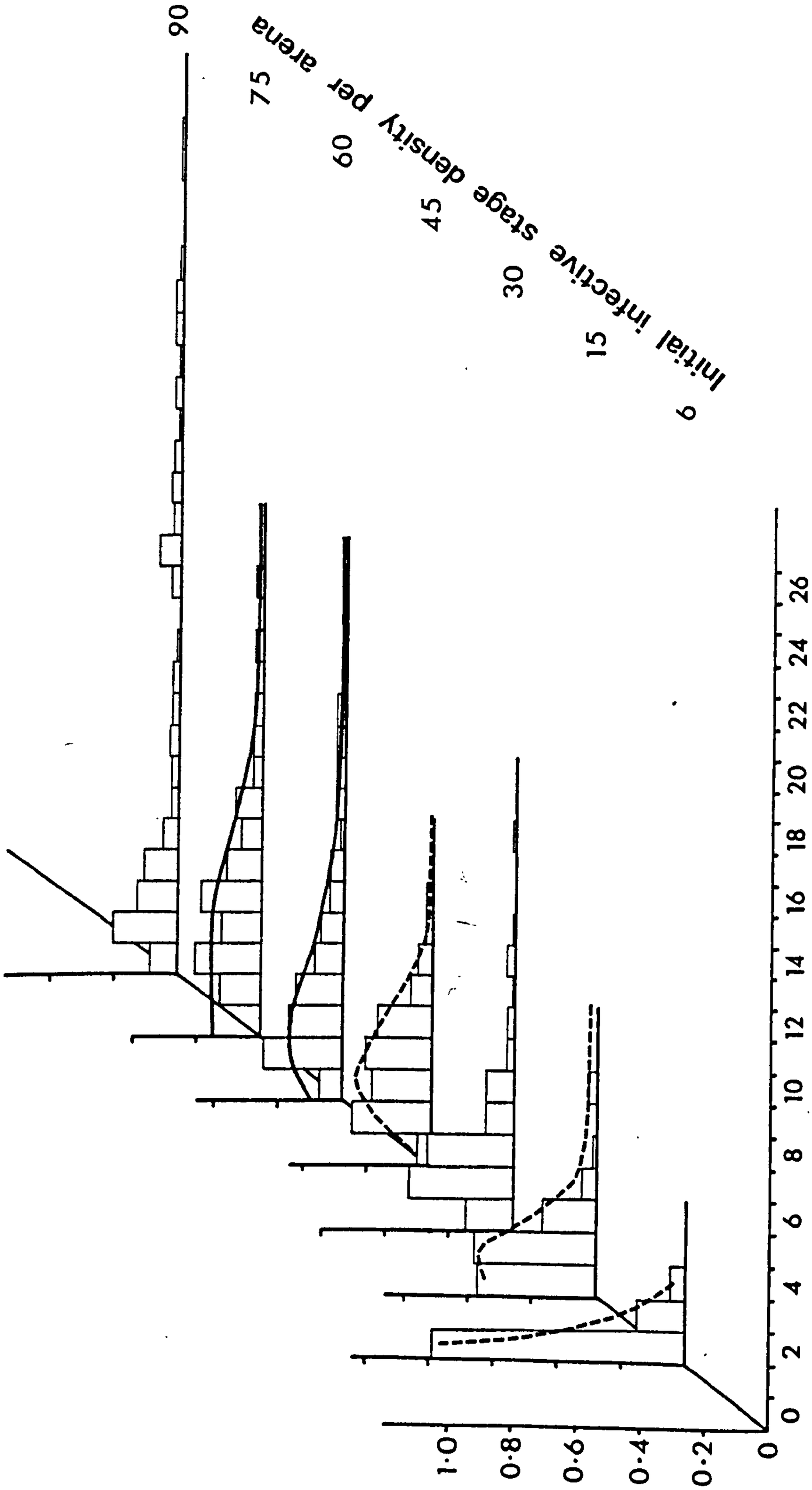
The arena volume was 3 ml, the exposure duration was four hours and the host density was one per arena.

I	χ^2 Poisson	degrees of freedom	χ^2 negative binomial	degrees of freedom
6	2.29'*	1	_$	-
15	2.25*	3	3.41*	3
30	56.64'	9	24.89'	8
45	3.63'*	7	3.06'*	6
60	57.45	7	11.82'*	11
75	15.60'*	10	4.43'*	9
90	421.85'	22	33.45'	21

* significant fit at the 5% level.

' alternative χ^2 test.

$ insufficient degrees of freedom.


Initial infective stage density per arena
9
15
30
45
60
75
90
Parasite burden (i)
0
2
4
6
8
10
12
14
16
18
20
22
24
26
Proportion of hosts with parasite burden (i)
0
0·2
0·4
0·6
0·8
1·0

2.4 (iii) Exposure Duration

The number of contacts between individuals in a freely mixing population is dependent upon two factors, the density of the individuals concerned and the length of time those individuals are allowed to mix.

When this argument is applied to a host-parasite system it is obvious that the duration of exposure of hosts to infective stages will be a major determinant, along with the respective densities of hosts and infective stages, of the level of parasitism attained. The relationship between the level of parasitism and exposure duration has been explored with respect to snail infection by miracidia (Anderson, 1978a), the infection of the fish Brachydanio rerio by the cercariae of the ectoparasitic digenean Transversotrema patialense (Anderson, Whitfield and Dobson, 1978) and to the infection of Tribolium castaneum by Hymenolepis diminuta (Keymer, 1980a). In each of these examples the initial density of infective stages was held constant (I_o), and the mean number of parasites recorded per host was found to rise to a plateau as the exposure period was increased. The height of each plateau was determined by several factors, the initial densities of infective stages, (I_o), and hosts, (H), the magnitude of the per capita infective stage death rate, (μ), and most important, the magnitude of the instantaneous rate of infection, (β), (per host per infective stage per unit volume per unit time).

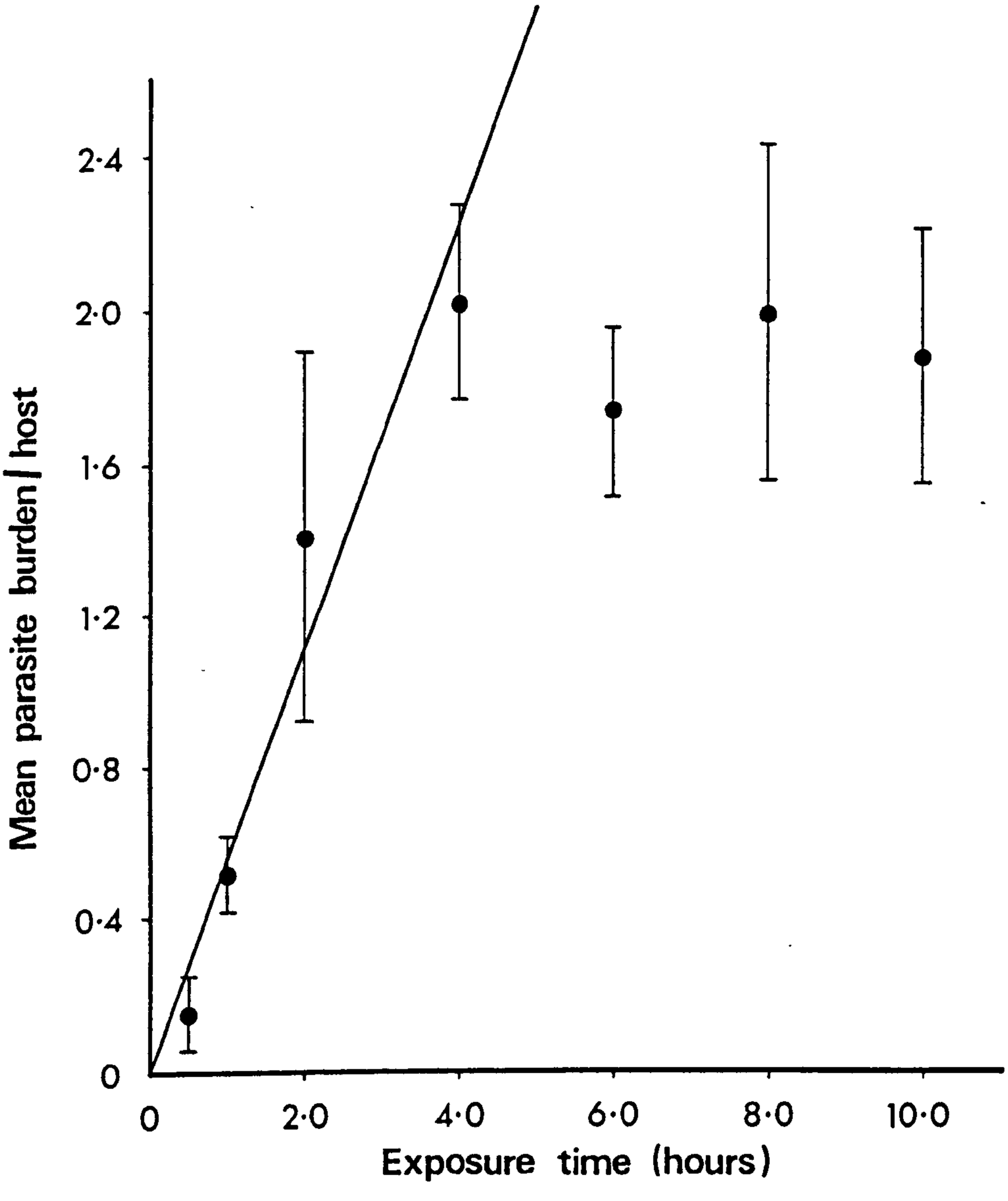
Published data relating exposure duration to the degree of parasitism for mermithid-host associations is unfortunately of insufficient detail to enable a valid estimate of the form of the relationship and therefore an experimental investigation of the effect of varying the exposure duration upon the parasite burden was initiated. The basic methods used have been described in section 2.4 and 2.4 (ii). The initial density of infective stages was kept constant at 30 per arena (10/ml) and exposure durations over the range 0.5 to 10 hours were used. The mean parasite burden per host was recorded and the results are shown in figure (2.4.7). The data shows that the mean parasite burden per host clearly rises to a plateau after the exposure duration has exceeded four hours,

Figure 2.4.7 The influence of the duration of exposure upon the mean parasite burden per host.

The points are observed data. The arena volume was 3 ml, the host density was one per arena and the initial infective stage density was 30 per arena.

The line is the prediction of equation (2.4.16), where,

β = 0.0193/host/infective stage/3 ml/hour
μ_2 = 0.0098/infective stage/hour
H = 1.0 hosts/arena
I_o = 30.0 infective stages/arena


Mean parasite burden / host
2·4
2·0
1·6
1·2
0·8
0·4
0
0
2·0
4·0
6·0
8·0
10·0
Exposure time (hours)

which might, at first, appear to show agreement with the examples quoted earlier. It may be seen, however, that the plateau, of about two parasites per host, is far below the potential level, dictated by the number of infective stages present and their mortality rate. An investigation of this was undertaken making use of equation (2.4.16) which may be used to model the situation.

$$M_{(t)} = \frac{\beta I_o}{(\beta H + \mu_2)} \left[1-\exp(-(\beta H + \mu_2)t)\right] \qquad (2.4.16)$$

(see section 2.4 (ii) eqn. (2.4.9) for derivation).

The mean parasite burden per host at time t, ($M_{(t)}$) is given when estimates of the instantaneous rate of preparasite mortality (μ_2) and the instantaneous rate of infection (β), obtained in sections 2.4(i) and 2.4 (ii) respectively, are substituted into the equation along with the initial infective stage density (I_o), the host density (H) and the exposure duration (t). The predicted relationship is shown in figures (2.4.7) and (2.4.8a), where it may be seen that the fit of the model to the data points is good only for the first four hours, with an increasingly large divergence thereafter. The only possible explanation of the observed pattern is that the rate of infection (β) is not constant as was assumed, but declines to effectively zero after the hosts have been exposed for four hours. The estimate of β obtained in section 2.4(ii) is therefore unsuitable for use when exposure durations exceed four hours in length.

An estimate of β for long exposure durations may be obtained by manipulating equation (2.4.16) and using the data shown in figure (2.4.7). As the duration of exposure increases ($t \to \infty$), equation (2.4.16) gives,

$$M_{(t)} \to \beta I_o/(\beta H + \mu_2) \qquad (2.4.17)$$

with the solution for $\beta_{(t=\infty)}$,

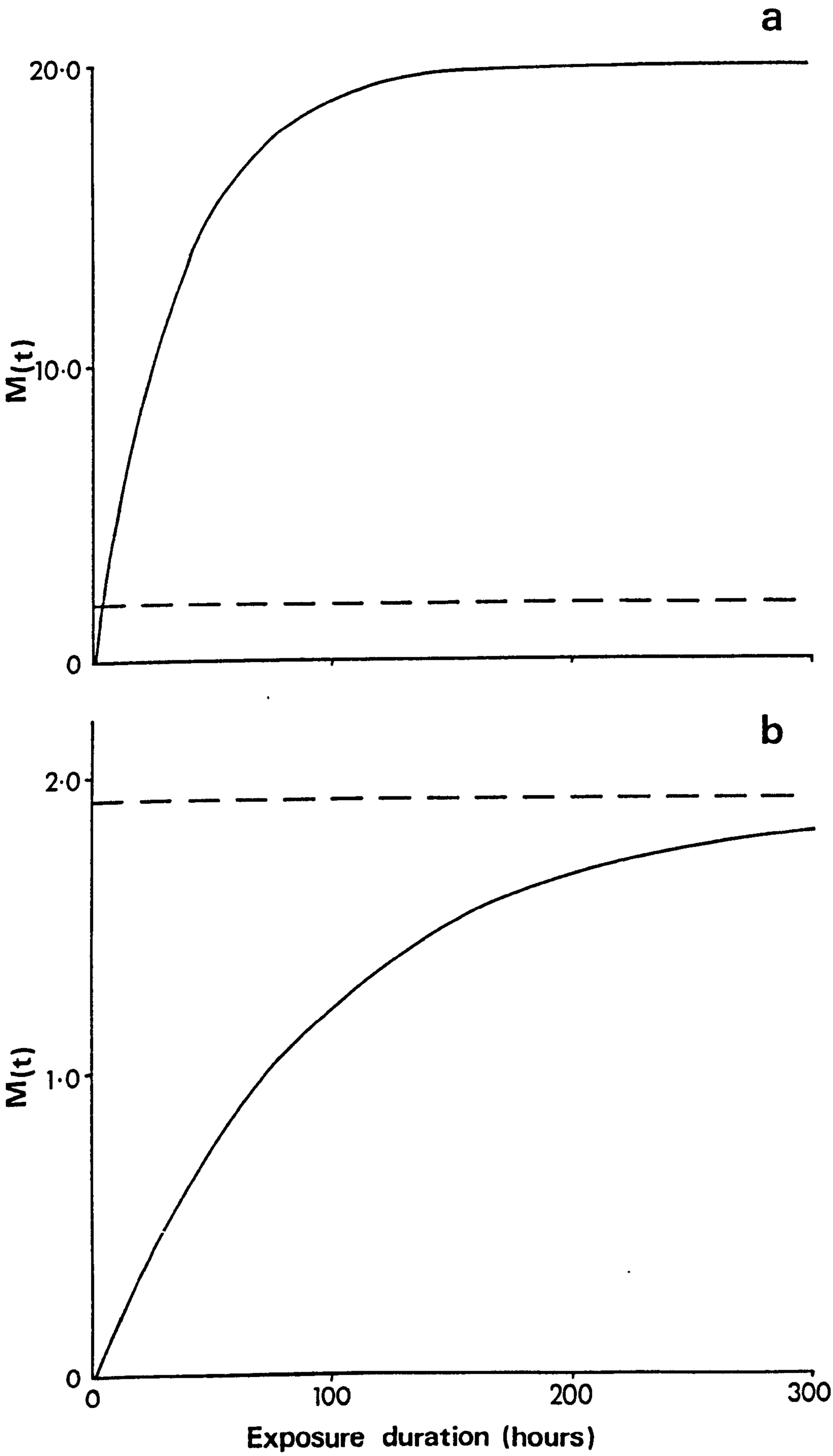
Figure 2.4.8 The relationship between the duration of exposure and the mean parasite burden per host at time t: the predictions of two models.

a. The prediction of equation (2.4.16) of the mean parasite burden per host ($m_{(t)}$), where,

β = 0.0193 /host/infective stage/3 ml/hour
μ_2 = 0.0098/infective stage/hour
H = 1.0 hosts/arena
I_o = 30.0 infective stages/arena

b. The prediction of equation (2.4.16), where,
β = 0.000664, derived from equation (2.4.18).
The other parameter values were as given above (2.4.8a).

The dashed line, in both cases, is the observed plateau in the mean parasite burden per host seen in figure (2.4.7).


a
20·0
10·0
0
M(t)
b
2·0
1·0
0
M(t)
0
100
200
300
Exposure duration (hours)

$$\beta = \frac{\mu_2 Mt}{I_o - HM_{(t)}} \qquad (2.4.18)$$

Values of the initial infective stage density, (I_o), and the host density (H) were set at the experimental values and the previous estimate of infective stage mortality, (μ_2), was used. The numerical value of $M_{(t)}$, the mean parasite burden per host at time t, as t approached infinity, was assumed to be the value at which the observed data formed the plateau in figure (2.4.7). This was estimated by taking the mean of the points at 4, 6, 8 and 10 hour exposure durations and was found to be 1.902 parasites per host. Using this method the instantaneous rate of infection was estimated at 0.000664 (per host per infective stage per 3 ml water per hour). This estimate of β is much lower than that estimated in section 2.4 (ii), the thirty-fold difference reflecting the difficulty in obtaining good estimates of transmission rates, even under ideal laboratory conditions. The cause of the observed difference in the two estimates of β is most probably due to a change in the actual rate of infection as the exposure time increased, although some other unknown cause dependant upon the experimental conditions cannot be ruled out.

The estimate of β obtained here, when substituted back into equation (2.4.16), gives a new prediction of the relationship between exposure duration and mean parasite burden per host, as portrayed in figure (2.4.8b). This prediction gives a much improved fit to the data for exposure times in excess of about six hours, but does greatly underestimate the value of the mean parasite burden for short durations of exposure.

It is apparent that there is, in reality, a fairly abrupt change in the rate of infection after the exposure duration has exceeded four hours. In addition to being difficult to model satisfactorily, this poses a major question as to why there should be such an abrupt change. The explanation of this phenomenon must be of a biological nature, based upon a change in either the host, infective stage or both together. The importance of this relationship to the dynamics of the system is paramount and a clear understanding of the situation

is essential.

There are several possible causes of such an abrupt change in the rate of infection. Firstly, the period during which the preparasitic nematodes are infective may be short, as seen in many digenean cercariae, resulting in a rapid decline in the rate of infection as the preparasites age. This possibility may be totally discounted on evidence presented by Petersen (1975) and that seen in section 2.4 (iv) herein. The second obvious possibility is that the infective stages show avoidance of hosts which are already infected, as seen in certain insect parasitoids. The ability of the infective stage to differentiate between infected and uninfected hosts would be the main assumption in this case. However reference to figure (2.4.6) shows that as infective stage density increases so does the degree of overdispersion, with some hosts harbouring very large numbers of parasites which effectively discounts the possibility of infective stages avoiding previously infected hosts. The final possibility relating to the infective stage behaviour is that only a small proportion of the preparasitic nematodes are infective. In a population of preparasitic nematodes the occurrence of a degree of variability in their ability to infect hosts would be expected but that only a small proportion of such a population be infective would not normally be expected. To test this hypothesis an experiment was conducted under the previously described standard conditions. Hosts were exposed to infection for four hours, at the end of which time the hosts were carefully removed with as little water as possible and were replaced with similar, but previously unexposed hosts, which were also exposed for four hours. The number of parasites acquired by each host was recorded and mean values for each of the two groups, the primarily and secondarily exposed hosts, were calculated. The results are displayed in table 2.4.2.

There appears to be a slight reduction in the infectivity of the preparasites to the secondarily exposed hosts, as measured by the mean parasite burden per host. This difference is however insignificant (Mann-Whitney U-test. $p=0.05$) and is by no means large enough to account for the decline in infectivity seen in figure (2.4.7). A small decline in infectivity would be expected between the primarily

and secondarily exposed hosts, based upon the natural variability in infectivity of the nematodes, those nematodes more adept at infecting a host would remove themselves more rapidly from the infective stage pool, causing a reduction in the average infection ability of the infective stage pool.

Table 2.4.2

The mean parasite burdens per host of two groups of hosts exposed consecutively to the same infective stages for four hours.

	Mean ± 95% CL	95% CL Range
Primarily exposed hosts	2.326 ± 0.265	2.061 - 2.591
Secondarily exposed hosts	1.947 ± 0.202	1.745 - 2.149

As aspects of the biology of the infective nematodes appear unable to explain the rapid decline in the rate of infection, the cause must be sought within the behaviour of the host. There are several possible ways in which the host may cause the observed reduction in the rate of infection. Firstly, there could be a loss of susceptibility to infection as the hosts aged within the first instar. Supposing a simultaneous egg hatch, the maximum difference in age between those hosts exposed for the shortest and longest duration could be 9.5 hours. However, when the actual egg hatching time is considered this potential 9.5 hour difference almost completely disappears. The eggs of the mosquito hatching up to an 8 hour period. This associated with the tendency to use younger hosts for the longer exposure duration (to avoid the hosts moulting to the second instar during exposure) makes it extremely unlikely that the age of the host had any bearing upon the observed phenomenon.

The second possible explanation of the observed decline in infectivity relates to a change in the susceptibility of the host to infection, enabled by some innate behavioural response of the host. This may be envisaged as an experience related phenomenon whereby the hosts become able to avoid infection either by an alteration of behaviour or by a reduction in their attractiveness. This change in host susceptibility,

however determined, may be obtained via two possible routes. There may be a threshold number of contacts between host and infective stages beyond which further contacts trigger a change in the host behaviour or attractiveness (or both) resulting in the rapid change in the rate of infection observed. This process working in isolation may be discounted, for, if the controlling factor was purely the number of host-preparasite contacts, then, assuming a linear relationship between host-preparasite contacts and infective stage density, a similar decline in the rate of infection would be expected as the density of the infective stages was raised; such is not the case (figure 2.4.4). The other possible route to a change in host susceptibility is that of the increasing experience of the host to the infective stages, the longer the exposure the more experience gained by the hosts. This may again be visualized as a change in behaviour and/or attractiveness with increasing experience, it is however difficult to envisage how this would operate or why it should be independent of the contact rate. Unfortunately data to test this hypothesis are, as yet, unavailable.

One further potential cause of the relationship under discussion is that the change in the host behaviour, or as is more likely in this instance, attractiveness, is dependent upon an alteration in the metabolism of the host. The hypothesis being that this process requires a period of time to become effective, a period of time which is seen as the four hour period between the initiation of exposure and the effective cessation of infection. This argument may also be applied in the somewhat unlikely event of the host producing a chemical inhibitor, which would act upon the infective nematodes. As both of these proposed methods again appear to be independent of the number or frequency of host-preparasite contacts, both of the proposals would require that initiation of the effect would occur almost immediately upon exposure (i.e. very few contacts would cause the stimulation of the host).

Until such time as further experimental investigation can be directed to solving this problem the actual cause of the relationship between the mean level of parasitism and exposure duration, depicted in figure (2.4.7), must remain a subject of speculation.

2.4 (iv) Preparasite Age

Transmission of parasites between hosts may be achieved in a variety of ways. Firstly, certain larval parasites are retained within an intermediate host awaiting transmission via a predator-prey link. Secondly, many parasite species produce free-living stages where transmission is achieved by contact with a susceptible host. Examples include eggs and cysts which gain entry to the host in a passive manner by ingestion and those forms where an active larval stage attaches to and penetrates susceptible hosts. Those stages which remain within the intermediate host often retain the ability to acquire nutrients from that host. Free-living infective stages, on the other hand, are usually small in size and non feeding. These free-living parasite transmission stages therefore possess a finite reserve of energy upon which they can call. The rate at which an organism uses its supply of energy will vary with the life-cycle of the organism and the prevailing environmental conditions. An active larval nematode will obviously use proportionately more of its reserves than will an encysted Fasciola hepatica cercaria and the rate of use will be greater at higher ambient temperatures. In addition the rate at which stored reserves are used need not necessarily be uniform, the organism may undergo specific alteration in behaviour in an attempt to reduce its activity and so prolong the period during which stored food reserves are available. An example of such a behavioural change is found in the cercariae of the digenean Transversotrema patialense, which show a marked decline in spontaneous swimming activity as they age (Whitfield, Anderson and Bundy, 1977), thus potentially extending both their life-span and infective period.

As non-feeding infective stages age there is often a decline in their ability to infect a potential host where the process of infection encompasses penetration and establishment within the host. It is, however, rarely possible to directly associate this decline in infectivity with the reduction in food reserves. This is because there will be many other physiological ageing processes occurring concomitantly. However, knowing the exact cause of any reduced infectivity when considering the population dynamics of a host-parasite relationship is of less importance

than understanding the relationship between infectivity and infective stage age.

Examples of infective stage age-dependent infectivity may be drawn from almost any parasitic group. The miracidia and cercariae of digeneans clearly show age related declines in infectivity (Olivier, 1966; Christensen, Nansen and Frandsen, 1976; Anderson, Whitfield and Mills, 1977; Prah and James, 1977 and Prechel and Nollen, 1979). A similar situation has been shown to occur relating to the eggs of digeneans (Kuris, 1980), cestodes (Keymer and Anderson, 1979) and the active, infective larval forms of nematodes, for example, Strongyloides ratti (Barrett, 1969), Haemonchus contortus (Rogers, 1940) Ancylostoma caninum (Rogers, 1939) and A. tubaeforme, (Croll and Matthews, 1973). In the case of the nematodes, the decline in the infectivity of the larvae is associated with a decline in both lipid content (the principal food reserve), and also in activity. Data available for R. culicivorax shows an age related decline in the activity and also in the infectivity of the preparasites (Petersen 1975), where activity was measured as the percentage of actively swimming nematodes found after given periods of time since hatching and infectivity was defined as the percentage infection recorded in hosts exposed to pre-aged infective stages. Note, however, that the percentage infection is a poor overall measure of infectivity (as used by Petersen, 1975). A better measure is the number of parasites established within the host when exposed under defined conditions to a fixed density of preparasites.

As discussed in a previous section (2.4 (ii)), the statistical distribution of the parasites within the host population is of great importance in the dynamics of the parasite population and for this reason the effect of preparasite age upon infectivity was investigated experimentally, in such a way that the parasite distribution could be ascertained. The experimental protocol used has been described in section 2.4 (ii), with preparasites hatched from eggs over a period of from two to eight hours and aged at 25°C until they reached the required, predetermined age. The estimation of the initial number of preparasites introduced into the arena was based upon the number of preparasites recorded as being alive, according to the criterion described in section

2.4 (i). The initial density of infective stages used was 10 per ml. with a standard exposure period of four hours. The number of parasites that established in each exposed host was recorded at the end of the exposure period, enabling the frequency distribution of the parasites to be ascertained together with the mean parasite burden per host.

The estimated values of the mean parasite burden per host are portrayed in figure (2.4 9a) and show a dramatic decline in the ability of the preparasitic nematodes to infect hosts once the infective stages had aged beyond 48 hours post-hatching. There is a similar and not unexpected trend in the percentage of hosts infected as preparasite age increased (figure 2.4.9b). There is good qualitative agreement between these results and those of Petersen (1975). The quantitative differences [between the results given in figure (2.4.9b) and Petersen's work] are most probably due to minor differences in the experimental conditions employed.

An examination of the distribution of the parasites within the host population shows that as the age of the infective stages increased there was a reduction in the degree of overdispersion of the parasites, i.e. fewer and fewer hosts were found harbouring large numbers of parasites as preparasite age increased, (figure 2.4.10). Frequency distributions fitted to the data showed a change in form from the negative binomial model, which describes overdispersion (aggregation) in the number of parasites per host to the Poisson model which describes parasites distributed at random between hosts. There is also a marked fall in the values of the variance-to-mean ratio of the number of parasites per host with increasing preparasite age (fig 2.4.11), which again indicates a preparasite age-dependent reduction in the degree of parasite aggregation. The preparasite age related fall in the mean parasite burden per host, the percentage of exposed hosts that became infected and in the degree of overdispersion in the parasite distribution are all linked and attributable to the same cause; namely that of the reduced infectivity of older infective stages. The reduced infectivity is most probably the result of a combination of the stresses caused by declining reserves of nutrient, and other physiological ageing processes. An examination of data generated by the use of aged preparasites which had been selected as being active (i.e. showing spontaneous activity and being able to

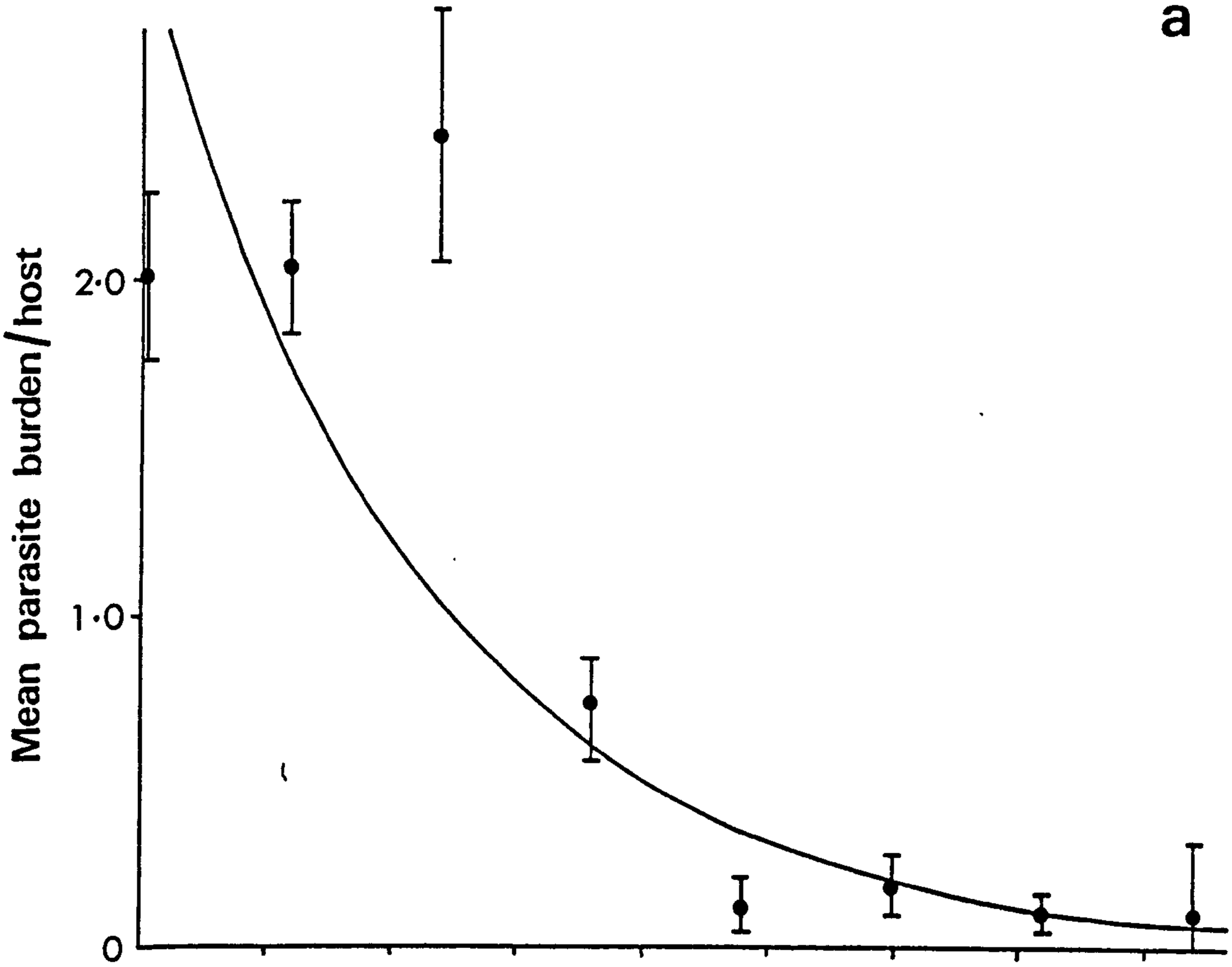
Figure 2.4.9 The influence of the age of the infective stages upon host infection.

a. The mean parasite burden per host. The points are observed data (± 95% confidence limits) and the line is a least-squares best-fit linear regression of lny on x, (a = 1.1089, b =-0.0224, r = -0.9255).

b. The percentage of exposed hosts that became infected. The points are observed data and the line is a least-squares best-fit linear regression of lny on x (a = 4.7089, b = -0.0153, r = -0.9322).

The arena volume was 3 ml, the host density was one per arena, the initial infective stage density was 30 per arena and the duration of exposure was four hours.

a


Mean parasite burden/host
2·0
1·0
0

b

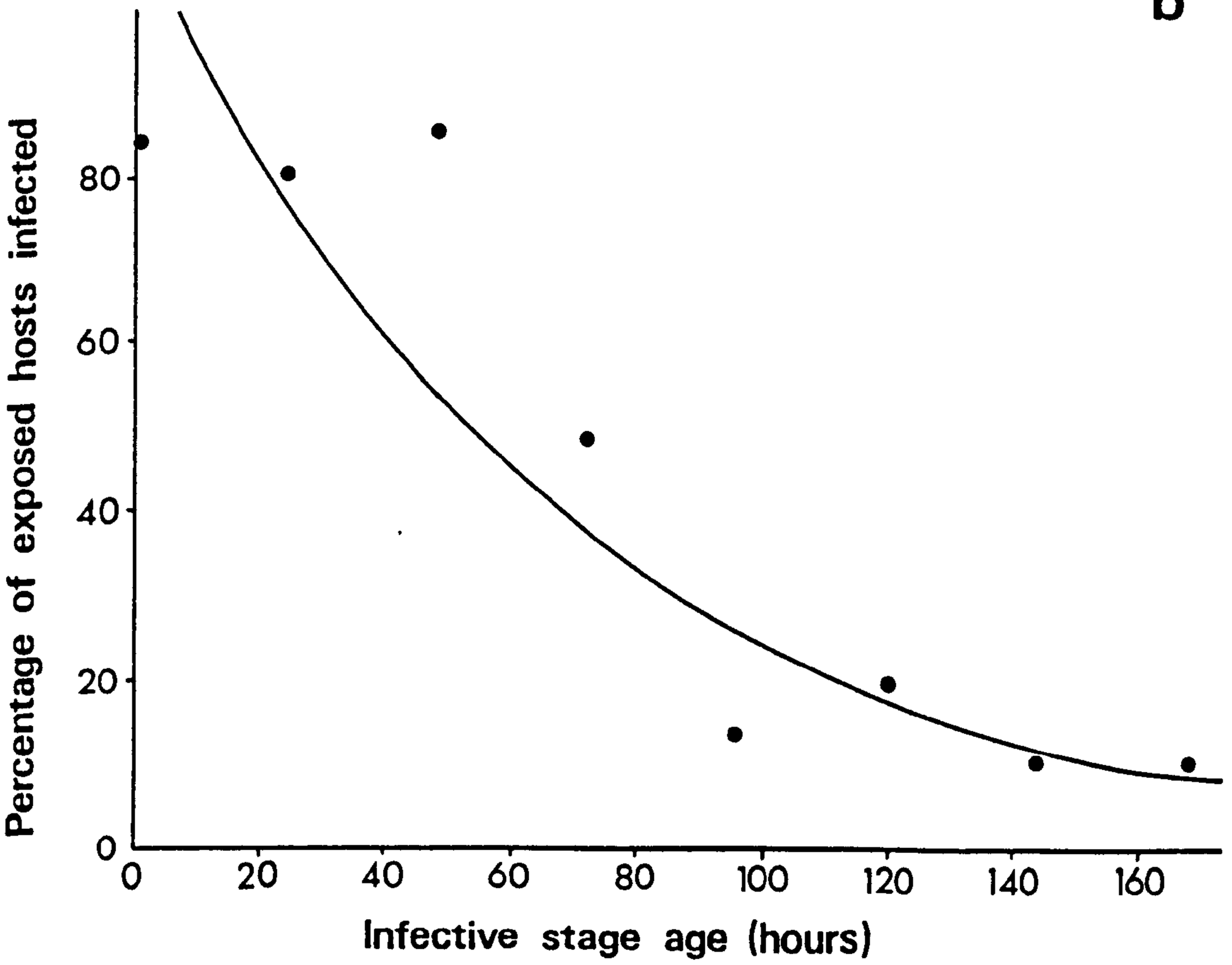

Percentage of exposed hosts infected
80
60
40
20
0
0
20
40
60
80
100
120
140
160
Infective stage age (hours)

Figure 2.4.10 The frequency distribution of parasites within the host population: the influence of infective stage age.

The solid lines show fitted negative binomial distributions and the dashed lines fitted Poisson distributions.

The arena volume was 3 ml, the host density was one per arena, the initial infective stage density was 30 per arena and the duration of exposure was four hours.

Age (hours)	χ^2 Poisson	degrees of freedom	χ^2 negative binomial	degrees of freedom
1	53.64'	9	24.89'	8
24	122.84'	8	26 73'	7
48	14.26	6	5.99*	7
72	1.86*	2	1.30'*	3
96	78.22#*	89	-	-
120	76.76#*	95	-	-
144	108.78#*	120	-	-
168	9.00+*	9	-	-

* significant fit at the 5% level.
' alternative χ^2 test.
\# χ^2 test on S^2/x (n>31). (Elliot, 1971).
\+ χ^2 test on S^2/x (n<31). (elliot, 1971).
\- variance < mean.

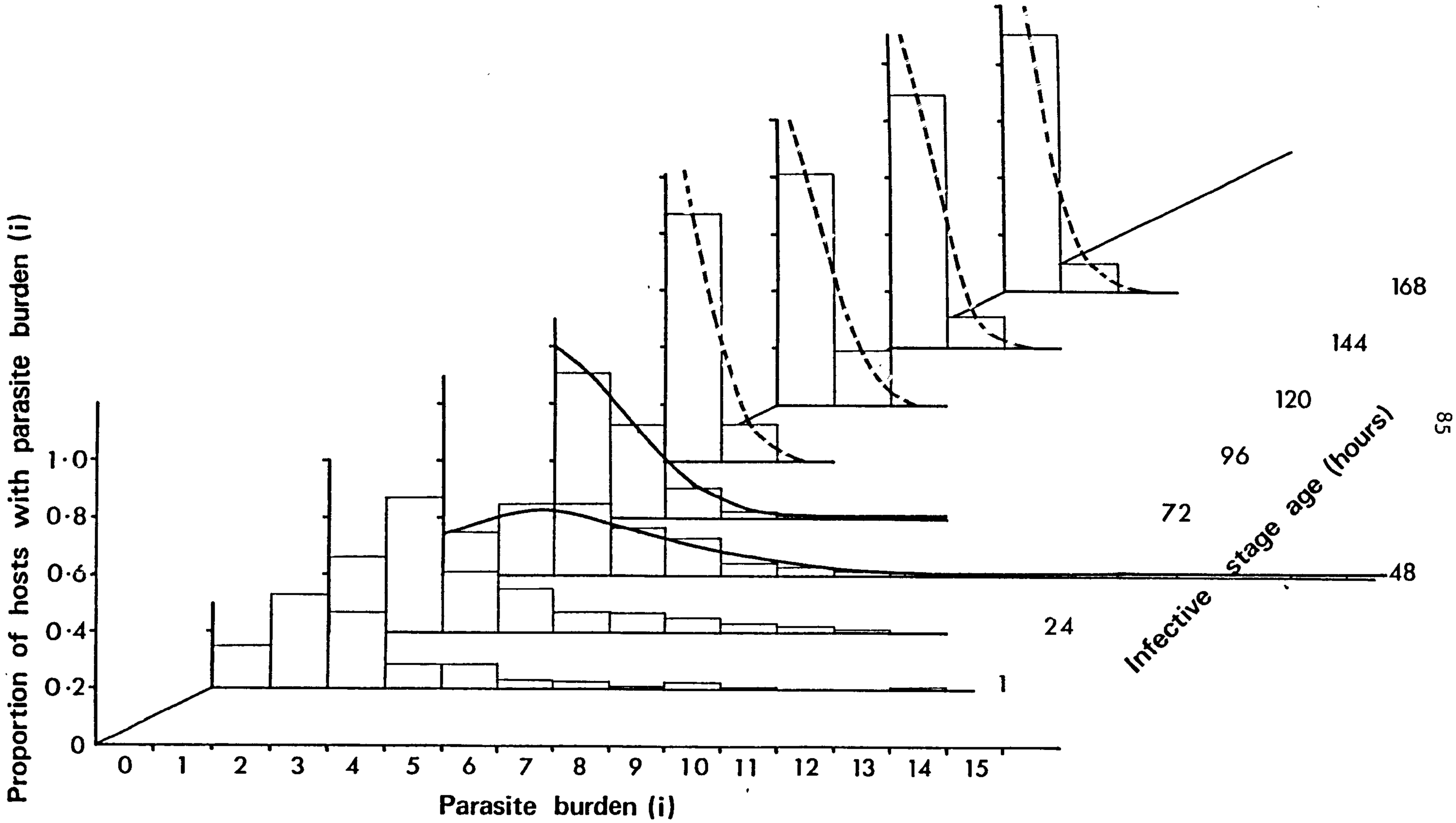
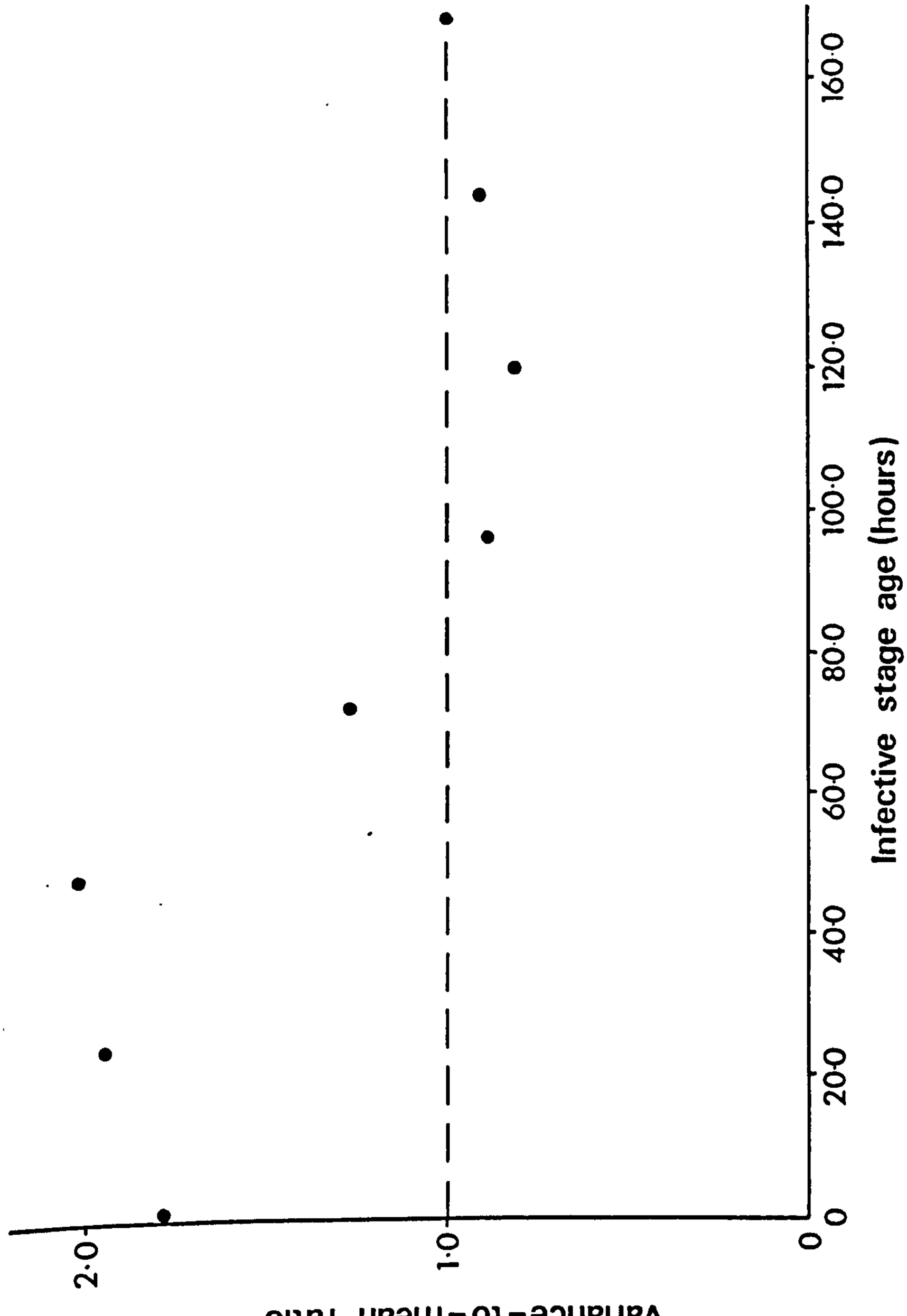

Proportion of hosts with parasite burden (i)
1·0
0·8
0·6
0·4
0·2
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Parasite burden (i)
168
144
120
96
72
48
24
1
Infective stage age (hours)

Figure 2.4.11 The influence of infective stage age upon the variance-to-mean ratio of the number of parasites per host.

The points are observed data. The dashed line represents the variance-to-mean ratio for a Poisson distribution.

The arena volume was 3 ml, the host density was one per arena, the initial infective stage density was 30 per arena and the duration of exposure was four hours.


Variance-to-mean ratio
2·0
1·0
0·0
0
20·0
40·0
60·0
80·0
100·0
120·0
140·0
160·0
Infective stage age (hours)

maintain their position in a water column), shows a similar pattern but with the degree of the changes in the measured parameters being consistently less. Thus the declines in mean parasite burden per host and in the percentage of exposed hosts which become infected are much less marked (figure 2.4.12a and b), with only a marginal reduction in the degree of overdispersion of the parasite population as indicated by the variance-to-mean ratio (figure 2.4.13). This is probably the result of either the more efficient utilization of stored food in these individuals or a greater initial amount of stored food, resulting in more energy being available at a given age. The explanation of why some preparasites should either have more stored food or use it more economically must be related to the inherent variability in biological systems, either due to genetic factors, or factors associated with parentage. For example, the experiences of the parent may influence the amount of nutrient stored in the larval offspring, especially as the females themselves exist off stored nutrients and may have been subjected to overcrowding and nutrient shortage during their own parasitic development.

There are two ways in which the infection process may be influenced by the age of the preparasite. The first is by a change in the efficiency of host location; the second, by a change in the efficiency of penetration and establishment. Although both may be of importance, the process of penetration is a highly active one and an old preparasite, which may be showing signs of reduced activity, would be less likely to be able to sustain the effort needed in maintaining contact with a host and of actually penetrating.

Although the reduction in overdispersion is related to the reduced infection ability found in older preparasites, the precise cause is the result of the stochastic nature of the infection process. As fewer infection events occur there will be less chance of a host picking up more than a very low parasite burden, which in turn will be insufficient to generate a high degree of overdispersion. The majority of successful infections will be the result of action by the younger members of the pool of infective stages. A conclusion that is strengthened when one considers that, assuming a constant rate of immigration of newly hatched preparasites there will necessarily be more younger stages present than

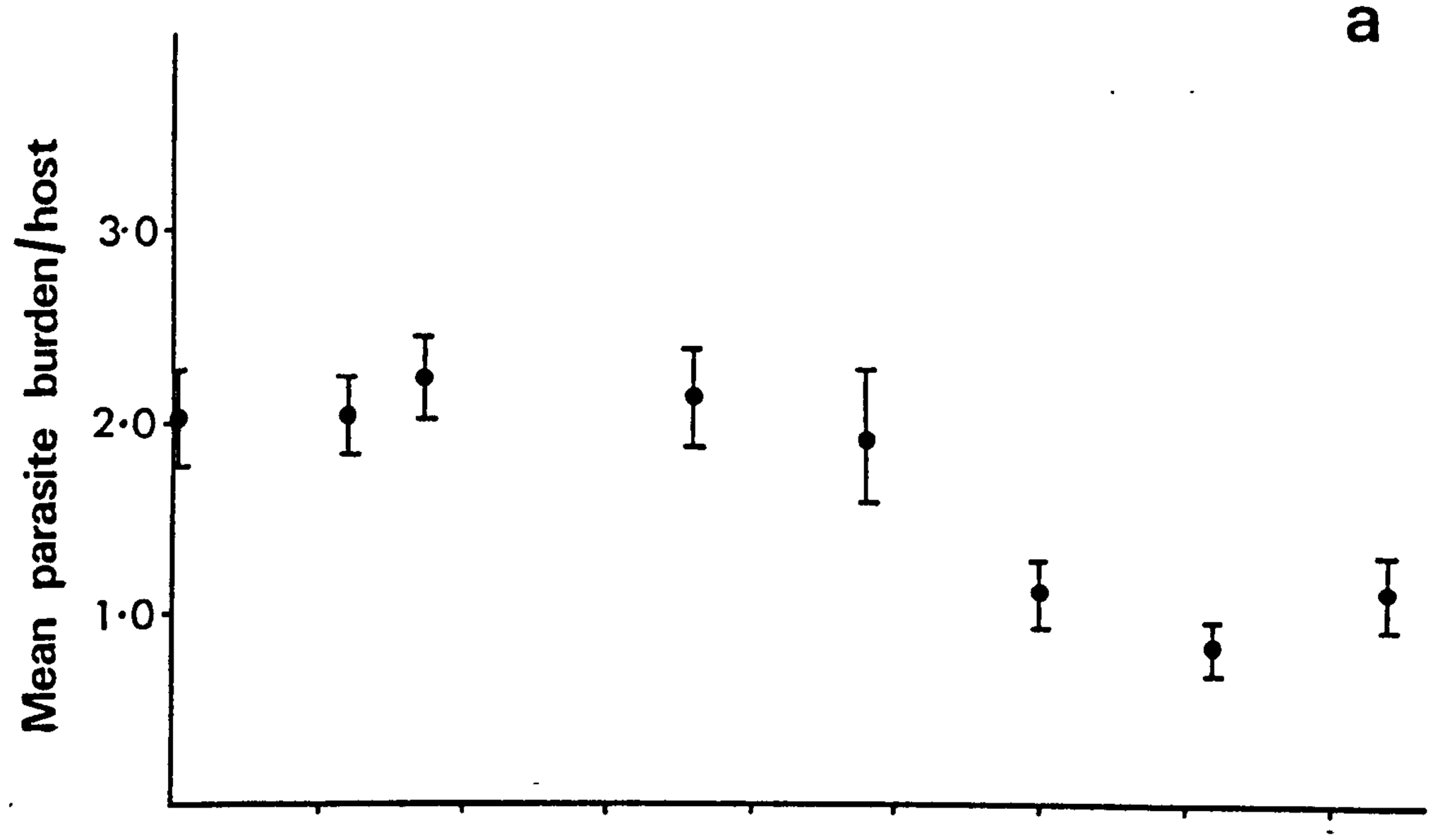
Figure 2.4.12 The influence of the age of spontaneously active infective stages upon host infection.

a. The mean parasite burden per host (± 95% confidence limits).
The points are observed data.

b. The percentage of exposed hosts that became infected.

The arena volume was 3 ml, the host density was one per arena, the initial infective stage density was 30 per arena and the duration of exposure was four hours.

a


Mean parasite burden/host
3·0
2·0
1·0

b

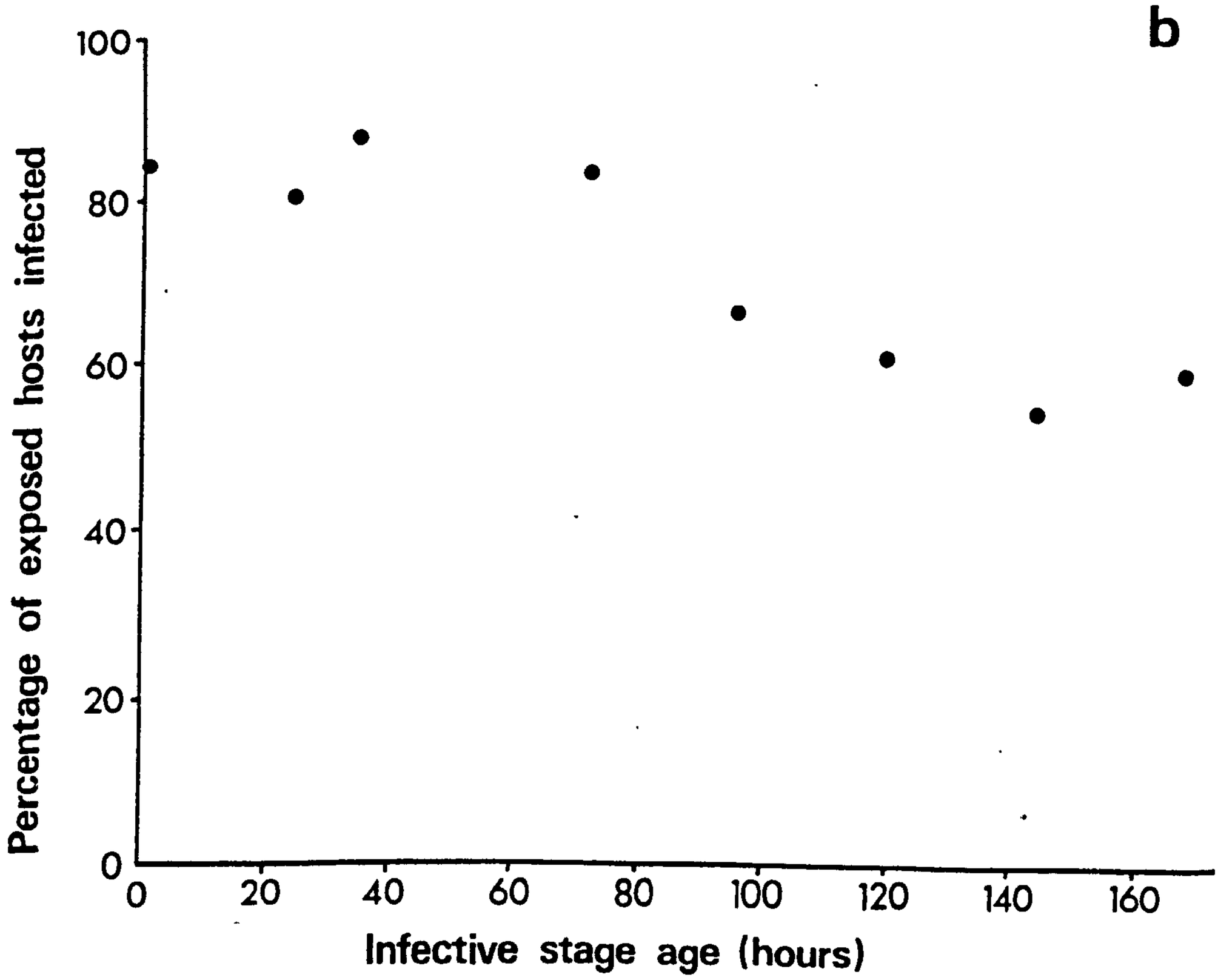
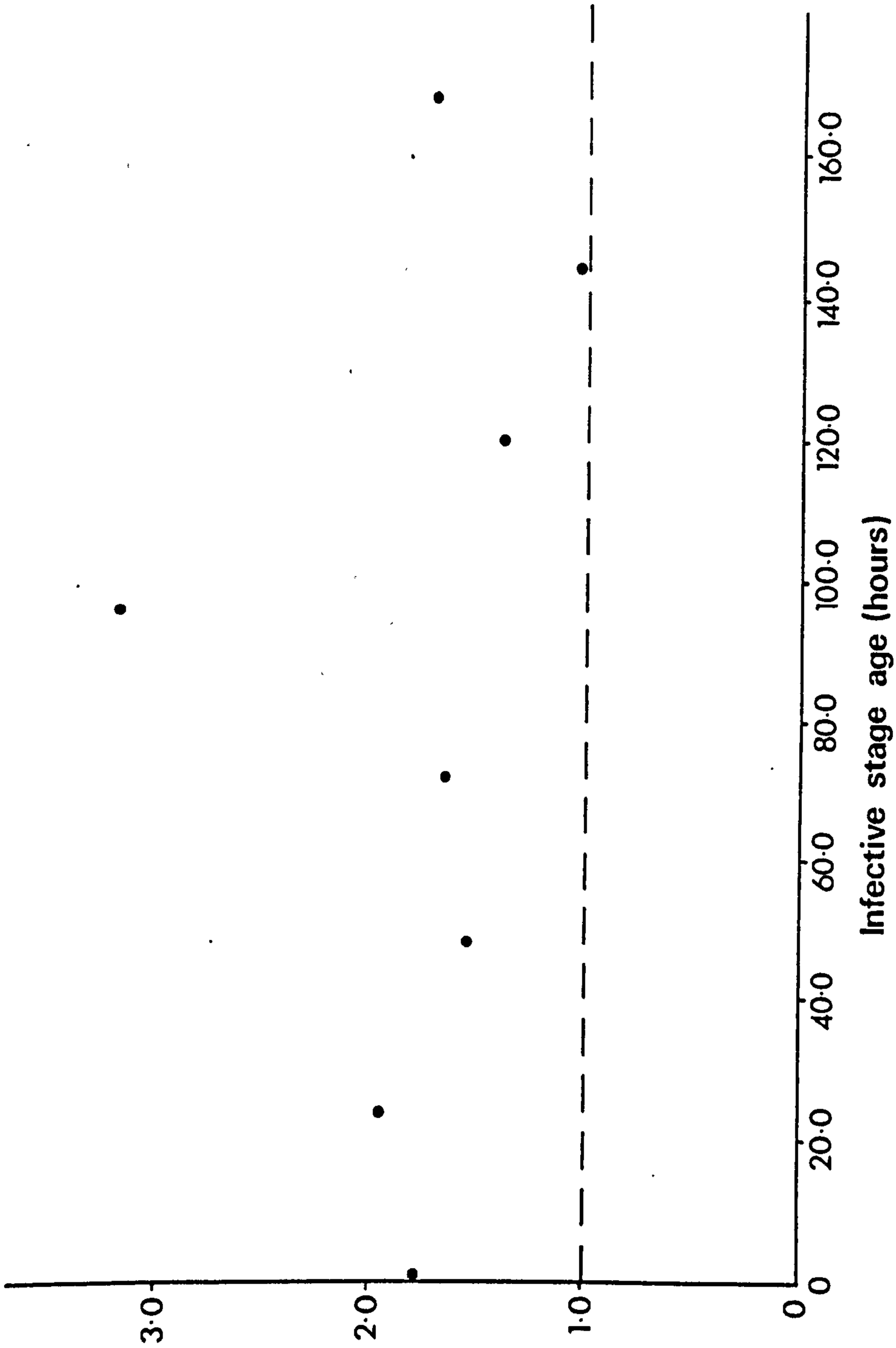

Percentage of exposed hosts infected
100
80
60
40
20
0
0
20
40
60
80
100
120
140
160
Infective stage age (hours)

Figure 2.4.13 The influence of the age of spontaneously active infective stages upon the variance-to-mean ratio of the number of parasites per host.

The points are observed data. The dashed line represents the variance-to-mean ratio of a Poisson distribution.

The arena volume was 3 ml, the host density was one per arena, the initial infective stage density was 30 per arena and the duration of exposure was four hours.


Variance-to-mean ratio
3·0
2·0
1·0
0
Infective stage age (hours)
0
20·0
40·0
60·0
80·0
100·0
120·0
140·0
160·0

older ones, since the death rate of preparasites increases exponentially with age (figure 2.4.2). Under conditions of constant imput of newly developed preparasites, the age distribution would be stable and the proportions in each age-class would therefore be identical to the age-dependent survival pattern portrayed in figure (2.4.1).

2.4 (v) Host Density

Changes in the density of hosts within the environment would be expected to have a major impact upon the transmission of a parasite within the host population. The rate of transmission is usually assumed to be directly proportional to host density (Bailey, 1975; Anderson, 1982a) In a situation where transmission is dependent upon contact, and contact occurs effectively at random, a doubling of either host or infective stage density will double the chance of contact between host and infective stage (see section 2.4 (ii)). This remains true only whilst the number of infective stages is not limiting. For a finite number of infective stages an increase in host density beyond a certain level will have no further effect upon the level of infection recorded.

Relationships of direct proportionality have been described under laboratory conditions for miracidia infecting snails (Lie, Heyneman and Kostanian. 1975; Anderson, Whitfield and Dobson, 1978; and Anderson, Whitfield, Dobson and Keymer 1978).

There are several factors which may impinge upon the relationship between host density and transmission efficiency. There may be a behavioural interaction between the hosts which either promotes or retards the rate of transmission, depending upon density. The following conditions may be regarded as being special cases of either of these broad categories. Factors which may promote transmission at raised host densities include, increased activity of the infective stages, due to the increased stimulation of more hosts (Anderson, 1978a); attraction of infective stages to an area of high host density, often seen in insect parasitoid-host interactions (Hassell, 1966; Murdie and Hassell, 1973). Predation of infective stages by the potential hosts may result in reduced transmission by the removal of infective stages from the environment, especially when infective stages are limiting in

number. However, the result of predation will depend upon the functional response of the host to the prey and also upon whether the process of predation has any tendency to increase the likelihood of infection taking place (i.e. placing the host in a spatial area occupied by an infective stage). Finally, a situation may arise where higher densities of hosts induce a change in host activity resulting in fewer infections taking place, particularly where physical disturbance by the host makes parasite-host contact less likely.

For a finite number of infective stages and a short duration of exposure, (one that effectively precludes infective stage deaths) the effect of increasing host density upon parasite transmission, (as measured by the mean parasite burden per host, $M_{(t)}$) is given by equation (2.4.12), which predicts an exponential decline in $M_{(t)}$ with increasing host density. Superficially, this type of relationship has been shown to occur in R. culicivorax infecting C. p. quinquefasciatus (Petersen, 1973a and b) and also for R. culicivorax infecting C. p. molestus (Kurihara, 1979). Further examination of the data presented by Petersen and Kurihara also showed an exponential decline in the value of the transmission coefficient (β) with increasing host density. Clearly this would accelerate the decline in $M_{(t)}$ predicted by equation (2.4.12), in which β was assumed to be constant.

Experiments were designed to investigate this phenomenon directly, the published data not being entirely suitable for detailed analysis.

The experimental conditions and protocol were as described in section 2.4 (ii), with, in this case, the density of hosts per arena (H) as the variable. The exposure duration used was four hours and the density of preparasitic nematodes (I_o), was thirty per 3 ml. arena. Host density was varied between one and twenty per arena.

The results showed a decline in the mean parasite burden per host with increasing host density (figure 2.4.14 a). The predictions of equation (2.4.12) do not, however, fit the data, consistently overestimating the level of infection.

Figure 2.4.14 The influence of host density upon infection of the host: 1.

a The relationship between host density per arena and the mean parasite burden per host. The points are observed data (± 95% confidence limits). The dashed line is the prediction of equation (2.4.12) where I_o = 30/arena, β = 0.0193/host/infective stage/3 ml/hour and t = four hours.

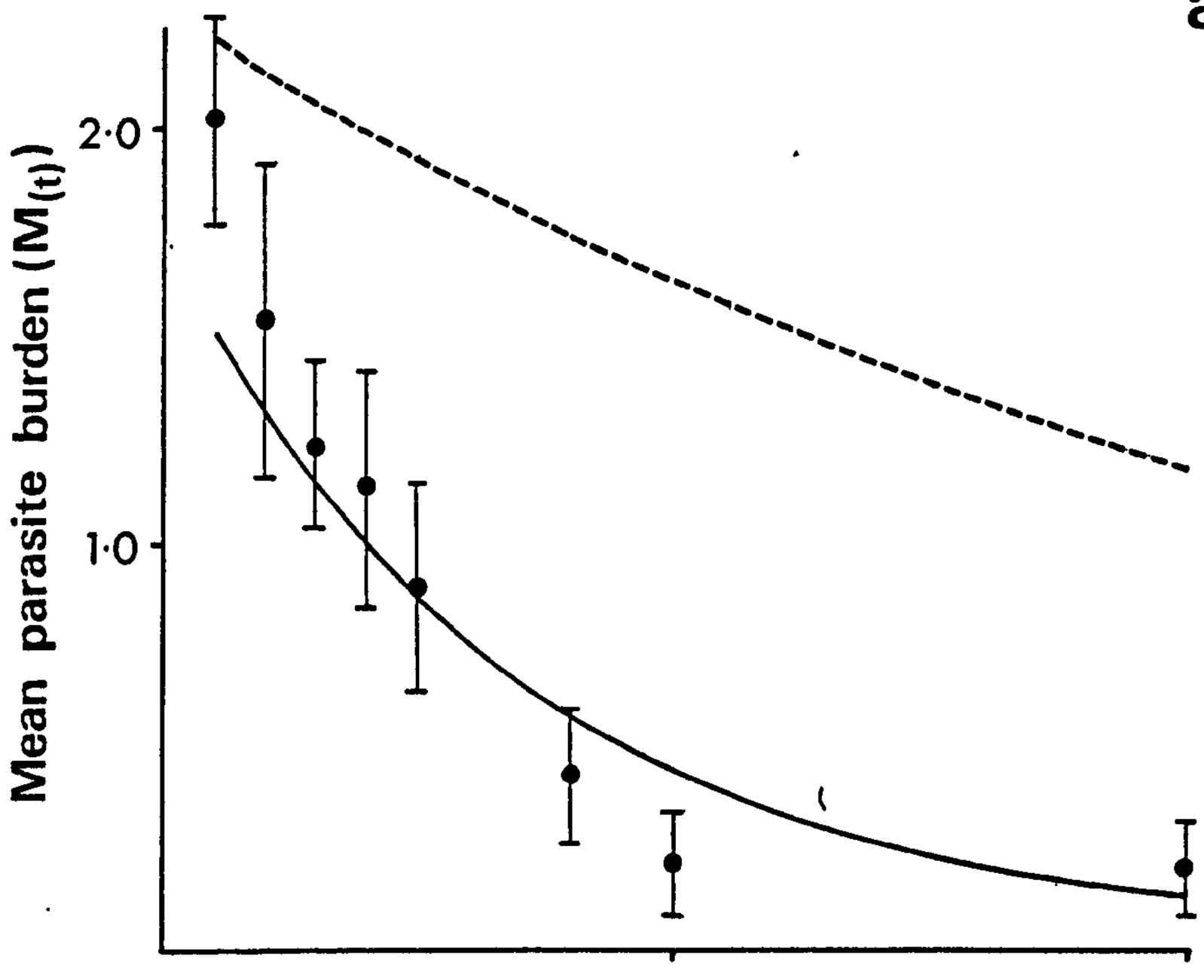
The solid line is the prediction of equation (2.4.12) where β, the transmission coefficient, varies with the host density, H (per arena) in the form $\beta = a \times 10^{bH}$, where a = 0.0149 and b = -0.0561. The other parameters are as given above.

b The relationship between the host density per arena and the transmission coefficient, β per host per infective stage per arena per hour.

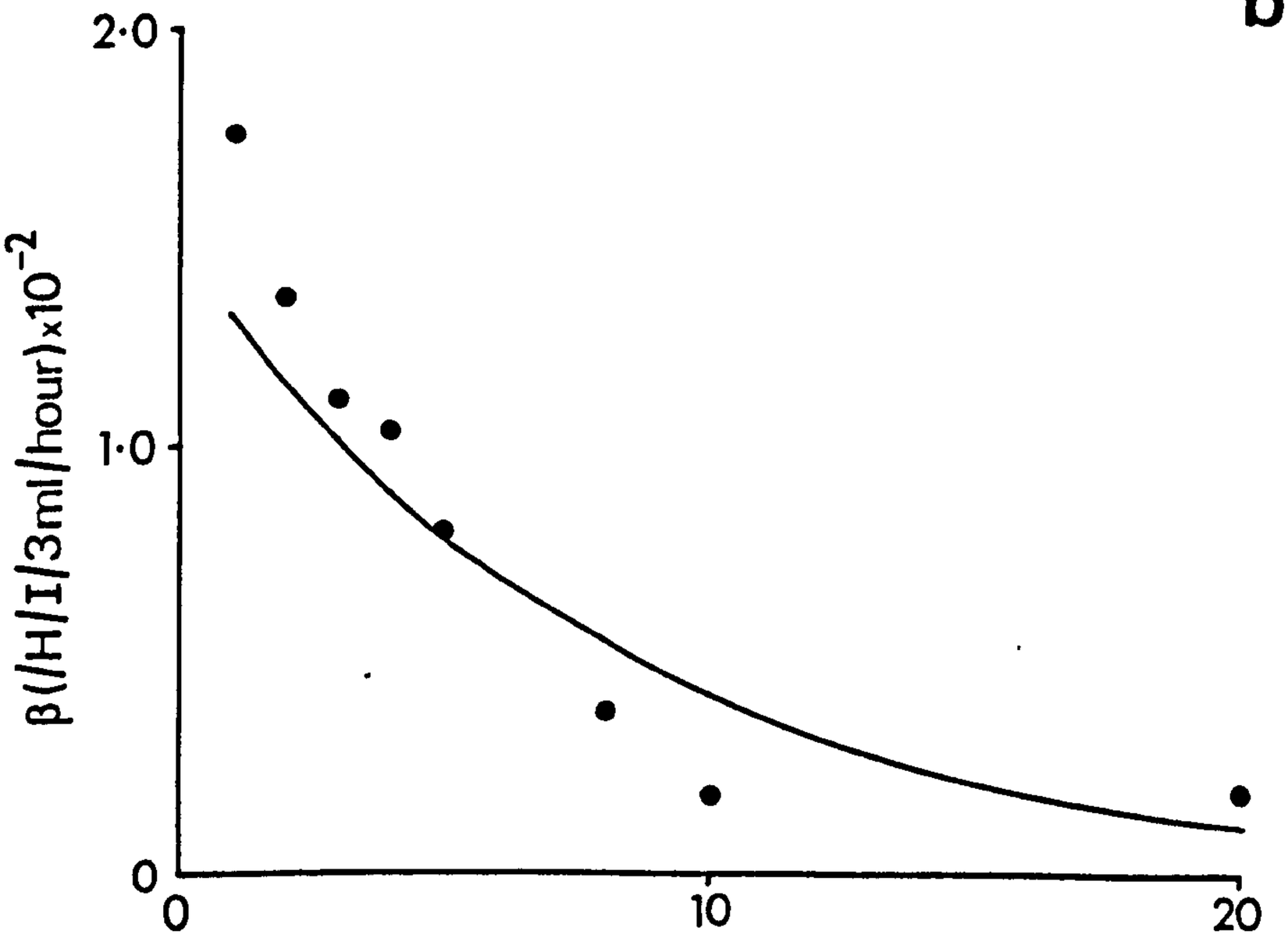
The points are values estimated from equation (2.4.19). where t = four hours, I_o = 30/arena, and $M_{(t)}$, the mean parasite burden per host, was the observed value at a given value of H, the host density per arena. The solid line is the prediction of the model, $\beta = a \times 10^{bH}$ as given above.

The arena volume was 3 ml.

a


Mean parasite burden ($M_{(t)}$)
2·0
1·0

b


β(/H/I/3ml/hour)$\times10^{-2}$
2·0
1·0
0
0
10
20
Host density (number per arena)

There are two potential contributory causes of the poor fit of the model predictions to the observed data. Firstly there is the natural variability in the infectivity of the preparasitic nematodes (as discussed in section 2.4 (iii)) whereby, with such a small pool of infective stages, the mean level of infectivity declines as those infective stages more adept at penetrating hosts leave the infective stage pool at an early stage during the exposure. The second factor is that there is a density-dependent host interaction which results in the observed fall in the mean parasite burden per host. The mode of the interaction is most probably one of mutual disturbance, as one host moves within the arena it contacts and disturbs other hosts which results in a chain reaction of further host movement and disturbance. Such disturbance would rise proportionately with host density, moving hosts providing a more difficult target for attachment and penetration by the parasite.

The result of these two processes is that, as the host density rises the instantaneous rate of infection β, (per host per infective stage per arena per hour) declines. The value of β at any given host density may be estimated from equation (2.4.19), which may in turn be derived from equation (2.4.12).

$$\beta = -\ln\left[\frac{1-HM_{(t)}}{I_o}\right] / Ht \qquad (2.4.19)$$

Values of β estimated in this way are presented in figure (2.4.14b), where it is clear that there is an exponential decrease in the transmission coefficient as the host density rises. The decline in β, seen here, corresponds to that reported by Petersen (1973a and b) and Kurihara (1979).

Given the logarithmic relationship between the transmission coefficient β and the host density (figure 2.4.14b), new predictions of the expected mean parasite burden per host can be made using equation (2.4.12) with β now as a variable. These predictions are shown as the solid line in figure (2.4.14a) and show a good fit to the data points.

The degree of dispersion of parasites within the host population appears to be consistently overdispersed as indicated by the variance-to-mean ratio, irrespective of host density, within the range examined (figure 2.4.15a). All frequency distributions of parasites, except that for the lowest host density, also show a statistically significant fit to the negative binomial distribution at the 5% level (table 2.4.3).

Table 2.4.3

Parasite distribution with increasing host density

Host density per arena	sample size (number of hosts)	Fit to negative binomial distribution	significance level (p)	variance-to-mean ratio
1	216	no	.01>P>.001	1.79
2	100	yes	.2	2.42
3	192	yes	.2>P>.1	1.63
4	100	yes	.5	1.84
5	100	yes	.5>P>.2	1.86
8	96	yes	.2	1.61
10	100	yes	.8>P.5	2.14
20	100	yes	.8>P.5	1.92

The constant level of overdispersion which is apparent, suggests that the reduction in the rate of infection occurs in such a way that the variance is reduced in direct proportion to the mean, maintaining the level of overdispersion of parasites within the host populations, despite a falling mean parasite burden. Previous sections in this chapter have

Figure 2.4.15 The influence of host density upon infection of the host: 2.

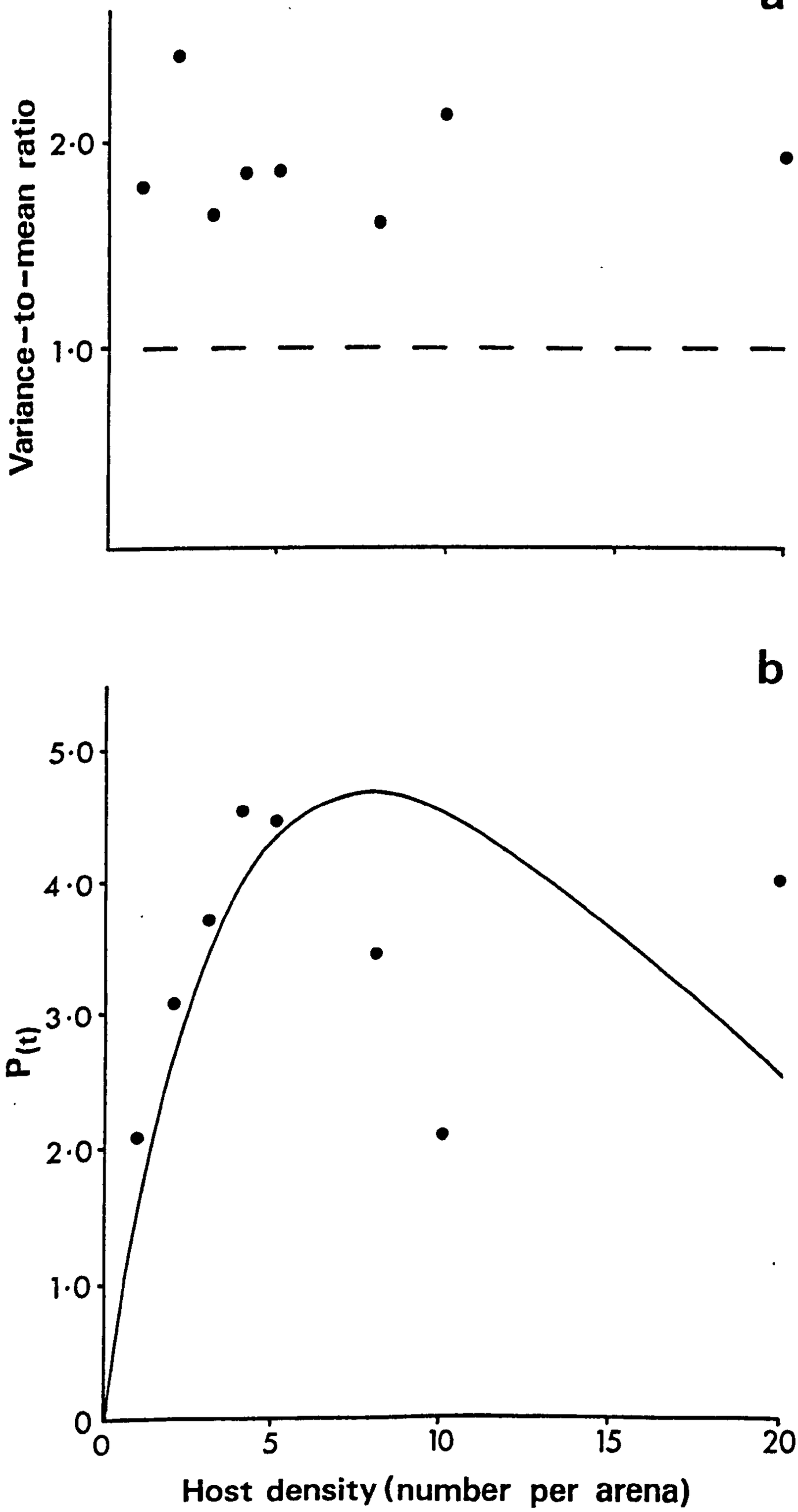
a The variance-to-mean ratio of the number of parasites per host.

The points are observed data, the dashed line represents the variance-to-mean ratio for a Poisson distribution.

b The total number of parasites within the host population per arena.

The points are observed data. The solid line is the prediction of equation (2.4.20) where I_o = 30/arena, t = four hours and β varies with H as seen in figure (2.4.14b).

The arena volume was 3 ml.


a
Variance-to-mean ratio
2·0
1·0
b
5·0
4·0
3·0
2·0
1·0
0
P(t)
0
5
10
15
20
Host density (number per arena)

shown that the degree of overdispersion tends to fall following a decrease in the mean parasite burden per host. The data portrayed in figure (2.4.15a) does not conform to this pattern, thus it appears likely that, in this case, the level of overdispersion is being maintained by the behavioural interaction between the hosts. The mechanism by which this selection occurs, is most probably, that those hosts which are least susceptible to infection benefit from the reduced frequency of infections at higher host densities to a greater extent than do those hosts which are more susceptible to infection. Thus, in the situation where fewer infections were taking place, causing a lower mean parasite burden, there would also be a tendency for less susceptible hosts to avoid infection more frequently than the more susceptible hosts. This would result in the maintenance of a consistently high level of overdispersion, despite the fall in the mean parasite burden per host.

Further light may be shed on the complexity of the relationship between host density and the dynamics of infection by examining the total number of parasites picked up by the hosts within an arena at each host density. Although there is a decline in the mean parasite burden per host with increasing host density (figure 2.4.14a), the relationship between the total number of parasites picked up per arena, ($P_{(t)}$), and host density, is less well defined. The observed data are presented in figure (2.4.15b) and show an initial rise in the total number of parasites acquired, ($P_{(t)}$), with increase in the host density. This is apparently followed by a decline in $P_{(t)}$ as the host density was further increased. Recourse to the model provides some insight, where the predicted values of $P_{(t)}$ were calculated using equation (2.4.20), derived from equation (2.4.12). The value of β was varied with H as seen in figure (2.4.14b).

$$P_{(t)} = I_o\,[1-\exp(-\beta H t)] \qquad (2.4.20)$$

The model predictions show the initial rise in $P_{(t)}$, seen in the observed data, and then a gradual decline caused by the decline in β as H increases. The shape of the curve is the result of opposing forces in the transmission process. As host density is increased there is a greater chance of contact between host and infective stage which promotes the number of infections that take place. Opposed to this is the interaction between hosts and the decline in the average infectivity of the infective stages as the pool of infective stages declines. This produces the conditions which result in an optimum density of hosts, given the limited number of available infective stages, at which the total number of parasites acquired during the exposure time peaks (figure 2.4.15b). The lack of agreement between the predictions of the model and the data point for the highest density of hosts may be due to chance variation as the sample size was reduced at this density, with only five replicates carried out.

The implications of this density-dependent constraint upon parasite establishment within hosts are considerable, with the ramifications affecting the dynamics of both the parasite and host populations. Such a relationship may well result in the regulation of the parasite population at high host densities, such that the potential effect of the parasite to depress the host population is severely reduced. In such circumstances more information is required to try to elucidate the importance of this complex relationship. Of particular relevance are studies which explore the behaviour and aggregation of both host and infective stage, particularly as aggregation has been shown to occur in populations of mosquito larvae (Hocking, 1953; Wada, 1965; Service 1968, 1977).

2.4 (vi) Host Age

It has been established by Petersen and Willis (1970) that the larval stage, or instar, of the host affects the percentage of hosts that become infected with R. culicivorax. These workers showed that the first three instars had similar susceptibilities, while the fourth instar was markedly less susceptible to infection. Poinar (1979) reports that pupae have seldom been found infected with R. culicivorax and adult mosquitoes never so.

The differences between the susceptibilities of different host instars is of importance because the host spends differing periods of time in each stage of its life-history (section 2.1) which may well affect the number of parasites establishing, particularly if significantly more time were spent in an instar with a very high or very low susceptibility.

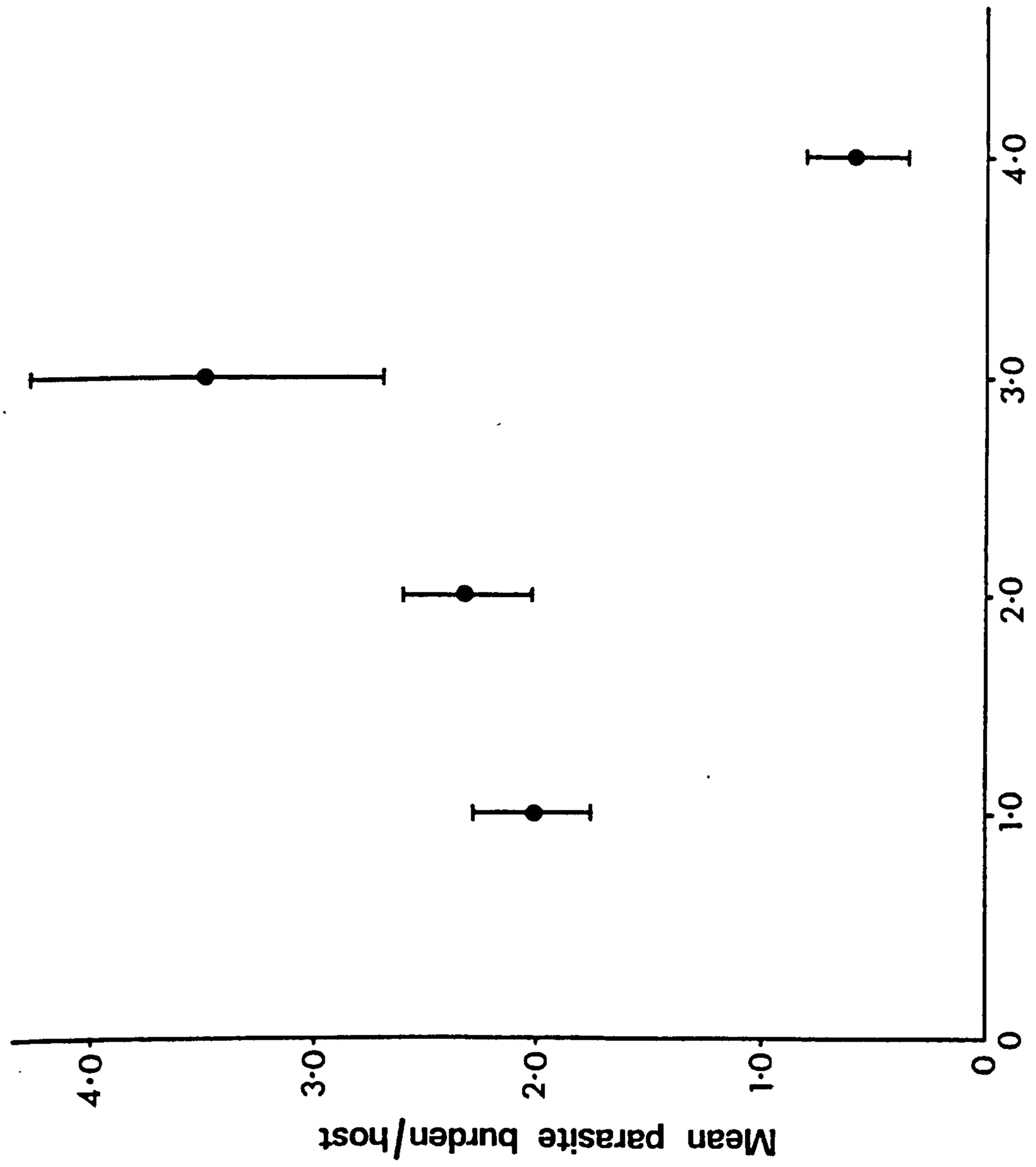
In order to quantify the relationship between host age and susceptibility in terms of the mean parasite burden attained experiments were designed where hosts were exposed under the previously described protocol. An initial infective stage density of 30 per arena (10/ml) was used with a four hour exposure period. Hosts in each of the four instars were individually exposed to infection and the mean worm burden was recorded for each instar. The results are presented in figure (2.4.16). It may be seen that there is little difference between the susceptibilities of first and second instars, the third instar is slightly more susceptible and the fourth instar is very much less susceptible; results which largely agree with those of Petersen & Willis (1970).

The reason for the initial increase in susceptibility with instar number which is evident in figure (2.4.16) is probably related to host size. Older hosts, being larger, present a better target for the infective stages. The marked reduction in susceptibility seen in fourth instar hosts is probably associated with a much thicker cuticle, together with the potential of this, the largest instar, to act as a predator of the infective stages. The larger instars also possess greater speed and mobility enabling them to avoid infection and remove infective stages which have attached.

Figure 2.4.16 The relationship between the age of the host (by instar) and the mean parasite burden per host.

The points are observed data (± 95% confidence limits).

The arena volume was 3 ml, the host density was one per arena, the initial infective stage density was 30 per arena and the duration of exposure was four hours.


Mean parasite burden/host
4·0
3·0
2·0
1·0
0·0
Host instar
0·0
1·0
2·0
3·0
4·0

From these results an estimate of the instantaneous rate of infection (β, per host per infective stage per unit volume per unit time) for each instar may be obtained. The data is replotted in the form, mean parasite burden per host (y) versus infective stage density (x) (see figure 2.4.4). The procedure for estimating β has been fully described in section 2.4 (ii) and the same method was used here. The estimates of β for each instar appear in table 2.4.4.

Table 2.4.4

Estimates of the transmission coefficient (β) for the four host instars

Instar	β (/H/I/3ml/Hr)
1	0.0193*
2	0.0201
3	0.0309
4	0.0048

* estimate from section 2.4 (ii).

These estimates reflect the changes in mean parasite burden for each instar. An overall, average rate of infection for the larval life-span of the mosquito, may be obtained when the rate of infection for each instar is multiplied by the proportion of the larval period spent in each instar (table 2.1.1) and the values summed. This method produces an estimate of the average value of β of 0.0174 (/host/infective stage/3 ml/hour). This estimate is the maximum value of β likely to occur under these experimental conditions. Use of this procedure eliminates the need to use separate estimates for each instar involved when modelling the transmission process (ch. 4).

It should be borne in mind that these estimates of β are in themselves averages of differing susceptibilities of hosts as they

age within each instar. Instars which are newly emerged, either from the egg or from the previous instar cuticle are highly susceptible to infection, their cuticle being untanned and therefore easily penetrated. These periods of extremely high susceptibility have purposely been excluded from this study, due to the problems involved in obtaining meaningful data relating to them. In spite of the high susceptibility of hosts at certain times after moulting there is no evidence of this causing an increased risk of host-parasite contact and as the proportion of each instar spent in this vulnerable state is very small it is most probable that, on average, there is little or no elevation in the mean worm burden per host due to this phenomenon. It might be expected however, that this situation may give rise to a few heavily infected hosts, increasing the degree of overdispersion in the parasite distribution.

It has been assumed, based on reports in the literature, that penetration of pupae is uncommon and of no major significance (Poinar, 1979). This apparent lack of susceptibility in pupae may be due to the infective stage failing to recognize them as hosts, or recognizing them as unsuitable. The somewhat thicker cuticle of the pupae may also have a significant role to play. There is one exception relating to the lack of importance attached to pupal penetration (and also penetration of late fourth instar larvae) and that is that penetrations at this stage may give rise to parasites within adult mosquitoes. Provided development takes place, this would enable the parasite to disperse from its original habitat. That adult mosquitoes have never been reported to be infected (Poinar, 1979) and the distribution of the parasite in the field is very localized suggests that this is not a common phenomenon. Results presented in section 3.2 do however show that adults can be infected and may well aid in parasite dispersal.

That the parasite is carried over into the adult is clearly unusual in this system and probably only occurs when the host is infected at such a late stage that the parasite is given insufficient time to moult and enter its rapid growth phase (section 2.2). This rapid growth phase restricts the development of the host, probably by the nutritional demands of the parasite. Similarly hosts infected with

Octomyomermis muspratti showed a significant increase in pupation when infected as third instars when compared with those infected as first or second instars (Petersen 1977).

2.4 (vii) Volume of the Infection Arena

It is important that the results generated by the experiments described in the previous sections bear the same relationship to each other as the size of the arena in which the hosts are exposed is increased. Clearly, if the rates of infection, and thus the observed mean parasite burden, change with the volume of the environment, (when the densities per unit volume are held constant), the use of a predictive model will be greatly hampered.

The effect of arena size has been investigated (Petersen & Willis 1970; Petersen 1973) and the results suggest that there was a decline in the number of infections that took place as the size of the arena was increased. However, in every case the density (per unit volume) of the hosts and/or the density (per unit volume) of the infective stages were changed as the volume of the infection arena was altered. This, and the use of the percentage of exposed hosts found infected as the measure of infection success, renders the data unsuitable for use in assessing the effect of arena volume upon the infection process.

The design of a suitable experiment to quantify the effect of volume upon infection is problematic. If the density of hosts and infective stagesare held constant, the mean parasite burden per host would be expected to be independent of the volume of the infection arena. However, as demonstrated in section 2.4 (v), host interaction can act to reduce the rate of infection. Thus, if the density of hosts was held constant as the arena volume was increased, there would be a concomitant increase in the number of hosts per arena. This may lead to increasing host interaction.

Figure 2.4.17 The influence of the arena volume upon the mean parasite burden per host.

The points are observed data (± 95% confidence limits).
The line is the prediction of equation (2.4.9) where,

β = 0.0193/host/infective stage/3 ml/hour.
I_o = 30/arena
H = 1/arena
μ_2 = 0.0098/infective stage/hour
t = 4 hours

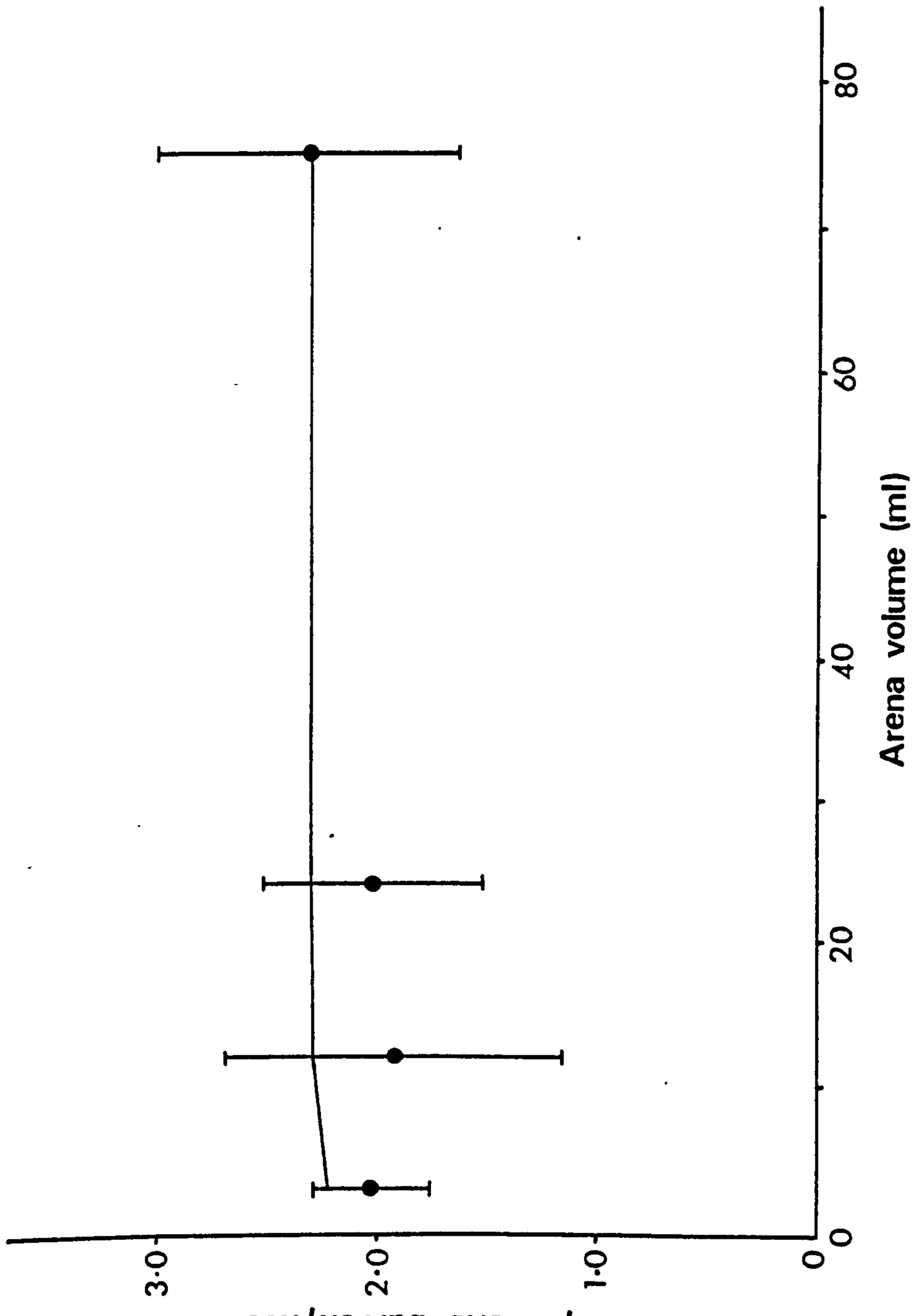
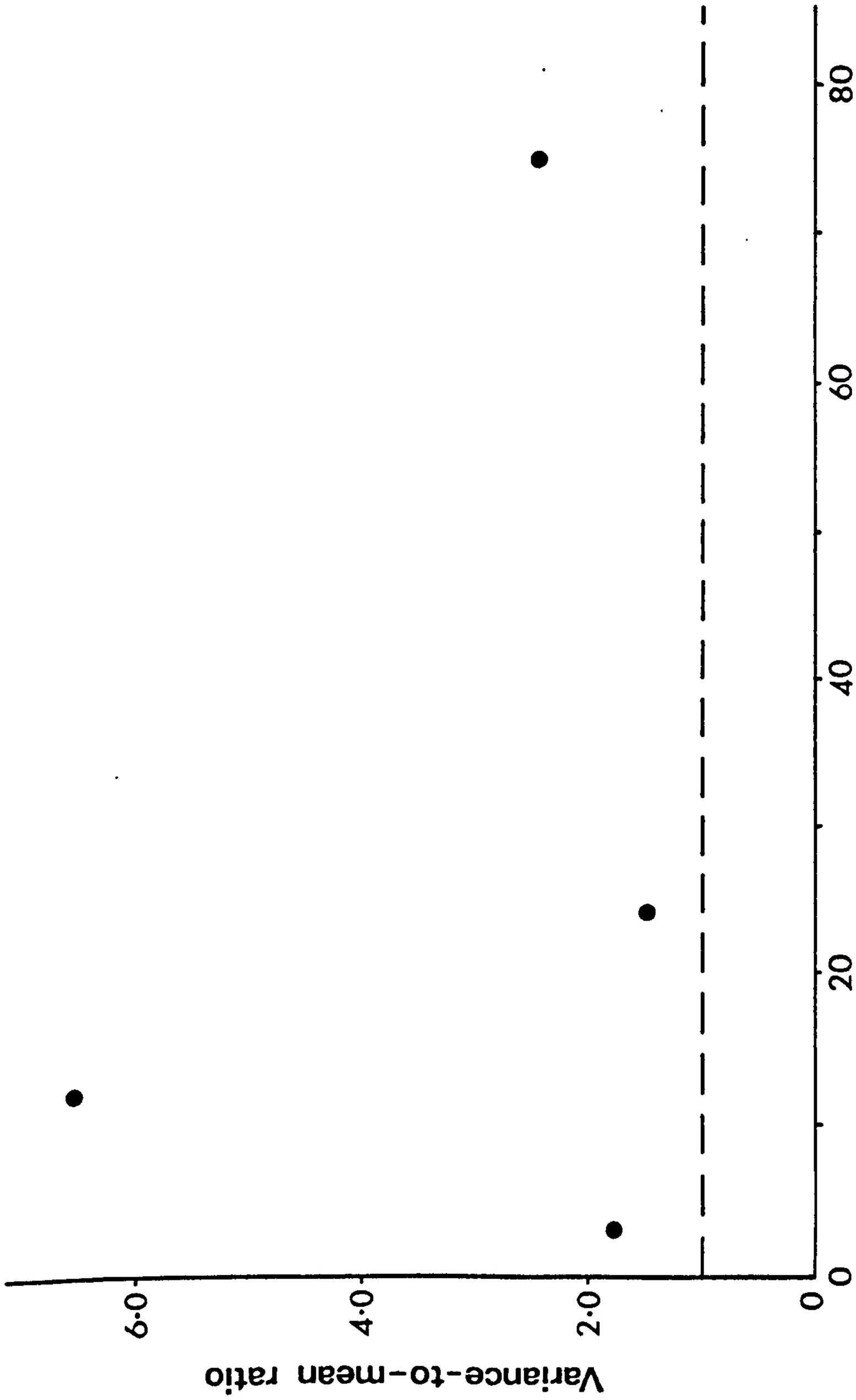

Mean parasite burden/host
3·0
2·0
1·0
0
Arena volume (ml)
0
20
40
60
80

Figure 2.4.18 The influence of arena volume upon the variance-to-mean ratio of the number of parasites per host.

The points are observed data. The dashed line represents the variance-to-mean ratio of a Poisson distribution.

The host density was one per arena, the initial infective stage density was 30 per arena and the duration of exposure was four hours.


Variance-to-mean ratio
6·0
4·0
2·0
0
0
20
40
60
80
Arena volume (ml)

Therefore, when the effect of arena volume upon infection was examined, the number of hosts per arena was held constant at unity. Arena volumes tested were 3, 12, 24 and 75 ml. A constant infective stage density of 10 per ml and a four hour exposure period were used.

The results portrayed in figure (2.4.17) show no significant change in mean worm burden per host as the volume of the arena was increased. Estimates of the expected mean parasite burden per host predicted using equation (2.4.9), (which takes into account the changing density of hosts with increasing arena volume) show an excellent fit to the experimental data. This provides the strongest possible evidence that the volume of the arena has no effect upon the infection process, provided that the density per unit volume of the infective stages remains constant.

This conclusion is supported when the variance-to-mean ratios of the mean parasite burdens are examined, where no trend in the degree of overdispersion is to be found (figure 2.4.18).

2.4 (viii) Discussion

The investigation of the transmission dynamics of any parasite is one of the most difficult areas in parasite population studies. This is largely due to the number of variables which bear upon the process, relating to the host (age, density, spatial distribution, nutritional status), the parasite (age, density, spatial distribution) and also the environment (spatial form of the habitat, temperature, pH, salinity, pO_2 etc.). In the laboratory many of these variables can be controlled thus enabling an understanding of the transmission dynamics to be built up.

Several factors have been shown to be of major importance in the dynamics of transmission in this parasite-host association. Firstly, the survival characteristics of the infective stage (a principle determinant of the infective stage density and age distribution) was shown to be strongly age-dependent (section 2.4 (i)), a pattern similar to other small non-feeding parasite transmission stages. It would be expected that this pattern would persist in the field, although the duration of the mean expected survival would vary with the intensity of the various pressures placed upon the population (predation, other pathogens and environmental stresses). The form of the survivorship curve also represents, at population equilibria, the stable age distribution of the nematode, which is of importance due to the decline in infectivity with increasing infective stage age (section 2.4 (iv)). Thus there is a need to be able to assess the mean age of the infective stages in order to estimate the average level of infectivity.

Infection critically depends upon host-infective stage contact, therefore, the densities of both infective stages and hosts are both very important in determining the level of transmission. A relationship of direct proportionality between the density of infective stages of the mean parasite burden per host (section 2.4 (ii)) indicates that, at least up to the highest density used herein, there is no density-dependent constraint upon the infection process (mediated for example by mutual interference between the infective stages). The lack of a density-dependent (i.e. regulatory) constraint which acts via the infective stage density indicates that the density of preparasites in the environment will be one of the major biotic factors that promotes the level of parasitism attained.

An estimate of the transmission coefficient, (β), from this directly proportional relationship, proved to be the maximum value of β likely to occur under such conditions, as the subsequent section (2.4 (iii)) showed that β was in fact not constant through time. A

second estimate of β, one reflecting a more normal exposure situation (large value of t) was made, although the precise cause of the change in the magnitude of the transmission coefficient with exposure time could not be accounted for.

The negative influence of increasing host density upon the value of the mean parasite burden per host (section 2.4 (v)) was not expected, a directly proportional relationship between the number of parasites establishing in a given time and the density of hosts is a common assumption in such situations. That the host is relatively so much larger than the infective stage, allied to the activity of the host and the aquatic habitat, does lend to the situation the potential of disturbance by the movement of the host. It is suggested, however, that the higher host densities used (up to twenty per arena) were very high relative to field estimates of larval density (see section 3.3). The density per unit area, the only unit usually reported in field studies, given that the area of the arena was approximately 1.9cm² varied from 5,200 hosts per m² (1 per arena) to 104,000 hosts per m² (20 per arena). Densities in the field tend to occur in the region of hundreds to a few thousand per m², exceptional circumstances are needed to produce the occasionally seen very high densities of tens of thousands of larvae per m². It is therefore suggested, even when the tendency of larvae to aggregate is taken into account (Hocking, 1953 ; Wada, 1965; Service, 1968; 1977), that in the field the effect of this host interaction would be much less pronounced. It is also probable that greater water depth would allow for altered behaviour at high density which may alleviate the disturbance effect together with the possible dispersal of hosts from areas of very high density.

One of the major features of an experimental system is to simplify as far as possible (whilst still reflecting its reality) the situation that one wishes to examine, in order to facilitate the examination process and allow useful observations to be made. It is, however, of paramount importance that the information gathered and

the deductions made from such investigations remain largely true in more complex situations, such as the field situation. That the same pattern of infection may be seen in much greater volumes of water that the standard 3 ml infection arena (2.4 (vii)) is a strong indication that the information gathered relating to the transmission of the parasite reported in this chapter is, as far as environment size is concerned, a fairly accurate reflection of the transmission process.

2.5 DYNAMICS OF THE PARASITIC STAGE

The survival characteristics and development time of the parasite within the host are important parameters affecting the dynamics of both parasite and host. Only a small proportion of the eggs laid in one generation will give rise to parasites within mosquito larvae (as shown earlier) and losses of the nematode during the parasitic phase will have a significant effect upon the dynamics of the parasite population. Similarly, the time delay between the infection of the host and emergence of the postparasitic nematodes may also be of significance. Long time delays have been shown to cause increasing degrees of instability in parasite populations (May & Anderson, 1978), resulting in fluctuations in parasite numbers tending towards cyclical behaviour and a greater likelihood of parasite extinction during troughs in population abundance.

With respect to mermithid-mosquito associations, the development of the parasitic stage of R. culicivorax has been investigated in A. aegypti by Gordon et al. (1974) and Gordon, Squires, Babie & Burford (1981); in C. pipiens by Petersen (1972), Platzer & Brown (1976), and Hughes & Platzer (1977). Aspects of parasite survival have been examined by Petersen (1972) and Gordon et al. (1981) for R. culicivorax, and by Petersen (1977) for Octomyomermis muspratti in C. pipiens.

Despite this body of literature, no serious attempts have been made to assess the significance of either the development time or survival of the parasite in terms of the general population dynamics of the parasite. The potential importance of any density-dependent parasite-induced premature host mortality has also been overlooked.

To redress this situation these important characteristics have been the subject of experimental investigation, in order to try to quantify the importance of each in the dynamics of the parasite population.

2.5 (i) Parasite survival

The initial importance of parasite survival characteristics is

self-evident; there are, however, three distinct causes of parasite loss that have to be considered. Firstly, parasites may die naturally within the host, due perhaps to some genetic or metabolic deficiency. Secondly, the host may mount a defence response resulting in the death of the parasite. Finally, the host may die prematurely, resulting in the death of its parasite population.

The natural death of parasites within the host could be expected to occur at a fairly constant, but relatively low level, probably associated with the moulting of the nematode and the metabolic switch to the active growth phase. This occurrence is almost impossible to investigate, in this or any other parasite system, due to the nature of the relationship and also due to the difficulty in differentiating between the effects of natural and host-induced parasite mortality.

Host-induced mortality arises due to an immunological response (specific or nonspecific) of the host to the parasite, usually taking the form of a cellular or humoral response, encapsulation and melanization respectively.

The host, C. p. fatigans, has, to date, not shown any sign of mounting an effective response to the presence of the parasite (see Poinar, 1979) but other host species have, (Petersen, Chapman & Willis, 1969; Ignoffo et al. 1973; Kerdpibule, Deesin, Sucharit & Harinasuta, 1974; Mitchell, Chen & Chapman, 1974; Petersen, 1975), both by encapsulation and melanization. During this study no parasites were found to be dead, dying or obviously affected by an immune response whilst in the host.

The death of the host, resulting in the death of its parasites, may occur in one of two ways. Either by the natural or environmentally influenced mortality of the host (predation, other pathogens, insecticides etc.), or by parasite-induced host mortality. Here a clear distinction must be made between the parasite-induced host death resulting in the release (emergence) of viable postparasitic nematodes, and parasite-induced mortality where the death of the host also leads to the death of the nematodes.

In other host-parasite systems, parasite induced host mortality has been shown to be dependent upon the density of the parasites, in either a linear or non-linear manner (see Anderson & May, 1978; Keymer, 1980b). The effects of linear and the more complex non-linear relationships between parasite burden and host survival are discussed by Anderson (1978b) and Anderson & May (1978). The difference between the effects of a linear and non-linear relationship are important enough to require a detailed investigation to ascertain the form of the relationship in this case. Data presented by Kurihara, (1979) suggest that the relationship is linear in form in this host-parasite association.

Three experimental regimes were used to investigate the effect of parasite burden on host survival. The first provided an estimate of the host mortality caused by the penetration and initial establishment of the nematodes. The protocol was, as in previous infection experiments, to expose 16 - 24 hour old 1st instar hosts to increasing densities of infective stages. Exposed hosts were examined 16 - 24 hours post-infection and the number of dead hosts were recorded. The data shown in table (2.5.1) strongly suggest that, as the intensity of exposure increases, as measured by greater infective stage density, there is a tendency for a greater percentage of the hosts to die. The change, evident when infective stage density is varied (table 2.5.1), can clearly be correlated with the linear increase in mean parasite burden per host as preparasite density increases (ch. 2.4 (vi)). The mortality is indubitably the result of the mode of infection. Mortality is caused by several factors, including loss of haemolymph due to penetration of the cuticle, trauma caused by the penetration process and possibly, secondary infections via the wounds.

Table 2.5.1

Mortality of hosts 16 - 24 hours post-infection for hosts exposed to a range of infective stage densities and two exposure times.

Exposure time (hours)	Infective stage density (/ml)	No. hosts exposed	% hosts dead
2	2	24	0
	5	48	2.1
	10	96	3.1
	15	48	0
	20	94	5.3
	25	-	-
	30	98	5.1
4	2	71	0
	5	47	0
	10	529	3.6
	15	-	-
	20	96	1.0
	25	-	-
	30	96	20.8

The second series of experiments consisted of the exposure of a large number of 16 - 24 hour old hosts to known densities of infective stages for a period of four hours. The exposed hosts were subsequently maintained in uncrowded conditions, as described in chapter 2.3 (ii). Immediately post-infection and at the same time on succeeding days, at least thirty of the exposed hosts were dissected and the number of parasites in each recorded. The mean number of parasites per host, the variance and the variance-to-mean ratio were calculated for each sample. Any significant parasite-induced premature host mortality would be seen as a fall in the variance-to-mean ratio in the sample immediately after the time at which the deaths took place. Figure (2.5.1) shows the results of these experiments in terms of the variance-to-mean ratios. There is little evidence of any density-dependent parasite-induced host mortality except in one of the experiments. The conditions under which the hosts were kept were, however, ideal for

Figure 2.5.1 The relationship between the age of the infection and the variance-to-mean ratio of the number of parasites per host.

The points are observed data.

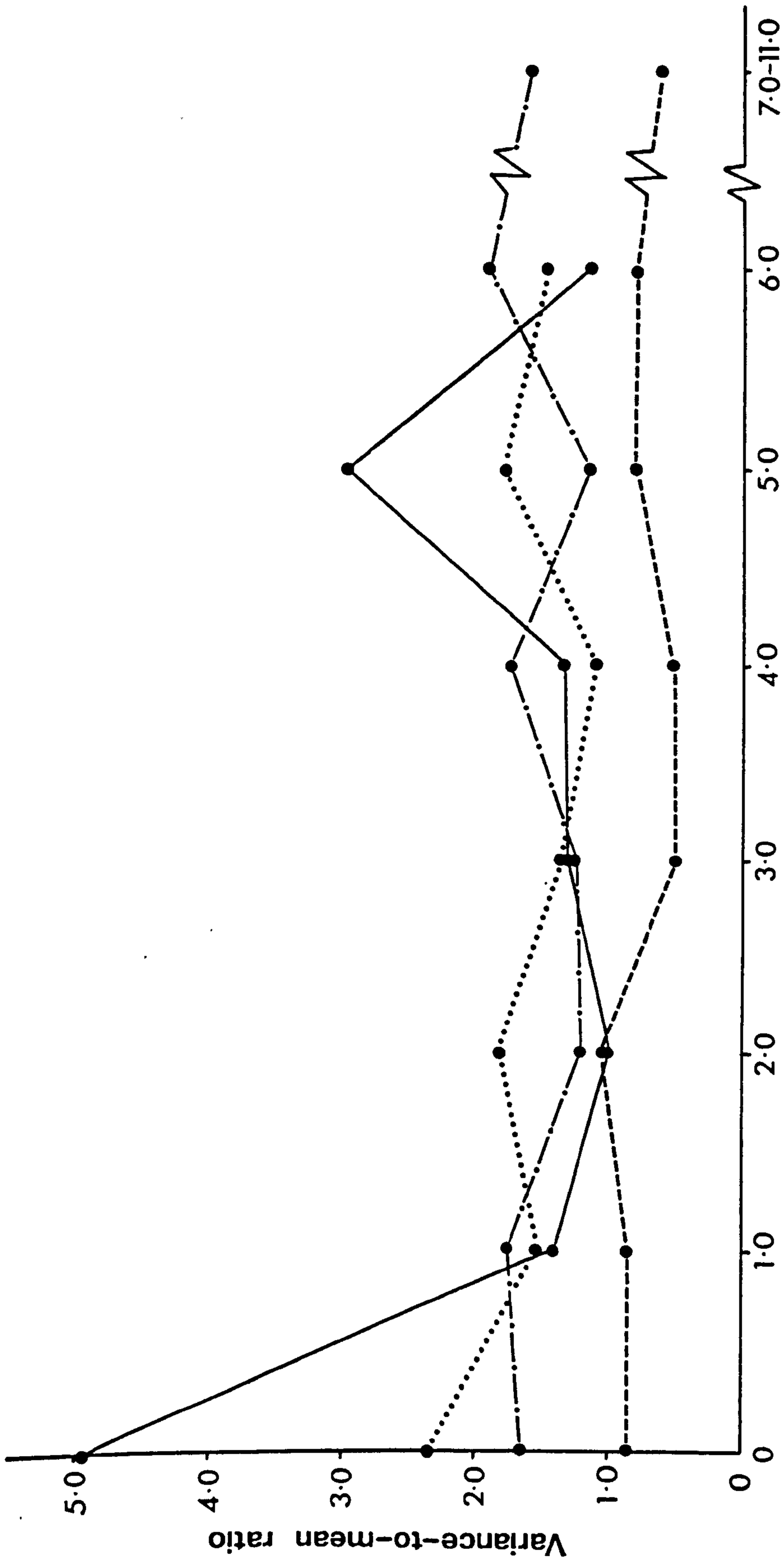
The lines indicate different host populations with the following mean parasite burdens per host at time t = 0,

1.033 parasites/host ————————

1.867 parasites/host ••••••••••••

2.700 parasites/host – – – – – – –

4.033 parasites/host —·—·—·—


Variance-to-mean ratio
0
1·0
2·0
3·0
4·0
5·0
Time post infection (days)
2·0
3·0
4·0
5·0
6·0
7·0-11·0

enabling most of them to survive. Under conditions of nutritional stress, strong density-dependent parasite-induced host mortality was demonstrated by Petersen, (1972). In addition, Gordon, et al. (1981) demonstrated that reduction in both quality and quantity of food given to infected mosquito larvae led to increased mortality of the hosts. This mortality, however, could not be directly linked to the parasites, as the effect of the various diets on uninfected hosts was not reported.

The third and final experiment consisted of exposing a known number of 16 - 24 hour old hosts to infective nematodes for up to eighteen hours at 25°C, with the object of producing a range of infection levels within different groups of hosts. The hosts were then maintained on a full diet at 25°C until either pupation or parasite emergence took place. The proportions of hosts that died prematurely were recorded. The mean parasite burden of those hosts that survived, either to produce viable postparasitic nematodes or to pupation was also recorded. The relationship between the proportion of the exposed hosts which died between infection and emergence/pupation, (i.e. those which died prematurely), and the final mean parasite burden at the time of emergence is linear and positive (figure 2.5.2), the slope of the line is however, quite shallow (b = 0.0238). This supports the hypothesis of density-dependent parasite-induced host mortality, although the density-dependence is clearly not strong under these conditions.

However, when identical experiments were performed at 15°C, 20°C and 30°C, no significant trend was apparent in the results (figure 2.5.3). It is possible that the relationship shown in figure (2.5.2) is erroneous, in that there is no density-dependent parasite-induced host mortality. However, taking into account published work (Petersen, 1972; Gordon et al. 1981), the relative sizes of host and parasite and the mode of parasite nutrition, there appears to be overwhelming evidence that density-dependent parasite-induced host mortality does occur; but at a low rate, ensuring development of the parasites before host mortality occurs. The possible reasons for lack of confirmation of density-dependent parasite-induced host mortality at temperatures other than 25°C are two-fold. Firstly, chance

Figure 2.5.2 The relationship between the mean parasite burden per host and the proportion of hosts that died between exposure to infection and emergence of the nematodes, at 25°C.

The points are observed data.

The line is the best-fit least-squares linear regression of y on x, where,
a = 0.0560, b = 0.0238 and r = 0.8708
(9df. p <.001).

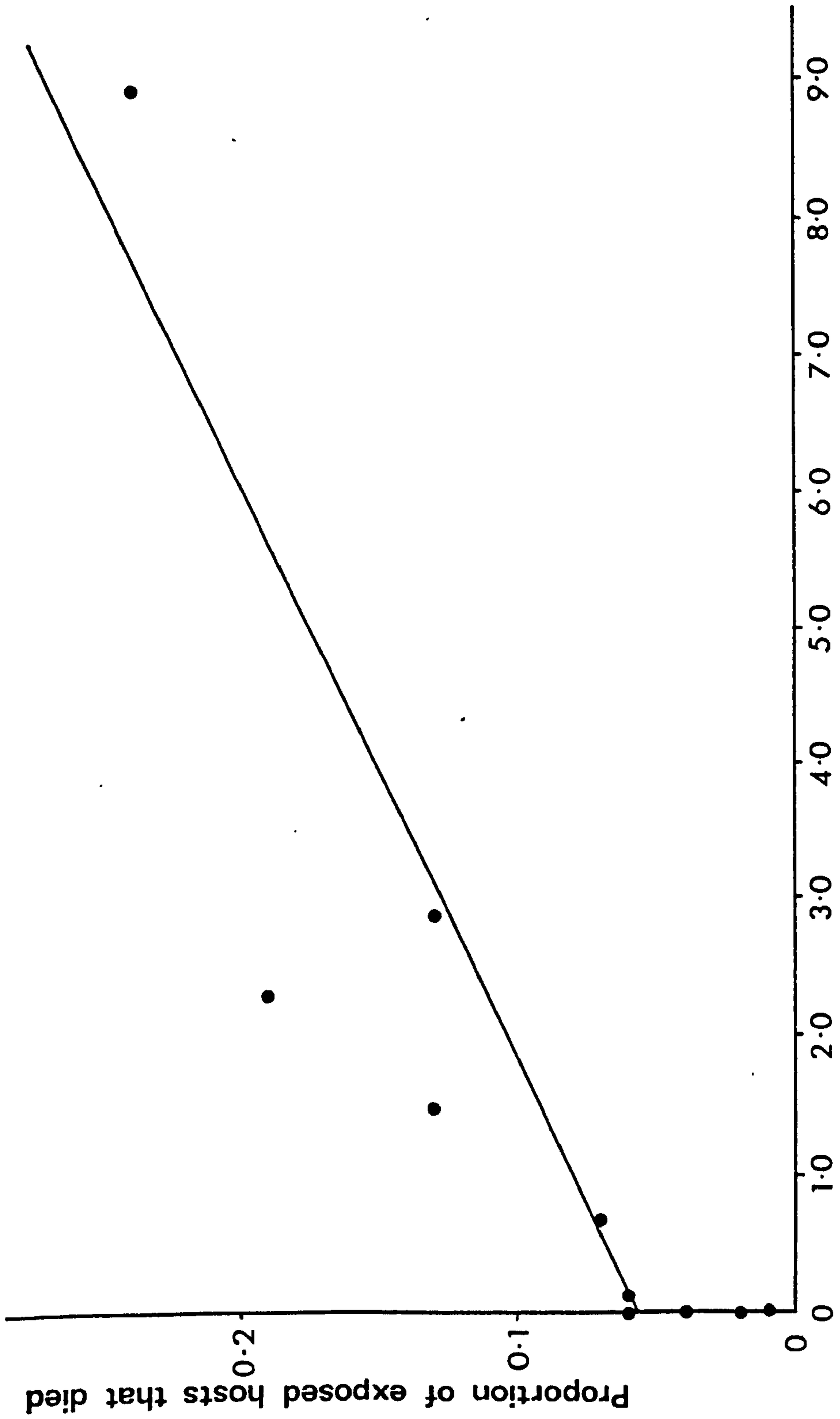

Proportion of exposed hosts that died
0·2
0·1
0
0
1·0
2·0
3·0
4·0
5·0
6·0
7·0
8·0
9·0
Mean number parasites/host

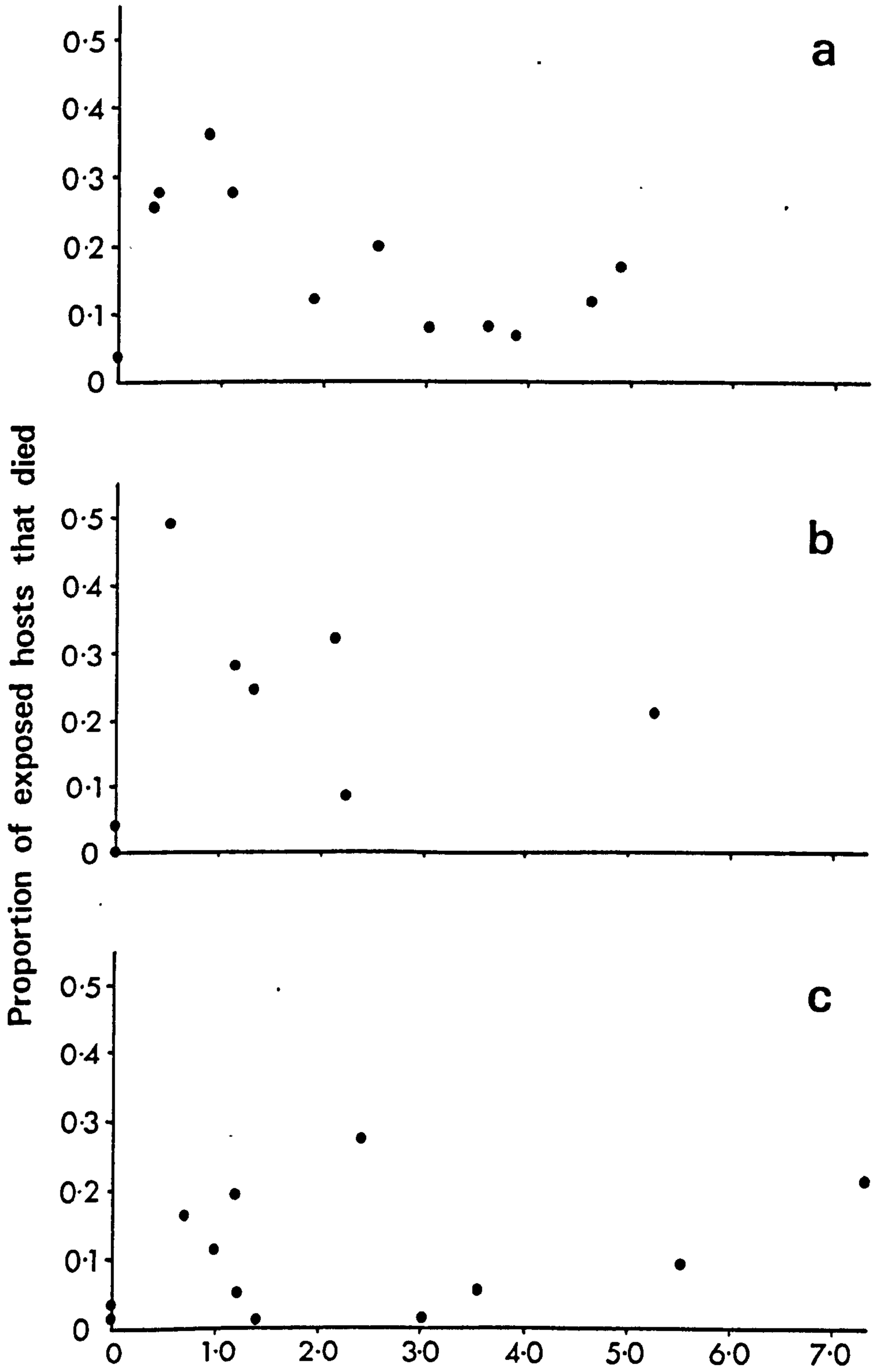
Figure 2.5.3 The relationship between the mean parasite burden per host and the proportion of hosts that died between exposure to infection and emergence of the nematodes at, 15°, 20° and 30°C.

The points are observed data.

a 15°C

b 20°C

c 30°C


a
b
c
0·5
0·4
0·3
0·2
0·1
0
Proportion of exposed hosts that died
0
1·0
2·0
3·0
4·0
5·0
6·0
7·0
Mean number parasites/host

events in sampling, linked with variability, both natural and due to slight differences in experimental protocol, masked what figure (2.5.2) shows to be a positive, but not very marked, relationship between host mortality and increasing parasite burden. Secondly, sub-optimal temperatures alter the physiology and behaviour of both host and parasite, which may either eliminate the relationship seen at 25°C, or more likely, increase chance variation ("noise") in host mortality, parasite mortality, and the effect of the parasite upon the host, causing the relationship to be masked. Further useful information might be gained from repeating these experiments in conditions other than ideal for the host, i.e. under stress due to crowding or a restrictive diet.

2.5 (ii) Length of the Parasitic Stage

The rate of development of invertebrates is dependent upon the ambient temperature and there frequently is an optimum temperature at which the rate of development is maximal. Such is the situation for both parasite and host under discussion here.

Although the rate of development of *R. culicivorax* is principally determined by the temperature, development time decreasing exponentially with increase in temperature (Platzer & Brown, 1976; Hughes & Platzer, 1977), there are other factors which can make a considerable difference to the development time. These factors include the quality and quantity of food provided for the host (Gordon *et al*; 1981) and the parasite burden per host (Petersen, 1972).

The importance of the length of the parasitic phase in the population dynamics of the nematode is intuitively obvious, the longer the parasitic phase, the longer will be the generation time of the nematode and, as such time delays increase, they tend to have a destabilizing effect upon the host-parasite interaction, often leading to parasite population cycles (May & Anderson, 1978). Further, increased time spent within the host increases the probability of the death of the parasites as a result of host mortality due, for example, to predators, other pathogens and insecticides.

It might also be expected that as different species of mosquito differ widely in their rates of development, in size and in the type of food they consume, the development of the parasite would be affected by the host species; this aspect of the biology of R. culicivorax has not been studied.

Data which relate to parasite development times are usually presented as the range from the start to the completion of worm emergence: in this form the data are unsuitable for use in quantitative comparisons to investigate factors which affect development time. For this reason, an experimental investigation of development times was carried out, in which the mean time to emergence was used to try to quantitatively evaluate the importance of factors which influence the length of the parasitic stage of the nematode.

Firstly, the mean development times of male and female parasites were investigated at 25°C. Hosts between 16 and 24 hours old were exposed, en masse and singly to a standard density of 10 preparasites per ml for four hours. After their removal from the arena containing the infective stages, hosts were maintained as described previously. The number and sex of the nematodes emerging were recorded daily (figure 2.5.4). The overall mean parasite burden per host was 1.75 and the proportion of females was 0.39 of the 1090 nematodes recovered. It may be seen from figure (2.5.4) that males emerge somewhat earlier than females, the mean time to emergence (post-infection) for males was 7.35 days and that for females was 8.57 days.

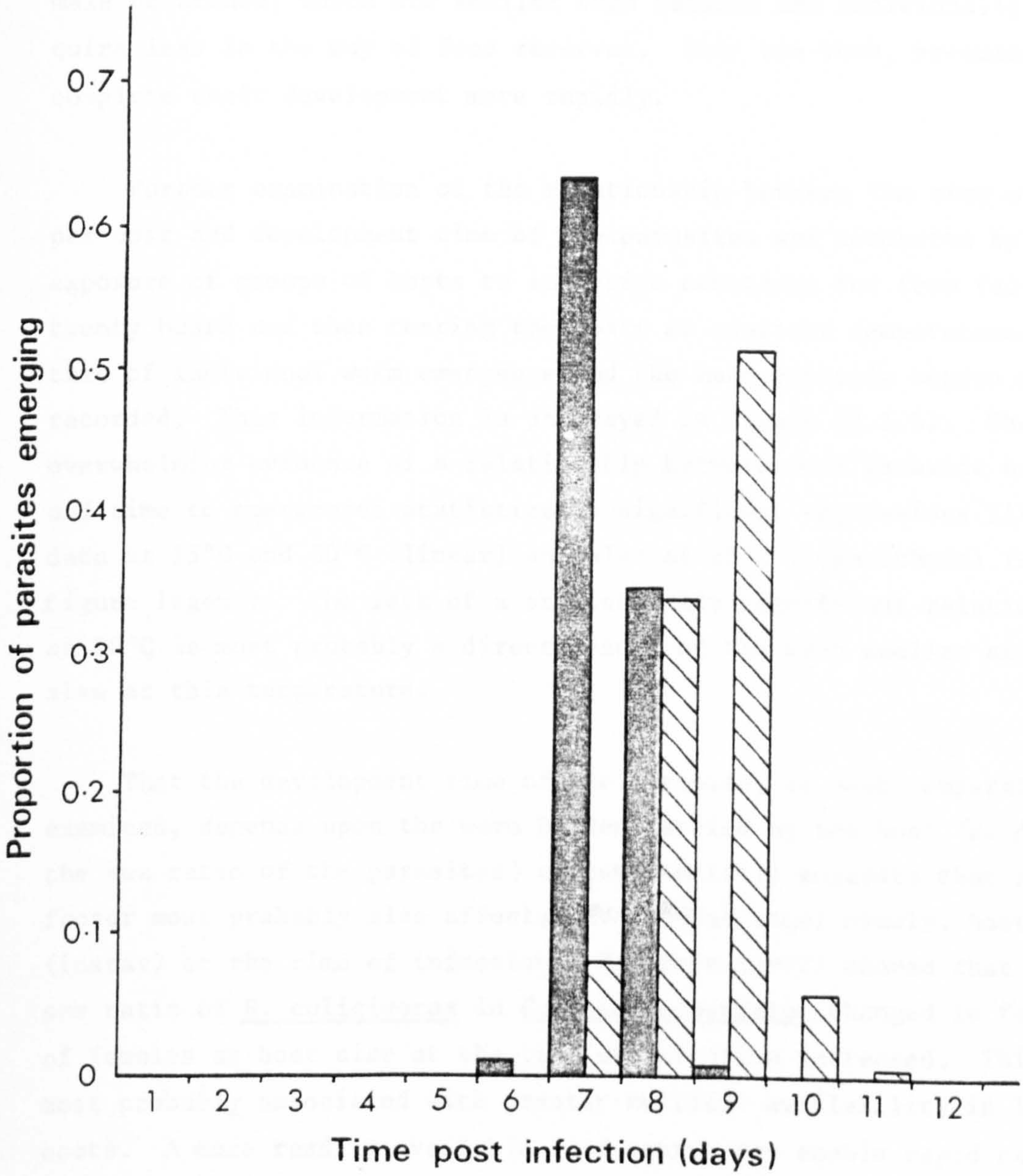
The difference in development time between male and female nematodes is most probably a result of the method of the sex determination of the nematodes, and it is the number of parasites which principally determines the development time in addition to the sex of the parasites (see chapter 2.6). Clearly, as Petersen (1972) has shown, development time is linked to parasite burden and there is a definable relationship between parasite burden and the sex determination of the nematode, these factors are all linked. It seems most probable that in this sequence of events there are two components working in tandem to delimit the period of the parasitic stage of the nematode, both of which are controlled by the number of parasites per host. Firstly,

Figure 2.5.4 The relationship between the age of the infection and the proportion of emergent nematodes.

Solid bars - male nematodes (n = 665)

Hatched bars - female nematodes (n = 425)

(overall mean parasite burden/host = 1.75).



Solid bars – Male nematodes

Hatched bars – Female nematodes

and most important a large number of parasites within a host will weaken that host more rapidly than fewer parasites, by both their activity and nutritional demands. This, in turn, will promote their emergence. Secondly, increasing parasite burdens give rise to more male nematodes, which are smaller than females and individually require less in the way of food reserves. They can thus, presumably, complete their development more rapidly.

Further examination of the relationship between the mean worm burden per host and development time of the parasites was conducted by the exposure of groups of hosts to infective nematodes for from four to twenty hours and then rearing the hosts at constant temperature; the time of individual worm emergence and the mean parasite burden were recorded. This information is portrayed in figure (2.5.5). There is overwhelming evidence of a relationship between mean parasite burden and time to emergence; statistically significant regressions fit the data at 15°C and 30°C (linear) and also at 25°C (logarithmic) (see figure legend). The lack of a statistically significant relationship at 20°C is most probably a direct result of the much smaller sample size at this temperature.

That the development time of the parasite, at each temperature examined, depends upon the worm burden carried by the host (as does the sex ratio of the parasites) circumstantially suggests that another factor most probably also affects development time; namely, host size (instar) at the time of infection. Petersen (1972) showed that the sex ratio of R. culicivorax in C. quinquefasciatus changed in favour of females as host size at the time of infection increased. This is most probably associated with greater nutrient availability in larger hosts. A more readily available food source may enable rapid parasite growth and a marginal shortening of development time.

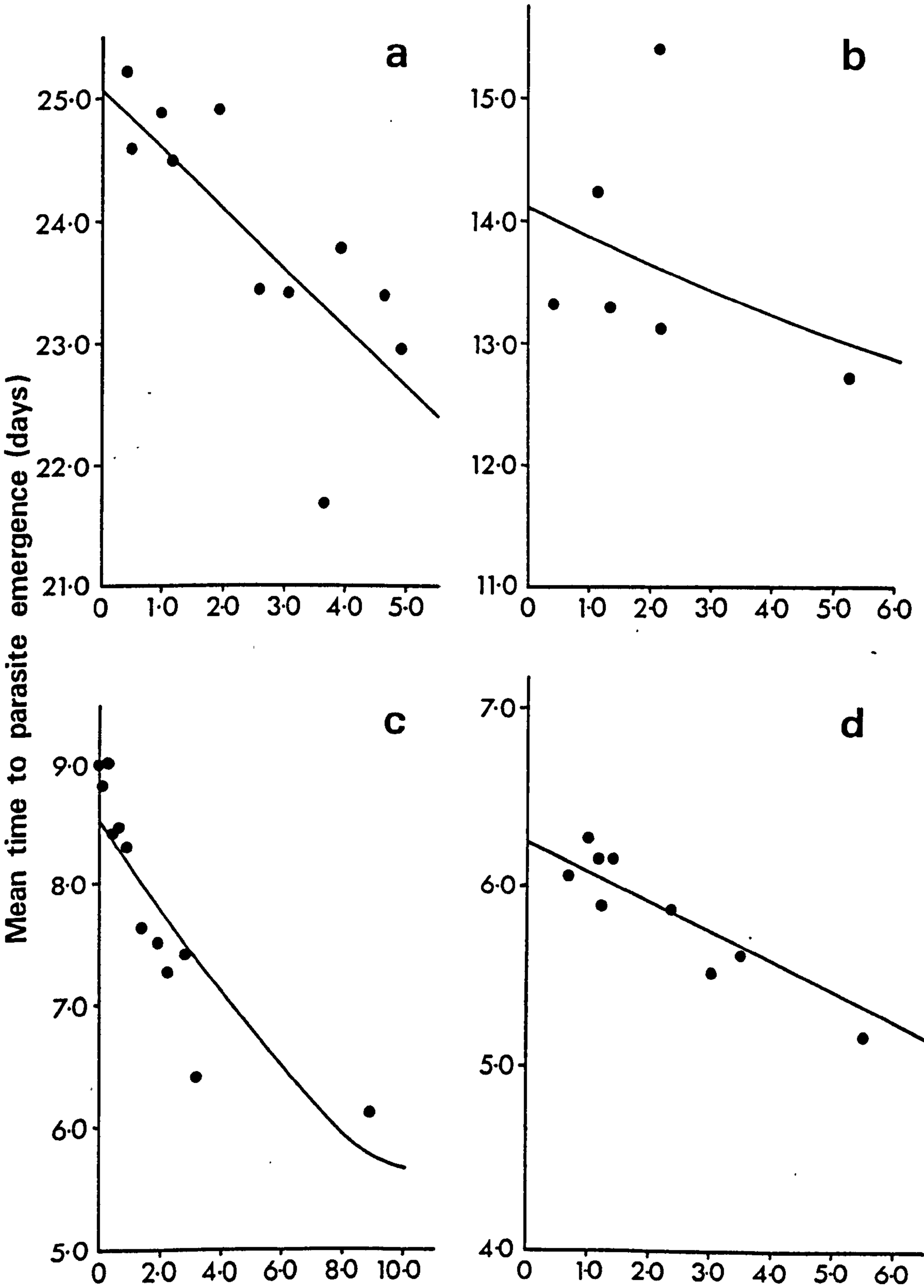
Host diet has also been shown to affect development times; restriction of host diet either in quantity or quality leads to lengthened parasite development times (Gordon et al.,1981), (the interaction between development time and the sex of the nematodes was not a complicating factor as the food quality was of such a low standard that in

Figure 2.5.5 The influence of the mean parasite burden per host upon the mean time to parasite emergence.

The points are observed data and the lines are, in a. and d. least-squares best-fit linear regressions of y on x, and in b. and c. regressions of lny on x.

a.	15°C	a = 25.0916,	b = -0.4802,	r = -0.7589
b.	20°C	a = 2.6527,	b = -0.0154,	r = -0.3751*
c.	25°C	a = 2.1446,	b = -0.0456,	r = -0.8767
d.	30°C	a = 6.2630,	b = -0.1670,	r = -0.9207

* not significant at the 5% level.


a
b
c
d
Mean time to parasite emergence (days)
25·0
24·0
23·0
22·0
21·0
15·0
14·0
13·0
12·0
11·0
9·0
8·0
7·0
6·0
5·0
7·0
6·0
5·0
4·0
0
1·0
2·0
3·0
4·0
5·0
6·0
0
2·0
4·0
6·0
8·0
10·0
Mean parasite burden/host

each test at least 88% of the parasites were male).

The effect of temperature upon the development of R. culicivorax has been examined by Hughes & Platzer (1977). Using C. pipiens as the host, they measured the median development times at both constant and fluctuating temperatures, they estimated the "developmental zero" temperature of the nematode, the rates of development (per capita per unit time) at different temperatures and the mean number of heat units (day-degrees) necessary to complete parasite development. This analysis however, did contain an important omission, in that the mean parasite burden per host was not reported and as has been shown, this can greatly affect the development time and is necessary for comparative studies. Because of the anomaly, and the use of median development times, an investigation was made of development of the parasite; the development of the host was examined simultaneously.

First-instar hosts were infected at 25°C or 30°C for between four and twenty hours and were then transferred to a constant temperature of 15°C, 20°C, 25°C or 30°C. Controls of uninfected hosts were simultaneously prepared. Hosts were fed ad libitum and isolated into 10-20 ml vials just prior to parasite emergence. The time of emergence and the number and sex of the nematodes were recorded. Also noted was the time of pupation of the uninfected hosts and control hosts. Hosts kept at 30°C were exposed at 30°C; those maintained at lower temperatures were exposed at 25°C. This was in order to enable high levels of parasitism to be achieved with relatively short exposure times, which cannot be achieved at lower temperatures due to the reduced activity of the infective stages. This introduced a degree of error into the estimation of the development data for 15°C and 20°C; however, this error was minimal, since the time spent at 25°C during infection was a small proportion of the total development time for both hosts and parasites when kept at the lower temperatures.

In an effort to create a base for direct comparison of similar analyses, the examination of the development of the parasite was restricted to hosts which produced only single female nematodes. The selection for study of single female parasites eliminated the complications of different mean worm burdens upon the developmental time

of the parasite and can also be justified, in that female development time is the determining factor in the time-delay resulting from this phase of the nematode life-cycle; the male worms emerging first.

The mean development times of single female parasites over the temperature range 15°C - 30°C are portrayed in figure (2.5.6a). Figure (2.5.6b) portrays similar data which relate to the development of the hosts (already 16 - 24 hours old). The form of these relationships is identical to that found by Hughes & Platzer (1977) for R. culicivorax and also for many insects (Varley, Gradwell & Hassell, 1973). The development times with changing temperature are described by a hyperbolic function of the form,

$$y = b\,\frac{1}{x} + a \qquad (2.5.1)$$

where the constants a and b may be estimated by a least-squares linear regression of y upon the reciprocal of x. For the parasite, a = 14.959, b = 621.707, with a correlation coefficient (r) of 0.996, which shows a very highly siginficant fit of the regression to the data ($p<0.001.3df$). Similarly for the host, a = -4.699, b = 284.919 and r = 0.995 ($p<0.001.3df$).

Having demonstrated that the development time-temperature curves may be described in both cases by a hyperbolic function, the per capita development rates may be obtained by taking the reciprocal of the development times predicted by the hyperbolic function (equation 2.5.1). These development rates, shown in figure (2.5.6a and b), are clearly linear and thus the developmental zero (T_o) may be found where the least-squares regression line intercepts the x axis ($T_o = -\frac{a}{b}$). The development zero for the nematode (single females) was found to be 12.0°C which is in relatively close agreement with that of 10.4°C reported by Hughes & Platzer (1977) and 12.8°C reported by Platzer & Brown (1976), especially when the different methods of estimation are taken into account. The developmental zero for the mosquito larvae was estimated at 8.0°C, some three degrees lower than the minimum development temperature reported for C. pipiens (Clements, 1963).

Figure 2.5.6 The influence of temperature upon the mean development times and rates of the parasite and host.

a Parasite: single females only (see text).

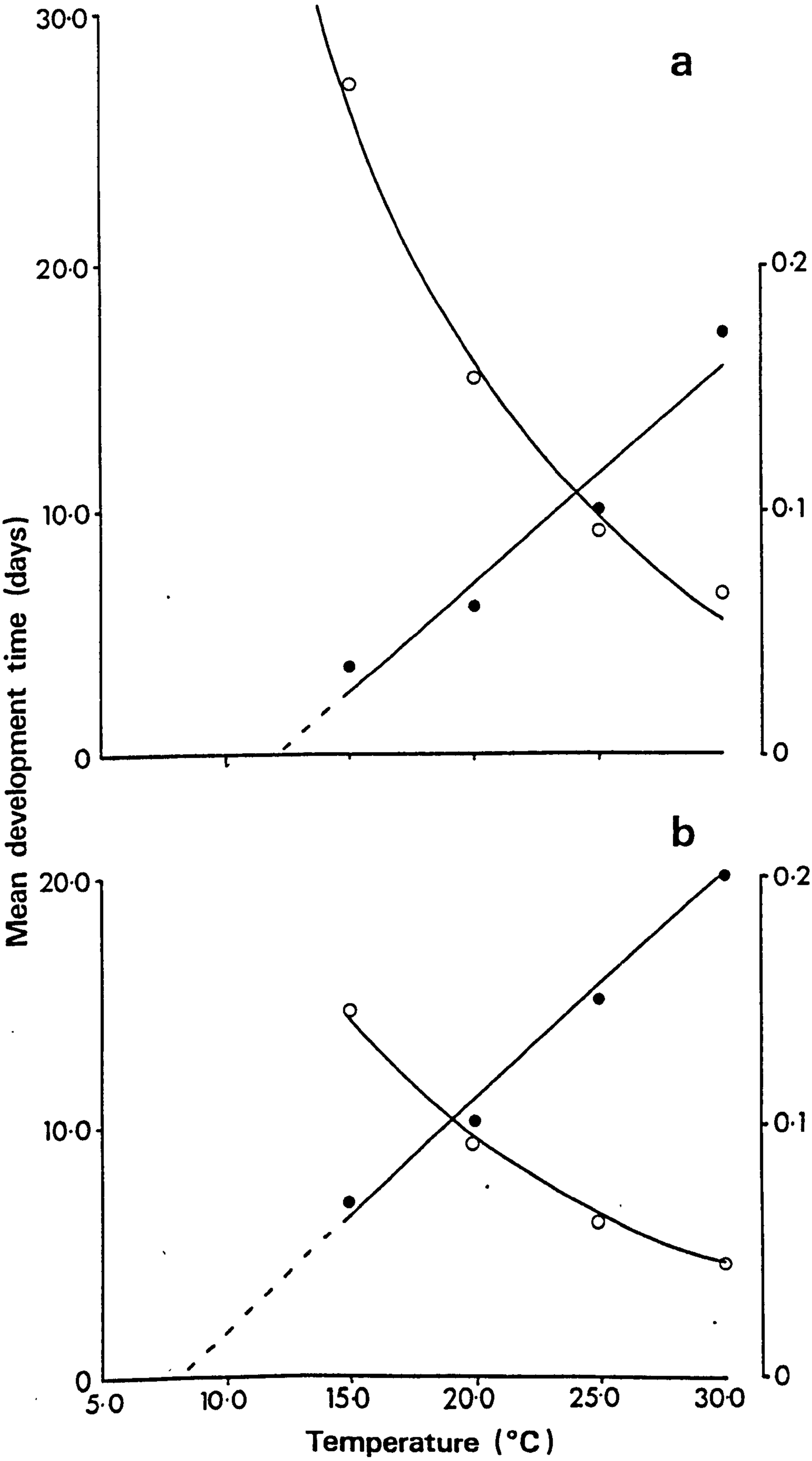
Open circles: estimated mean time (y) from infection to emergence (in days), at each temperature (x). The line is the predictions of equation (2.5.1), where, a = -14.959 and b = 621.707.

Solid circles: estimated mean development rates (/capita/day) given by 1/y. The line is the least-squares best-fit linear regression of 1/y on x (a = -0.1072, b = 0.0089, r = 0.9710, $0.01 > p > 0.001$).

b Host.

Open circles: estimated mean development time (in days), for twenty-hour old hosts to pupation. The line is the predictions of equation (2.5.1), where, a = -4.699 and b = 284.919.

Solid circles: as (a) above, where, a = -0.0740, b = 0.0092, r = 0.9930, $p < 0.001$.


a
b
30·0
20·0
10·0
0
0·2
0·1
0
Mean development time (days)
(per capita per day)
Mean development rate
5·0
10·0
15·0
20·0
25·0
30·0
Temperature (°C)

Having obtained estimates of the developmental zero, the development rate ($\frac{1}{t}$) is given by the formula,

$$\frac{1}{t} = b\ (T - T_o) \qquad (2.5.2)$$

where b is the slope of the linear regression of the observed development rates with temperature, T is the ambient temperature and T_o is the developmental zero (Varley et al; 1973).

The number of day-degrees (D°), often referred to as heat units, necessary for the completion of development is given by,

$$D^\circ = (T - T_o)t \qquad (2.5.3)$$

where T and T_o are defined above and t is the development time. The mean number of day-degrees necessary for development of the parasite within the temperature range 15°C - 30°C (taking estimates at each whole degree) is 117.4 ± 3.9 (S.E.). This again shows close agreement with the figure of 122.2 ± 3.6 (S.E.) reported by Hughes & Platzer (1977). The mean number of day-degrees required for host development was estimated at 110.5 ± 1.1 (S.E.9, which is less than that required by the parasites, the host showing a more rapid development at each temperature examined (cf. figures 2.5.6a and b).

The development times, and therefore rates, of the host show good agreement with those given by Shelton (1973) for the higher temperatures, but he recorded considerably shorter development times at the lower temperatures than those reported here. These differences are most probably due to differences in experimental method. Shelton (1973) recorded the mean time to first pupation, while the mean values are presented here. Also differences in food supply and the possibility that Shelton (1973) used wild mosquitoes may be important, since these would probably be better able to acclimate to the less favourable temperatures than the inbred laboratory strain used in this study.

2.5 (iii) Discussion

Data presented in this section demonstrate that R. culicivorax is quite capable of prematurely killing the host, C. p. fatigans. There are several mechanisms by which this can occur, but in each case there is a strong link with the mean parasite burden per host. Ignoring the effect of parasite burden upon the sex of the nematode, which will be examined fully in the next section, it is seen that for a given mean parasite burden the more overdispersed (aggregated) the parasites, the greater will be the number of parasites killed as a result of the density-dependent, parasite-induced host mortality. In other words, the population of nematodes will, to some extent, be regulated by deaths as a result of this process and as suggested, such density-dependent regulatory controls have a stabilizing effect upon the dynamics of populations.

Figure (2.5.2) demonstrates that the parasite-induced host mortality varies linearly with parasite burden, indicating that the density-dependent regulatory effect will occur at a low level. This conclusion is also supported by the relatively shallow slope of the line. Where non-linear density-dependent regulators occur, they can have a very severe limiting effect upon the population concerned, and in extreme cases may destabilize the population, (Anderson, 1980).

Clearly, the effect just outlined will reduce the population of postparasitic nematodes entering the environment, but reference to section 2.6 will show that of those parasites lost as a result of this form of mortality, the vast majority will be males, because of the large mean parasite burdens necessary to effect the parasite-induced host mortality. Thus, the effect is most pronounced at high levels of parasitism, when there is already excess production of male nematodes. Thus, even the loss of many males will not greatly affect the egg imput into the next generation. Hence, the effectiveness of this density-dependent control upon the parasite population may be of limited significance.

The importance of the developmental time delay of the parasitic stages of the nematode within the host will be further discussed in

Chapter 4. Those factors which affect the length of this time delay have been examined and, as above, the most important features of the relationship are the mean worm burden per host and the statistical distribution of the parasites. These, of course, act as moderators upon the developmental delay set by the ambient temperature.

In addition, it is clear that the statistical distribution of the parasites within the host population is of profound importance in the dynamics of the parasite and thus of its host.

2.6 SEX DETERMINATION

The observed sex ratio of a population is determined by many factors. In the majority of organisms, the sex ratio of a given generation is predetermined by genetic characteristics which control the ratio of male to female offspring during early development. However, in some organisms selective advantages have been gained by having their sex ratio determined by environmental rather than genetic factors. It is to this subject that the following sections are addressed.

2.6 (i) Environmental Sex Determination

The sex ratio of a species is frequently of great importance in relation to the rate of production of offspring. Too much bias in favour of either sex may result in a reduction in the overall birth rate of a population. For a given species, the frequency of each sex has been and still is frequently regarded as being more or less a constant, despite much empirical evidence to the contrary (Clausen, 1939; Fisher, 1958).

Under non-varying environmental conditions one might expect an optimum (usually 1:1) sex ratio to become established (Maynard Smith, 1982). Perturbations may induce a change in the sex ratio away from the optimum. Such perturbations fall into two categories, physical, which will always tend to be density-independent and biological, which are frequently density-dependent in action. The mode of action of a given perturbation may be of two forms, either (1), to alter the sex ratio of the existing population, usually by differential rates of mortality acting upon the sexes or (2) to change the sex ratio of the offspring which will form the next generation.

Physical environment-induced change in the sex ratio of organisms is commonplace, where, for example climatic variations result in chance alterations in the sex ratio of the existing populations. Physical factors can also be responsible for altering the sex ratio

of offspring, as seen in certain reptiles, where the sex ratio of a generation of offspring is determined by the temperature at which eggs are incubated (Bull, 1980).

The influence of the biological environment upon sex ratio is frequently closely linked to the density of the species under consideration, this being a major variable in determining important parameters such as food availability and mating probabilities.

Population density, (like the physical environment), can influence sex ratio either in the current or succeeding generation, but in addition, the influence may be dependent upon either the total population density or the relative population densities of the two sexes.

High or low densities of one or other of the sexes have been demonstrated to be able to alter the sex ratio in a wide range of organisms, either inducing sex changes in the current population, as seen in some teleost fish (Shapiro, 1980) or more usually by altering the sex ratio of the offspring. In this context the sex ratio of offspring has been demonstrated to be directly affected by; (i), the density of pollen on the stigma in Silene dioica (Correns, 1928) and Rumex acetosa (Rychlewski & Zarzycki, 1975), the proportion of male offspring varying inversely with pollen density; (ii), the sex ratio of the parents, as for example in the guppy (Poecilia reticulata) where the sex ratio of the offspring varies inversely with that of the parents (Geodakin , Kosobutsky & Bileva, 1967); (iii), the occurrence of haplodiploidy, where unfertilized eggs produce female and fertilized eggs male offspring, commonly found to occur in arthropods (Clansen, 1939; Flanders, 1956) and finally (iv), delayed fertilization, caused by a lack of male partners giving rise to a preponderance of male offspring, a widespread mechanism found in several genera of insects (Seiler, 1920; James, 1937; Hannah, 1955), the trout Salmo iridens (Mrsic, 1923) and the anuran Rana esculenta (Hertwig, 1912).

Examples of the effect of total population density upon sex ratio may also be drawn from a number of animal groups. For example,

crowding in parasitic species resulting in the development of a preponderance of males has been demonstrated in plant parasitic nematodes (Ellenby, 1954; Koliopanos & Triantaphyllou, 1972), entomophilic nematodes (Christie, 1929; Parenti, 1962; Welch, 1965; Petersen, 1972 and 1977; Ezenwa & Carter, 1975) and also in parasitic copepods, (Malaquin 1901, cited by Christie, 1929).

Other environmental factors shown to be of importance in sex ratio determin ation include the availability of sunlight in certain orchids (Dodson, 1962; Gregg 1975); organism size in orchids (Gregg 1975), parasitic wasps (Charnov, Las-den Hartogh, Jones & van den Assem, 1981), molluscs (Hoagland, 1978) pandalid shrimps (Charnov, Gotshall & Robinson, 1978; Charnov, 1979) and entomophilic nematodes (Petersen, 1972; Poinar, 1979); host size in both parasitic hymenoptera (Charnov et al,1981) and entomophilic nematodes (Petersen, 1972 and 1977); and also whether they are the first or second wasp to parasitize a given host in Nasonia vitripennis (Werren, 1980).

Environmental sex determination is clearly a common phenomenon, occurring in a wide range of organisms and having many different causative agents. It is a major influence in the reproductive success of those organisms concerned and may also have significant implications relating to the population dynamics of the organisms.

A more detailed examination of the phenomenon in mermithids reveals that the situation, although apparently simple, is in fact rather complex. Initially the sex ratio of the parasites is determined by the number of parasites within a host (Petersen, 1972 and 1977; Enzenwa & Carter 1975); low burdens preferentially forming female parasites and high burdens male parasites. Speculation as to how parasite burden affects the sex ratio has covered a wide range of possible mechanisms (Welch, 1965) but evidence is now available to demonstrate that nutrient availability is one of the principal determinants (Petersen, 1972; Gordon et al, 1981). There is also evidence that nutrient availability, mediated by a temperature dependent rate of metabolism, influences sex determination in reptiles,

where there is a finite quantity of nutrient available in the egg (Ferguson & Joanen, 1982). In a host little larger than the parasite(s),the nutrient available to each parasite will be determined by two factors; the number of parasites within the host competing for what nutrient is available and the rate at which the host itself acquires nutrient. Other important factors, which also presumably operate via nutrient availability are host size and species (Petersen, 1972 and 1977).

If the population dynamics of R. culicivorax and the effect the parasite has upon its host population are to be understood then the environmental sex determination which affects the parasite must be fully investigated. The effects of parasite burden, the statistical distribution of parasite numbers within the host population and environmental temperature are examined in the following sections.

2.6 (ii) The Effects of Parasite Burden and the Statistical Distribution of the Parasites

The relationship between the sex ratio and the parasite burden of mermithids, measured at the individual level, is well documented (Christie, 1929; Parenti, 1962; Petersen, 1972, 1973a and 1977; Enzenwa & Carter 1975). In every instance there is a reduction in the proportion of nematodes differentiating as females as the parasite burden increases. Given this, the relationship at the population level will be dependent upon two factors; the mean parasite burden per host and the statistical distribution of the parasites within the host population. Unfortunately, the majority of published data relating to this topic does not include the complete frequency distributions or in some cases the mean parasite burdens. Hence it was necessary to carry out a detailed experimental investigation.

The experimental regime consisted of exposing a known number of 16-24 hour old first instar hosts to infective nematodes for up to eighteen hours. Hosts were then maintained as described in section 2.3 (ii) at 25°C. Five days post-infection (p.i.) hosts were isolated in vials with 10-20 ml water; the water was changed daily. The number and sex of the nematodes which emerged were recorded, as was the day

p.i. of their emergence. The numbers of uninfected hosts (those which pupated) were also recorded. The mean parasite burden per host for each host cohort was calculated by dividing the total number of emergent nematodes by the number of hosts that survived until either parasite emergence or pupation (i.e. the zero frequency class was included in the calculation of the mean).

The relationship between the sex ratio of the postparasitic nematodes, portrayed as the proportion of the total number of nematodes which developed as females and the mean parasite burden per host is depicted in figure (2.6.1). There is a distinctive exponential decay, in the proportion of nematodes that developed as females as the mean parasite burden per host increased; this demonstrates a strongly density-dependent relationship between the mean parasite burden and the sex ratio of the nematode.

It is evident from figure (2.6.1) how important the mean parasite burden is, but, the statistical distribution of the parasites has not so far been considered. For the data presented in figure (2.6.1) the values of the dimensionless parameter k of the negative binomial distribution ranged from 4.93 to 0.31. As a consequence of this, the fitted curve in figure (2.6.1) shows the expected relationship for the average degree of dispersion shown by the data points. Differences in the statistical distributions of the parasites would not be expected to change the qualitative form of the relationship but may well affect the quantitative aspect. From the data presented in figure (2.6.1) it is unfortunately impossible to determine the effect of the differing degrees of overdispersion upon the sex ratio.

A frequency histogram of the proportion of parasites emerging as females from hosts with specific parasite burdens (i) shows that as the parasite burden rises there is a very rapid decline in the proportion of female nematodes. Eventually, in hosts bearing seven or more parasites no females are formed (figure 2.6.2). This demonstrates the severity of the density-dependent constraint upon the sex ratio of the parasite. Given this information, the Poisson and negative binomial frequency distributions were used to assess the effect of the

Figure 2.6.1 The influence of parasite burden upon the sex ratio of the nematode: mean parasite burden at 25°C.

The points are the observed proportions of the nematode populations that developed as females. The line is the least-squares best-fit linear regression of ln y on x, (a = -0.1661, b = -0.5164, r = 0.9798).

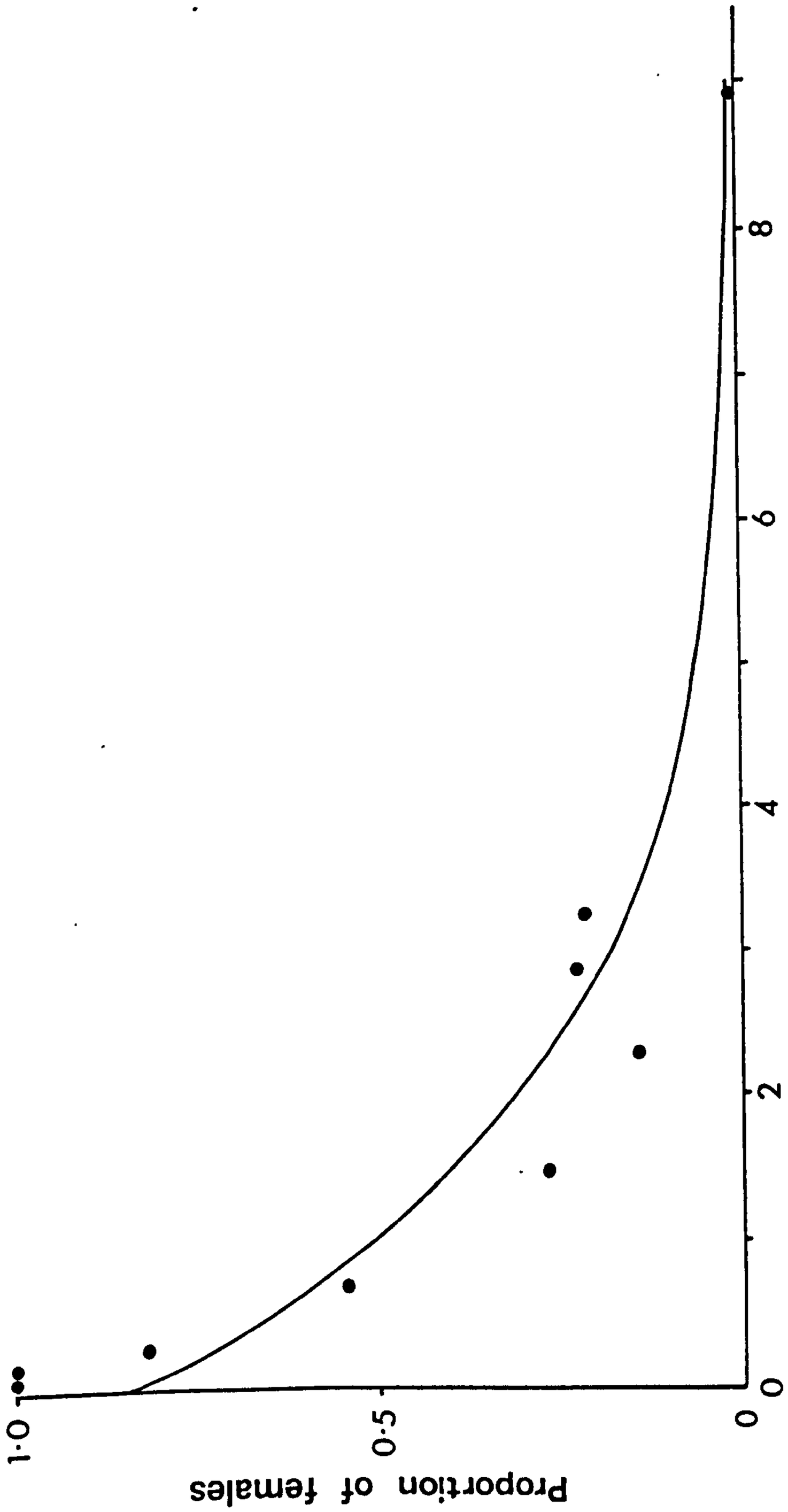
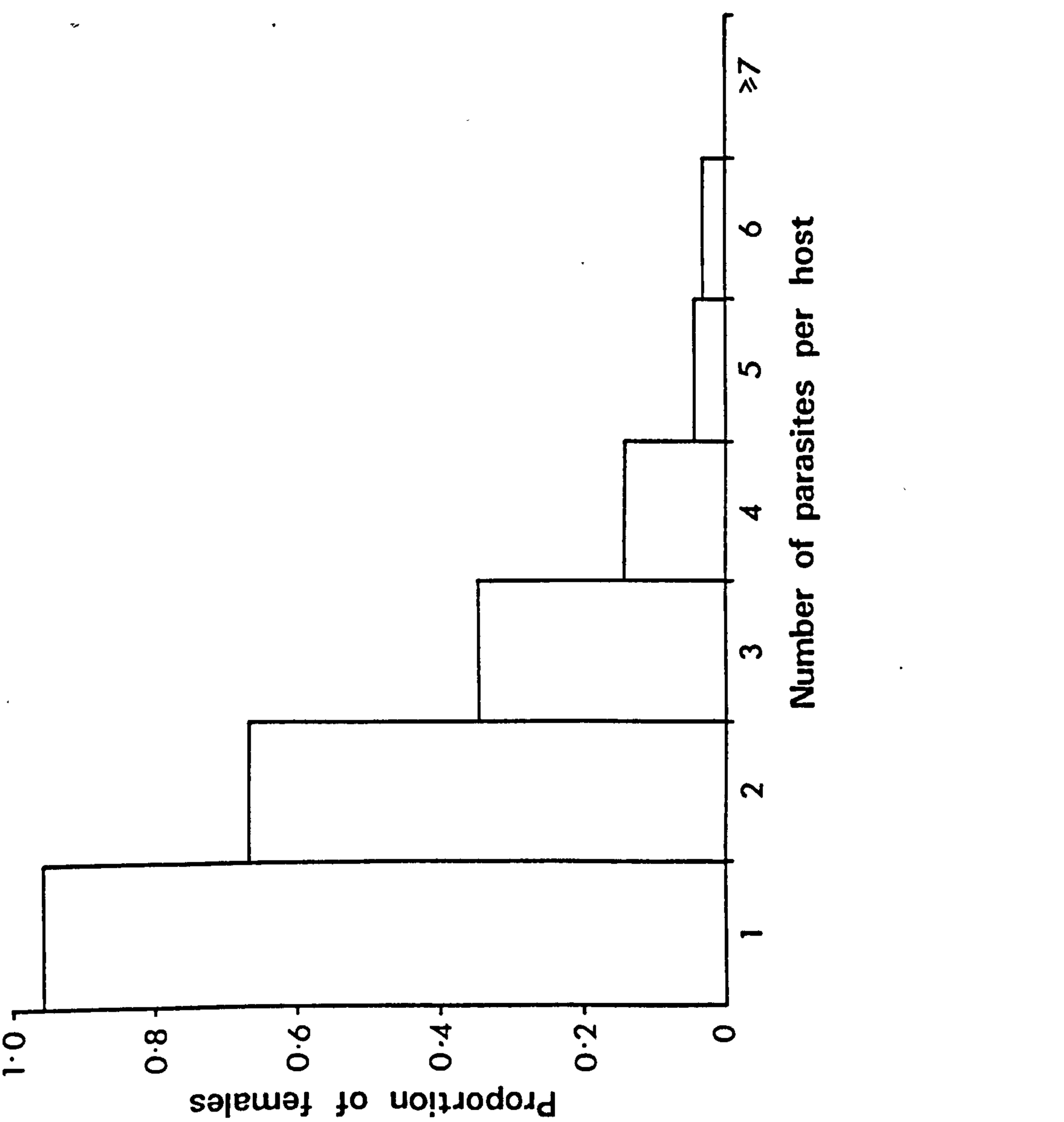

Proportion of females
1·0
0·5
0
0
2
4
6
8
Mean parasite burden

Figure 2.6.2 The influence of parasite burden upon the sex ratio of the nematode: parasite burdens in individual hosts, at 25°C.

The observed proportion of the nematodes that developed as females from hosts with a given parasite burden (i).


Proportion of females
0
.2
.4
.6
.8
1
Number of parasites per host
1
2
3
4
5
6
≥7

degree of dispersion upon the sex ratio of the nematodes. This may be accomplished by setting a value of m, the mean parasite burden per host and, in, the case of the negative binomial distribution, a value of the parameter k as well. The expected number of hosts with i parasites may then be calculated for a given host population size. The expected number of hosts with i parasites multiplied by i gives the number of nematodes of both sexes produced by hosts with that parasite burden. If the number of nematodes is then multiplied by the proportion expected to be female according to parasite burden, i, (as indicated in figure 2.6.2) then the proportion expected to be female from that level of parasite burden will be obtained. When repeated for levels of i from one to six and summed, the proportion of the total parasite population expected to be female is obtained. The numerical values of the total parasite population and the population of female parasites will be determined by the size of the host population.

This procedure was carried out for a range of values of mean parasite burden per host (m) and differing degrees of dispersion as measured by the parameter k, using both Poisson and negative binomial models. The results are portrayed in figure (2.6.3). It is clear that as the parasite distribution is varied from random to highly overdispersed, for a given mean parasite burden the sex ratio is altered in favour of male parasites. The effect is most noticeable at low mean parasite burdens, a fact borne out by figure (2.6.4), which shows the relationship between the proportion of female nematodes and the parameter k of the negative binomial distribution. This simple technique therefore has shown that decreasing values of k, corresponding to increasing overdispersion in the parasite distribution, have a highly detrimental effect upon the proportion of the parasite population which develops as females.

Something not immediately apparent from either figure (2.6.3) or (2.6.4) is that, for a fixed host population size and fixed value of k, as the mean parasite burden per host (m) increases (and the proportion of nematodes becoming females declines), the number of female nematodes initially increases rapidly. This is due to the

Figure 2.6.3 The influence of parasite burden upon the sex ratio of the nematode: mean parasite burden, at 25°C (expected).

The expected proportion of the nematode population that would develop as females at different mean parasite burdens per host (m), (see text for method). The dotted line is the predictions when the parasites are distributed at random (Poisson) amongst the hosts and the solid lines are when the parasites are aggregated in a negative binomial manner, for fixed values of k.

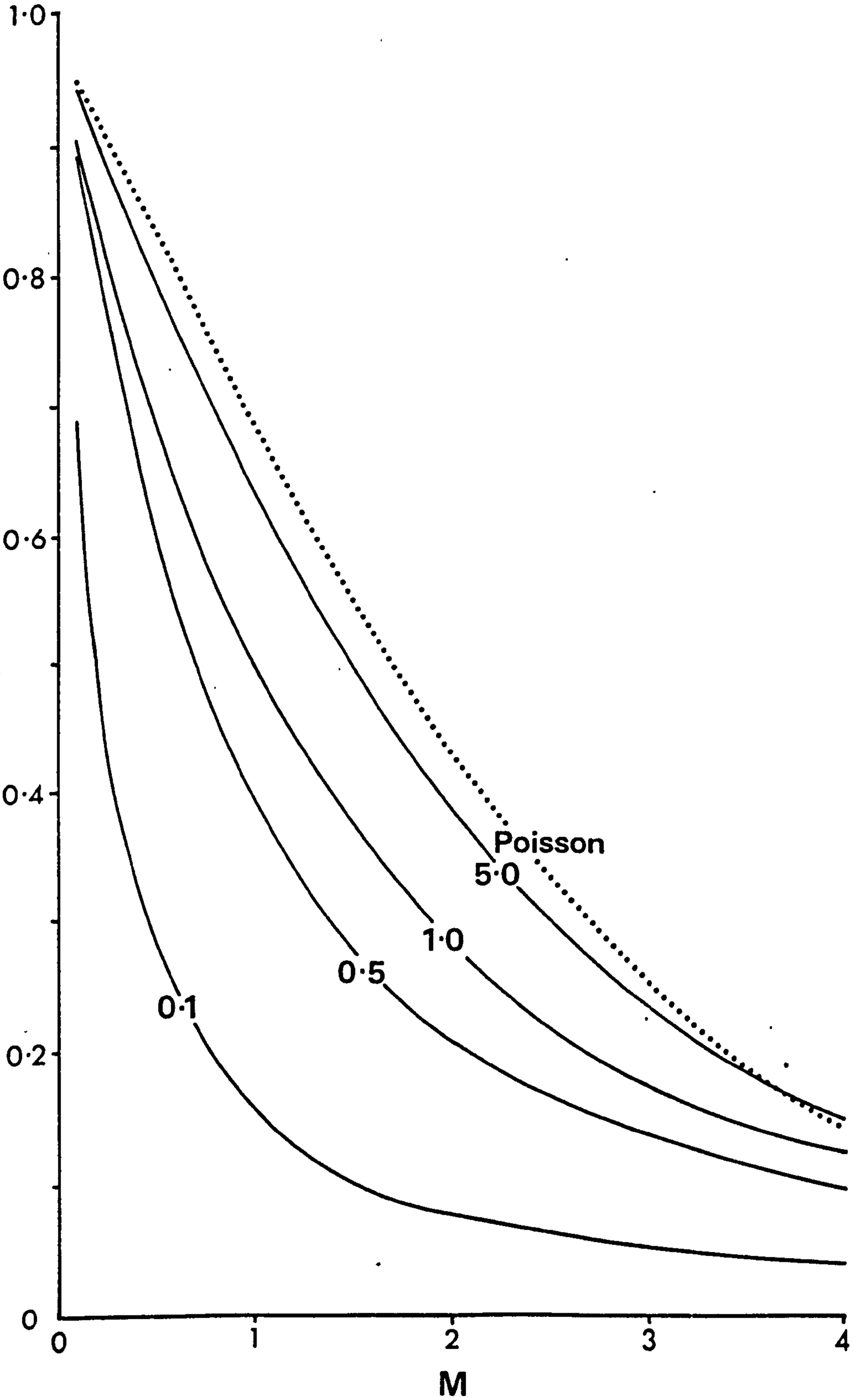
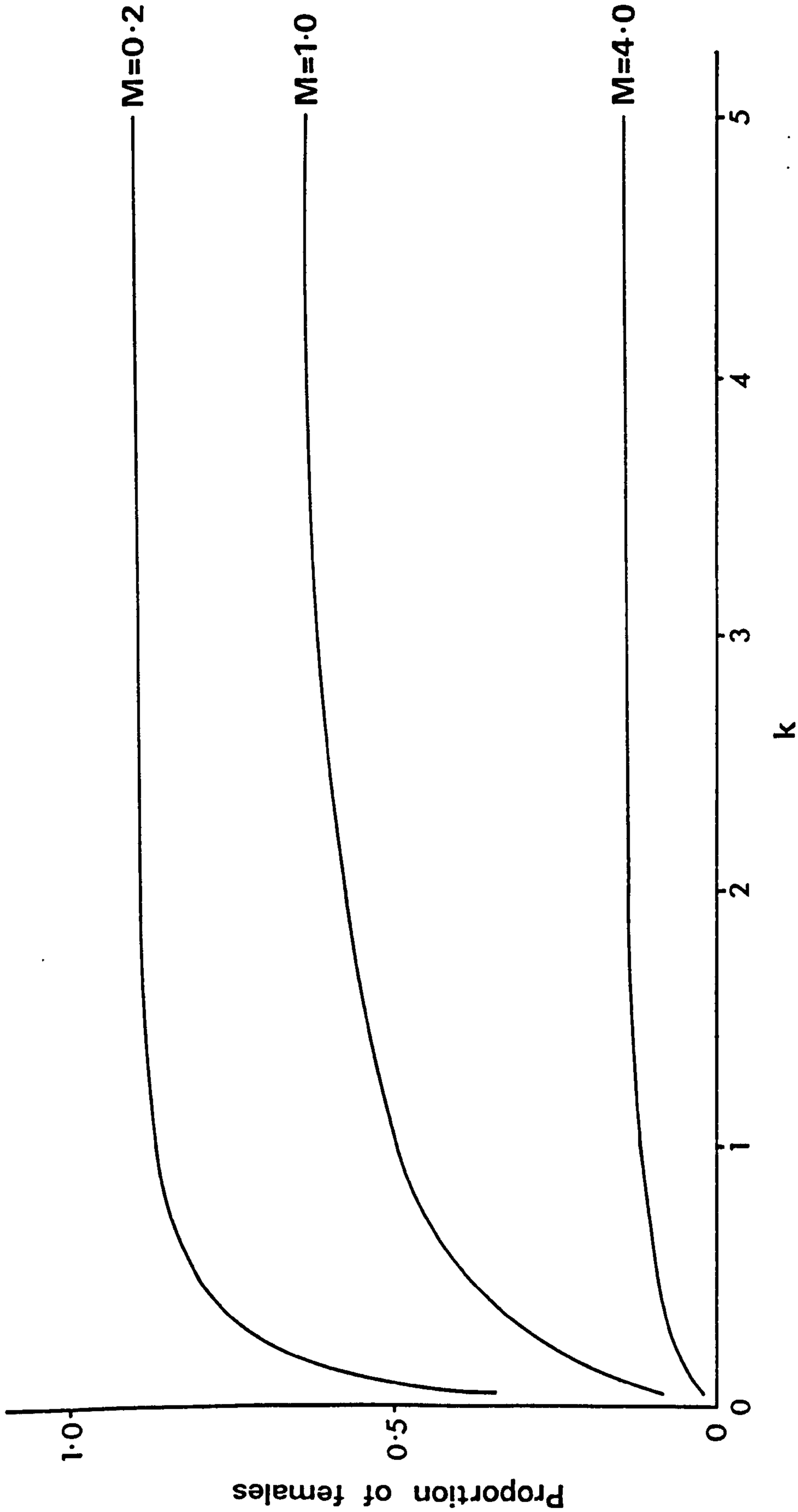

1·0
0·8
0·6
0·4
0·2
0
Proportion of females
0·1
0·5
1·0
5·0
Poisson
0
1
2
3
4
M

Figure 2.6.4 The influence of parasite distribution upon the sex ratio of the nematode: mean parasite burden, at 25°C.

The expected proportion of the nematode population that would develop as females given different degrees of overdispersion (k values, of the negative binomial distribution). Each line is for a different (fixed) mean parasite burden (M).


M=0·2
M=1·0
M=4·0
5
4
3
2
1
k
1·0
0·5
0·0
Proportion of females

decreasing proportion of the population that is female referring to a rapidly growing population size (as the parasite population increases with m). As the mean parasite burden continues to rise there occurs a peak in female numbers followed by a decline, due to an increasing proportion of the hosts carrying parasite burdens which produce very few or no female nematodes,(figure 2.6.5). Clearly,beyond this turnover point in female numbers,the density-dependent constraints acting upon the parasite population become increasingly severe.

It may be seen from figure (2.6.5) that the inter-relationships between m, k and the female parasite population are most evident when the parasite population is dispersed at random throughout the host population, and becomes less evident as the parasite distribution becomes more overdispersed.

No account is taken of differing host mortality rates at different parasite burdens due to the parasite burden being assessed at the time of emergence. If the mean parasite burden was estimated prior to nematode emergence then some compensation must be made with respect to premature host mortality.

Throughout this section the sex ratio of the nematode has been examined in terms of the female population. The reproductive output of a generation of parasites is totally dependent upon the number of female nematodes present in that generation, provided that adequate numbers of males are available for mating purposes. An examination of the curves of the proportion of parasites that are females, (figure 2.6.5) shows that the only time male abundance may be in any way limiting is at very low mean parasite burdens associated with a parasite distribution of approximately random form. However, it is unlikely due to the formation of males at low parasite burdens (figure 2.6.2), the life-span of males (figure 2.7.2a) and their polygamous behaviour, that there would ever be a shortage of males to mate with females.

As the reproductive output and hence the size of the next parasite generation is so dependent upon the magnitude of the female population, given suitable conditions for population growth the parasite population will tend towards the level at which most female parasites are

Figure 2.6.5 The predicted relationship between the mean parasite burden per host and both the number of female nematodes emerging and the proportion of the nematodes that developed as females.

The relationship is depicted for increasing degrees of overdispersion from the Poisson (random distribution) through a range of values of k from the negative binomial distribution.

(see text for method of prediction).

——— Number

..... Proportion

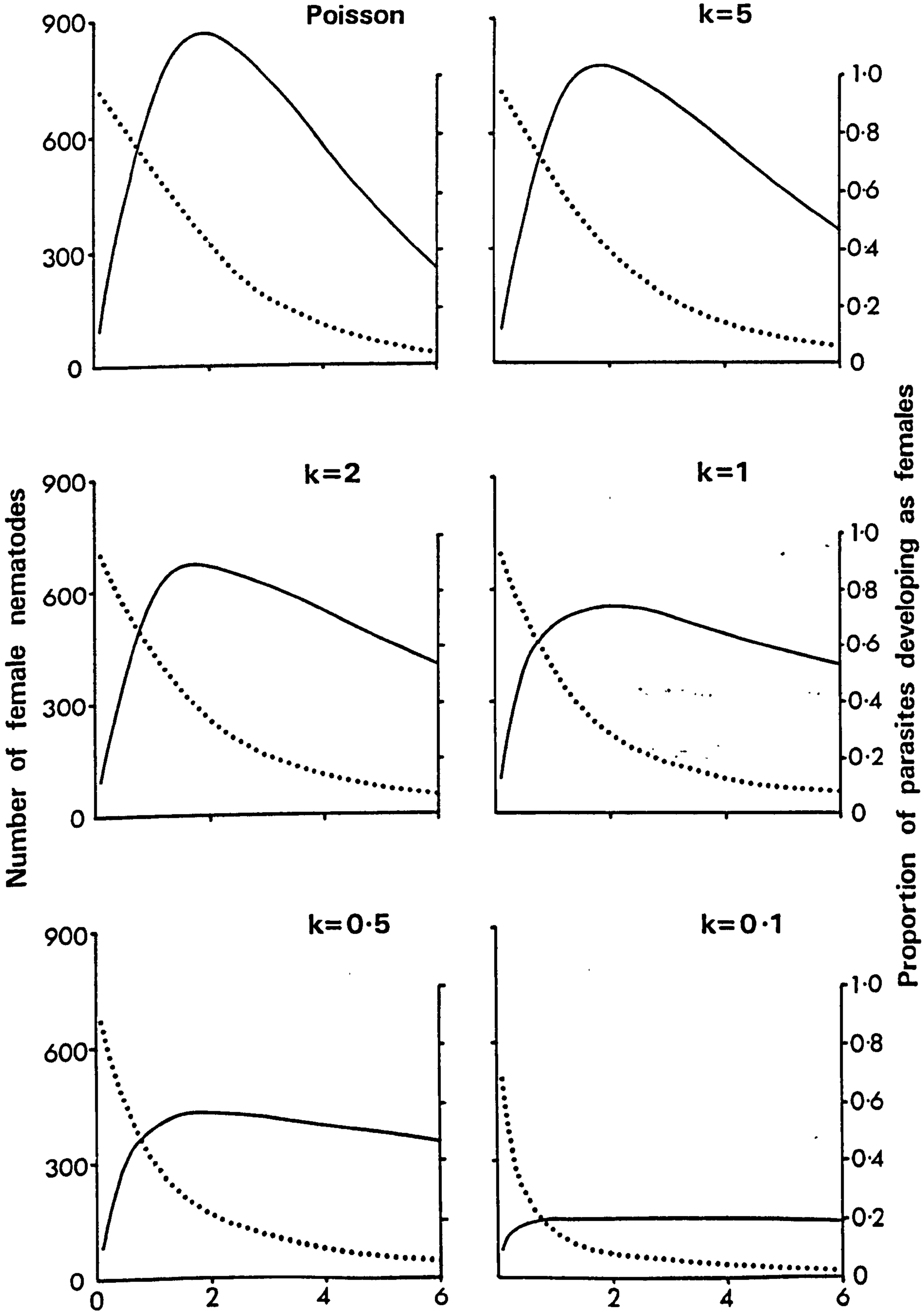

Poisson
k=5
k=2
k=1
k=0·5
k=0·1
900
600
300
0
1·0
0·8
0·6
0·4
0·2
0
2
4
6
Number of female nematodes
Proportion of parasites developing as females
Mean parasite burden

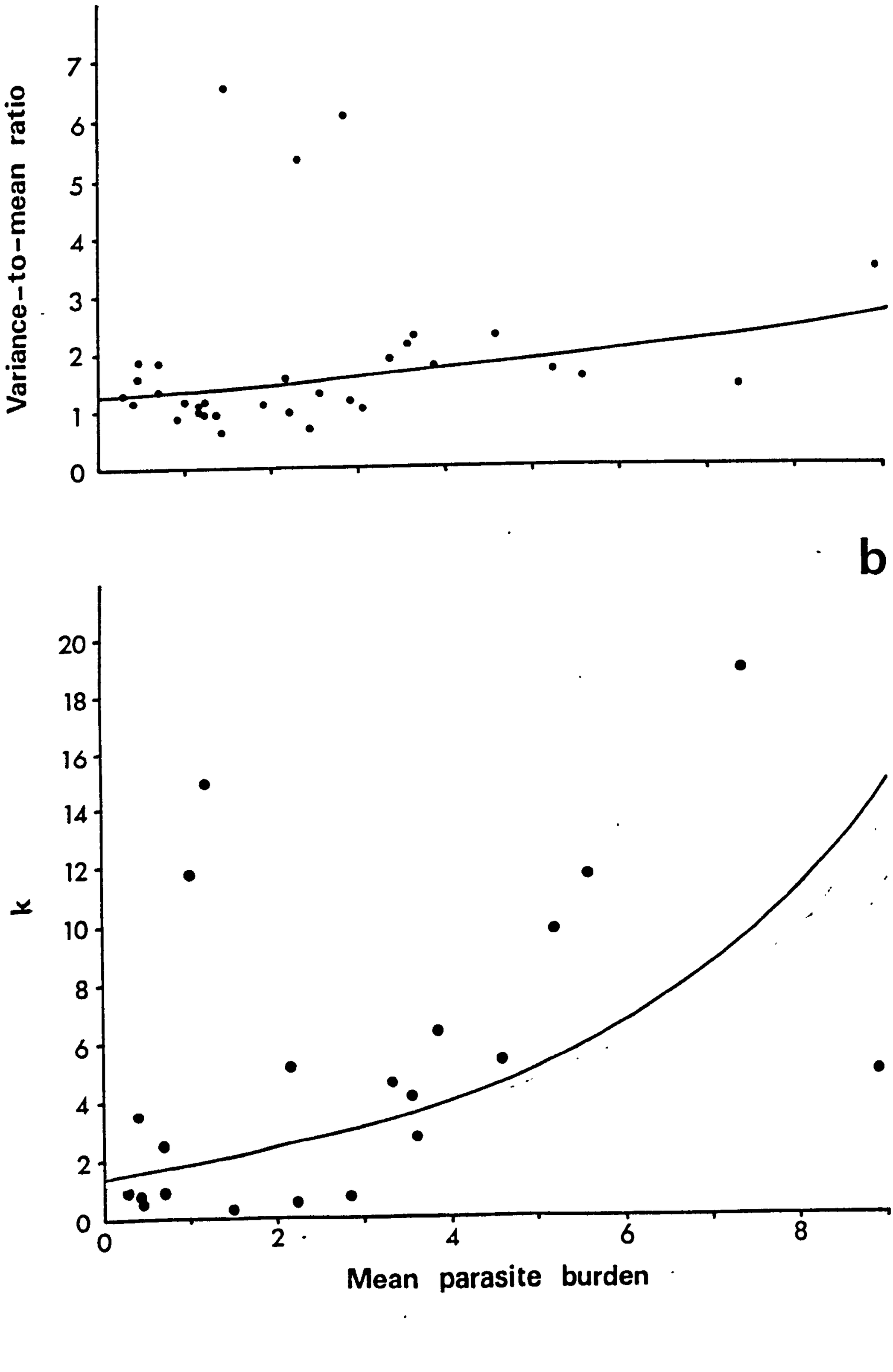
Figure 2.6.6 The relationship between the mean parasite burden and the degree of parasite aggregation.

The points are observed data and the lines are least-squares best-fit linear regressions of lny on x.

a. The variance-to-mean ratio
   (a = 0.1965, b = 0.0865, r = 0.3041*)

b. The parameter k of the negative binomial frequency distribution
   (a = 0.2952, b = 0.2669, r = 0.5181)

* not significant at the 5% level.


a
Variance-to-mean ratio
0
1
2
3
4
5
6
7
b
k
0
2
4
6
8
10
12
14
16
18
20
0
2
4
6
8
Mean parasite burden

produced. Increases in the mean parasite burden beyond this point will lead to progressively fewer females, fewer eggs and an eventual fall in the mean parasite burden. The inference may therefore be drawn from figure (2.6.5) that the mean parasite burden in a host population infected with R. culicivorax will tend towards two parasites per host, a level maintained by the density-dependent regulatory action of the environmental sex determination.

Data relating to the distribution, mean parasite burden and sex ratio of Romanomermis spp. within naturally infected host populations is scarce but that available does show a measure of agreement with the predicted values, as does the data for other mermithid species (table 2.6.1). The field data show relatively good agreement with that expected for a parasite showing a moderate degree of overdispersion with a tendency to maximise the number of female nematodes produced. Discrepancies can largely be accounted for due to differences in parasite and host species. Thus U. sapphirina is a smaller host than P. confinnis and the same parasite in the same locality produced a smaller proportion of the population as female nematodes from the former host (table 2.6.1).

To put the relationship between the degree of overdispersion and the parasite sex ratio into perspective, laboratory estimates of the negative binomial parameter k range from 0.14 to 18.89, with the majority falling between 0.5 and 3.0. At these levels of overdispersion considerable impact upon the sex ratio would be expected, particularly at low mean parasite burdens (figure 2.6.5).

The mechanisms which generate the overdispersion in the distribution of the parasites are various, but two of the most important contributory factors are the essentially random nature of the host-parasite contact, linked with host heterogeneity in susceptibility to infection (see section 2.4 (ii)). Intuition might suggest that the degree of overdispersion would increase as the mean parasite burden per host increased. The available evidence relating to this topic is however conflicting. Figure (2.6.6a) shows the relationship between the degree of overdispersion, portrayed as the variance-to-mean

Table 2.6.1

The proportion of female nematodes that emerged from field collected hosts, with mean parasite burdens and a guide to the level of over-dispersion.

Parasite sp.	Host sp.	m	k	p.♀	Source
Romanomermis sp.	Aedes nigripes	1.36	→ ∞	0.63	Galloway & Brust, (1976)
Romanomermis sp.	Ae. communis/ churchillensis	2.05	3.96	0.16	Galloway & Brust, (1976)
Romanomermis sp.	Psorophora confinnis	2.71	3.70	0.56	Petersen et al., (1968)
Romanomermis sp.	Uranotaenia sapphirina	-	-	0.19	Petersen et al.,
		-	-	0.31	(1968)
R. nielseni	mixed, (mostly Aedes spp.)	-	-	0.23	Tsai &
		-	-	0.54	Grundman,
		-	-	0.38	(1969)
		-	-	0.48	
Hydromermis churchillensis	Ae. communis	0.48	0.52	-	Welch, (1960)
H. palustris	Cladotanytarsus sp.	0.61	0.47	-	Hominick & Welch, (1971)
Gastromermis deltensis	Harnischia sp.	0.32	-	-	Hominick & Welch, (1971)
G. viridis & Isomermis wisconsinensis	Simulium vittatum	0.76	0.99	-	Phelps &
		0.61	0.76		Defoliart,
		0.51	1.23		(1964)
		1.12	0.94		

m = mean parasite burden per host

k = parameter of the negative binomial distribution

p.♀ = proportion of the nematodes that were female

ratio, plotted against the mean parasite burden per host for all temperatures (15°, 20°, 25° and 30° C). There is a slight trend towards increasing overdispersion with increasing mean parasite burden. On the other hand, figure (2.6.6b) portrays the same relationship except that the degree of overdispersion is represented by the parameter k of the negative binomial, where there is a clearly inverse relationship between the degree of overdispersion and the mean parasite burden. From such conflicting evidence, it is impossible to draw any conclusions about the relationship between the dispersion pattern and mean parasite burden. However, it should be noted that the sample shown in figure (2.6.6a) is without obvious bias, using all available data, whilst that portrayed in figure (2.6.6b) used only data which showed a statistically significant fit to the negative binomial model at the 5% level and thus excludes all data where the variance-to-mean ratio was less than or equal to unity.

The field and laboratory evidence presented, supports the hypothesis proposed by Couturier (1963) and Welch (1965) that the environmental sex determination shown by mermithids may be one mechanism by which the parasite population is regulated. The qualitative and quantitative effects of the environmental sex determination upon the magnitude of the parasite population shown does, in fact, have considerable implications in relation to the density-dependent regulation of parasite numbers and the establishment of an equilibrium mean parasite burden per host. This aspect of the relationship will be further discussed in chapter four.

2.6 (iii) The Effect of Temperature

To a poikilothermic animal the ambient temperature is of prime importance in determining rates of survival and reproduction, generation time and many other features of its ecology.

The effect of temperature upon R. culicivorax has been examined in terms of preparasite survival (section 2.4 (i)), infection (Brown & Platzer, 1977; Galloway & Brust, 1977), development time (Platzer

& Brown, 1976; Hughes & Platzer, 1977) and field reports relating to activity (Petersen & Willis, 1971) and overwintering (Nickle, 1979). There is however no published data relating to the effect temperature may have upon the sex ratio of R. culicivorax.

Having investigated the effects of mean parasite burden per host and the form of the statistical distribution of the parasites within the host population upon the sex ratio of the nematode at 25°C (section 2.6 (ii)), the same experimental design was used at different temperatures to assess the effects, if any, of temperature upon sex ratio. The temperatures examined were 15°C, 20°C and 30°C, the infection and rearing procedure were as described in section 2.6 (ii), with the following differences. Hosts were exposed at 25° and then transferred to 15° and 20°C, while those at 30°C were exposed and kept at this temperature throughout.

The observed relationships between the mean parasite burden per host and the proportion of nematodes differentiating as females are portrayed in figure (2.6.7) for the three temperatures (c f. figure 2.6.1 for 25°C). It may be seen that the form of the relationships is the same for each temperature, suggesting that the same processes are in operation. The fitted curves may be used to give a quantitative comparison of the sex ratio at any given mean parasite burden between the temperatures (figure 2.6.8). There is no clear trend in sex ratio with change in temperature in this data, however, no account of the possible differences in the statistical distribution of the parasites at the different temperatures has been made.

Examination of the frequency histograms of the proportion of nematodes emerging as females from hosts with a given parasite burden ,i, shows that there is little difference between 20°, 25° and 30°C (figures 2.6.9b, 2.6.2 and 2.6.9c respectively) but that generated at 15°C shows an increased tendency for female formation at all but the lowest level of parasite burden. The tendency being most noticeable at the higher worm burdens. This could clearly help to account for the elevated level of female formation seen at 15°C as portrayed in figure (2.6.8) but is totally unable to explain the comparatively

Figure 2.6.7 The influence of temperature upon the sex ratio of the nematode: mean parasite burden per host.

The points are the observed proportions of the nematode populations that developed as females. The lines are least-squares best-fit linear regressions of ln y on x.

a $15^{\circ}C$ (a = -0.0149, b = -0.2840, r = 0.8979)

b $20^{\circ}C$ (a = 0.0621, b = -0.4772, r = 0.9266)

c $30^{\circ}C$ (a = 0.1616, b = -0.5134, r = 0.9843)

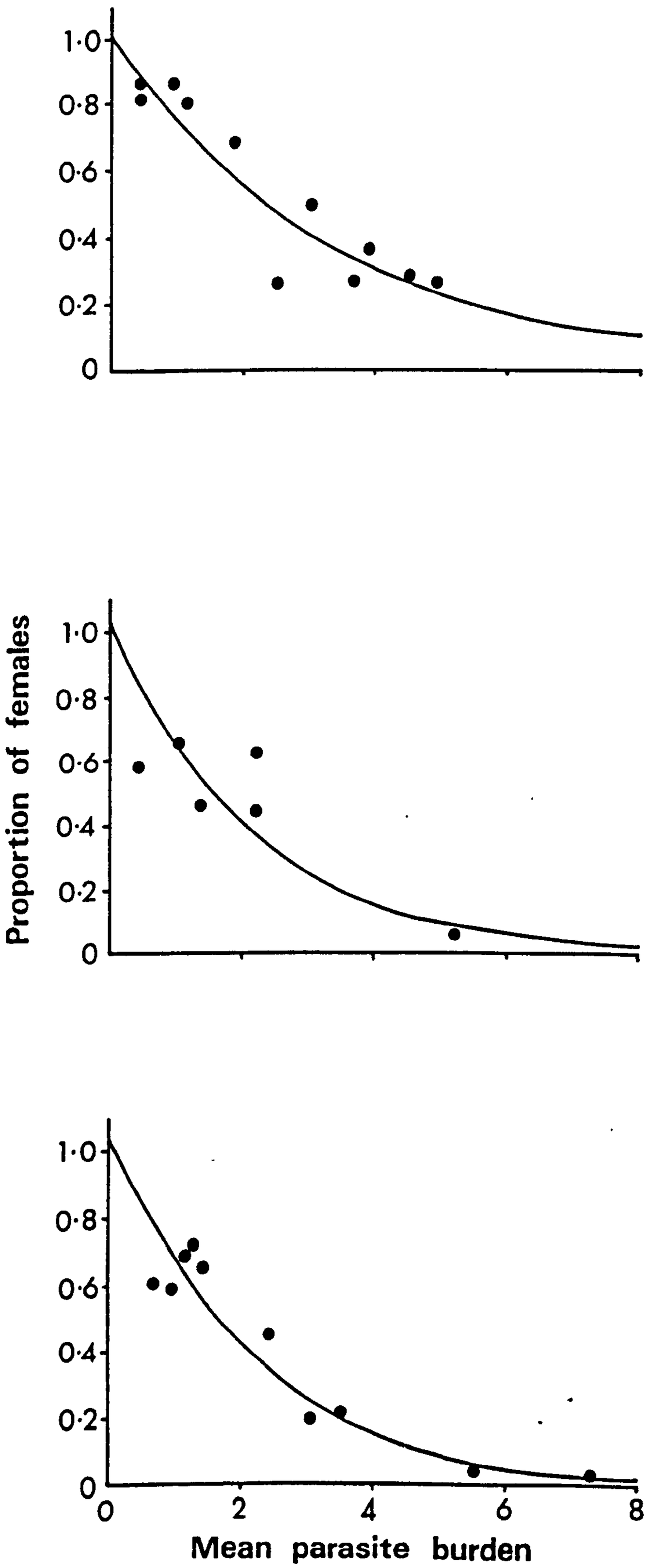

a
1·0
0·8
0·6
0·4
0·2
0
b
Proportion of females
1·0
0·8
0·6
0·4
0·2
0
c
1·0
0·8
0·6
0·4
0·2
0
0
2
4
6
8
Mean parasite burden

Figure 2.6.8 The influence of temperature upon the sex ratio of the nematode: undefined parasite distribution.

The predictions of the regressions, fitted in figures (2.6.1) and (2.6.7), of the proportion of the nematode population that would develop as females at four temperatures.

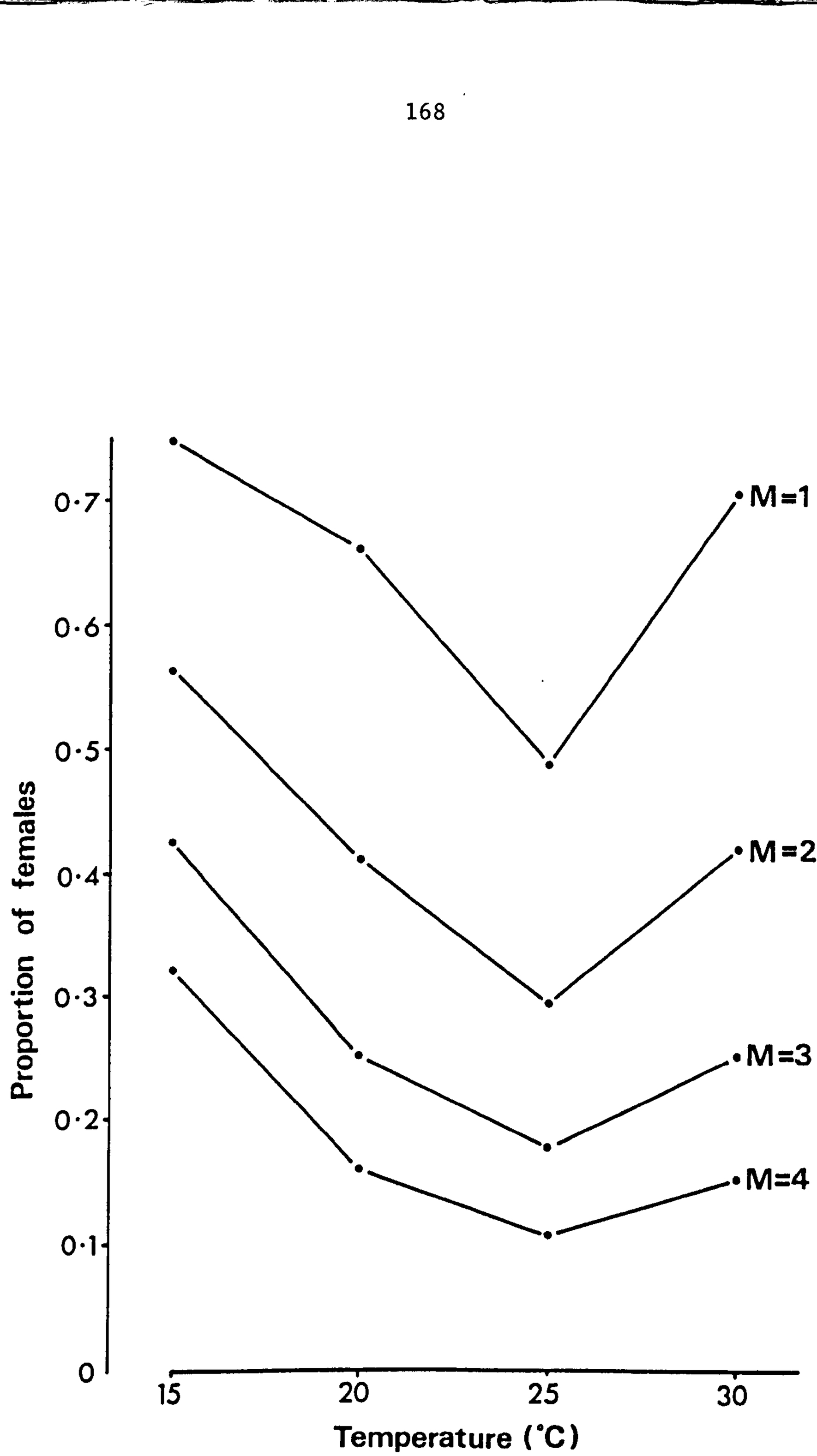

Proportion of females
0·7
0·6
0·5
0·4
0·3
0·2
0·1
0
M=1
M=2
M=3
M=4
15
20
25
30
Temperature (°C)

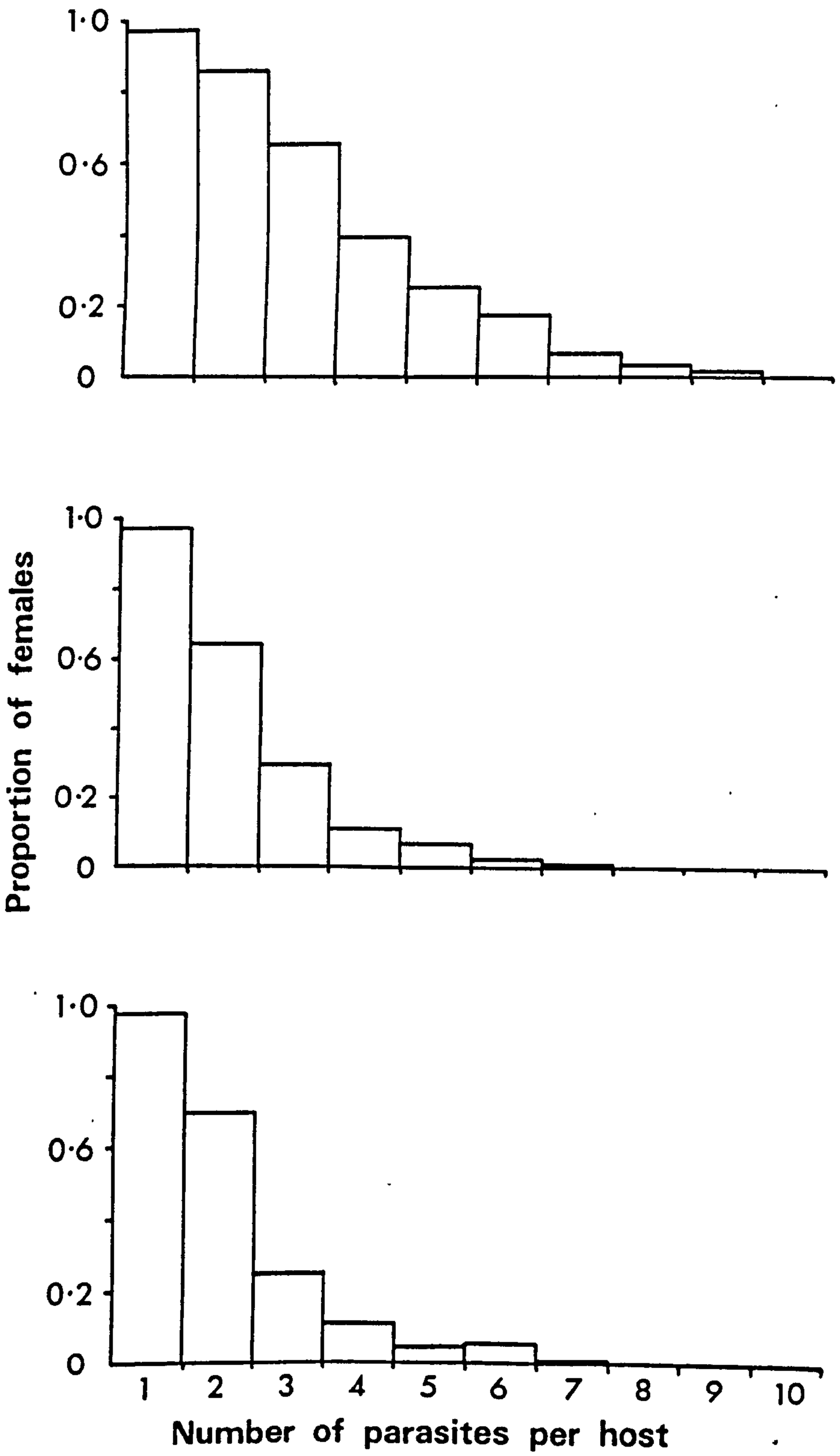
Figure 2.6.9 The influence of parasite burden upon the sex ratio of the nematode: parasite burdens in individual hosts, at three temperatures.

The observed proportion of the nematodes that developed as females from hosts with a given parasite burden (i).

a $15^{\circ}C$.

b $20^{\circ}C$.

c $30^{\circ}C$.


a
1·0
0·6
0·2
0
b
1·0
0·6
0·2
0
Proportion of females
c
1·0
0·6
0·2
0
1
2
3
4
5
6
7
8
9
10
Number of parasites per host

low proportion of females produced at 25°C also seen in this figure.

As clearly shown in section 2.6 (ii), the statistical distribution of parasite numbers within the host population can greatly influence the sex ratio of the parasite. Data presented as the mean parasite burden per host versus the female proportion of the parasite population, shows a relationship which is dependent upon the statistical distribution of the parasites for each data point. Thus the fitted curves in figures (2.6.1) and (2.6.7) show the expected relationship for the average degree of dispersion shown by the data points. If one erects a null hypothesis for the temperature-sex ratio relationship, i.e. there is in reality no difference between the sex ratio of nematode populations produced at different temperatures over the range 15° to 30°C, then it would be expected that the average degree of dispersion of parasites would vary inversely with the trend in sex ratio with changing temperature (figure 2.6.8). A crude estimate of the average degree of dispersion shown by the data portrayed in figures (2.6.1) and (2.6.7) may be obtained by averaging the variance-to-mean ratios of each of the data points for each temperature. The relationship between the female proportion of nematodes at each temperature and the average variance-to-mean ratio, is shown in table 2.6.2 where it is clear that there is an inverse relationship between the average degree of dispersion and the female nematode proportion obtained from the fitted curves (figures 2.6.1 and 2.6.7).

Table 2.6.2

The relationship of the mean level of overdispersion measured as the average variance-to-mean ratio (Av. $S^2/\bar{x}$) and the proportion of female nematodes formed at different mean parasite burdens (m) over a range of temperatures (T, °C).

		Female Proportion of Nematodes			
T	Av. $S^2/\bar{x}$	m=1	m=2	m=3	m=4
15	1.39	0.75	0.56	0.43	0.32
20	1.40	0.66	0.41	0.25	0.16
25	3.77	0.49	0.29	0.18	0.11
30	1.19	0.70	0.42	0.25	0.15
L.R.	-	*	NS	NS	NS

(L.R. shows the level of significance to a fitted linear regression).

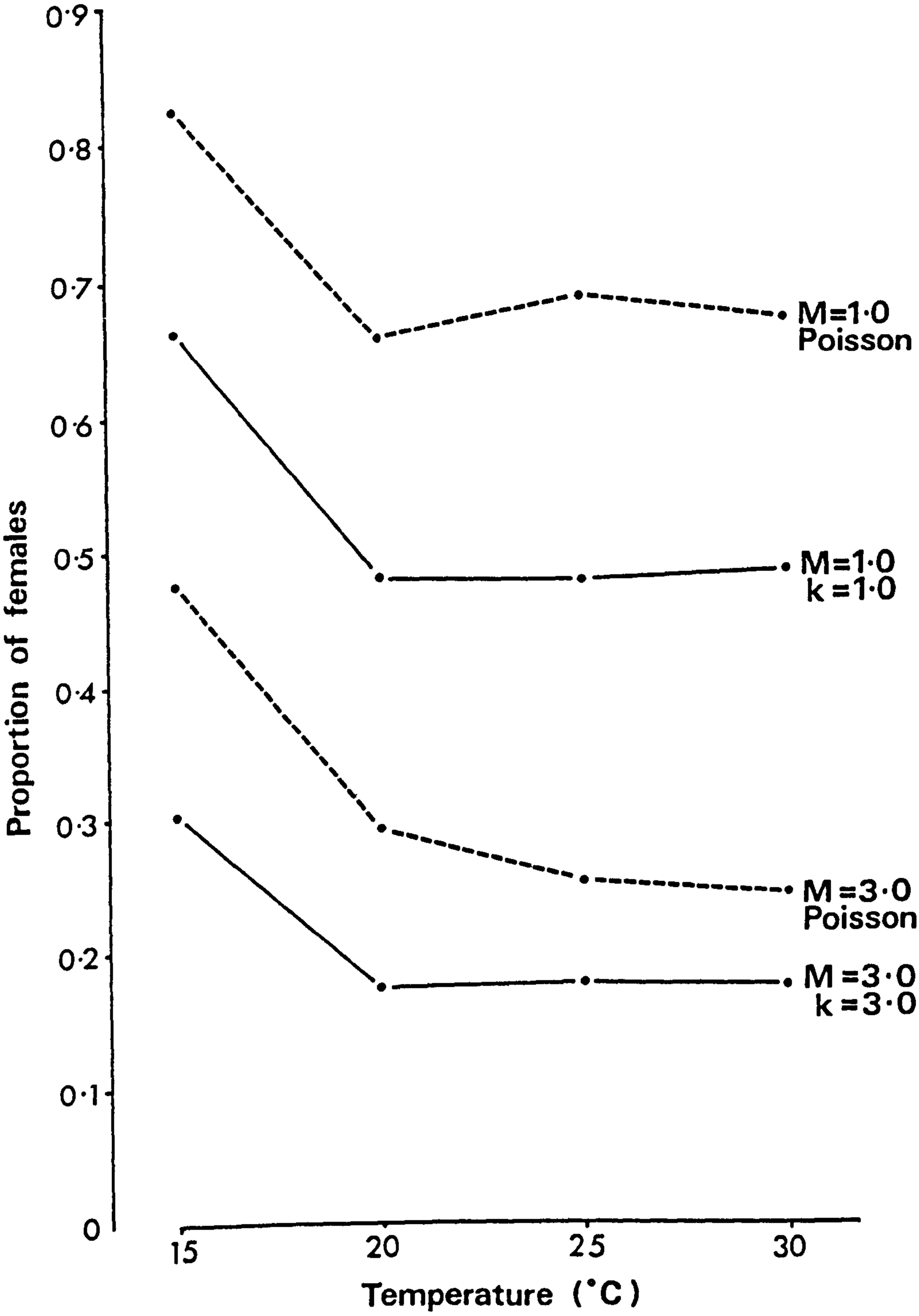
At each mean parasite burden (m) there is an inverse trend between the average variance-to-mean ratio and the female proportion of the nematode population, but there is a statistically significant linear relationship only at m=1 ($r=0.94$; $0.02>p>0.01$).

The most important point to note is that the very high average variance-to-mean ratio seen at 25°C corresponds well with the comparatively low female worm production observed at this temperature. There is thus some evidence in the experimental data to suggest that there is no real difference between nematode sex ratio at different temperatures.

A comparison of the expected female proportion of the nematode population at the four temperatures where the statistical distribution of parasite numbers within the hosts are identical, eliminates the effect of differing degrees of dispersion and gives a true picture of the effect of temperature upon sex ratio. This may be accomplished using the method described in section 2.6 (ii) to produce figure (2.6.3), where the frequency histograms (figure 2.6.9) are used in conjunction with theoretical frequency distributions to produce estimates of the female proportion of the nematode population under differing conditions of mean parasite burden and statistical distribution. A selection of such data is presented in figure (2.6.10) and clearly shows that the very low female proportion of the nematode population seen at 25°C was due totally to the relatively high degree of overdispersion shown in the original data. Figure (2.6.10) shows that there is no identifiable effect of temperature upon the sex ratio of the nematode at 20°, 25° and 30°C, but there is a marked difference at 15°C. It may therefore be suggested that temperature does influence the sex ratio of the parasite, but only at relatively low temperatures.

Figure 2.6.10 The influence of temperature upon the sex ratio of the nematode: defined parasite distribution.

The predictions of the sex ratio of the parasite given fixed distributions (Poisson and negative binomial) and the observed relationship between parasite burden and sex ratio seen in figures (2.6.2) and (2.6.9) (see text for method). k is the parameter of the negative binomial distribution.


0·9
0·8
0·7
0·6
0·5
0·4
0·3
0·2
0·1
0
Proportion of females
M=1·0
Poisson
M=1·0
k=1·0
M=3·0
Poisson
M=3·0
k=3·0
15
20
25
30
Temperature (°C)

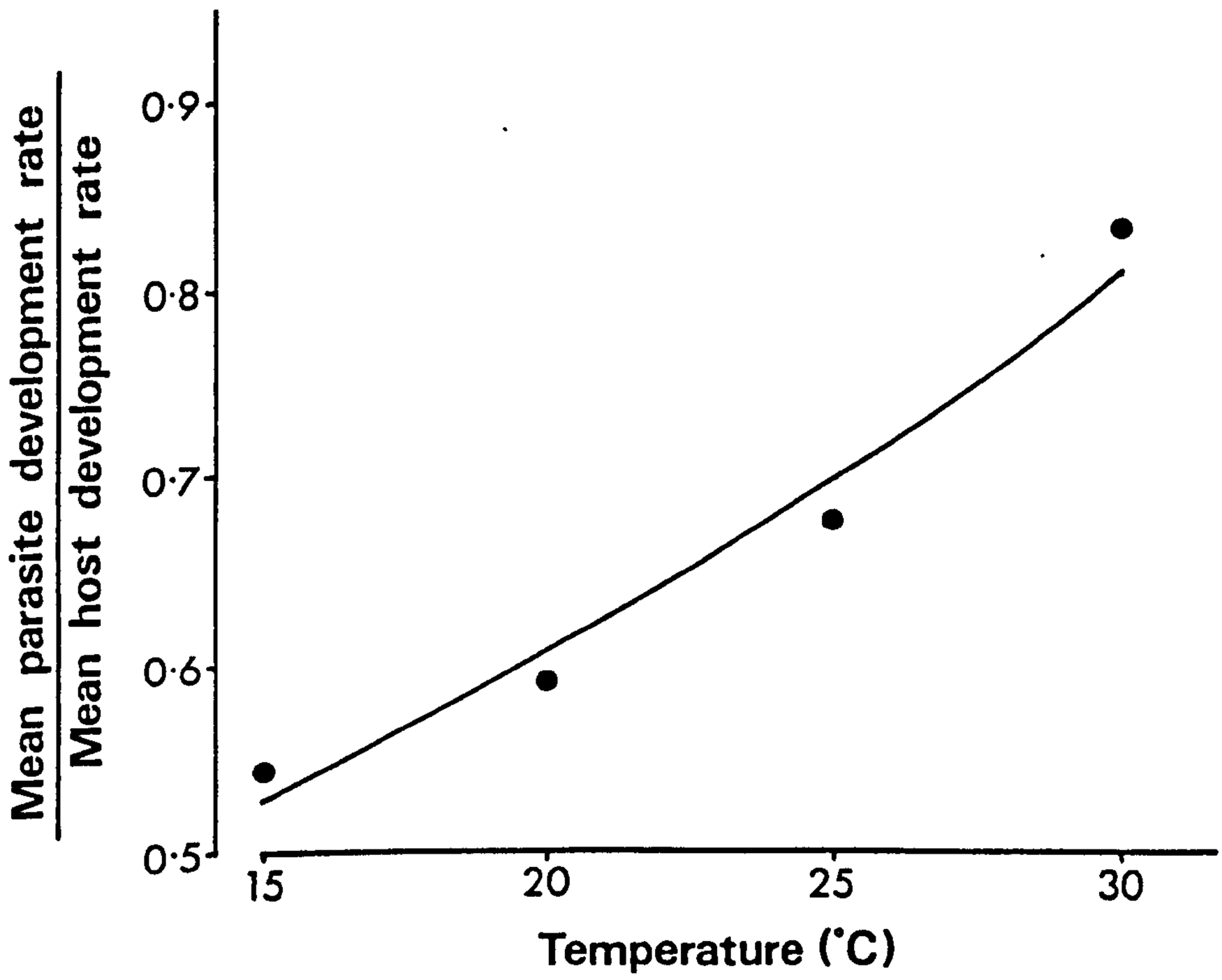
There are many mechanisms by which temperature may affect the sex ratio of an organism, but in this instance, with overwhelming evidence of a causative relationship between parasite sex ratio and nutrient availability the effect of temperature upon nutrient availability to the parasite may be of prime importance.

The most obvious effect of temperature upon such a host-parasite system is to define the rates of development of both host and parasite (figure 2.5.6). It might be supposed that the feeding rate of the host would show the same temperature dependency as the host development rate. Assuming such a relationship, as the development rate of the parasite slows at lower temperatures, more nutrient will become available to the parasites as the rate of host feeding (≡ development) declines at a slower rate (see figure 2.5.6). This provides a basis for the increase in female parasite production observed at the lowest temperature examined (figures 2.6.9 and 2.6.10); There would also be an expected gradual effect as temperature changed, which is not visible in figure (2.6.10). There are two factors which may have a bearing upon this situation, firstly, relatively small temperature-induced changes in the sex ratio of the nematode would be prone to masking by stochastic variation in one or a combination of the important factors which influence the determination of the sex ratio of the nematode. Secondly, the relationship between the parasite development rate and temperature has been assumed to be linear, when there is a distinct non-linear tendency in the relationship (figure 2.5.6a). This non-linearity would tend to increase the difference between the host feeding and parasite development rates in a non-linear manner, as the temperature falls. This may be seen in figure (2.6.11) where the ratio of the observed parasite development rate to the host development rate is plotted against temperature, the relationship being best described as a logarithmic increase (as opposed to a linear increase). This would tend to make proportionately more nutrient available to the parasites at the lower temperatures.

From the evidence presented, it may be deduced that the environmental temperature does affect the sex ratio of <u>R. culicivorax</u> at lower

Figure 2.6.11 The difference between the development rates of host and parasite: the influence of temperature.

The points are the ratios of the mean per capita rates (per day) of development of the parasite to the host. The developmental rates were estimated from the fitted lines (solid circles) in figure (2.5.6). The line is the least-squares best-fit linear regression of ln y on x ($a = -1.0702$, $b = 0.0285$, $r = 0.9831$).


Mean parasite development rate
Mean host development rate
0·9
0·8
0·7
0·6
0·5
15
20
25
30
Temperature (°C)

temperatures. The way in which temperature affects sex determination is most probably via nutrient availability although the direct action of temperature upon the physiology of sex determination cannot be excluded.

2.6 (iv) Discussion

Environmental sex determination of the type demonstrated in *R. culicivorax*, occurs throughout the mermithid nematode group and is potentially a very strong density-dependent regulator of the parasite population. The number of parasites per host has long been known to play an important role in determining the sex ratio of mermithid nematodes, whilst the importance of the statistical distribution of the parasites within the host population has remained largely unexplored. It has been shown here, that the statistical distribution of the parasites is of paramount importance in the process of sex ratio determination. The dependence of the sex ratio determination upon the statistical distribution of the parasites has clear implications for any future experimental investigations into the effects of biological or physical factors upon the sex ratio of mermithids. Ignoring the effects of the degree of dispersion of the parasites may well lead to erroneous results. It might also be suggested that field workers take more note of the statistical distributions of parasites then has hitherto been the case.

From published data, it is apparent that the levels of parasite burden of *Romanomermis spp*. in the field vary greatly, but with the majority of reported levels being less than three parasites per host. Whilst field distributional data is scarce, that which is available shows that the typical distribution is one of low to medium over-dispersion, with occasional high levels of aggregation and random distributions also occurring. The field data therefore displays what might be described as a surprisingly good empirical fit to the predicted situation, where the distribution and mean parasite burden tend to maximise the female nematode population. The significance of this in terms of the population dynamics of the parasite is self evident; the magnitude of the succeeding generation of parasites is to a large extent dependent upon the number of female nematodes formed in

the current infection. All factors which tend to reduce the number of female nematodes issuing from a host population will also tend to reduce the magnitude of the next parasite generation. Such factors include both very high and very low levels of mean parasite burden, high degrees of parasite aggregation within hosts and nutritional stress upon the host brought about by low nutrient imput, inter-and/or intra-specific (host) competition.

Such density-dependent regulation in the magnitude of the parasite population, when occurring in conjunction with the over-dispersion of parasite numbers usually seen, would be expected to lead to a situation where the parasite population tends towards an equilibrium level (see Anderson & May, 1978). How this important population behaviour is affected by other aspects of the biology of both the parasite and host will be reviewed in chapter four.

The effect of the environmental temperature upon the sex ratio of R. culicivorax is not dramatic but is most probably of importance at low temperatures. The parasite tends to have lower rates of infection at lower temperatures (Brown & Platzer, 1977; Galloway & Brust, 1977) which will lead to smaller parasite populations. This tendency will be opposed by the increased proportion of the nematode population differentiating as females at lower temperatures, effectively increasing the range of temperatures at which the nematode can exist.

It should be borne in mind that the effect of the developmental temperature upon the sex ratio of R. culicivorax reported herein was investigated under conditions of constant temperature. Diurnally fluctuating temperature (as would be expected to occur in the field) may complicate the relationship (Hughes & Platzer, 1977).

The advantage to the individual parasite of having environmentally determined sex, bearing in mind that the selection for such a process must occur at the level of the individual, is related to the reproductive potential of members of the two sexes of differing size. High parasite burdens produce smaller parasites than do low parasite bur-

dens, there is therefore a correlation between large size and female parasites and similarly, small size and male parasites. Charnov & Bull (1977) and Charnov et al. (1981) have suggested that where sex determination is largely dependent upon the size of the organism then there must be a significant selective advantage to one or both of the sexes in being either large or small. In this instance, it is clear that the individual gains more by being a large female than by being a large male. The biological interpretation of this is that as the number of eggs produced by a female is determined by the initial size of the (non-feeding) adult, a larger female has a greater potential egg output than a smaller female. As the size of the individual declines fewer and fewer eggs will be able to be produced by a female and eventually a point is reached when the individual will gain more in reproductive terms as a male than as a female. Thus, at the individual level, it is of greater selective advantage to be an average male rather than a below average female. The occurrence of environmental sex determination of this type therefore benefits the individual by maximising its reproductive potential.

2.7 DYNAMICS OF THE POSTPARASITIC NEMATODES

Upon emergence from the host, postparasitic R. culicivorax adopt a free-living mode of existance. They drop to the bottom of the aquatic environment and burrow into the substrate. There, after a suitable interval they undergo moulting, mate and oviposit. The rate at which these activities occur is of major importance in the population dynamics of the nematode as they cause considerable time delays in the completion of the life-cycle and are thus important determinants of the average generation time. Also of importance are the survival characteristics of both the juvenile and adult post-parasites. Heavy mortality at these stages in the life-cycle would seriously reduce the size of the egg population and therefore the next generation of nematodes.

Published data relating to the survival of postparasitic R. culicivorax is mostly anecdotal, referring either to overwintering at low temperatures (Nickle, 1979) or to survival under laboratory mass rearing regimes, where infection by the parasitic fungus Catenaria anguillulae Sorokin can cause severe mortality (Platzer & Brown, 1976; Dhillon & Platzer, 1978; Platzer & Stirling, 1978; Stirling & Platzer, 1978).

Investigation of the dynamics of moulting in R. culicivorax has been conducted by Petersen (1975) and also Imbriani & Platzer (1981), who showed that the rate at which juvenile postparasites moulted was dependent upon the gaseous conditions under which they were kept; mean time to moulting increasing with decreasing oxygen availability.

The time delay between moulting to the adult stage and the occurrence of mating has so far not been considered, usually being included in the time delay until egg formation or oviposition. This aspect of reproduction is of particular importance, especially when it might be expected that the average time to mating will be at least partially dependent upon a variable sex ratio.

2.7 (i) Postparasitic Juvenile Survival

The survival characteristics of the juvenile nematodes are of

major importance, and factors which tend to reduce nematode survival must be assessed in order to be able to estimate the proportion of both male and female juveniles surviving until they are of reproductive age.

An experiment was designed to measure both the rate of mortality and the rate of maturation of juvenile postparasites. Newly emerged postparasites, collected over a six hour period, were placed into one of two plastic trays according to sex. Each plastic tray was of 30 ml volume and contained approximately 10 ml of standard sand substrate and 10 to 15 ml distilled water. After placing the nematodes into the trays, they were covered with a lid and kept at 25°C in a 12:12 hour light:dark regime. Every 24 hours the nematodes were removed from the containers and examined, those which had died or moulted were removed whilst the remainder were replaced into their container. This procedure was repeated on three occasions using 14 male/4 female, 19 male/21 female and 20 male/39 female nematodes on each occasion. The three replicates were summed giving a sample size of 53 male and 64 female nematodes.

During the development period, up to and including moulting, no male nematodes died and only one of the female nematodes died; which was found dead on day seven post-emergence. The cause of death was not ascertained, but infection as a result of repeated handling can not be excluded.

The survival of the juvenile nematodes over the period up to and including moulting, a maximum of twelve days, was very close to one hundred percent, thus suggesting that under good field conditions there may be no significant premoult mortality.

There are, however, other factors which must also be taken into account when considering postparasite survival, as for example the fungus C. anguillulae which as previously stated can cause considerable mortality. Predation by both vertebrates and invertebrates may also make significant inroads upon the survival of postparasites in the field, depending upon the relative densities of both nematode and

predator and the availability of alternative prey. Environmental desiccation may also play a role in survival in certain seasonal geographical regions but must be considered as a catastrophic event.

Despite these potential losses, the survival of postparasitic juvenile R. culicivorax is most probably very good under usual field conditions. Mortality at this stage in the life-cycle probably does not account for a significant proportion of the post-parasitic population.

2.7 (ii) Moulting to the Adult Stage

The time delay between parasite emergence and moulting is important because the longer this period is, the greater will be the average generation time and the smaller will be the proportion of nematodes that survive to reproduce.

An earlier investigation into the moulting ecology of R. culicivorax produced data which suggested that the mean time to moulting for males was 33.5 days and for females was 43.9 days (Petersen, 1975). Imbriani & Platzer (1981) report mean development times for female R. culicivorax from 10.6 to 32.4 days as the quantity of atmospheric oxygen above the substrate was decreased.

The experiment described in the previous section (2.7 (ii)) enabled the time of moulting of individual nematodes to be recorded. The proportion which moulted on successive days post-emergence is shown in figure (2.7.1a) with the cumulative proportion moulted portrayed in figure (2.7.1b). The most obvious feature to be seen in these figures is that the male nematodes tended to moult earlier than the females. Males moulted over a five day period from six to ten days post-emergence, while females moulted from day six to day twleve post-emergence. The mean time to moult for males was calculated at 7.79 days and for females at 8.56 days.

The slightly shorter premoult period shown by males agrees with the results of Petersen (1975). The large discrepancy between the

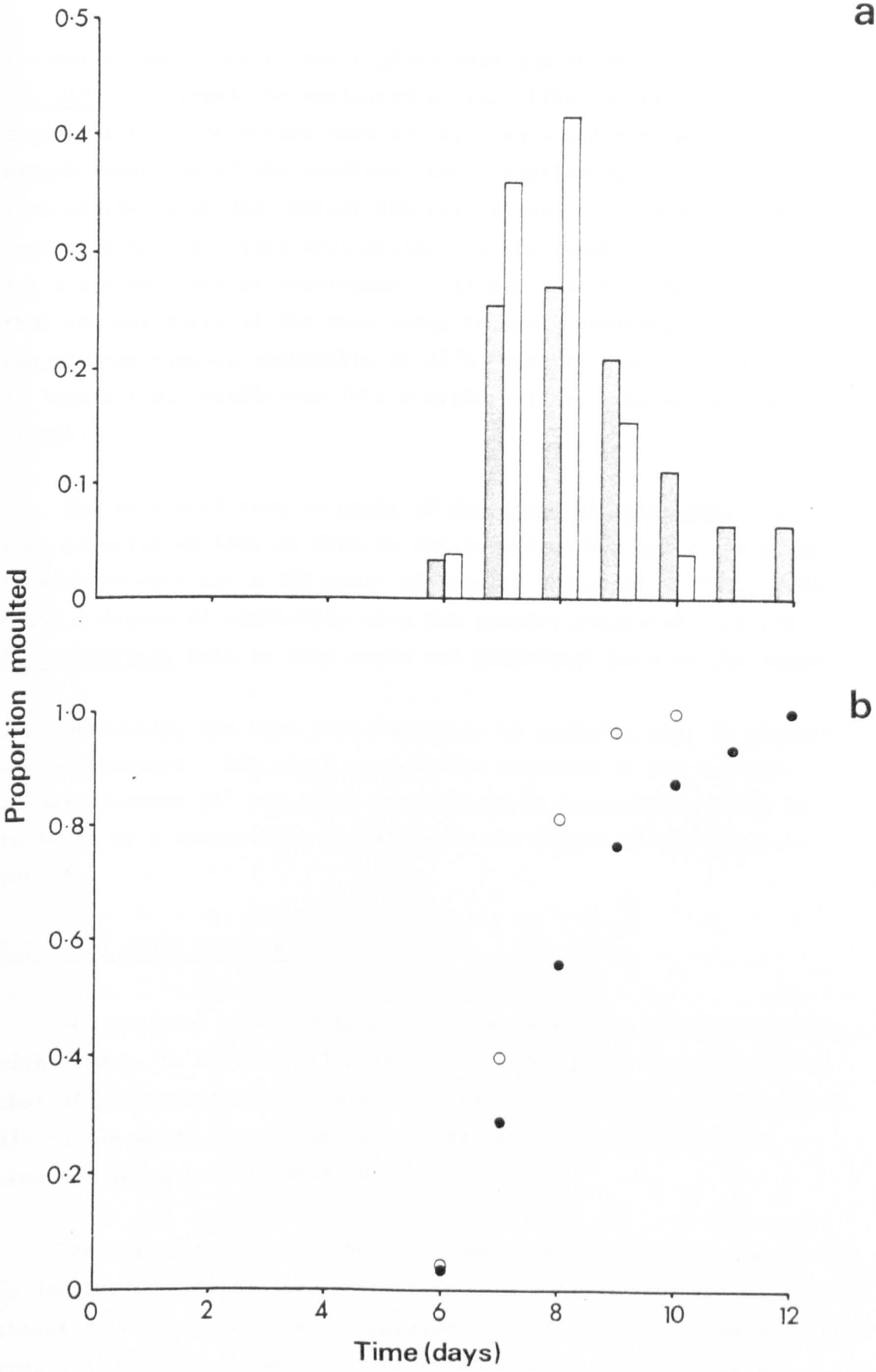
Figure 2.7.1 Time to moulting for post-parasitic juvenile nematodes.

a The proportion of nematodes that moulted daily, post-emergence from the host.

Solid bars - females (n = 63)
Open bars - males (n = 53)

b The cumulative proportion moulted at time t post-emergence.

Solid circles - females.
Open circles - males.


a
0·5
0·4
0·3
0·2
0·1
0
moulted
Proportion
b
1·0
0·8
0·6
0·4
0·2
0
0
2
4
6
8
10
12
Time (days)

estimated mean times to moult given here and those of Petersen, op. cit. can largely be explained by the different experimental regimes used. The method used by Petersen would have allowed oxygen depletion of the substrate thus lengthening the development time of the nematodes, whilst the method used in this study would tend to maximise oxygen availability to the nematodes, allowing for a maximal rate of development. It may therefore be assumed that the estimates of the mean times to moult presented here are the minimum figures obtainable at 25°C, with the time increasing in less aerobic conditions (which might well be expected in the field).

The estimated time to moult of Octomyomermis muspratti has been reported at four or five to thirteen days for males and eight to sixteen days for a 90% moult of females (Petersen, 1978b). This shows a degree of similarity with the results presented here for R. culicivorax both in time scale and difference between the sexes.

Obviously, the time from emergence to moulting will be temperature dependent. All times quoted were measured by the various authors between 24° and 28°C. Any change in temperature would be followed by a comcomitant variation in the length of the premoult period.

2.7 (iii) Adult Survival

The position of adult R. culicivorax and other adult mermithids with respect to investigation into their ecology is very similar to that of the postparasitic juveniles, with those aspects of the adult life-cycle which contribute to the reproductive success of the nematode being largely unknown.

The mortality rate of the adult nematodes is of major importance, in females it defines what proportion will successfully lay eggs and therefore the size of the egg population, while in males the mortality rate will determine the number of females mated and the rate at which mating occurs, thus having a great influence upon the reproductive.

success of the adults.

As the adults are non-feeding, their pattern of survival would be expected to be very similar to that seen in the non-feeding pre-parasitic stage (section 2.4 (i)), where the rate of mortality is age-dependent. To test this hypothesis survivorship experiments were carried out.

Eighty unmated male nematodes were selected at random from a population which had moulted during a 48 hour period; they were placed into two 30 ml plastic trays with approximately 10 ml of standard sand substrate and 10 to 15 ml of distilled water. Each tray containing forty nematodes, was covered and kept at a constant 25°C in a 12:12 hour light:dark regime. This procedure was repeated for eighty unmated female nematodes. In both cases the nematodes were known to be unmated due to the sexes being separated prior to moulting. The number of nematodes of each sex remaining alive was ascertained approximately once a month, the water being completely changed at this time. The criterion of death was failure to move spontaneously, or, after stimulation with a dissecting needle. On no occasion was evidence of egg development seen in the females and no deposited eggs were seen, strongly suggesting that copulation is required for reproduction in this species.

The survivorship curves for the male and female nematodes are portrayed in figure (2.7.2). In each case the form of the curve is clearly age-dependent, the pattern being similar to the age dependent survival model of Anderson & Whitfield (section 2.4 (i) equation 2.4.3).

The longevity shown by unmated males would enable them to survive periods of unfavourable conditions and when there were no females about, thus increasing their chances of being reproductively active when conditions improved or females became available. This may be of particular importance in seasonal environments where the first infection of a new season will tend to be of low intensity (due to temperature) producing mostly females, where there would be a considerable advantage if males were already present in the environment, having overwintered, ready to fertilize females.

Figure 2.7.2 Survival of unmated adult nematodes.

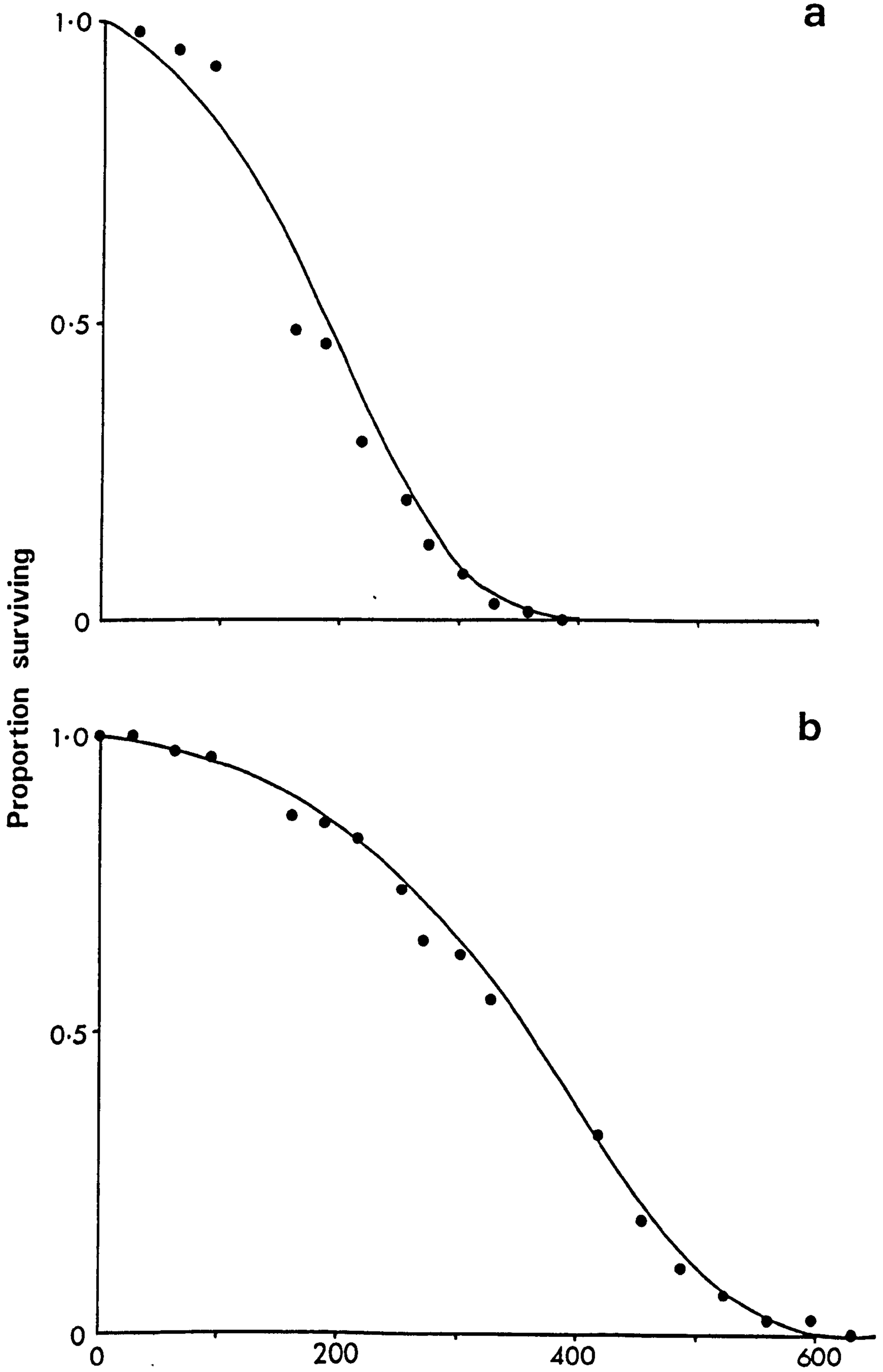
a males (n = 80)

b females (n = 80)

The points are observed data. The lines are the predictions of an age-dependent survival model (equation 2.4.3) where,

a a = 0.001153, b = 0.010418

b a = 0.00338, b = 0.007936


a
b
1·0
0·5
0
Proportion surviving
0
200
400
600
Time (days)

The mean expected life-span (see section 2.4 (ii))of unmated males was found to be considerably less than that for unmated females, 185.7 and 334.6 days respectively. This difference in expected life-span is almost certainly a result of the difference in size and therefore in the quantity of energy stored by the two sexes.

The longevity of unmated females is also of importance and for the same reasons as in males, enabling them to survive through unfavourable conditions or times when males are scarce and still produce numerous viable eggs upon the improvement of conditions.

Obviously, the older the female at the time of mating the fewer, on average, will be the number of eggs produced. With a mean expected life-span of 334.6 days it may be assumed that an average female would still be able to produce substantial numbers of eggs up to about 200 days post-moult. There would also be a clear advantage to larger than average females in this situation, again emphasising the selective advantage of producing large females (see section 2.6).

The survival characteristics of mated females may have important consequences for the egg population and were therefore investigated, also at 25°C. Newly mature (moulted) females, selected at random from a population of females isolated from males immediately after emergence from the host, were placed with males for twenty-four hours, at the end of which time the females were isolated. Females which showed no egg development within a twenty-one day period were excluded from the analysis. When individual females showed egg development they were transferred to 30 ml containers as previously described. Two replicates were carried out, one of eight and one of twenty-nine female nematodes, the results being summed to provide an adequately large sample size. The nematodes were examined every second day to assess survival and remove the accumulated egg population to prevent any potential inhibition of egg laying or infection due to the crowding of a large number of eggs.

Although this regime causes a bias in favour of those females which mate most readily, it is unlikely that this would influence their

subsequent survival.

The survival characteristics of mated females are portrayed in figure (2.7.3), and are of a similar age-dependent form to that seen in both unmated males and females, although the fit of the Anderson-Whitfield model is less satisfactory.

The effect of the nutritional demands of egg development and oviposition can clearly be seen in comparing the survival of mated and unmated females. The survival of the two groups was very similar until oviposition was complete and there was then a rapid decline in the population of the mated females. This is also reflected in the mean expected life-spans, that for mated females was 66.9 days, whilst for unmated females it was 334.6 days. The very rapid fall in the proportion of mated females surviving after forty days is indicative of the small quantity of usable energy reserves retained after egg development and oviposition, which is essentially complete after twenty to thirty days post-mating (see section 2.8).

The extended survival of some five percent of the female nematodes may reflect either an increased retention of nutrient within the trophosome, possibly by the larger females, or a higher level of efficiency in metabolising what nutrient remains. Provided that this behaviour does not withhold nutrient from egg development, it will be of little significance as there can be no significant role that "exhausted" females can play in the population biology of the nematode. Some benefit may be accrued from surviving post-ovipositional females by providing suitable meals for predators thus enabling unmated and ovipositing females to suffer a lower rate of predation. However, such benefit would most likely be minimal as the greater density of suitable prey caused by the presence of the post-ovipositional females would tend to attract more predators. In addition the presence of such females may reduce the rate of mating (section 2.7 (iv)), as Imbriani & Platzer (1981) have shown that males will copulate with post-ovipositional females, temporarily inhibiting them from successfully mating with unmated females.

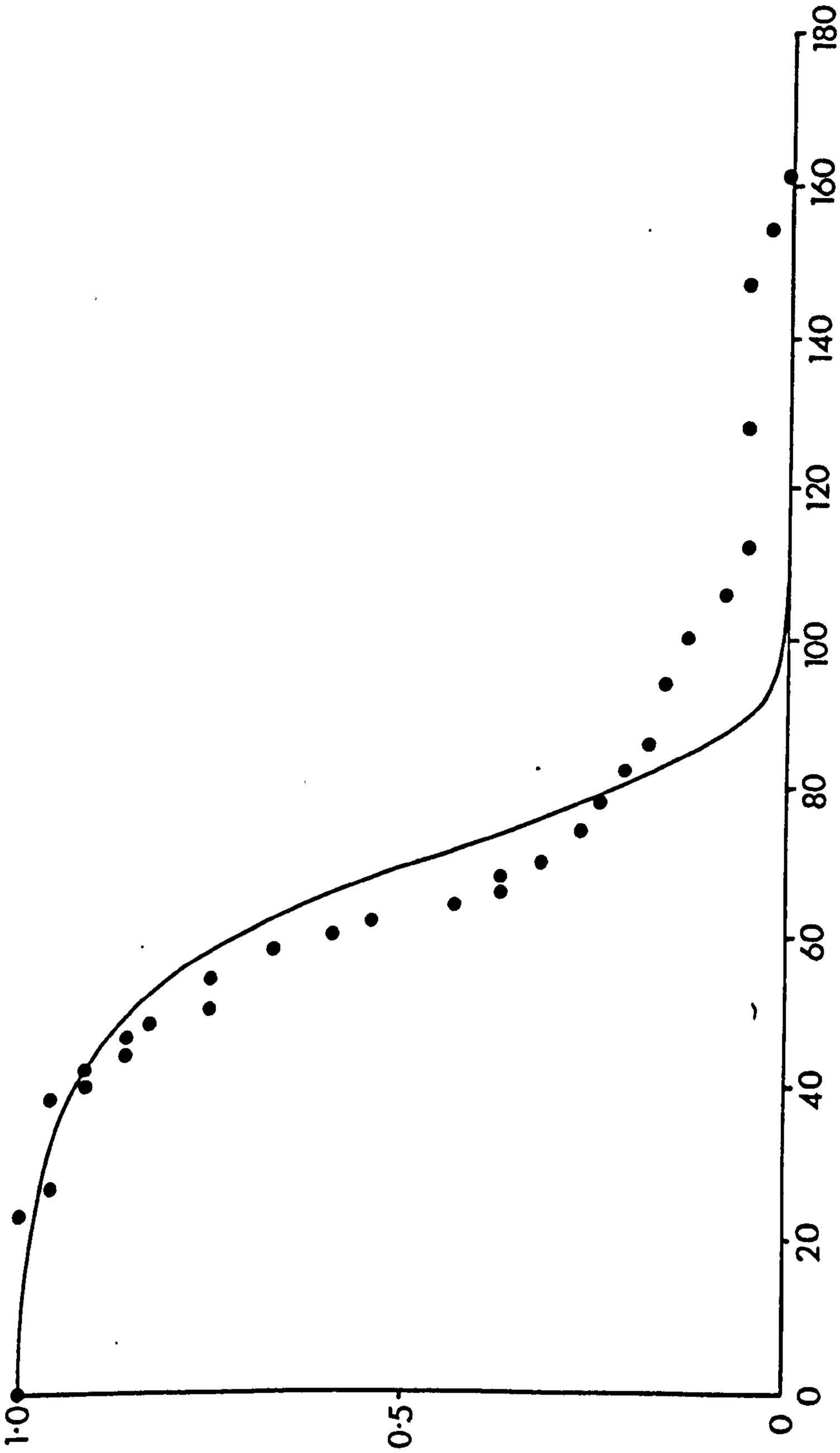
In the field, mating would not be synchronous and would not occur

Figure 2.7.3 Survival of mated adult female nematodes.

The points are observed data. The line is the prediction of an age-dependent survival model (equation 2.4.3) where,

a = 0.000288, b = 0.074834

The sample size was 37.


Proportion surviving
1·0
0·5
0·0
0
20
40
60
80
100
120
140
160
180
Time (days)

immediately post-moult in all females, thus,there would be some level of female mortality before completion of oviposition. The extent of such mortality would be dependent upon the prevailing environmental conditions, the availability of males (i.e. the sex ratio) and the ability of the male and female nematodes to find each other.

The survival characteristics of mated males were not investigated because it was deemed unlikely that mating would seriously affect the survival of the male, particularly when supplied with such a relatively large energy reserve. If there was any tendency for mating to decrease the survival of the male nematodes it would be largely balanced by the male concerned having already contributed genes to the next generation during mating.

It is abundantly clear that the survival characteristics of adult R. culicivorax have evolved to enable each nematode to maximise its reproductive potential, depending upon its size, benefiting the nematodes at both the individual and population level.

It should be borne in mind that the survival characteristics described here are for laboratory populations kept under conditions which are likely to maximise survival. In the field other factors would act upon the nematodes tending to alter (and mostly to reduce) their survival (particularly climatic conditions, predation and other parasites).

2.7 (iv) Mating Characteristics

For R. culicivorax the mating process begins with the emergence of the parasites from the host. Having emerged and burrowed into the aquatic substrate the nematodes have then to locate a member of the opposite sex with which to mate after moulting has taken place. This is probably made easier by the habit of the hosts of congregating together, especially at environmental boundaries, thus nematode deposition would tend to occur in certain areas.

Advantages would clearly be gained by nematodes aggregating into

heterosexual groups in the substrate thus markedly increasing the probability of mating. Such group forming behaviour was frequently seen in culture vessels and has also been reported to have been observed in a long term experiment, where the nematodes congregated about the roots of aquatic plants (Nickle, 1979). Clearly there must be some mechanism which allows individuals to find each other and thus form such groups. The most probable mechanism is a chemical attractant released by one or both sexes, which attracts members of the other or both sexes. Further work is required to demonstrate the presence, source and identity of such an attractant.

The newly emerged postparasites have a premoult period of seven or eight days in which they can congregate into groups before they are able to reproduce, thus reducing any delay in mating associated with mate location.

Having initiated or joined a group and also having moulted, the nematodes proceed to mate; mating even being observed to occur before the female has completely shed her juvenile cuticles.

There are three major unanswered questions related to the mating behaviour of R. culicivorax. Firstly, it is not known whether the males are monogamous or polygamous, the latter being the usual assumption; secondly, the rate at which a male can copulate with and fertilize a female, and from this; thirdly, the effect that the sex ratio has upon the proportion of females mated within a certain time span.

To investigate these questions an experimental regime was conceived which consisted of separating the sexes of unmoulted (and therefore virgin) nematodes and maintaining them until moulting had occurred. One mature male and from one to ten mature female nematodes were then placed into a 3 ml well in a plastic tissue culture plate containing approximately 2 ml sand substrate and enough distilled water to cover the sand. The tissue culture plate was then kept at a constant 25°C in a 12:12 hour light:dark regime. After 96 hours the nematodes were removed and the males discarded. The females were isolated into small vials with approximately 0.5 ml sand and

1 to 2 ml distilled water, each vial being so marked that the female contained within could be assigned to its own particular group. The vials were examined daily for oviposition, which was the criterion used to show that mating had occurred. The onset of oviposition was never observed to occur later than thirteen days post-separation and examination was continued for at least this length of time.

The experiment just described assumes that mating is required for egg production, a sound assumption as no egg development or oviposition was ever recorded for unmated female R. culicivorax throughout their life-span (section 2.7 (iii)). Also, no egg development or oviposition was seen in virgin O. muspratti (Petersen, 1978b).

At the time of male removal pairs of worms in copulo were physically separated. Of a total of thirty-two females thus separated none died and twenty-three (72%) proceeded to produce eggs.

The information obtained from this series of experiments provides answers to the questions posed earlier. As the number of females per male was increased so the proportion of those females mated within the 96 hour period of male association fell (figure 2.7.4a). Also of note is that even with a 1:1 sex ratio only 80% of females mated successfully. The exact reason for this cannot be identified from these experiments but is most likely the product of males and females not coming into contact, also, there is the possibility that some pairs were unable to mate successfully and others were sterile.

If the data are presented in a slightly different form (figure 2.7.4b) two further points emerge. Firstly that males are polygamous and secondly, that there is a maximal rate at which the males mate with females. With respect to the polygamy, within the 96 hour period the maximum number of females mated by one male was three, but there is no reason to believe that this is the upper limit of male polygamy, indeed given more time a male would be expected to mate with an increasing number of females. The data presented in

Figure 2.7.4 The influence of the sex ratio of the adults upon mating success.

a The decline in the proportion of successfully mated females (indicated by oviposition) with an increase in the number of females per male.

The points are observed data and the line is the least-squares best-fit linear regression of ln y on x (a = -0.1159, b = -0.1720, r = 0.9781).

b The rate of mating, indicated by the number of mated females per male, over a range of values of sex ratio.

The points are observed data and the line is the least-squares best-fit linear regression of y on ln x (a = 0.9728, b = 0.3674, r = 0.8370).

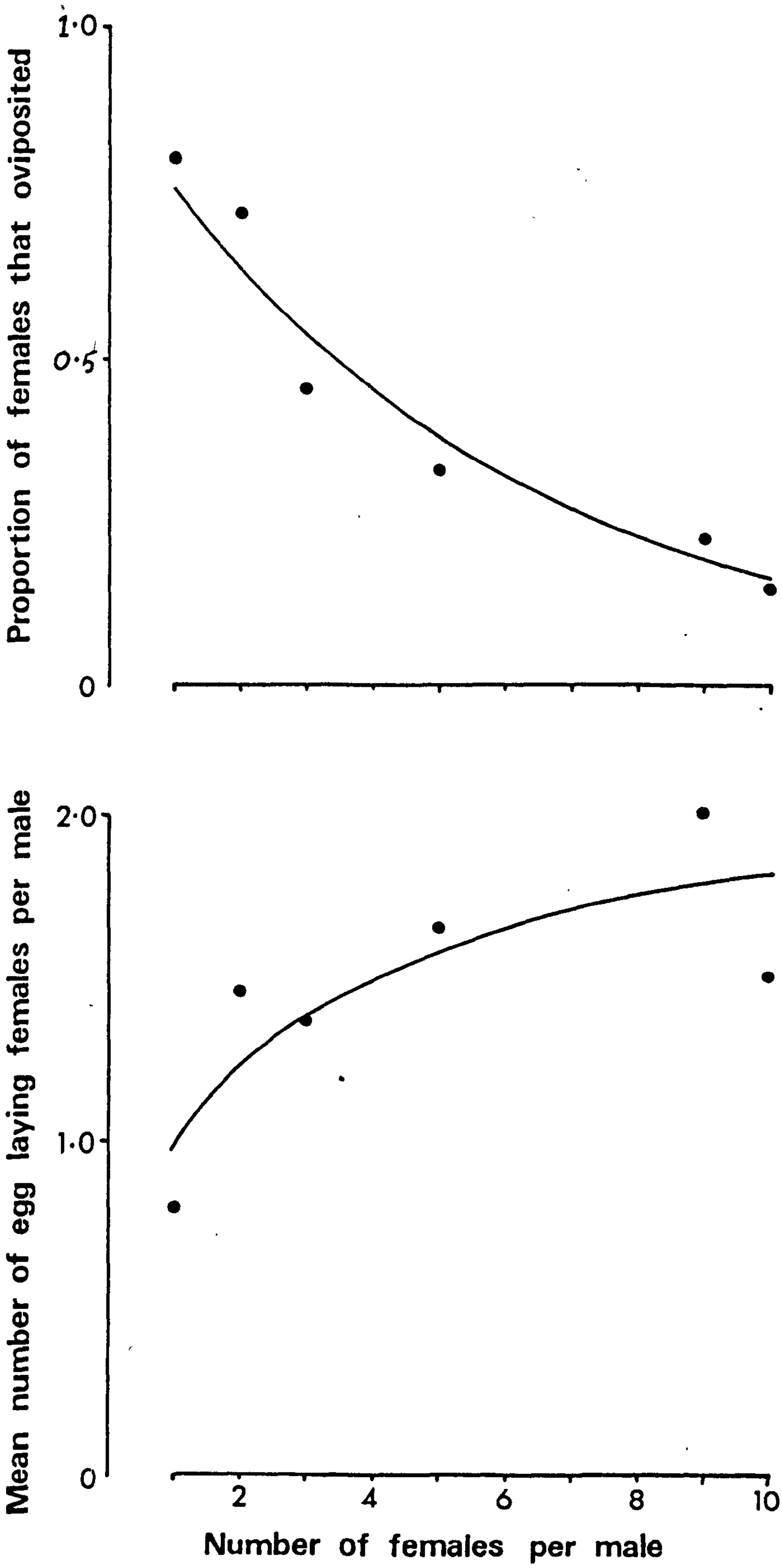

a
Proportion of females that oviposited
1·0
0·5
0
b
Mean number of egg laying females per male
2·0
1·0
0
2
4
6
8
10
Number of females per male

figure (2.7.4b) shows that the mean number of females mated by a male within the 96 hours, increased asymptotically to a value of approximately two as the number of available females was increased. This gives a maximum rate of mating of 0.5 females/male/day, a rate which will decline as the number of available females declines (although the proportion of females mated will increase through time).

The notion of a maximal rate at which mating can occur hinges upon the concept of a handling time associated with the act of mating each female. This is identical to the handling time concept as applied to host or prey finding by predators and parasitoids (Rogers, 1972; Hassell, Lawton & Beddington, 1977) and may be visualized as the sum of the time spent in locating a female and the time spent in copulating with that female. From the maximal rate of mating, the minimum handling time can be estimated and in this case is two days. It is not possible, at this juncture, to assign a specific proportion of the handling time to the two elements of search and copulation.

As the hosts tend to congregate together, the postparasites aggregate into groups and with at least a seven day period before maturation of the emergent nematodes the principal determinant of the magnitude of the time delay until oviposition will be the sex ratio of the nematodes, as at constant temperature all the development rates (moulting, egg formation) will be constant. In the field it is not only the sex ratio of the current generation which is of importance, the presence of unmated females and sexually active males from previous generations will exert a considerable influence upon the characteristics of mating.

The effect that the overall sex ratio has upon the mean time to mating may be assessed if one assumption is made. This assumption is that the relationship between the proportion of female nematodes mated and the length of time that they are exposed to males is linear. If this assumption is accepted, then the mean time to mating of females under varying conditions of sex ratio may be calculated. This was carried out and the data are presented in table (2.7.1).

Table 2.7.1

The mean time to mating of female R. culicivorax given fixed sex ratios.

Sex ratio (♂:♀)	Mean time to mating (Days)
1:0.75	2.56
1:1	2.67
1:2	3.17
1:3	3.76
1:5	5.31
1:10	12.55

The estimates of the mean time to mating for females given in table (2.7.1) will tend to be under estimates, due to the assumed linear relationship between the time spent in a mixed sexual community and the proportion of females mated tending to become asymptotically non-linear as time progresses as has already been described. However, the estimates do provide an approximate time scale upon which to base future deductions as to the influence of the sex ratio upon the delay in mating and the impact this has upon the magnitude of the mean generation time of the nematode.

Three further factors may also affect the rate of successful mating. As mentioned in section 2.7 (iii), the presence of post-ovipositional females will tend to reduce the rate of successful mating by distracting males into unfruitful copulations. This aspect was not taken into account in the estimation of the mean times to mating presented in table (2.7.1), although the effect of males mating with the same female more than once was to some extent, as a consequence of the experimental design. Both of these factors will tend to reduce the rate of successful mating. The third factor is the gaseous environment; Imbriani & Platzer (1981) have demonstrated that increasingly anaerobic conditions lead to an increase in the mean time between moulting and oviposition. The actual effect that anaerobic conditions have upon the mating characteristics in relation to the egg development time has not yet been quantified.

2.7 (v) Egg Development

Once mating has occurred there is a further time delay, associated with the formation of ova and their fertilization prior to egg deposition by the female. The length of this delay is likely to be temperature dependent.

Estimates of the average length of the time delay between mating and initial oviposition may be extracted from the literature. However since this aspect has never been examined in isolation some caution in interpretation is necessary. The most comprehensive study of the postparasitic development of R. culicivorax to date (Imbriani & Platzer, 1981) provides an estimate of the mean time between moulting and initial oviposition of 8.25 days at 27°C under aerobic conditions and with a male to female ratio of 1:0.75. Given this information the mean expected time between moulting and mating, given in table (2.7.1) would be 2.56 days. Thus the mean time between mating and initial oviposition would have been 5.69 days. Imbriani & Platzer op. cit. also demonstrated that the length of time between moulting and oviposition increased as the environment became more anaerobic; it is not known what proportion of this increase is attributable to egg development time increasing and what to increased delays in mating, although increases in both may be expected.

Data presented by Petersen (1975) giving an estimate of the mean time between moulting and observable egg production in R. culicivorax of 38.78 days could not be used to estimate the average delay between mating and egg production because the sex ratio of the adults was unknown and therefore that portion of the 38.78 days taken up with mating could not be estimated. It is clear however that the egg development time in this instance must have been considerably longer than the other available estimate. This may well be a consequence of differing oxygen availabilities under the different experimental conditions.

In a study of O. muspratti (Petersen, 1978b), the mean time between

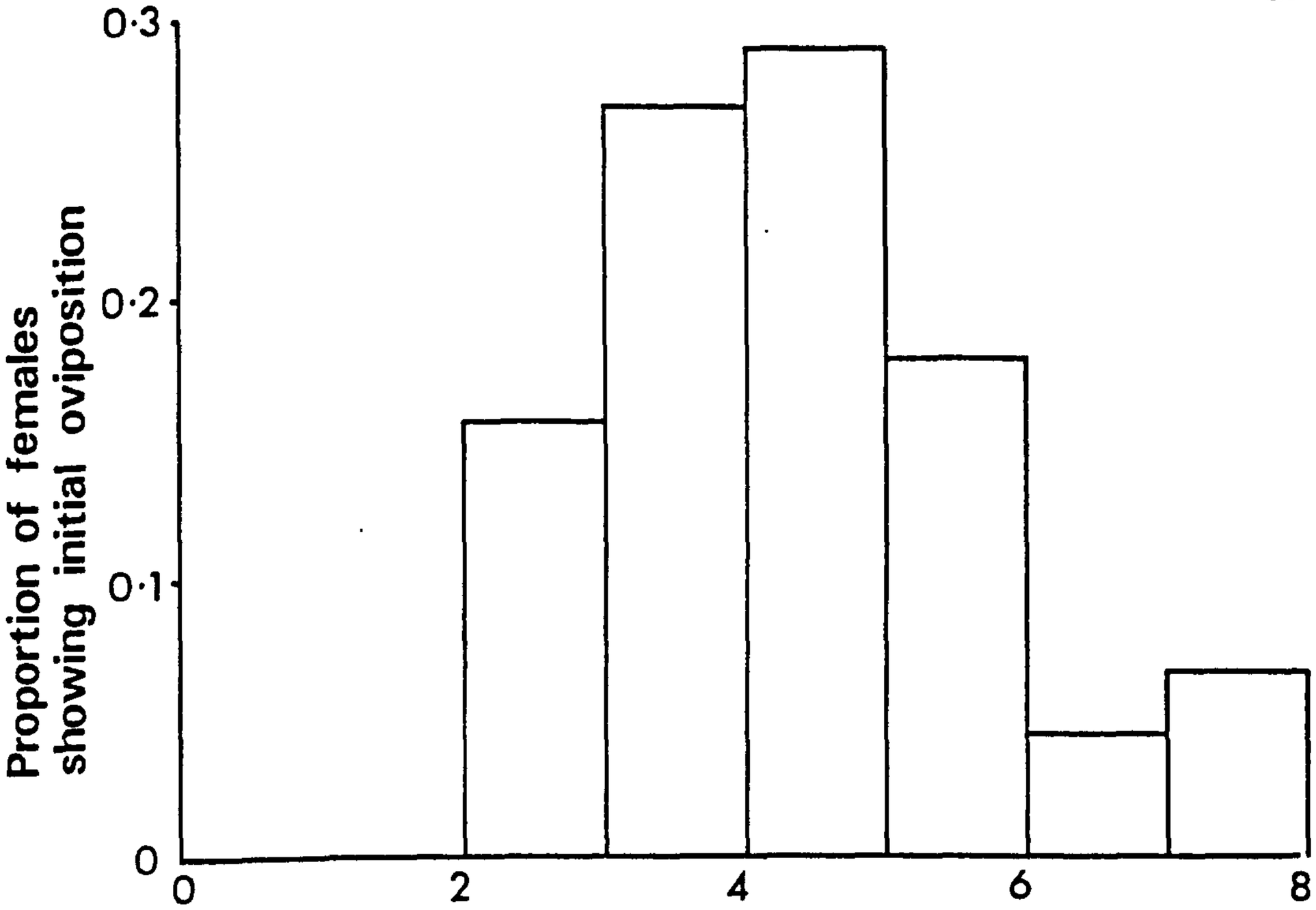
Figure 2.7.5 The time delay between mating and initial oviposition.

a The observed relationship between the time post-mating and the proportion of females that initiated oviposition.

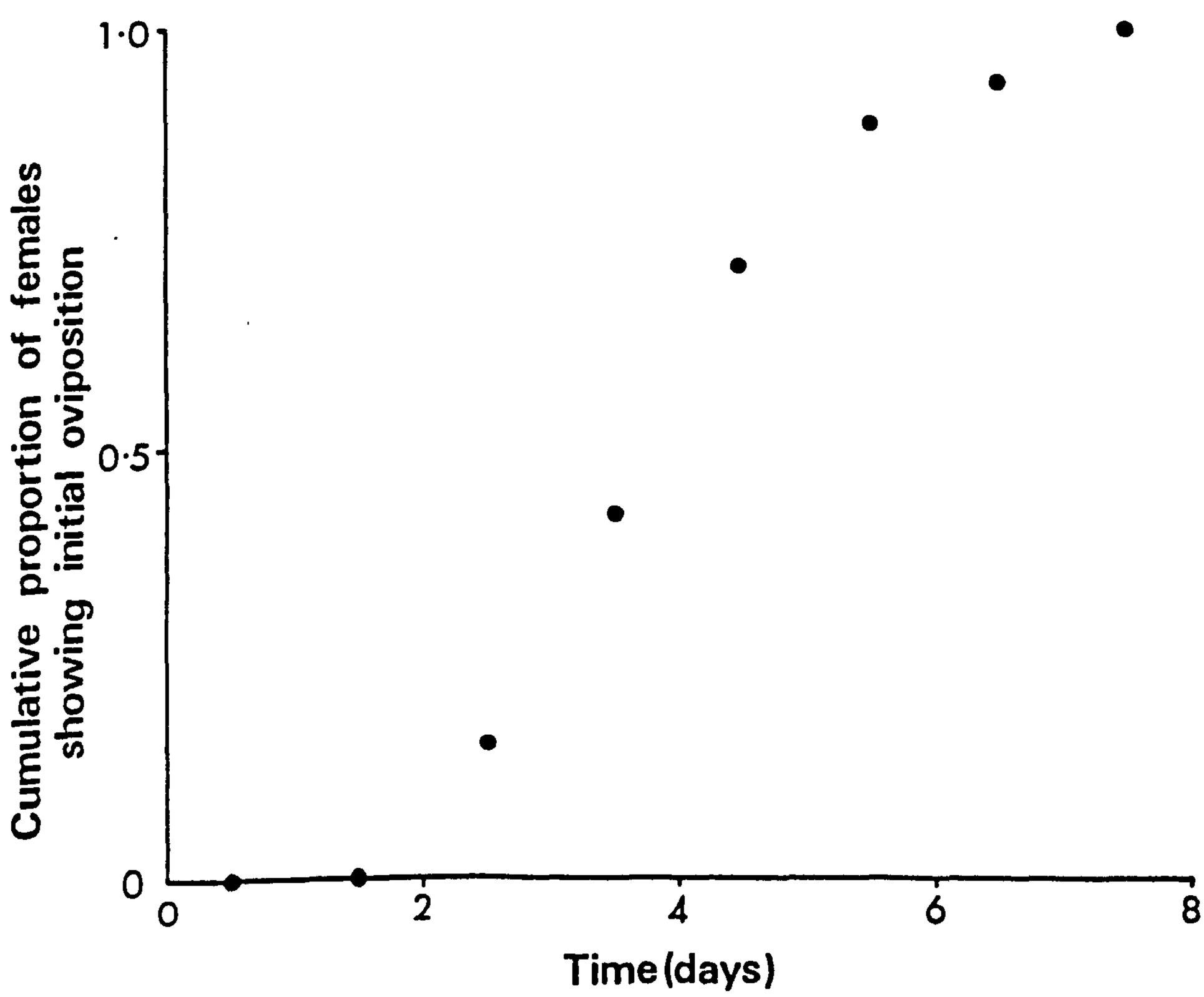
b The observed relationship between the time post-mating and the cumulative proportion of females that had initiated oviposition.

The sample size was 45 females.

a


Proportion of females showing initial oviposition
0·3
0·2
0·1
0
0
2
4
6
8

b


Cumulative proportion of females showing initial oviposition
1·0
0·5
0
0
2
4
6
8
Time (days)

mating and initial oviposition, assuming the mating characteristics are similar to those of R. culicivorax may be crudely estimated to be in the region of 5 to 9 days.

A direct estimate of the mean time between mating and initial oviposition without having to make assumptions about the delay due to mating and sex ratio is likely to be more accurate.

An experiment was organised consisting of isolating male and female postparasites until they had moulted. Males and females were then placed together for twenty-four hours at 25°C with a 12:12 hour light:dark regime. At the end of the twenty four hour period the males were discarded and the females isolated into small vials containing 0.5 ml sand and enough distilled water to cover the sand. Each vial was carefully examined every twenty-four hours when the time of oviposition was noted. Females which had not oviposited by day thirteen post-separation were regarded as unmated. The midpoint of the twenty-four hour mating period was taken as t=zero. Five replicate experiments were conducted with twelve, fourteen, seven, two and ten nematodes producing eggs. The data were pooled to give a sample size of forty-five and are portrayed in figure (2.7.5). The relationship is clearly time-dependent, with a mean time between mating and initial oviposition of 4.39 days. This estimate agrees favourably with that of 5.69 derived from the published data of Imbriani & Platzer (1981), although the two degree temperature difference between the experiments would slightly influence the two estimates.

2.7. (vi) Discussion

Under conditions where predation is absent and infectious agents are discouraged, the survival of postparasitic juvenile R. culicivorax is very nearly one hundred percent. The low level of mortality observed appears to be associated with cuticle shedding, where the inability to completely shed the juvenile cuticles leaves the way open for infection to take place. The period during which such juvenile mortality may take place is very restricted, being delimited by the

rate at which moulting progresses, thus minimising the possibility of juvenile deaths. It is proposed that, even under field conditions, postparasite juvenile mortality will play an inconsequential role in the population dynamics of the parasite under most conditions, unless there is particularly heavy predation.

The moulting pattern of R. culicivorax has been shown to be relatively simple, moulting being a necessary prelude to mating. The explanation of why males moult on average one day earlier than females can only be surmised but it is most probably a size related phenomenon, the smaller the nematode the less time is needed in physiological preparation prior to moulting.

The importance of the availability of atmospheric gasses, particularly oxygen, to the development of postparasites, demonstrated by Imbriani & Platzer (1981), may have major implications when considering populations living in less than ideal conditions; in stagnant pools for example. The ability of the nematode to develop and reproduce under such conditions, though at a slower rate, is of considerable adaptive advantage and greatly increases the potential number of sites it may colonize.

The various aspects of the population biology of the adult nematodes must all be viewed in terms of the need of each individual to maximise its reproductive success. In the case of the female, the survival characteristics show that having been provided with a large food store for reproductive purposes, the female may use the food reserve, in the absence of a mate, to extend its life-span to such an extent as to very much increase its probability of mating. Although its reproductive output would be reduced, this is of great selective advantage, as, in the absence of this attribute, the female may die before finding a mate.

The potential longevity of males shows the effort expended in provisioning them and suggests that it would be very wasteful if they were only to mate once and die. This has been shown not to occur, the males making full use of their longevity by being polygamous.

In examining the survival of mated females it became obvious that enough sperm could be stored by the female (probably from a single copulation) to fertilize all the eggs produced, assuming that all eggs laid were fertile. However, a lack of sperm may be a contributory factor in some females retaining nutrients after oviposition, there being little point in producing infertile eggs.

The mating behaviour of such animals is rarely easy to investigate experimentally; however several important characteristics have been explored. These include the demonstration of male polygamy and the effect that the sex ratio has upon the rate at which mating progresses. Most important, an estimate of the average time to mating has been obtained, with this time being relatively short for the sex ratios expected to occur in the field.

An important concept in population biology is the mean generation time, defined as the average time taken by an organism to replace itself in the next generation. It is important to distinguish between the average and minimum generation time (see section 2.9). The mean generation may be broken down into its various components allowing each to be assigned a measure of importance in terms of its length and also its variability. For R. culicivorax the mean time between emergence from the host and initial oviposition may contribute significantly to the mean generation time of the nematode. This time delay is made up of three components, the mean time from emergence to female moulting (8.56 days), the mean time from moulting to mating (2.67 days, assuming a 1:1 sex ratio) and the mean time between mating and initial oviposition (4.39 days), producing a sum of 15.62 days for the average time between emergence and initial oviposition. This estimate may be compared with those calculated for R. culicivorax from the data of Imbriani & Platzer (1981) at 19.05 days (27°C, 1:0.75 male:female) and Petersen (1975) at 38.78 days and for O. muspratti (Petersen, 1978b) of between 15.10 and 19.10 days (1:2, male:female). Possible reasons for the magnitude of the estimate obtained from Petersen's R. culicivorax data have already been put forward, the other estimates all show a remarkable degree of similarity, despite the differences in the experimental regimes.

2.8 DYNAMICS OF THE POPULATION OF EGGS

The magnitude of the population of eggs of R. culicivorax and its rate of change with respect to time, are, (like the populations of the parasitic nematodes, preparasites and postparasitic juveniles) defined by three rate parameters; the rate of immigration (birth), the rate of emigration (hatching, maturing) and the rate of mortality. Accurate estimates of each of the three rates are required if an understanding of the dynamics of the egg population is to be realised.

Quantitative estimates of these rates enable an assessment to be made as to their relative importance and also enables a further component of the mean generation time to be estimated. In this section experiments aimed at estimating each rate are described.

2.8 (i) Birth Rate

The rate at which eggs are released into the environment by female nematodes forms another link in the chain of events which control the parasite population and also contributes to length of the mean generation time. The eggs are released in an unembryonated condition, the net rate at which eggs complete embryonation is dependent upon the environmental temperature and the birth rate.

Romanomermis culicivorax is the only mermithid for which an estimate of the average number of eggs per day is available (Petersen, 1975). However, as estimates of other characteristics presented by Petersen in this paper have shown less than complete agreement with estimates presented here and in other publications (see sections 2.7 (ii), (iv)), it was deemed necessary to measure the birth rate experimentally. The method adopted was to take newly mature adult female R. culicivorax and isolate them into small petri dishes containing approximately 5 ml distilled water. Two adult male nematodes were then placed with each female until mating had been observed or egg development initiated, whereupon the males were removed. The isolated females were transferred to clean petri dishes every twenty-four hours, enabling regular changes of water and those eggs deposited

to be counted. The experimental conditions were 12:12 hours light:dark at a constant 25°C throughout.

After several days of oviposition many of the nematodes began turning opaque and later showed evidence of fungal infection. Eventually, all females died before completing oviposition. The experiment was repeated, with the same sequence of events occurring. Repeat experiments utilizing aged water from a fish tank were also unsuccessful, even when the pH was lowered to 5.2 by the addition of the physiological buffer M.E.S. (2-(N-morphilino)ethane sulfonic acid). Acidification has been proposed as a method of combating infections of Catenaria anguillulae (Stirling & Platzer, 1978; Platzer & Stirling, 1978). The failure of this suggests that either the pH was not reduced to a low enough level or the parasite was a different species or strain. Finally, an antifungal agent (Fungizone) was added to the fish tank water at rates of 0.25, 0.5 and 1.0 ml per 100 ml, but without success. In one further trial, using aged fish-tank water alone, one female did survive until the completion of oviposition, as evidenced by the depleted state of the trophosome (Petersen, 1975). This success could not however be repeated due to the continual infection problems. Previous workers have also had difficulty in maintaining postparasitic R. culicivorax without a substrate (Platzer & Brown, 1976), but others have reported some successes (Petersen, 1975; Finney, 1980).

The proportion of eggs laid per day and the cumulative proportion of eggs laid by the single female are portrayed in figure (2.8.1 a and b) and show typical time (age) dependent curves, which are surprisingly smooth for an unreplicated result. This data may be compared with that reported by Petersen (1975) and portrayed in figure (2.8.2 a and b). Despite some difference there is a marked degree of similarity in the two data sets, Petersen's data being based on forty-eight females. The range of individual female egg release was 1388 to 4431, with a mean of 2480 eggs per female over an eight to twenty-seven day period. The mean time to oviposition calculated from this data was 6.59 days. This compares with the 2381 eggs laid by the single female (figure

Figure 2.8.1 The birth rate of R. culicivorax: 1

a The observed relationship between the time (post-initiation of oviposition) and the proportion of the total number of eggs laid per day.

b The observed relationship between the time (post-initiation of oviposition) and the cumulative proportion of eggs laid.

The sample size was one female. The total number of eggs laid was 2381.

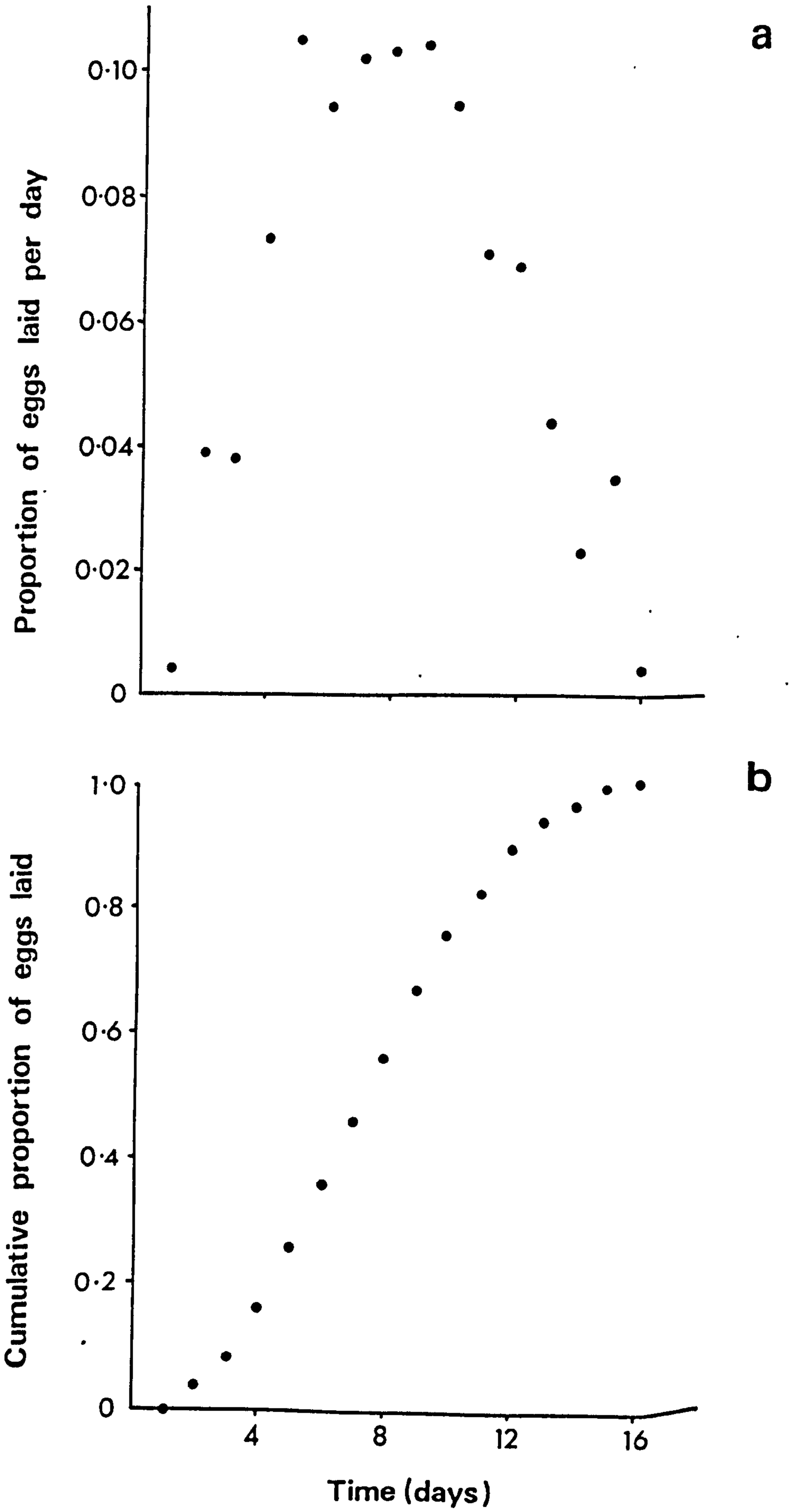

a
Proportion of eggs laid per day
0·10
0·08
0·06
0·04
0·02
0
b
Cumulative proportion of eggs laid
1·0
0·8
0·6
0·4
0·2
0
4
8
12
16
Time (days)

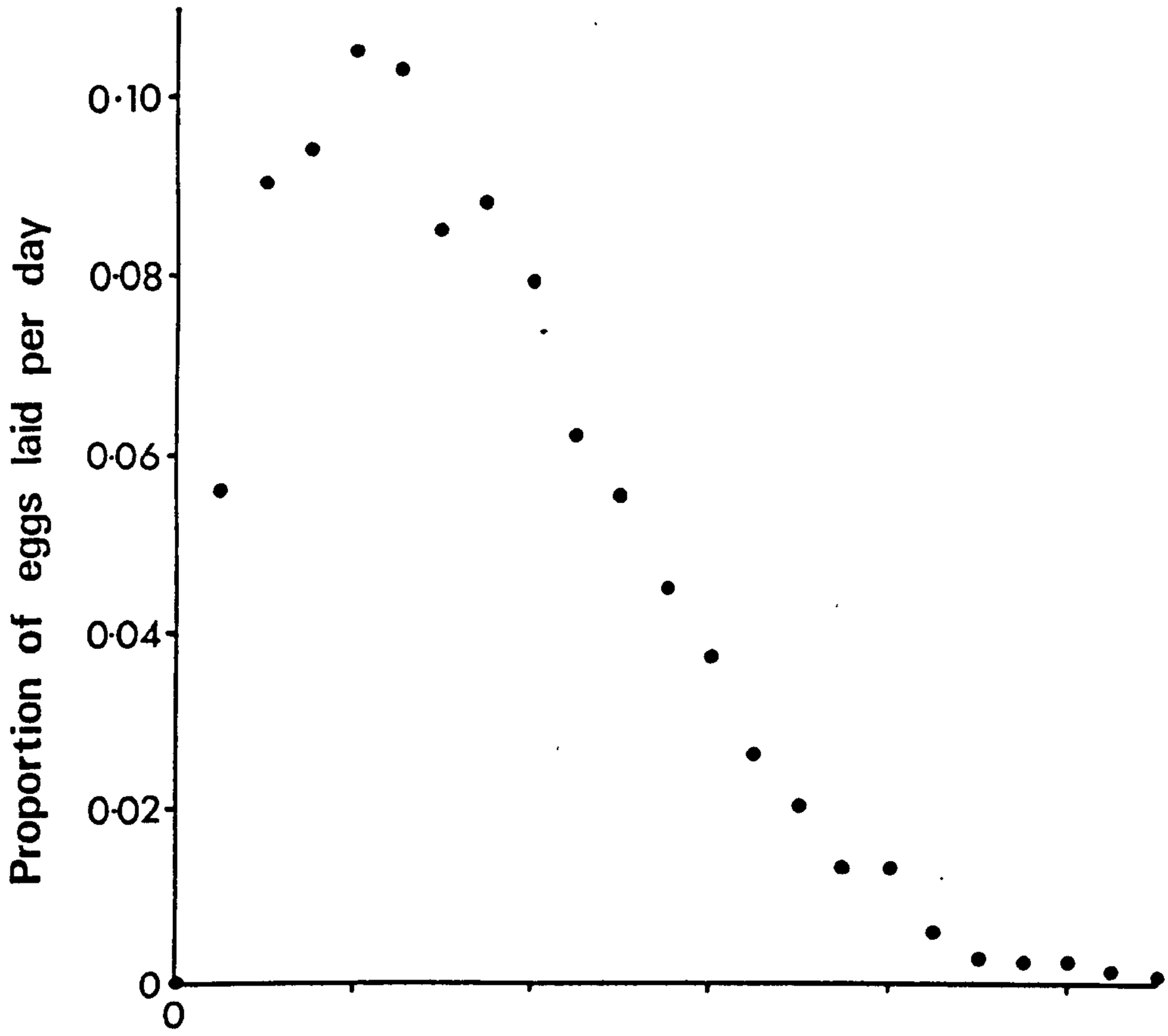
Figure 2.8.2 The birth rate of R. culicivorax: 2

a The observed relationship between the time (post-initiation of oviposition) and the proportion of the mean number of eggs per female laid per day.

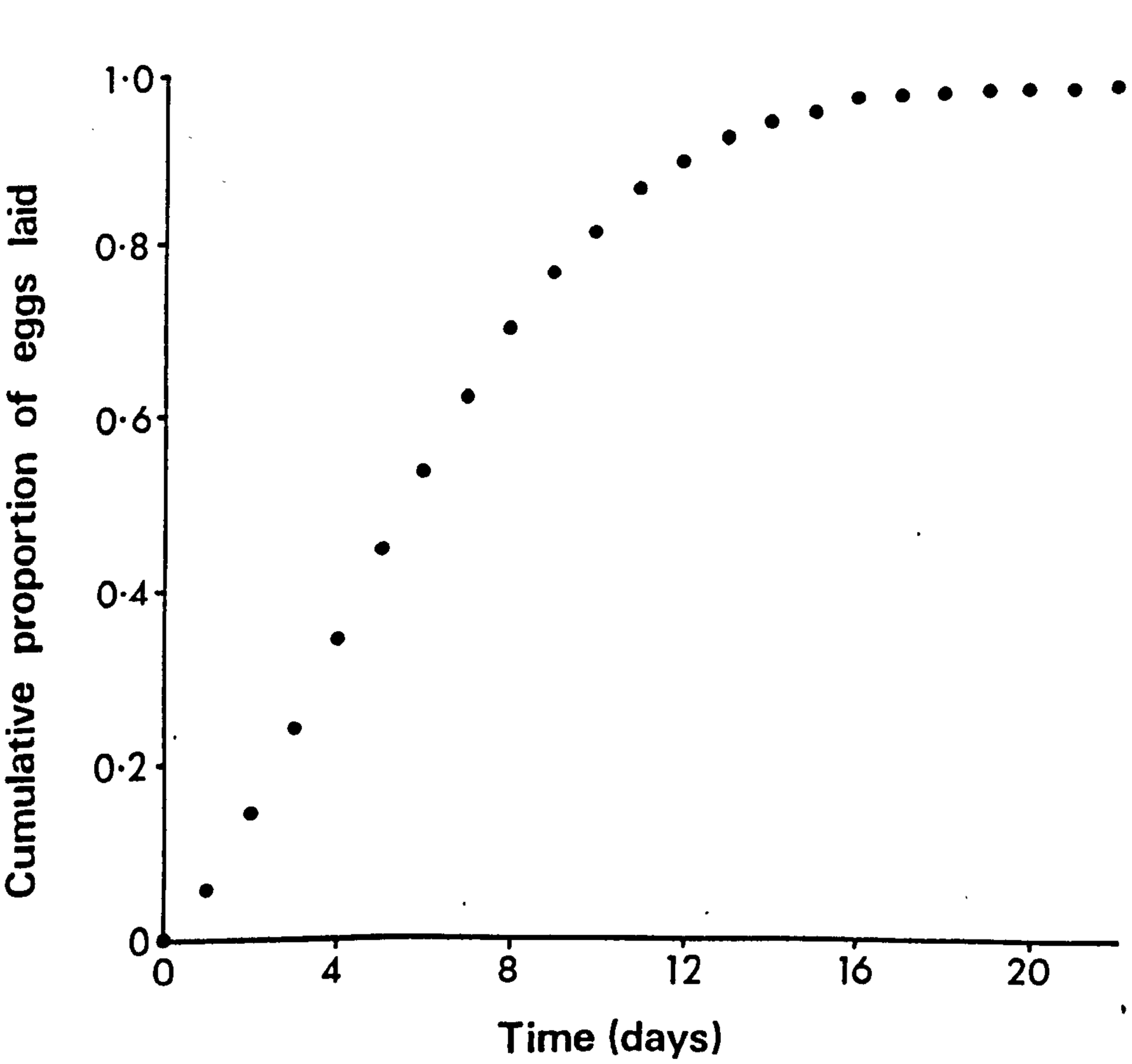
b The observed relationship between the time (post-initiation of oviposition) and the cumulative mean proportion of eggs laid.

Data from Petersen (1975). The sample size was 48 females with a mean egg output of 2480.

a


Proportion of eggs laid per day
0·10
0·08
0·06
0·04
0·02
0
0

b


Cumulative proportion of eggs laid
1·0
0·8
0·6
0·4
0·2
0
0
4
8
12
16
20
Time (days)

2.8.1) over a sixteen day period with a mean time to oviposition of 8.08 days. Both the number of eggs and the time scale for oviposition compare favourably, and are also similar to time scale estimates obtainable from the data of Imbriani & Platzer (1981) for R. culicivorax, and from Petersen (1978b) for O. muspratti.

As all available investigations to date show similar results and due to the unsoundness of relying on data obtained from a single individual the data presented by Petersen (1975) was used for all further analysis of birth rate.

If the rate of oviposition per female per day is assumed to be a constant, the mean birth rate may be estimated by dividing the mean total egg output by the period during which eggs were produced. The period of egg deposition was taken as eighteen days, the point at which 98% of eggs had been laid. The birth rate was therefore estimated as 137.78 eggs per female per day. This estimate gives a mean time to egg deposition of 9.0 days which, although slightly longer, is a fair approximation to that observed.

Finally, it would seem likely that the birth rate is dependent upon temperature, but this has not, as yet, been examined.

2.8 (ii) Rate of Hatching

The eggs of mermithids such as R. culicivorax are laid in an unembryonated condition and, therefore, need a certain length of time in which to develop before they are able to hatch. There may be a further delay, if, for example, the eggs do not hatch immediately upon embryonation. Little published work exists relating to the natural hatching rate of mermithid eggs although some data is available for induced (mass) hatching caused by flooding mature eggs kept in a moist substrate (Petersen, 1975).

Thornton & Brust (1979) reported that the embryonation time of R. communensis was dependent upon temperature, with development to the late coil stage taking 16.5 days at 20°C and proportionally longer at lower temperatures; development failing to occur at 5°C. Embryonation

in R. culicivorax at 25°C might well be expected to take a number of days and add significantly to the mean generation time.

To investigate the time taken by eggs of R. culicivorax to develop and hatch spontaneously at 25°C two experiments were designed. Two experimental regimes were used to enable a cross check to be made, a large sample size to be used and the mortality characteristics of the eggs to be assessed.

The first experiment consisted of the placement of several ovipositing females into a small petri dish containing approximately 5 ml of distilled water at 25°C. After twenty-four hours the nematodes were removed and (ninety-seven) eggs were transferred and placed singly into 0.3 ml wells of a tissue culture plate, each well containing distilled water. Distilled water was used in an effort to reduce or prevent the growth of filamentous algae which would have hampered observation of the eggs. The trays were kept at a constant 25°C in dark, humid conditions, to reduce algal growth and evaporation respectively. The water was replaced periodically. The start of the experiment (t = 0) was taken as halfway through the oviposition period, i.e. twelve hours before the adult females were discarded. Each egg was examined every second or third day until either death or hatching occurred. Death was deemed to have taken place if the egg became opaquely white, unrecognisable in shape or when fungal infection was observed.

Once the preparasitic nematodes had vacated their egg shells the empty eggs were extremely difficult to see. However, as the eggs were kept individually in small volumes of water the actively swimming nematodes were easily observed. Hatching was only noted as having taken place if a free, either living or dead preparasite was found. Five of the original ninety-seven eggs were lost due to algal growth and were discounted from the analysis. Seventy-four eggs hatched over an 84 day period, there occurring a small initial burst of hatching immediately embryonation was complete on days thirteen and fourteen, with three eggs hatching. There was then a long period, some twenty-eight days during which only four eggs hatched, forming a relative lull in the hatching of eggs. This may well be the explanation of

why Thornton & Brust (1979) recorded an initial 5% of 1000 eggs of R. communensis hatched immediately post-embryonation at 15°C and 20°C, with no further hatching being observed, for they only continued observations for some sixteen days post-embryonation at 20°C and ten days at 15°C, which would not have taken them past such a lull in hatching.

In the second experiment, fifteen female nematodes were isolated in small petri dishes containing approximately 5 ml of distilled water. After twenty-four hours the females were removed and discarded, the petri dishes were maintained in the same way as just described for the earlier experiment. Every second or third day those preparasites which had hatched were counted and removed. This was continued for ninety-nine days, until no unhatched eggs remained. Over the ninety-nine days 893 preparasites successfully emerged from the original (unknown) number of eggs. The length of the maximum observed time to hatching of eggs in the two experiments was remarkably similar, 81.14 and 99.14 days in each case, with the longer period observed, not unexpectedly, when using the larger number of eggs. The data from the two experiments were pooled to give a sample size of 967 eggs. The proportion hatched per day and the cumulative proportion hatched are portrayed in figures (2.8.3 a and b).

One of the most obvious features in figure (2.8.3 a) is that there is still an initial burst of hatching immediately post-embryonation followed by a relative lull, followed by the major period of hatching.

The mean time to hatch (from oviposition) was established as 53.15 days, the reciprocal of this giving the mean instantaneous hatch rate (σ_1) of 0.0188 per egg per day.

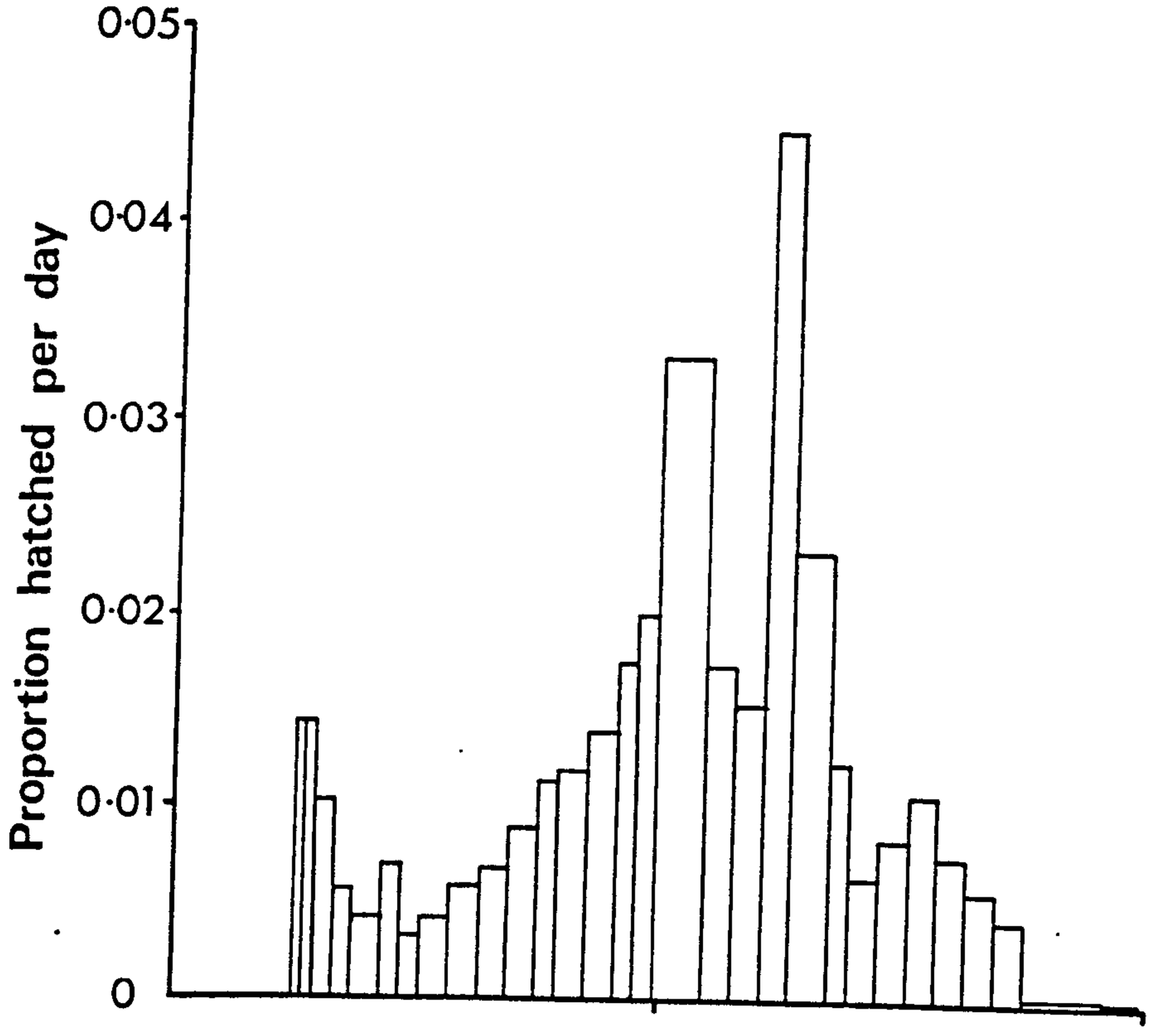
An alternative technique for estimating the mean time to egg hatch, and therefore the instantaneous rate of hatching, utlilizes the predictive nature of the time-dependent survival model of Anderson & Whitfield (1975), (equations 2.4.1 - 2.4.5), where the proportion of eggs remaining unhatched replace the proportion of

Figure 2.8.3 Spontaneous rate of hatching of eggs, at 25°C.

a The relationship between time (post-oviposition) and the proportion of eggs hatched per day.

b The relationship between time (post-oviposition) and the cumulative proportion of eggs hatched.

The sample size was 967 eggs.


a
0·05
0·04
0·03
0·02
0·01
0
Proportion hatched per day

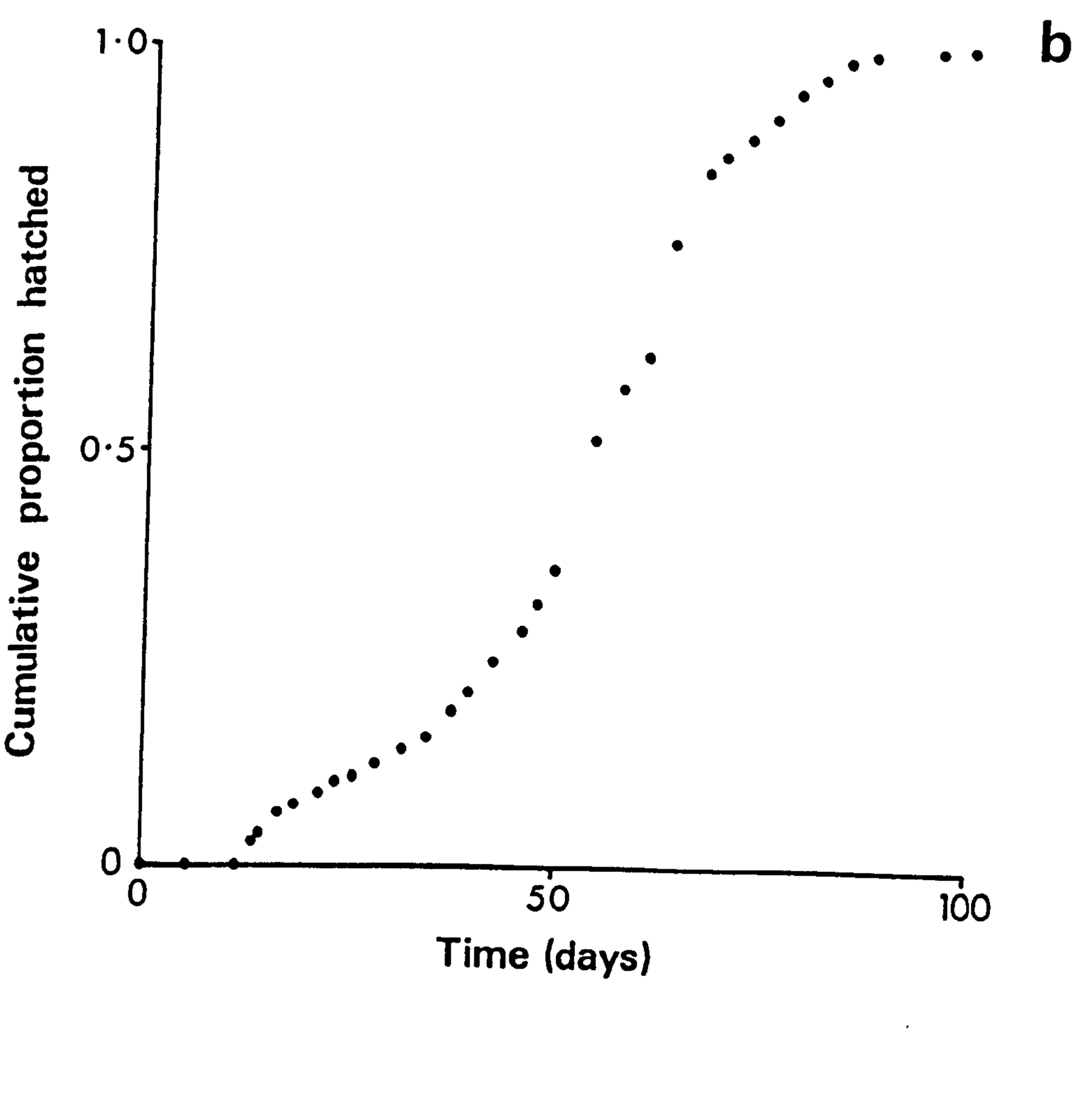
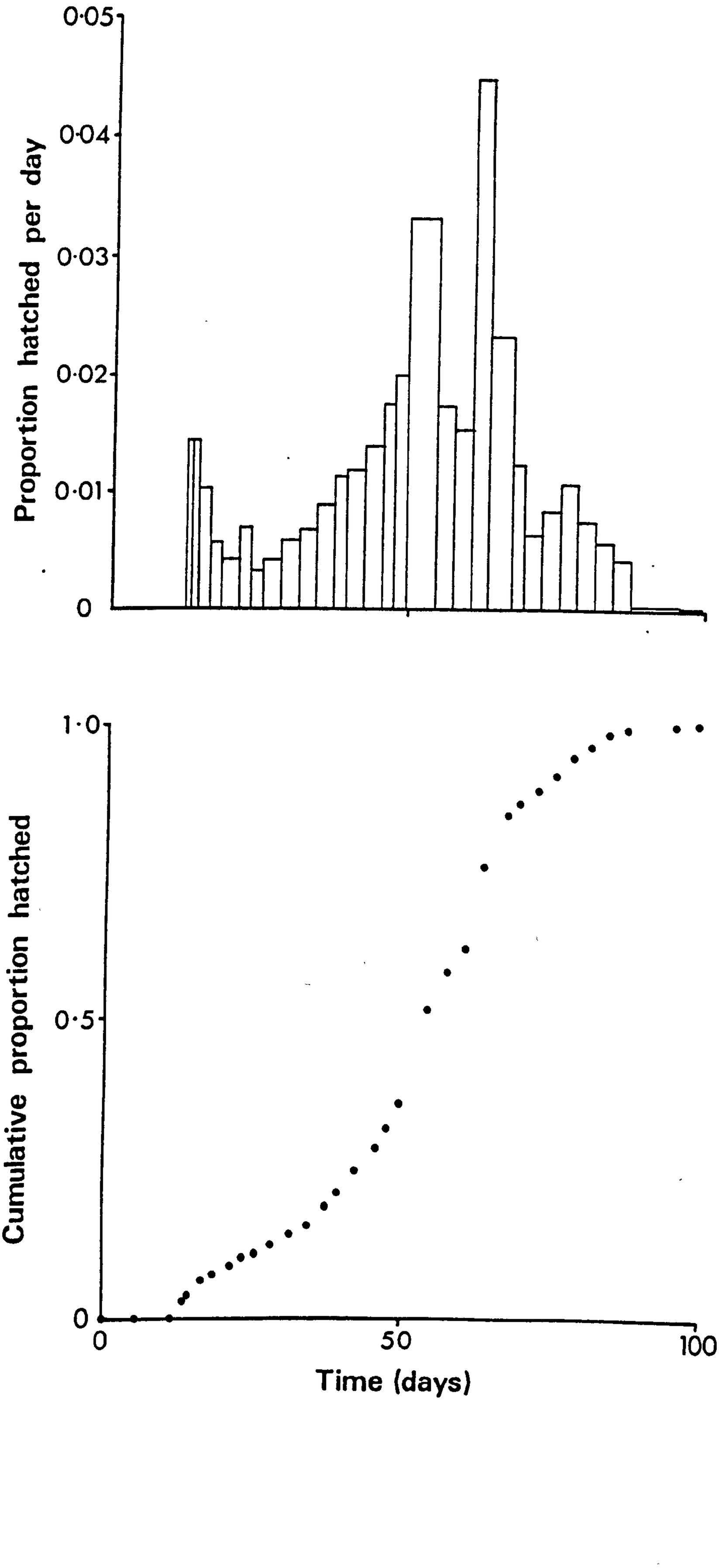

b
1·0
0·5
0
Cumulative proportion hatched
0
50
100
Time (days)

Figure 2.8.3 Spontaneous rate of hatching of eggs, at 25°C.

a The relationship between time (post-oviposition) and the proportion of eggs hatched per day.

b The relationship between time (post-oviposition) and the cumulative proportion of eggs hatched.

The sample size was 967 eggs.


a
0·05
0·04
0·03
0·02
0·01
0
Proportion hatched per day
b
1·0
0·5
0
Cumulative proportion hatched
0
50
100
Time (days)

animals remaining alive. This technique gives an equation for the instantaneous rate of hatching at time t ($\sigma_{1(t)}$) of

$$\sigma_{1(t)} = 0.002079.\exp(0.054571t) \qquad 2.8.1$$

The regression line (of the $\log_e$ transformed values of $\sigma_{1(t)}$) shows a very good fit to the data points with a correlation coefficient (r) of 0.9810 ($p<.001$). The area under the subsequent predicted curve of the proportion of eggs that remained unhatched at time t was calculated, giving a value for the mean time to hatching of 51.93 days. This produces an estimate of the mean instantaneous rate of hatching (σ_1) of 0.0193 per egg per day.

The length of time between an egg being deposited and it's spontaneous hatch is therefore considerable and will form a large proportion of the mean generation time (section 2.9). The only study where mean time to egg hatch has been reported gives estimates for R. culicivorax eggs of between 9.5 and 11.7 days (Imbriani & Platzer, 1981); however, no direct comparison may be made as these workers failed to make clear whether they were measuring a spontaneous or induced hatch rate. They also gave no indication of the number of eggs used or the range of development times.

When kept under conditions where the substrate is damp but not flooded, R. culicivorax eggs embryonate but do not appear to hatch in any significant numbers. When flooded with water a mass hatching occurs, a technique developed for mass rearing programmes (Petersen & Willis, 1972; Petersen, Willis & Chapman, 1978). The biological basis for such behaviour is most probably as an adaptation to a seasonal environnment where changes in water levels may leave deposited eggs above the water line. In such environments, large scale precipitation initiates mosquito breeding and an abundance of suitable hosts results. The advantage of mass hatching in the eggs of R. culicivorax becomes apparent, in that it will place large numbers of infective stages into the environment which will shortly

contain abundant hosts. This type of behaviour has been observed in the field and there are suggestions that the induced hatching may be specifically to capitalize on the rapid breeding floodwater mosquitoes (Petersen et al, 1968). This behaviour may be of considerable importance in some areas where pools are present for only a small proportion of the year, but support fairly large parasite and host populations. To return to the laboratory aspect of this behaviour, it has been reported that cultures induced to hatch, if returned to storage will again produce a flush of infective stages on future floodings after several weeks (Petersen & Willis. 1972). It is unclear as to whether this hatching, occurring at successive floodings, is due to the hatching of only a proportion of those eggs already present or to more eggs being deposited by females present in the culture. Although both processes almost certainly contribute to the situation it is probable that the latter is the more important.

Synchronous hatching is not seen in all mermithids (e.g. R. communensis (Thornton & Brust, 1979), R. nielseni (Finney, 1978) and O. muspratti (Petersen, 1981)).

2.8 (iii) Egg Survival

The rate of mortality of the eggs of R. culicivorax is likely to be a major determinant of the dynamics of the egg population and will thus affect the population dynamics of the nematode throughout the entire life-cycle. The egg is a very vulnerable stage, lying unprotected in the substrate it makes an easy target for both predators and parasites. The average length of time taken by an egg to develop and hatch is comparatively long, which may result in significant egg losses even with only a low daily rate of egg mortality.

The first experiment described in the previous section (2.8 (ii)) enabled the survival characteristics of a cohort of ninety-two R. culicivorax eggs to be investigated at 25°C. A total of eighteen of the ninety-two eggs (19.6%) died, the remainder successfully

hatched. There was a distinct bimodal pattern in the egg mortality, an initial peak occurring shortly after oviposition and a second peak occurring towards the end of the hatching period (figure 2.8.4). The interpretation put upon this bimodial pattern of mortality is that a proportion of the eggs laid were infertile and rapidly succumbed to bacterial and/or fungal attack. This is supported by the observation that 45% of egg mortality occurred within five days of oviposition. There was then another group of eggs which although fertile were genetically or physiologically incompetent and failed to follow a normal embryological development. Two of the eighteen eggs which died were observed to follow a course of development which resulted in malformed embryos, both of which died towards the end of the embryonation period. The magnitude of this early peak in mortality may have been slightly elevated as a result of damage caused whilst handling the eggs, although this is thought to be minimal.

Following the initial peak in egg mortality, there was a period when the embryonated eggs were hatching where no egg mortality was observed. It was only towards the latter half of the hatching period that egg mortality was again observed to occur. It is presumed that this was largely due to the death of preparasites within the eggs which had exhausted their energy reserves, either because they were originally under-supplied or because they had been over active.

The average instantaneous rate of mortality (μ_1) per egg per day may be estimated provided that the instantaneous rate of egg hatching (σ_1 per egg per day) and the proportion of eggs which survive to hatch (p) are known.

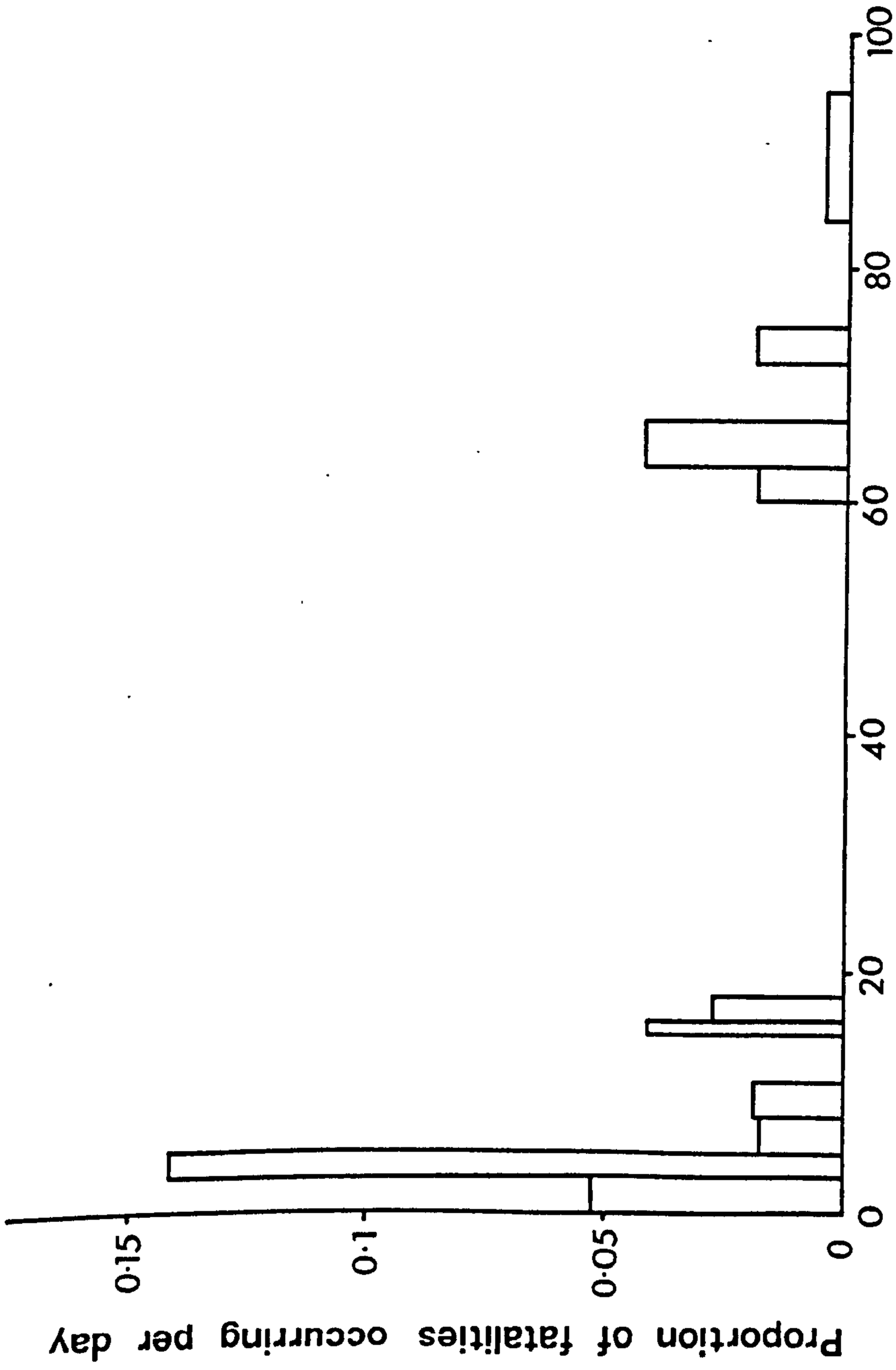
$$p = \sigma_1/(\mu_1 + \sigma_1) \qquad 2.8.2$$

thus
$$\mu_1 = (\sigma_1/p) - \sigma_1 \qquad 2.8.3$$

with seventy-four of ninety-two eggs surviving, $p = 0.8043$ and σ_1 estimated in section 2.8 (ii) is 0.0193 (/egg/day); the mean instantaneous rate of egg mortality is 0.0047 per egg per day.

Figure 2.8.4 The relationship between the age of eggs and the proportion that died per day, at 25°C.

The sample size was 18 eggs.


Proportion of fatalities occurring per day
0·15
0·1
0·05
0
0
20
40
60
80
100
Time (days)

The assumption that the rate of egg mortality is constant will be seen to be a gross biological simplification; but in essence has little effect upon the dynamics of the nematode in that as long as the proportion of eggs surviving to hatch and the rate at which they hatch remain the same, the exact timing of egg mortalities is of little consequence.

In the field it may be assumed that much higher rates of mortality frequently operate, due to a number of causes, of both climatic and biotic origins.

2.8 (iv) Discussion

For a population of nematodes of constant size (i.e. at a population equilibrium), the positive and negative rate parameters controlling the magnitude of each sub-population must balance. Thus, the birth rate under such circumstances must be exactly matched by the hatching and mortality rates in order for the population of eggs to remain constant. Long term variance away from such equilibrium would tend to result in a change in the dynamics of the nematode population and also in its magnitude.

Under constant conditions, the mean number of eggs deposited per female would be expected to remain constant with the rate of hatching being principally determined by the temperature and the endogenous rhythm of the eggs. Thus, the main variable and prime determinant of this stage in the life-cycle will be the rate of egg mortality. Factors affecting egg mortality will also be far harder to control than say temperature which largely controls both birth and hatching rates.

Egg mortality even under ideal laboratory conditions is relatively high, accounting for some 19.6% of eggs laid. The mean number of eggs per female which actually manage to produce preparasitic nematodes is therefore less than 2000. The magnitude of this figure (and of the mean egg output of females) is rather uncharacteristic of parasites, which frequently produce vast numbers of eggs in order

to overcome the massive losses incurred during transmission. Parasites, as a result, often have a reproductive potential orders of magnitude greater than that of their host. For example certain ancylostomes may lay up to 20,000 eggs per day (Smyth, 1976) and may also have a long expected life-span; adult Ancylostoma duodenale having an expected life-span of one year (Anderson, 1980). The comparatively low reproductive potential of R. culicivorax appears even more extraordinary when compared with the reproductive potential of their hosts. Under similar laboratory conditions the mean number of eggs laid per female C. p. fatigans is 507.4 (Gomez et al, 1977). This suggests that the infective stages have considerable success in host location leading to a smaller than expected loss during transmission.

The rate of spontaneous hatching over a period of some three months would be expected to have several consequences. Firstly, if hosts were unavailable, for whatever reason, the wastage in terms of unsuccessful preparasites would be kept to a minimum. Secondly, if hosts were available, nonsynchronous hatching would tend to avoid excessive multiple parasitism by keeping the density of infective stages in the environment down to moderate levels. Finally, during periods of host abundance, full use of available hosts may not be made. The rather special case of flooding causing synchronous hatching would tend to produce the opposite effects to those just described for synchronously hatching eggs, but the advantages of fully utilizing hosts which may only be available for a short period are self evident.

2.9 GENERATION TIME AND REPRODUCTIVE RATE

The dynamics of a population will be largely dependent upon the mean generation time of the organism concerned. The mean generation time (T_c) is defined as the average time taken by an organism to replace itself in the next generation.

The stability of a population to perturbation is reduced as the generation time increases relative to the natural return time of that population (Maynard Smith, 1974; Southwood, 1981). The duration of stability is also dependent upon the magnitude of T_c, in relation to the length of time the habitat remains favourable (Southwood, 1981). There is, in addition, a tendency for cycles in population abundance to occur when the effect of density-dependence is delayed, as is the case when there is a long generation time (with many forms of density-dependence) (May, 1981).

Such factors as those outlined above, all affect the parasite population. In addition, however, they also affect the level of parasite-induced depression of host abundance. It is intuitively obvious, that a parasite with a generation time that is substantially less than that of its host will be able to respond to changes in host density very rapidly. This will tend to promote stability in the host-parasite association. For a parasite with a substantially longer generation time than that of its host, the converse will be true, with a tendency towards periodic, and possibly chaotic, changes in parasite abundance and, thus, in host population depression.

An estimate of the mean generation time of R. culicivorax may be obtained by summation of the mean developmental times of the various stages. A list of the development times, estimated at 25°C, is given in table 2.9.1.

Table 2.9.1

The mean developmental times of the various stages in the life-cycle of R. culicivorax at 25°C.

Mean time to:	Mean time (days)	Source
Egg hatch (from birth)	51.93 (53.15*)	2.8 (ii)
Infection**	0 - 4.25	2.4 (i)
Emergence of female parasites	8.57	2.5 (ii)
Moult (females)	8.56	2.7 (ii)
Mate	2.67 - 12.55+	2.7 (iv)
Initial oviposition	4.39	2.7 (v)
Birth	6.59	2.8 (i)

* estimates obtained by different techniques (see text).

** the mean time to infection must fall within the range of zero to the maximum time of preparasite survival.

\+ dependent upon the sex ratio, 1:0.75 - 1:10 (♂:♀).

The minimum mean generation time (T_c) at 25°C is, therefore, 82.7 days. The maximum value of T_c, under these conditions is 98.1 days. It should be noted, however, that the time taken by females to moult, mate and develop eggs may be considerably longer than that given in table 2.9.1 (see section 2.7). The mean time between egg hatch and infection is likely to fall towards the lower end of the 0 to 4.25 day range, due to the decline in both survival and infectivity of the preparasites with age (sections 2.4 (i) and 2.4 (iv)).

The mean generation time of the host, C. p. fatigans, is much shorter than the that of the parasite. Gomez et al. (1977) gives a value for T_c of 44.68 days for C. p. fatigans. This clearly implies that the parasite, with its longer generation time, may have some difficulty in depressing the host population in a regulatory manner.

It has been shown that, hosts with a high reproductive potential

are better able to withstand the impact of pathogens at the level of the population (Anderson, 1980). The reproductive potential of mosquitoes is undoubtedly high, with large values of both the intrinsic rate of natural increase (r) and of the net replacement (reproductive) rate (R_o). Estimates of r and R_o for C. p. fatigans are 0.1138 and 161.36 respectively (Gomez et al., 1977). For comparative purposes, estimates of these parameters for the parasite would be useful. However, their estimation requires life-table data which is very difficult to obtain for the parasite, notably the probability of infection.

A crude idea of the relative reproductive potential of an organism may be obtained by examining the observed reproductive output and the mean generation time. The mean egg output of female C. p. fatigans is 507.4 eggs per female (Gomez et al., 1977) compared with 2480 eggs per female for R. culicivorax (Petersen, 1975). Thus, given the differences in T_c, the reproductive potential of the parasite may be estimated to be about 2 to 3 times that of the mosquito for a given period of time. Although greater than that of the host, the reproductive potential of the parasite is not large enough to inspire confidence in its regulatory ability of the host population. This is particularly evident when the losses of the parasite during transmission are considered; a factor frequently responsible for the reproductive output of parasites being orders of magnitude greater than that of their hosts.

For a parasite to persist within a host population, transmission must occur at a rate at least as great as the rate of loss of the parasites (i.e. there is a transmission threshold). For the transmission threshold to be exceeded, the basic reproductive rate (R) of the parasite must exceed unity (see Anderson, 1982b). The basic reproductive rate, R, may be obtained from model (see section 4.1), where,

$$R = \frac{\sigma_1\sigma_2\lambda s\beta H\alpha}{(\beta H+\mu_2)(\mu_1+\sigma_1)(\mu_4+\sigma_2)\mu_5(\mu_3+\mu_6+\delta+\alpha)} \qquad (2.9.1)$$

(the parameters are defined in table 4.1.1), H is the host population density.

It may be seen that R is the ratio of the rate parameters involved in parasite transmission to the parameters of parasite mortalities and emigration rates. This reflects the basic, two process form of the population dynamics: an input balanced by an output.

CHAPTER 3 : HOST POPULATION DYNAMICS

3. HOST POPULATION DYNAMICS

The population ecology of mosquitoes in their natural habitats is not as well understood as it might be. Although it has been shown that, in some species at least, up to 95% of eggs laid fail to produce adult mosquitoes, the factor(s) responsible for such mortality have yet to be identified (Lakhani & Service, 1974; Service, 1981).

For the effect of a parasite population upon its host population to be understood and quantified, excessive and unexplained host mortality must be avoided. Thus, laboratory studies, where mortality factors can be controlled, are imperative. Ideally, both the immediate impact of the introduction of a parasite upon the host population, and also the longer term effects should be examined. Field studies related to the impact of entomophilic nematodes upon their hosts deal mostly with the initial effect of the parasite upon the host population, as does much laboratory work (see Poinar, 1979). With reference to R. culicivorax, experiments have also centred upon the immediate reduction in the host (larval mosquito) population, usually in terms of the percentage infected. There have also been studies to determine whether the nematode could persist in the host population/environment. There are a few studies where an initial mermithid release has been followed for a season (Petersen & Willis, 1972; Woodward, 1978; Creighton & Fassuliotis, 1980; Brown Westerdahl, Washino & Platzer, 1982). In all of these studies, the host input (birth rate) was unknown and almost certainly highly variable, making quantitative deductions difficult and prone to error.

To provide data upon which to base a predictive model, an experimental approach was adopted to measure the effects of the parasite upon the host population under controlled conditions. The study naturally divided into two categories, that which dealt with the host in the absence of the parasite (a control experiment), and that which dealt with the interacting host and parasite populations.

The experiments are described in the succeeding two sections. The experimental outline is given in section 3.1 but the reasons for the protocol adopted are explained in section 3.2. This is because the methods were largely determined by the problems associated with the estimation of the impact of the parasite upon the host population.

3.1 IN THE ABSENCE OF PARASITES

Under laboratory conditions, where abundant food is available and where rates of mortality are very low compared with field conditions, a population of mosquitoes would be expected to grow exponentially until, either some artificial control was applied or crowding led to an increase in mortality and/or a decrease in fecundity. Such conditions would not provide a good framework upon which to base a series of experiments. Also, with problems associated with providing blood meals for experimental adult mosquitoes, in estimating the mosquito population and because of the fluctuating input of eggs under such conditions, a more controlled approach was taken.

The experimental regime employed an aquatic environment able to support the whole parasite life-cycle and the aquatic stages of the mosquito. Known numbers of young mosquito larvae were then introduced and emergent adults collected. With a fixed rate of input the numbers of adults which emerged provided a suitable method of assessing the impact of the parasite upon host abundance.

The arena used was a 13 litre plastic aquarium, with a 2 cm deep layer of sand/gravel substrate and 7.5 litres of water. The water was changed by a constant flow system at a rate of approximately 7.5 litres per day. The outflow was covered by a fine stainless steel mesh which prevented the loss of the mosquito larvae. The inflowing water used was aerated tapwater of neutral to slightly alkaline pH at room temperature (c. 19°C). The tank was provided with an aquarium heater/thermostat, set to maintain a constant water temperature of 25°C.

Once set up, the tank was allowed to equilibrate for 3 weeks. At the end of the third week 100 sixteen-to-twenty four hour old mosquito larvae were introduced. Eggs were not used due to the difficulties associated with counting the eggs and separation of eggs bonded into an egg raft. Newly hatched larvae were not used because of the risk of mortalities associated with the counting

procedure. Mosquito larvae in the same age range were introduced at the same rate in subsequent weeks (i.e. 100 per week). Wholemeal breadcrumbs were provided on a daily basis, the amount varying with the number and age of the larvae. The tank was covered to prevent the escape of emergent adult mosquitoes, which were collected on a daily basis. The number and sex of the adults collected were recorded and grouped corresponding to the period at which the larvae were introduced; this was essentially a weekly total.

The experiment was run for 31 weeks with 2796 adult mosquitoes collected in total; these comprised of 1451 females and 1345 males. It was found that, on average, males emerged approximately one day earlier than females. The number of adults emerging from each batch of larvae introduced into the arena is portrayed as the percentage of larvae which emerged as adults in figure (3.1.1).

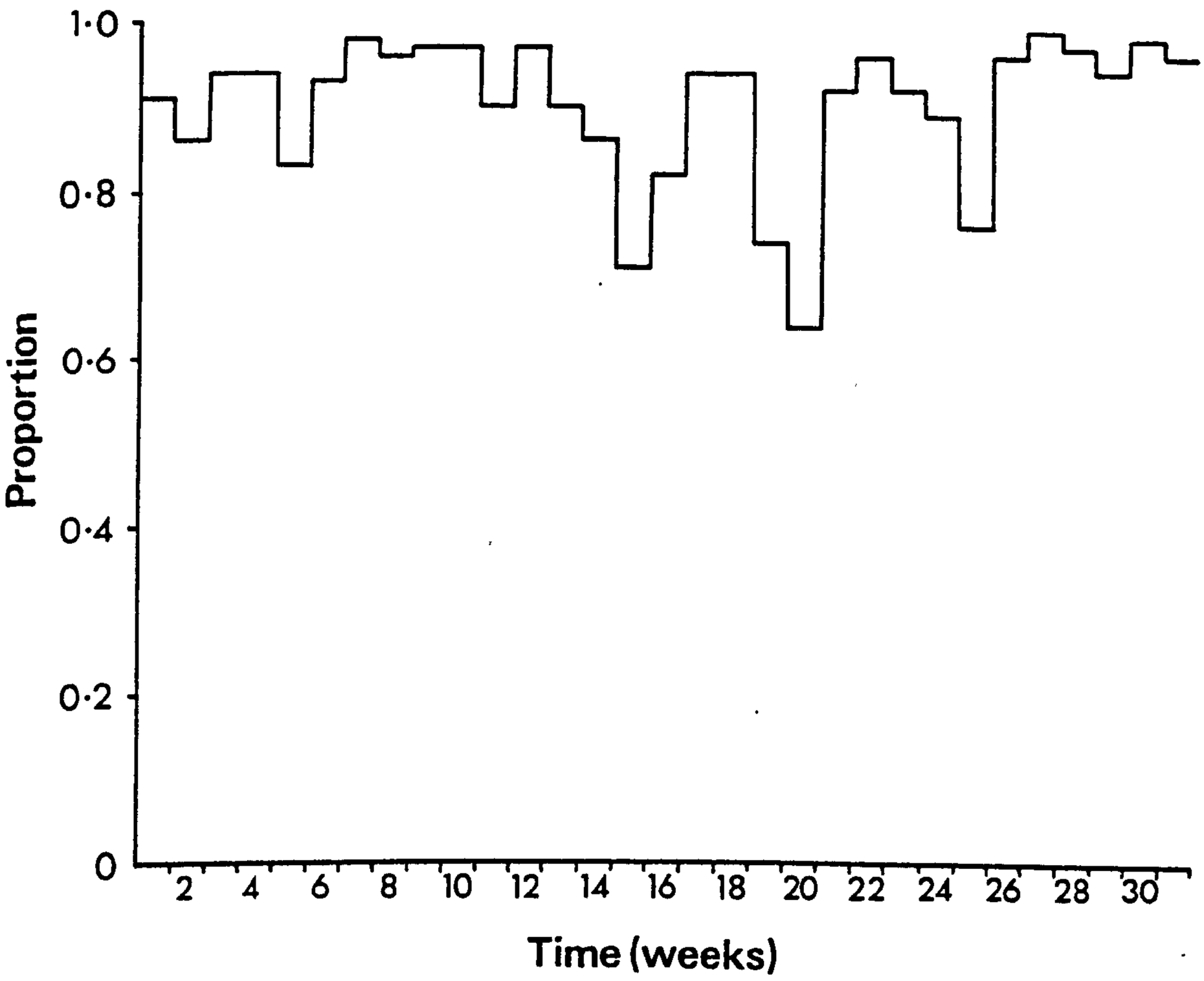
A mean proportion of 0.9019 (±0.0319 95% CL) of introduced larvae developed successfully to the adult stage. This figure is far in excess of field survival estimates (Lakhani & Service, 1974; Service, 1981), but compares well with other laboratory estimates (Shelton, 1973).

A comparison of the mean number of male and female mosquitoes which emerged from the weekly larval imputs (F-test, t-test) showed no significant difference at the 5% level, suggesting that the sex ratio of emergent adult mosquitoes under the prevailing conditions was approximately 1:1.

The number of adult mosquitoes which emerged during this experiment may be compared with the predictions of a simple model based on laboratory estimates of the mean rates of larval and pupal development and mortality. Where the input (immigration) of mosquito larvae is a constant (Λ/unit time), then, the rate of change in the larval mosquito population (H) with respect to time may be

Figure 3.1.1 Depression in adult mosquito emergence by the parasite: the control.

The sequential proportions of the weekly introductions of 100 twenty-hour old mosquito larvae that successfully emerged as adults (see text). The mean proportion that emerged was 0.9019 (± 95% C.L.of 0.0319).


1·0
0·8
0·6
0·4
0·2
0
Proportion
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
Time (weeks)

described by

$$dH_{(t)}/dt = \Lambda - H_{(t)}(\mu_6+\sigma_3) \qquad (3.1.1)$$

where $H_{(t)}$ is the density per unit volume of mosquito larvae at time t, μ_6 is the instantaneous rate of larval (host) mortality (/larva/unit time) and σ_3 is the instantaneous rate of pupation (/larva/unit time). Thus, for constant values of Λ, μ_6 and σ_3, the equilibrium host population per unit volume (H*) is

$$H^* = \Lambda/(\mu_6+\sigma_3) \qquad (3.1.2)$$

Where μ_7 is the instantaneous rate of pupal mortality (/pupa/unit time) and σ_4 is the instantaneous rate of adult emergence (/pupa/unit time), the rate of change of the pupal population (C) with respect to time is given by

$$dC_{(t)}/dt = \sigma_3 H_{(t)} - C_{(t)}(\mu_7+\sigma_4) \qquad (3.1.3)$$

where $C_{(t)}$ is the density of the pupae per unit volume at time t. Following the same arguments laid out above, at equilibrium, the population of pupae (C*) is

$$C^* = \sigma_3 H^*/(\mu_7+\sigma_4) \qquad (3.1.4)$$

Therefore, at equilibrium the number of adults emerging per unit time (N) is given by

$$N = \sigma_4 C^* = \sigma_4\sigma_3\Lambda/(\mu_6+\sigma_3)(\mu_7+\sigma_4) \qquad (3.1.5)$$

With the standard conditions of volume and unit time taken to be the arena volume (7.5l) and the day (24 hours) respectively, estimated values of the four rate parameters were calculated. These values and data sources are presented in table 3.1.1. The estimates of the development rates (σ_3 and σ_4) were obtained by taking the inverse of the mean developmental time of each stage, while the instantaneous rates of mortality were estimated using equation (2.8.3). It should be noted that all rates are average rates and thus, are independent of the age of the stage concerned.

Table 3.1.1

The proportion surviving, the development and mortality rates of larvae and pupae of C. p. fatigans.

Stage	Proportion surviving	Developmental rate (/capita/day)	Mortality rate (/capita/day)	Source
larvae (16-24 hrs to pupation)	0.9800	0.1608 (σ_3)	0.0033 (μ_6)	Ch. 2.5.
pupae *	0.8975	0.4923 (σ_4)	0.0562 (μ_7)	Gomez et al.,(1977)

* estimated at 26°C.

The slightly elevated temperature used by Gomez et al. (1977), one degree higher than the standard 25°C used in this study, was assumed to make no significant difference in the quantitative value of the pupal rate parameters.

To reflect the conditions of the experimental tank with an input of 100 mosquito larvae per week, Λ, the daily immigration rate of larvae was set at 14.29 (larvae per day). If the above parameter

values are used, equation (3.1.5) gives the number of emergent adults per day (N) as 12.56, which represents a proportion of 0.8795 of the introduced larvae developing to adults. This predicted value agrees with the observed proportion of 0.9019 (±95% CL. 0.0319). It may thus be assumed that the model is a reasonable mimic of changes in host density under the given experimental conditions.

3.2 PRESENCE OF THE PARASITES

The experimental investigation of the effect of the presence of the parasite R. culicivorax upon populations of the host C. p. fatigans was carried out in four aquariums. Each was set up as described in the previous section. Once the tanks had been set up, each was inoculated with a known number of newly emerged juvenile male and female postparasitic nematodes. A three week period was then allowed to elapse before the introduction of hosts. This enabled the nematodes to moult and mate, and for subsequent egg production, development and hatching to occur.

Each tank was inoculated with nematodes in a 2:1 male-to-female ratio. Tank (1) contained two female nematodes; tank (2), ten females; tank (3), thirty females and tank (4), one hundred females. The nematodes introduced into tanks (1) and (2) were placed together, whilst those introduced into tanks (3) and (4) were scattered over the substrate.

Hosts were introduced into each tank as described in the previous section. All hosts, both for use in the experiments described here and in the previous section, were taken from a population of larvae hatched from several egg rafts. This procedure was adopted to prevent inter-tank differences in host susceptibility to infection. The control (sect. 3.1) and experimental tanks were run concurrently.

The estimation of the effect of the nematode upon the host population can be measured in one of several ways. The most accurate and direct method would have been to take periodic samples of hosts and measure the proportion infected, the parasite burdens and the statistical distribution of the parasites within the host sample. In order to accurately estimate these factors, the sample size would have had to have been of such a magnitude as to cause repeated perturbations of the system by the removal of parasites which would otherwise have contributed to the succeeding generations. The effect would have been to artificially inflate the mortality rate of the parasite. Thus, an indirect

method of measuring the impact of the parasite was adopted. Of the available indirect methods, the production of imago mosquitoes from the tanks was chosen, due to the ease of measurement, the low level of potential errors and the additional benefit of being able to directly measure the effect of the parasite upon adult mosquito production and sex ratio. This approach did, however, necessitate the use of a mathematical approach, described in the previous section, to investiage the effect of the presence of the parasite upon the host population and to estimate the equilibrium mean parasite burden per host. Due to the success of this method in the previous section, this was not considered to be a significant drawback.

Emerged adults were periodically examined to see if they were infected with the nematode.

The proportion of larvae introduced each week which successfully developed to the adult form are shown for the four tanks in figure (3.2.1 a-d). When compared with figure (3.1.1), the presence of the nematode can be seen to have resulted in a substantial depression in the percentage of emergent adults, the depression being periodic in occurrence. Table (3.2.1) shows the values of the mean proportion that developed to the adult form in the four experimental tanks and also for the control. Each value of the proportion of introduced hosts surviving to adulthood in the experimental tanks, were shown to be significantly different from that seen in the control tank at the 0.1% level. (For method see Sokal & Rohlf, 1969). The adult production from each experimental tank was also shown to be significantly different from every other one (except (1) and (2)) at the 5% level.

Figure 3.2.1 Depression in adult mosquito emergence by the parasite.

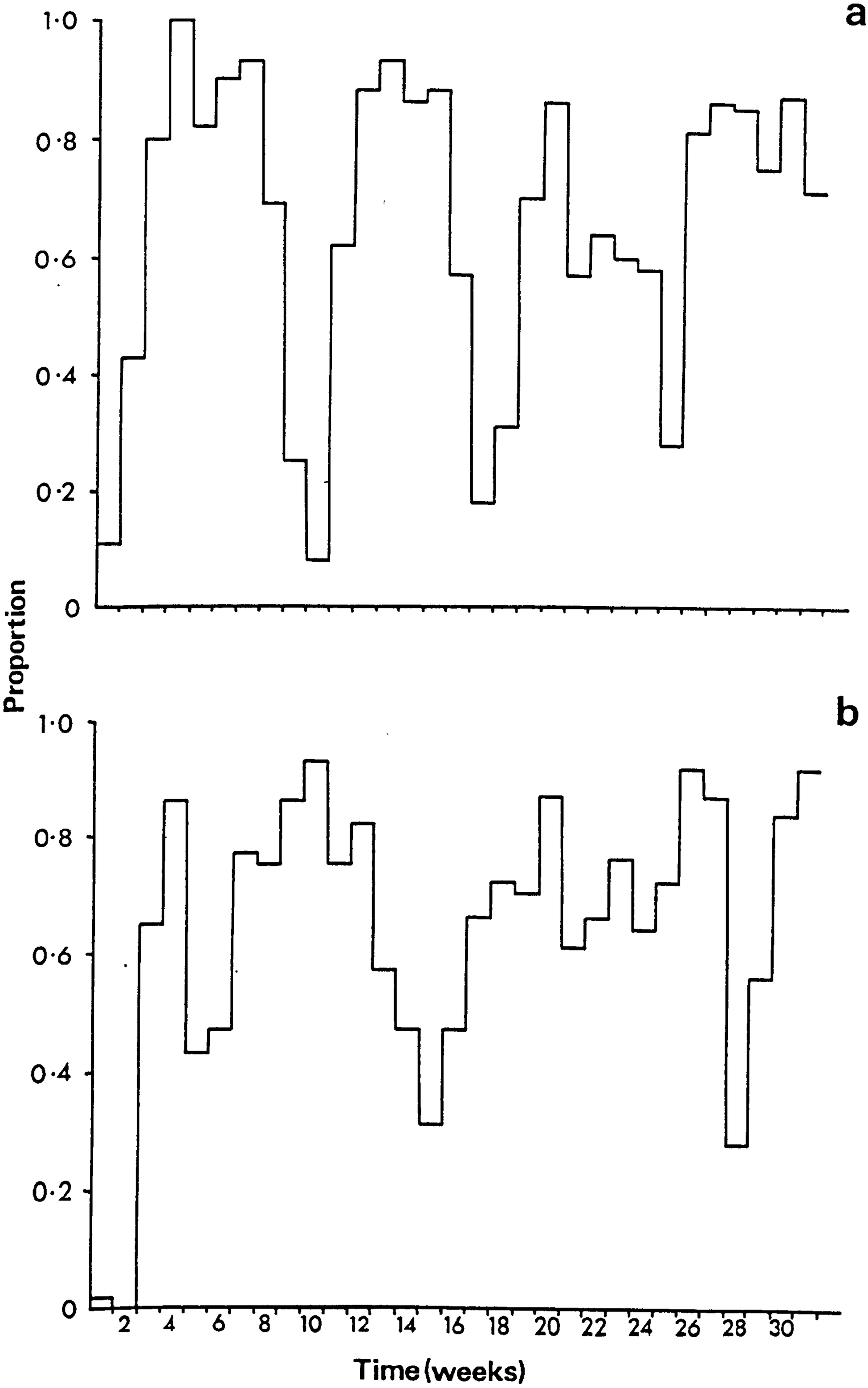
The sequential proportions of the weekly introductions of 100 twenty-hour old mosquito larvae that successfully emerged when exposed to infection by R. culicivorax (see text).

a Initial nematode innoculum of 2 ♀ and 4 ♂ .
Mean proportion that emerged ($\bar{x}$) = 0.6555
(± 95% C.L. of 0.0965).

b Initial innoculum 10 ♀ and 20 ♂ .
$\bar{x}$ = 0.6374 ± 0.0887

c Initial innoculum 30 ♀ and 60 ♂ .
$\bar{x}$ = 0.4554 ± 0.1340

d Initial innoculum 100 ♀ and 200 ♂ .
$\bar{x}$ = 0.5013 ± 0.1345


a
1·0
0·8
0·6
0·4
0·2
0
Proportion
b
1·0
0·8
0·6
0·4
0·2
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
Time (weeks)

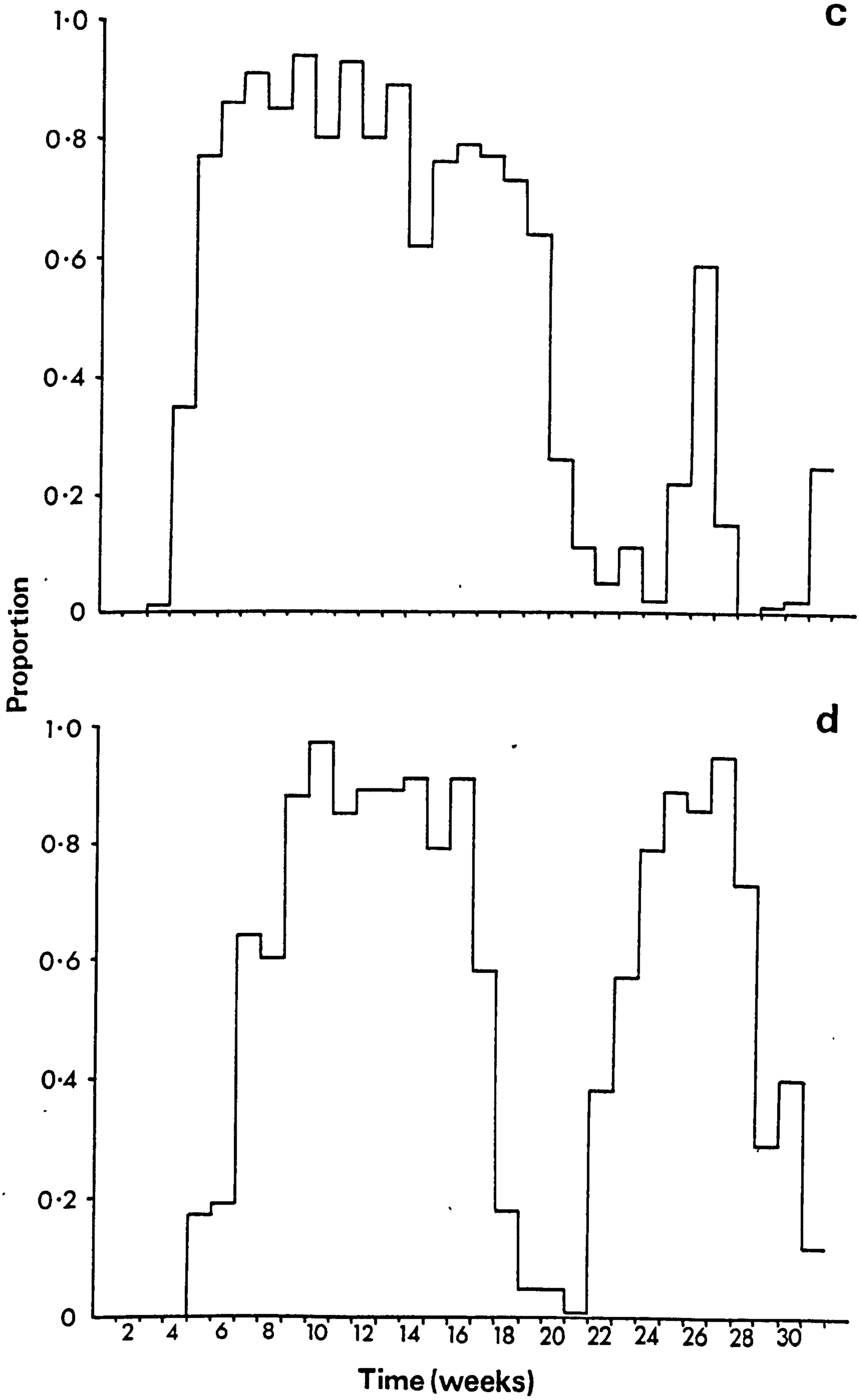

c
1·0
0·8
0·6
0·4
0·2
0
Proportion
d
1·0
0·8
0·6
0·4
0·2
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
Time (weeks)

Table 3.2.1

The mean proportion of hosts, introduced into control and infected tanks, that developed to adults.

	Control	(1)	(2)	(3)	(4)
$\bar{x}$	0.9019	0.6555	0.6374	0.4554	0.5013
± 95%	0.0319	0.0965	0.0887	0.1340	0.1345

It would be expected, that under constant conditions of temperature and host abundance, the population of parasites within a closed system would reach a dynamic equilibrium. Assuming that there was only one equilibrium point, there would then be no difference in the mean output of adult mosquitoes from the system, irrespective of the initial number of nematodes introduced. That such a similarity between the four tanks was not observed suggests that either there is more than one equilibrium level under the prevailing conditions, or that the equilibrium state was not reached in some or all of the experiments, due to insufficient elapsed time. Given the length of the mean generation time of the nematode (section 2.9), it is quite probable that the thirty-one week experimental period was insufficient to allow for equilibration of the system. Analysis of the latter few weeks of experimentation which might be expected to show an increasing similarity between the levels of adult production in each tank, proved invalid, due to the magnitude of the fluctuations and the limited amount of data available (see figure 3.2.1).

The periodic nature of the depression of host abundance seen in figure (3.2.1) may reflect a tendency of the parasite population to cycle in abundance. Cycles, whether stable or damped may result from the very severe density-dependent regulation imposed by environmental sex determination (see ch. 2.6). In this instance, it is more probable that the observed periodicity in host depression is generated as a result of the interaction between the periodic input of hosts, the environmental sex determination and the relatively

long mean parasite generation time. The latter process is probably of greatest significance. Conclusions relating to possible cyclic population behaviour in this association cannot, however, be validly drawn from the data. Cyclic behaviour may well be greatly altered or eliminated as a result of diurnal and seasonal environmental fluctuations in the field.

For due consideration to be given to the impact of the parasite upon the host population, knowing the degree of total population suppression is, by itself, insufficient. Selective infection, by the parasite, of either male or female hosts would greatly alter the ability of a given host population to respond to the presence of the parasite. This is true irrespective of whether the effect is a result of a real parasite preference for one sex or the other, or whether it is produced by differential susceptibility to infection between the host sexes.

The sex ratio of the emergent adult mosquitoes from the control tank was 1:1 (see section 3.1). For each of the four experimental tanks the mean number of emergent male and emergent female mosquitoes showed no significant difference, (i.e. sex ratios were 1:1), suggesting that there is no differential parasite-induced mortality between the sexes.

Periodic examination of the emergent adult mosquitoes revealed that some of them were infected with nematodes. Of 186 adult male and 150 adult female mosquitoes examined, 6.8% were found to be infected. The nematodes were small and at an early stage of development. The mean parasite burden was 0.092 parasites per host (P/H). Of the 23 infected mosquitoes 15 were male and 8 were female; comparison by a 2 x 2 contingency table showed no significant difference in the number of infected male and female mosquitoes ($0.8>p>0.7$). A comparison of the mean parasite burdens of male (P/H = 0.1129) and female mosquitoes (P/H = 0.0667) also showed no significant difference (normal deviate; $0.1>p>70.05$). Despite the lack of support for the hypothesis that there are host sex-related differences in parasite burdens, it was considered feasible that such a phenomenon

may occur but may only be detectable under conditions of high or low levels of exposure to infection. The 336 adult mosquitoes examined were divided into two groups: (1) those exposed in high and (2) those exposed in low risk situations. High risk conditions were defined as periods of time when the number of emergent adult mosquitoes obtained from one weekly input of larvae, from one tank, fell below half of the mean number of adults per input recovered from that tank. Similarly, low risk infection periods were those when more than half the mean number of emergent adults per input emerged. The results are shown in table (3.2.2). χ^2 and t-test analyses showed no significant difference between either the number of males and females infected or the parasite burdens of the two sexes; in both instances $p>0.05$. This shows a very different pattern compared with that seen in natural (mixed) mermithid infections of adult Prosimulium mixtum, where 11.7% of females and no males were found to be infected (Mokry & Finney, 1977).

Table 3.2.2

Infection levels in adult mosquitoes.

	Low Risk		High Risk	
No. Examined	313		23	
No. Infected (%)	12 (3.8)		11 (47.8)	
mean P/H	0.045		0.739	
	♂	♀	♂	♀
No. Examined	171	142	15	8
No. Infected	7	5	8	3
mean P/H	0.047	0.042	0.867	0.500

The data presented in table 3.2.2 clearly shows that there is no differential mortality or susceptibility to infection between the two host sexes over the normal host development periods. It should be noted, however, that males develop faster than females, and at

lower temperatures female development may be extended significantly longer than that of the males, resulting in extended exposure periods.

One aspect of this type of host-parasite association which has received little attention,are the ways in which the nematode may be able to invade new localities. Welch (1960; 1965) reported parasitic nematodes in aquatic larval hosts being carried over into the adult host. Welch stated that this would assist the dispersal of the parasite throughout the environment. Table 3.2.2 shows that even under low levels of exposure,3.8% of emergent adults carry nematodes in this association, whilst at high levels of exposure, 47.8% of adults were found to harbour parasites. It is unfortunately, not known whether or not such nematodes can develop to maturity, but obviously, if development can take place and the mosquito can support the nutritional demands placed upon it, then the probability of dissemination to new sites will be greatly increased. In addition to the possibility of increased dispersal of the nematode, the presence of the parasite also raises new considerations about the impact of the parasite upon the host population. The presence of the nematodes, particularly if development is initiated, will put an additional stress on the adult mosquitoes, reducing their life expectancy and probably adversely affecting fecundity as well. An elevated level of adult mosquito mortality may, under certain circumstances, be of profound importance in limiting both the magnitude and rate of growth of a depressed host population. Further investigation of these factors is required.

The experiments have directly measured the effect of parasitism upon the number of emergent adult mosquitoes. Based solely upon these results,the effect upon the host (larval mosquito) population can only be surmised. However, by extending the mathematical approach used in section 3.1, much improved understanding of the effect of parasitism upon the populations of larval and pupal mosquitoes may be obtained. The approach taken was that of Anderson & May (1978).

Equation (3.1.1) describes the rate of change in the host

population in the absence of parasites and modification of this equation for a situation where infections occur is straightforward. Of the various parameters influencing the association (again reflecting the situation in the experimental tanks), the rate of host immigration, Λ, (hosts/day) remains the same, as does the natural per capita rate of host mortality per day (μ_6), which is assumed to affect both parasitized and unparasitized hosts equally. The per capita rate at which hosts pupate (σ_3) will also remain the same. However, pupation only takes place in unparasitized hosts. The proportion of hosts which remain uninfected will be determined by the statistical distribution of parasite numbers within the host population.

Investigation has shown (section 2.4) that two frequency distributions, the Poisson (random) and negative binomial (overdispersed), adequately describe the range of parasite distributions observed. The zero class, p(0), (i.e. the proportion of hosts with no parasites) for the Poisson distribution is given by exp(-m), where m is the mean parasite burden per host. For the negative binomial distribution, the zero class is dependent upon the mean (m) and also the parameter k, which is an inverse measure of the degree of parasite overdispersion or aggregation, where p(0) is given by $(1+m/k)^{-k}$. Thus, the per capita rate at which hosts from an infected population pupate, is given by $p(0)\sigma_3$.

The principal effect of the parasite upon the host is to induce host mortality, either as a result of the emergence of the fully developed parasites or due to the damage or stress caused by the infection before nematode development is completed (section 2.5). The former is easy to measure experimentally and a per capita rate of host mortality, α, per day, may be determined. Estimates of α were obtained by taking the inverse of the mean time to emergence of the nematode (figure 2.5.5). This provides an estimate of the mean value of α for a given host cohort with a given mean parasite burden. Clearly, this means that α will only operate upon infected hosts. Thus, by following the above argument, the proportion of infected hosts is given by 1 - p(0), with the per capita rate of parasite

emergence-induced host mortality being α (1-p(0)). The net rate of parasite-induced host mortality is not constant but varies with the mean parasite burden, M (section 2.5) in a linear manner such that $\alpha = a+bM$ where a and b are the intercept and slope of the regression line of α on M (table 3.2.3).

Table 3.2.3

The intercept (a) and slope (b) of the least-squares best-fit linear regression of the rate of emergence-induced host mortality (α/host/day) on the mean parasite burden per host,(M), at four temperatures.

Temperature (°C)	Intercept a	Slope b	Correlation coeff. (r)	Sample size (n)
15	0.0374	0.0010	0.7538*	7
20	0.0695	0.0012	0.5159	6
25	0.1085	0.0056	0.8747***	9
30	0.1523	0.0055	0.9613***	10

* $p<0.05$ *** $p<.001$

The rate at which infected hosts die as a result of parasitism (mortality distinct from that due to nematode emergence) is clearly dependent upon the individual parasite burden of each host (ch. 2.5). If it is assumed that the pathogenicity of the parasite to the host is a constant, δ, the rate at which hosts with a parasite burden of i die will be δi. Thus, the net rate of loss of hosts from a host population, $H_{(t)}$, will be

$$\delta H_{(t)} \sum_{i=o}^{\infty} i.p(i) \qquad (3.2.1)$$

where p(i) is the probability that a given host will contain i parasites, and where $\sum_{i=0}^{\infty} i.p(i)$ is the average number of parasites

per host (Anderson & May, 1978). The per capita rate of host mortality is then δM.

The estimation of δ, the measure of parasite pathogenicity is (as noted by Anderson & May (1978)) not straightforward. It requires a knowledge of the parasite burdens (i) of individual hosts, which can only be obtained by destructive sampling or by individual infection regimes (with the assumption that there is no natural parasite mortality) linked with measurements of the survival characteristics of hosts with burdens of i parasites. Because of the extreme difficulty of obtaining such direct estimates of δ, 'order of magnitude' estimates were made indirectly. The method adopted in this study, was to use the survival data for host cohorts with different mean parasite burdens (figure 2.5.2). A value of the instantaneous rate of host mortality, μ, (/host/day) was calculated for each cohort of infected hosts (equation 2.8.2). Given that these estimates included the natural rate of host mortality ($\mu_6 = 0.0033$), this was subtracted from each cohort estimate of μ to give an instantaneous rate of parasite-induced host mortality, ($\hat{\mu}$), for each cohort. The estimates of $\hat{\mu}$ from each cohort were then divided by the mean parasite burden of the relevant cohort to give cohort estimates of δ, from which a mean value of δ(/host/parasite/day) was obtained (table 3.2.4).

At this point, it should be noted that there are several inherent inaccuracies involved in this method of estimation of δ. First, the estimate should be based upon a sum of the individual effects but is instead based upon the average effect of the parasites. Second, the values of M used were all underestimates of the effective value of M. This is because M was measured upon completion of the parasite development period and thus excluded the parasite burden of those hosts which died during the development period as a result of infections; a group which would be likely to contain proportionatelymore heavily infected hosts.

Table 3.2.4

Estimates of the pathogenicity of the parasite to the host (δ/parasite/host/day) and the natural host mortality rate (μ/host/day).

Temperature (°C)	Number of Cohorts	Mean δ	S^2 (δ)	μ_6
15	11	0.0175	0.0005	0.0029
20	5	0.0340	0.0017	0.0022
25	7	0.0076	0.0010	0.0033
30	10	0.0143	0.0003	0.0079

The range of the estimated values of δ for the different temperatures is quite large (table 3.2.4) and may reflect the level of accuracy of the parameter estimation technique. It may also reflect an optimum temperature at which the host is best able to cope with the presence of the parasite, where a balance occurs between the length of the parasite developmental period and differences between the nutritional demands of the parasite and the ability of the host to fulfil these requirements.

The accuracy of this method of estimating δ can, to some extent, be tested by comparing predictions of the proportion of hosts dying under defined conditions with experimental data. Using an average development time for infected and uninfected hosts, according to the proportions of each expected with given parasite distributions and values of M (see ch. 2.5). The predicted proportion of a host cohort dying during the developmental period was calculated. Assuming a linear relationship between parasite burden and host mortality, the proportion of hosts dying is given by $(\delta M + \mu_6)t$, where t is the mean development time in days.

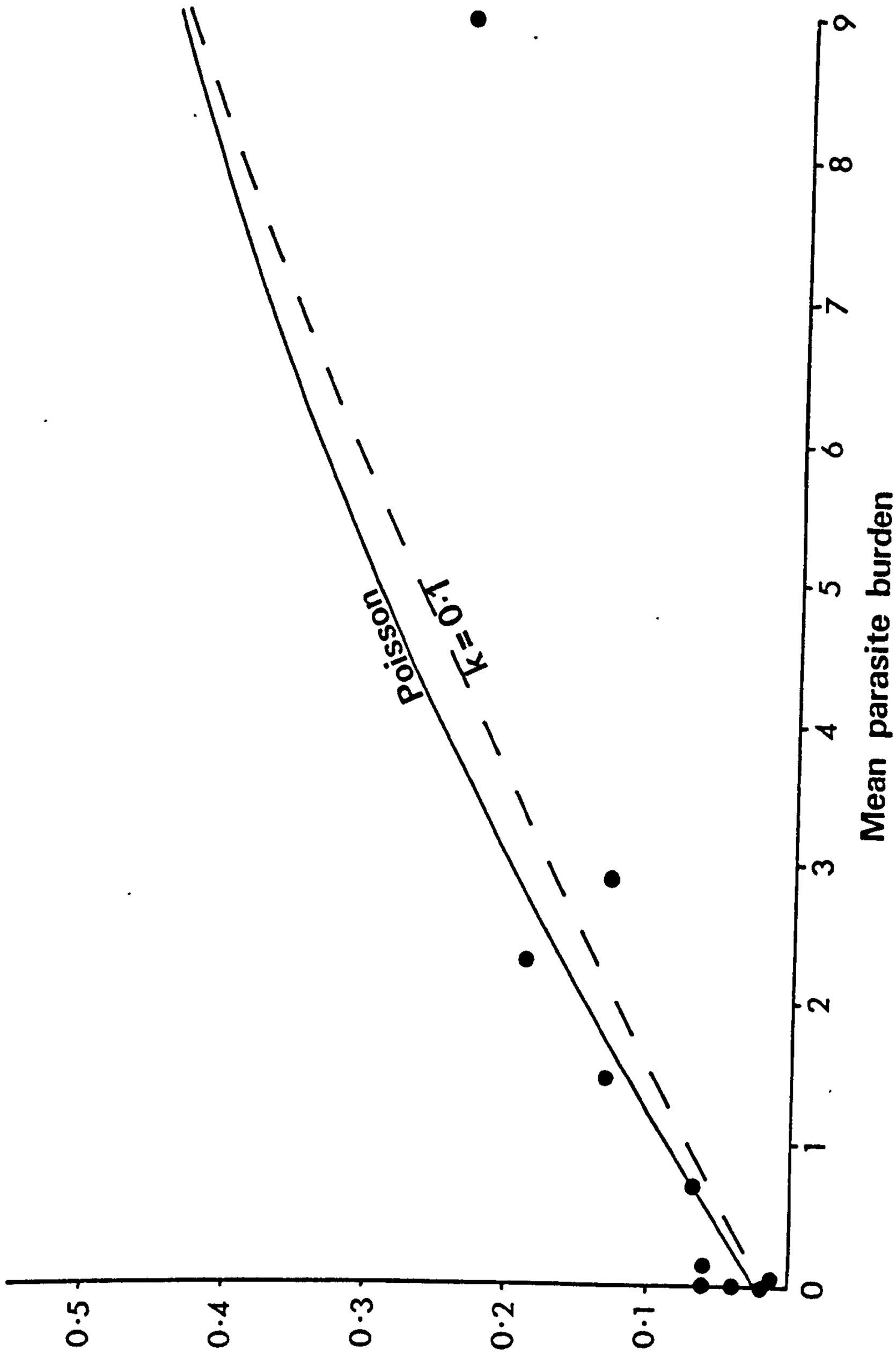
Predictions for two distinct parasite distributions, over a range of values of mean parasite burden, M, are shown with the experimental data in figure (3.2.2). The predicted values show

Figure 3.2.2 The influence of the mean parasite burden per host upon the mortality of hosts: observed and predicted results.

The points are the observed proportion of hosts that died during development as a result of infection.

The solid line is the expected proportion of hosts dying when there is a linear relationship between parasite burden and host mortality and a Poisson distribution of parasites among hosts. The proportion dying was calculated from $(\delta M + \mu_6)t$ where δ (the pathogenicity of the parasite to the host) was 0.0076, μ_6 (the natural host mortality rate per host per day) was 0.0033, t was the mean development time (see text) and M was the mean parasite burden per host.

The broken line is the same as the solid one except that the distribution of the parasites was assumed to be overdispersed (negative binomial, k = 0.1).


Poisson
$T_K = 0{\cdot}1$
Proportion dying
0·5
0·4
0·3
0·2
0·1
0
1
2
3
4
5
6
7
8
Mean parasite burden

relatively good agreement with the observed data, except for the tendency to overestimate the proportion dying at the higher values of M. This is acceptable if one considers that such high values of M are unlikely to occur in the field and are very difficult to induce in the laboratory (hence the paucity of data at high values of M). Given that the predictions show a reasonable fit to the data, the estimates of δ may be taken as order of magnitude estimates for the four temperature regimes employed in the experiments.

With the two additional terms of host mortality (α and δ) the equation for the rate of change of the host population, (equation 3.1.1), becomes

$$dH_{(t)}/dt = \Lambda - H_{(t)}(\mu_6+p(0)\sigma_3+\alpha(1-p(0)) + \delta M_{(t)}) \quad (3.2.2)$$

Therefore the equilibrium host population (H^*) is given by,

$$H^* = \Lambda/(\mu_6+p(0)\sigma_3+\alpha(1-p(0)) + \delta M^*) \quad (3.2.3)$$

and the equilibrium pupal population, C*, by

$$C^* = \sigma_3 H^* p(0) /(\mu_7+\sigma_4) \quad (3.2.4)$$

If it is assumed that the number of pupae affected by parasites is small, or that the effect of parasitism upon the pupae is insignificant, then equation (3.1.5), which gives the rate (per day) at which adult mosquitoes emerge, becomes

$$N = \sigma_4 C^* = \sigma_4 p(0)\sigma_3 \Lambda / (\mu_6 + p(0)\sigma_3 + \alpha(1-p(0)) + \delta M)(\mu_7 + \sigma_4) \quad (3.2.5)$$

Using the laboratory estimates at 25°C, of the rate parameters in equations (3.2.3), (3.2.4) and (3.2.5), solutions were found for H*, C* and N under different conditions of mean parasite burden and parasite distribution (figure 3.2.3 a-c). It is clear from these results, that, increasing mean parasite burdens lead to a reduction in the number of adult mosquitoes emerging, whilst the equilibrium number of hosts initially increases and then falls. The rise and fall in the magnitude of H* with increasing values of M* is the result of the interaction of two processes. Firstly, infected hosts, particularly those with low parasite burdens, have greatly extended life-spans (figure 2.5.5). Longer mean development times give rise to an increase in the number of hosts at any one time. This effect is counter balanced by the increasing effect of premature parasite-induced host mortalities as M* is increased, effectively shortening the mean host life-span and leading to the decline in M*.

The relationship between C* and M* is such that the numbers of pupae decline as M* increases, in direct proportion to the decrease in the value of the proportion of uninfected hosts, p(0) (i.e. as the pool of uninfected hosts declines so does the number of pupae which are drawn from that pool at a constant rate).

The decline in the magnitude of the number of emergent adults (N/week) with increasing M* is also dependent upon the size of the pool of uninfected hosts, which then have to pass through the pupal stage.

If the distribution of the parasites within the host population is changed from random (Poisson) to overdispersed (negative binomial), it can be seen from figure (3.2.3) that the qualitative patterns of relationship between H*, C*, N and M* remain unchanged. There are, however, noticeable changes in the numerical values of the popu-

Figure 3.2.3 Depression in mosquito abundance by the parasite: predictions of the model.

a The relationship between the equilibrium mean parasite burden (M*) and the equilibrium host population density per arena (H*). The predictions of equation (3.2.3) given a range of parasite distributions (Poisson, dotted line; negative binomial, solid line). The dashed line is the magnitude of H* in the absence of the parasite (equation 3.1.2).

b The relationship between M* and the equilibrium population density of pupae per arena (C*). The predictions of equation (3.2.4) given a range of parasite distributions (Poisson, dotted line; negative binomial, solid lines). The dashed line is the magnitude of C* in the absence of the parasite (equation 3.1.4).

c The relationship between M* and the mean number of emergent adults per week at equilibrium (N). The predictions of equation (3.2.5) given a range of parasite distributions (Poisson, dotted line; negative binomial, solid lines). The dashed line is the magnitude of N in the absence of the parasite (equation 3.1.5).

σ_3 = 0.1608/host/day

σ_4 = 0.4923/pupa/day

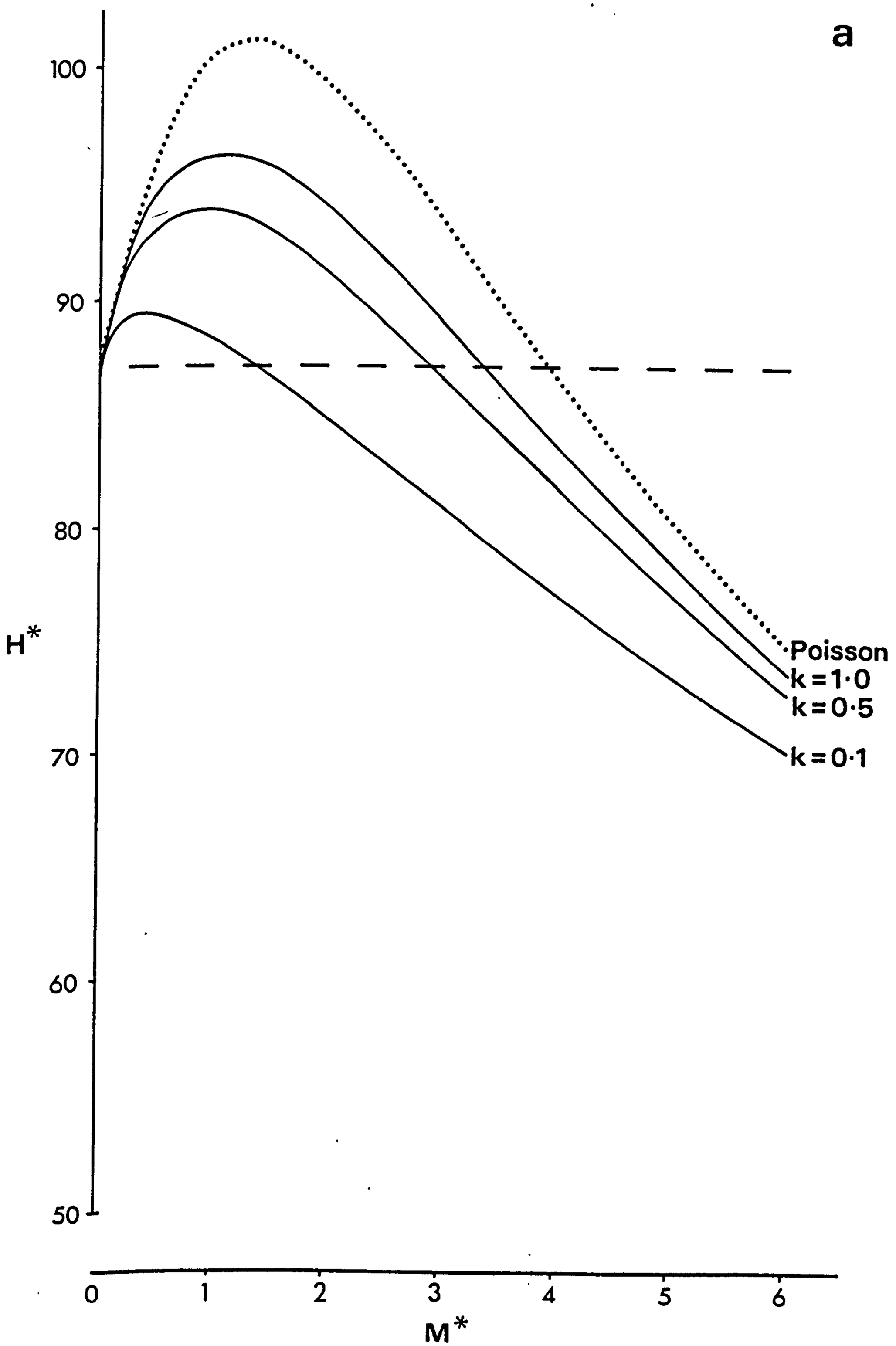
μ_6 = 0.0033/host/day

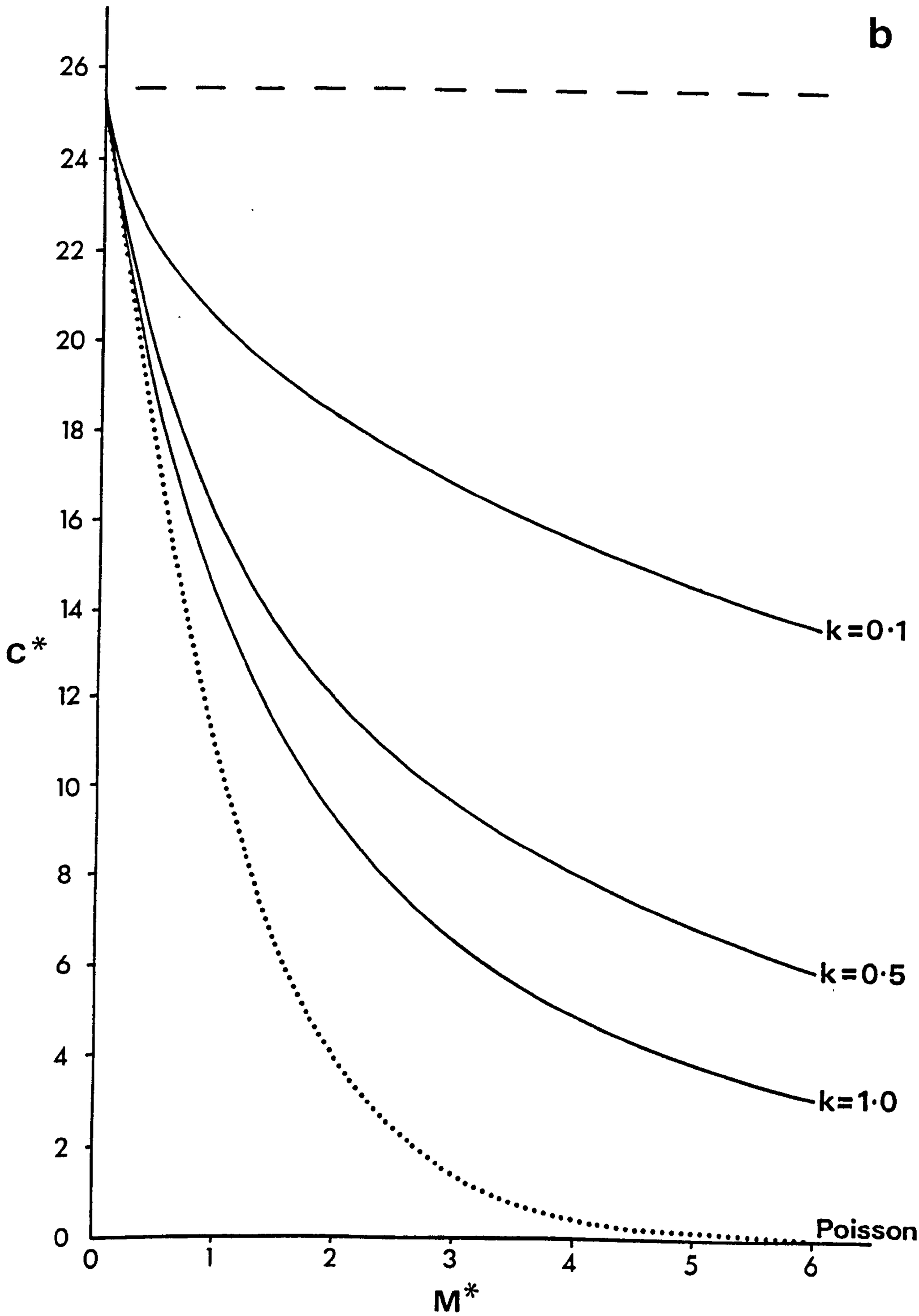
μ_7 = 0.0562/pupa/day

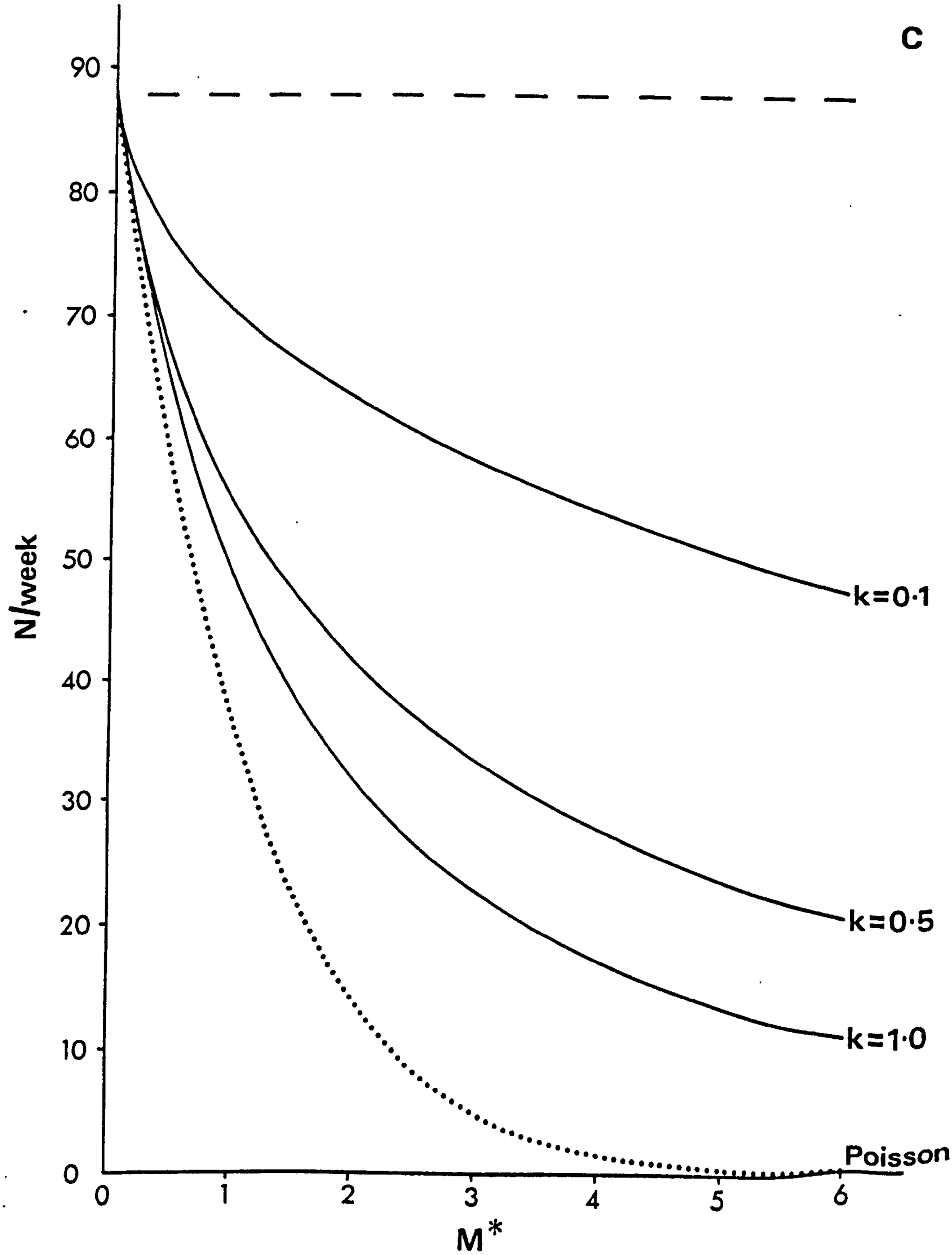
Λ = 14.2 hosts/day

α = 0.1085 + 0.0056 M*/host/day

δ = 0.0076/host/parasite/day


a
100
90
80
70
60
50
H*
Poisson
k = 1·0
k = 0·5
k = 0·1
0
1
2
3
4
5
6
M*


b
26
24
22
20
18
16
14
12
10
8
6
4
2
0
C*
0
1
2
3
4
5
6
M*
k=0·1
k=0·5
k=1·0
Poisson


C
90
80
70
60
50
40
30
20
10
0
N/week
k=0·1
k=0·5
k=1·0
Poisson
0
1
2
3
4
5
6
M*

lations. With an increase in the level of overdispersion, fewer hosts harbour a greater proportion of the parasites, thus, for a given mean, a greater proportion of hosts will be uninfected (larger p(0)). This has two consequences. First, it leads to a reduction in the mean host life-span by creating more hosts with shorter life -spans (both uninfected and heavily infected hosts). This,in turn, leads to a decline in the value of H* due to the increase in the host emigration and death rates. The increase in the proportion of uninfected hosts leads to increased immigration into the pupal stage, thus, increasing C* and N.

It should be borne in mind that this is a rather crude approach and is only valid when the populations are at equilibrium. This is because dynamic changes in one factor will lead to concomitant changes in other factors. Thus an increase in the degree of overdispersion will not only affect the factors outlined above but will cause increased parasite losses due to premature parasite-induced host mortality, which in turn, will cause a decline in both M* and the degree of overdispersion. Therefore, investigation at fixed values of M* and fixed statistical distributions, though of much benefit in understanding the host-parasite interaction, must be interpreted with caution.

From the results obtained in the experimental tanks, values of N (the number of adults/week) may be estimated for each tank, by dividing the average number of adults which emerged per batch by the mean development time of larval and pupal mosquitoes. Given these estimates of N, an estimate of the value of M* prevalent in each tank may be obtained by the numerical solution of equation (3.2.5). Estimates of the value of M* thus obtained, for different statistical distributions of parasiteswithin the host populations, are presented in table 3.2.5. These estimates are subject to the error that the tanks had probably not reached equilibrium states. However, the range of estimated values of M*, from the four tanks, does provide a good estimate of the range within which M* would be expected to fall. As the statistical distribution of parasites tends to fall in the Poisson to moderately overdispersed range

(k>0.5; see table 2.6.1), the expected value of M* for the experimental system under investigation would be between 0.57 and 2.24 parasites per host.

Table 3.2.5

Values of the equilibrium mean parasite burden per host, M*, obtained from equation (3.2.5) for a range of parasite distributions.

Tank	1	2	3	4
N	65.55	63.74	45.84	50.13
Distribution				
Poisson	0.57	0.60	0.96	0.86
Negative binomial				
k=5.0	0.60	0.63	1.05	0.93
k=1.0	0.74	0.79	1.49	1.29
k=0.5	0.96	1.04	2.24	1.87
k=0.1	3.54	3.91	9.37	7.71

Despite the simple assumptions incorporated into the model, the inherent errors in parameter estimation and the somewhat artificial tank ecosystem, the estimated range of M* shows very close agreement with the range of mean parasite burdens recorded for Romanomermis spp. and other mermithids in the field (table 2.6.1).

3.3 DISCUSSION

In addition to providing quantitative estimates of certain population parameters, the results presented in this chapter have highlighted several important factors in the Culex- Romanomermis, host-parasite relationship.

The effect of mermithids in depressing both pupal and emergent adult densities, has (because of the host-parasite relationship resulting in host death) been predicted in many studies (Welch, 1964; Phelps & Defoliart, 1964; Welch, 1965; Petersen & Willis, 1971). There are, however, no quantitative data available to support this view.

In the form presented here, theory and experimentation have been used in an integrated way, to quantify pupal and adult depression. It may be shown that parasitization by R. culicivorax causes elongation of the larval phase in the mosquito life-cycle,by comparing the rate of development of mosquito larvae (Shelton 1973) with that of the parasite (section 2.5 (ii)). The effect of this change in the life-span of the host upon the magnitude of the host population, has not previously been investigated (although it has been discussed in terms of sampling bias when examining levels of parasitism in the field (Welch, 1965)). Clearly, in field populations, elongation of the life-span of hosts will tend to lead to an increase in the proportion of those hosts suffering mortality, either as a result of predation, other pathogens,or due to adverse environmental conditions. The implications of extended life-spans for infected hosts are many and cause difficulty in interpreting the impact of parasitism on host adundance. There will be a tendency for increased host densities to: (1) increase the risk of predation, by attracting predators to denser patches of prey; (2) allow host densities to cross threshold levels, allowing epidemic outbreaks of disease. Such possibilities should however be kept in perspective bearing in mind the magnitude of the population change and its duration. The above effects may be exacerbated or alleviated by differential rates of

predation upon infected and uninfected hosts. This type of phenomenon is not uncommon in host-parasite associations (van Dobben, 1952; Herting & Witt, 1967; Lester, 1977; Bethel & Holmes, 1979; Camp & Huizinga, 1979). Obviously, if either the infected or uninfected class was significantly preferred by predators, then the effect of predation upon the host and parasite populations could be considerable. This is an extremely complex area of ecology, involving many variable parameters and it is not proposed to deal in depth with it here. However, a preliminary investigation of differential host predation was carried out and is presented and discussed in appendix (IV). These factors will also have a bearing upon the parasite population, especially as infected hosts have a greater expected life-span than uninfected hosts and will therefore tend to be in the environment longer, allowing a proportionately greater length of time for adverse environmental factors to act.

It has previously been reported, that, under certain conditions, nominally larval parasitic mermithids may be carried over from the larval host into the adult stage (Welch, 1965; Curran, 1981). Unfortunately, however, the number of adults affected and the parasite burdens in adult hosts were not always reported. The levels of adult parasitization reported here, (table 3.2.2) imply that the direct effect upon the adult population may be a significant one. This is in addition to the enhanced probability of parasite dispersal to new sites. Field data on adult infections with mermithids are rare and are confined to simuliid hosts. Mokry & Finney (1977) reported 11.7% of female Prosimulium mixtum infected with mermithid nematodes (with no males found parasitized); developed nematodes were seen to emerge from the adult female hosts. Curran (1981) reported less than 1% of adult simuliids infected with Gastromermis metae. The need for river dwelling mermithids to infect adults has been interpreted in terms of combatting downstream drift of both infected hosts and infective stages (Welch, 1964; Colbo & Porter, 1980).

No preferential male or female infection, or parasite-induced

mortality was found in this study, supporting one of the basic assumptions in many host-parasite relationships, that of an essentially random infection process.

The simple deterministic model, described in section 3.1, was shown to adequately reflect the mean level of adult mosquitoes emerging from an immigration-death/emigration system in the absence of parasites. Simple elaboration of the model, to incorporate the effects of parasitism upon the host population under otherwise identical conditions, provided predictions of the magnitude of both host and parasite populations. Problems of parameter estimation where direct measurement proved difficult or impractical, were largely overcome using indirect or estimation methods which were validated by comparing model predictions to observed data.

The theoretical approach enabled detailed investigations of the effects of parasitism upon the larval, pupal and adult mosquito population to be made. Such investigations, if attempted experimentally, would be time consuming and difficult to execute, even under laboratory conditions. Perhaps more importantly, the model enabled quantitative estimates of the mean parasite burdens to be made, without disrupting the parasite or host populations by destructive sampling. The translation of predicted values of host and parasite population levels to a field situation, obviously entails the incorporation of many more variables, both of a biological nature (in terms of host reproduction, egg survival and development, time delays and density-dependent constraints) and also environmental factors. Preliminary studies, examining the field status of parasites and hosts have been carried out for mermithid-host associations (Curran, 1981). Such studies need to be built upon and supported by rigorous laboratory and theoretical investigations.

One further observation may be made in relation to the predicted values generated by the model. The distribution of the parasites within the host population is of great importance in determining the host population size and the number of uninfected hosts developing

via the pupal stage to become adults. This is in addition to the effects of the statistical distribution of parasites upon the development time of the parasites (ch. 2.5) and upon the sex ratio of the nematode (ch. 2.6). Prevailing levels of mean parasite burden per host and overdispersion in the field (table 2.6.1), suggest that, the depression of the adult mosquito population would fall into the region of 30-80%, bearing in mind that the data presented in table (2.6.1) are point samples in time and do not necessarily reflect the equilibrium mean parasite burdens or average degree of overdispersion of the populations.

For any realistic conclusions to be drawn from the model predictions (especially in terms of understanding the field situation), account should be taken of the density of hosts. Given that, in the absence of infection, the equilibrium host population (H*) predicted by the model is 87.05 (for an arena with a water surface area of 22.0 cm by 32.5 cm),the density of hosts was 1218 hosts per m^2. This rose to 1399 hosts per m^2 when H* rose to 100, due to the presence of the parasite. Field data on mosquito larval densities are not readily available, but documented accounts show larval densities to vary greatly with species, habitat-type and season. Some typical larval densities in the field are as follows: for Culex tarsalis in California, USA, 129 per m^2 (Hagstrum, 1971); Anopheles stephensi in Pondicherry, India, 600-800 per m^2 (Kaur & Reuben, 1981); in the sub-alpine Pyrenees, France, Aedes cataphylla, 5000-8000 per m^2 and Ae. communis 700-1200 per m^2 and in the Carmargue, France, Ae. detritus 400-800 per m^2 (Croset, Papierok,Rioux, Gabinaud, Cousserans & Arnaud, 1976). For C. p. fatigans in effluent drains, Faridabad, India, reported levels of larval density range from 4,600 per m^2 to 68,600 per m^2 (Rajagopalan, Menon, Mani & Brooks, 1975). The densities that occurred in this study are, therefore, not unusual and may well be expected to occur in the field. Those habitats which support very high densities of mosquito larvae are frequently eutrophic and/or polluted; situations which do not normally favour transmission of R. culicivorax.

CHAPTER 4 : THEORETICAL STUDIES OF THE POPULATION DYNAMICS OF R. CULICIVORAX

4.1 THE BASIC MODEL

The basic model of this host-parasite association has two main components. The first element is an equation that describes the rate of change of the host population through time, presented and developed in the previous chapter (equation 3.2.2). The second element, is an equation system describing the rate of change in population abundance of the various stages in the parasite life-cycle. This element will be described in the following paragraphs, together with a discussion of the equilibrium properties of the model.

A series of linked, first order differential equations was formulated to describe the rate of change through time of the five parasite populations (eggs through to adults). As with the host equation, (3.2.2), the model is constructed to maximise tractability without sacrificing biological realism.

Each population was assessed in terms of the various, net demographic rates which contribute to its overall rate of change in abundance through time. These component rate processes are broadly divisible into input (birth, development and immigration) and output (death, development and emigration) rates, which pertain to each stage. The detailed submodels for each stage are described below.

The basic model is developed assuming the parasites to be distributed in a random (Poisson) manner amongst the hosts.

Eggs.

The population of eggs at time ($E_{(t)}$), is a function of the rate at which the eggs are produced by the adult nematode population, the rate at which they hatch and their rate of mortality. This may be formalized as

$$dE/dt = \lambda s A_{(t)} - E_{(t)}(\sigma_1 + \mu_1) \qquad (4.1.1)$$

where $A_{(t)}$ is the magnitude of the adult nematode population at time t. The rate parameters are defined as; λ, the birth rate of eggs (/female/unit time), s, the proportion of adults that are female, σ_1, the rate of egg hatching (/egg/unit time) and μ_1, the rate of egg mortality (/egg/unit time). At equilibrium (dE/dt=0) the magnitude of the egg population (E*) is given by,

$$E^* = \lambda s A^*/(\sigma_1+\mu_1) \qquad (4.1.2)$$

where A* is the equilibrium adult nematode population.

Infective Stages.

Similarly, the population of infective stages at time t, ($I_{(t)}$), is determined by the rate at which they are formed (i.e. the per capita egg hatch rate, σ_1/unit time) and the mean length of time they remain in the environment. The latter rate is determined by their per capita rate of mortality (μ_2/unit time), plus the rate at which they leave the infective stage pool to become parasites. Thus,

$$dI/dt = \sigma_1 E_{(t)} - I_{(t)}(\beta H_{(t)}+\mu_2) \qquad (4.1.3)$$

where $H_{(t)}$ is the host population size at time t and β is the instantaneous rate of infection (/host/infective stage/unit volume/unit time). This assumes a linear relationship between host density and the net rate of infection. Equation (4.1.3) has the equilibrium solution,

$$I^* = \sigma_1 E^*/(\beta H^*+\mu_2) \qquad (4.1.4)$$

where I* and H* are the equilibrium population densities of the

infective stages and hosts respectively.

Parasites.

The rate of change in the population of parasites is determined by the rate at which infections take place and the rate of parasite loss. The former is dependent upon the densities of both host and infective stage, the latter upon the rate at which parasites develop to become postparasites and the death rate of the parasites(inclusive of natural parasite mortalities and those attributable to both parasite-induced and natural host mortalities).

The input into the parasite population has already been defined as one of the loss terms in the dynamics of the infective stage population, namely $\beta H_{(t)} I_{(t)}$.

The net rate at which natural parasite mortalities occur is a function of the instantaneous parasite death rate (μ_3/parasite/unit time) and the magnitude of the parasite population at time t ($P_{(t)}$), viz. $\mu_3 P_{(t)}$.

The rate at which natural host mortalities contribute to the overall parasite mortality will, on average, be dependent upon the mean parasite burden per host, $M_{(t)}$, where the net rate of parasite mortality is

$$\mu_6 H_{(t)} M_{(t)} \qquad (4.1.5)$$

where μ_6 is the instantaneous rate of host mortality (/host/unit time). Clearly, if $P_{(t)}$ is the parasite population at time t,

$$M_{(t)} = P_{(t)}/H_{(t)} \qquad (4.1.6)$$

and the net rate of parasite mortality attributable to natural host deaths (equation 4.1.5) becomes,

$$\mu_6 P_{(t)} \qquad (4.1.7)$$

Given that δ is the constant that determines the pathogenicity of the parasite to the host (as defined in the previous chapter, /parasite/host/day), the level of 'premature' host mortality caused by the parasites is directly related to the parasite burden of individual hosts. Specifically, the net rate at which hosts die given by equation (3.2.1) as $\delta H_{(t)} \sum_{i=0}^{\infty} i.p(i)$, which assumes a linear relationship between parasite burden and host mortality. Hosts which die as a result of parasitism, will also result in the death of those parasites which they harbour. The resultant net rate of parasite loss, again assuming a linear relationship between parasite burden and host mortality, will be,

$$\delta H_{(t)} \sum_{i=0}^{i=\infty} i^2.\, p(i) \qquad (4.1.8)$$

where $\sum_{i=0}^{i=\infty} i^2.p(i)$ is equivalent to the square number of parasites per host and is therefore dependent upon the statistical distribution of the parasites within the host population. Anderson & May (1978) derive this expression in general and give precise definitions for several probability distributions, with that for the Poisson distribution being $M+M^2$, thus equation (4.1.8) becomes,

$$\delta H_{(t)} (M_{(t)} + M^2_{(t)}) \qquad (4.1.9)$$

The final term in the equation, determines the rate at which parasites leave the parasite population to become postparasitic

juveniles. This is dependent upon the instantaneous rate of parasite-induced host mortality produced by the emergence of the nematodes (α/host/day). This has been shown to vary with the mean parasite burden per host (section 2.5). The estimates of α presented in section 2.5 were (obviously) only for those hosts that were infected, thus, the rate at which parasites emerge from a host population must contain a term denoting what proportion of the population is infected. This term is $1-p(0)$, where $p(0)$ is the unparasitised proportion of the population. The net rate of parasite emergence is, therefore,

$$\alpha H_{(t)} M_{(t)} (1-p(0)) \qquad (4.1.10)$$

When equations (4.1.7 and 9) are combined with the natural net parasite mortalities and the net rate of infection, the rate of change in the proportion of parasites through time can be modelled as follows,

$$dP/dt = \beta H_{(t)} I_{(t)} - P_{(t)} (\mu_3+\mu_6) - \delta H_{(t)} M_{(t)} M^2_{(t)} - \alpha H_{(t)} M_{(t)} (1-p(0)) \qquad (4.1.11)$$

Juveniles.

The rate of change in the population of postparasitic juvenile nematodes ($J_{(t)}$) is determined in a similar manner to that of the populations of eggs and infective stages. The input term is the same as the development term in equation (4.1.11), with a juvenile mortality rate and an output (development) rate to adult nematodes. Thus, where μ_4 is the rate of juvenile mortality (/juvenile/unit time) and σ_2 is the rate of moulting to the adult stage (/juvenile /unit time),

$$dJ/dt = \alpha H_{(t)} M_{(t)} (1-p(0)) - J_{(t)} (\mu_4 + \sigma_2) \qquad (4.1.12)$$

With the equilibrium solution,

$$J^* = \alpha H^* M^* (1-p(0)) / (\mu_4 + \sigma_2) \qquad (4.1.13)$$

where J* is the equilibrium juvenile population density.

Adults.

The final equation in this system expresses the rate of change in the adult population through time ($A_{(t)}$), where μ_5 is the adult mortality rate (/adult/unit time). The expression is a simple immigration-death model.

$$dA/dt = \sigma_2 J_{(t)} - \mu_5 A_{(t)} \qquad (4.1.14)$$

with the equilibrium solution,

$$A^* = \sigma_2 J^* / \mu_5 \qquad (4.1.15)$$

Substitution of the equations for the equilibrium populations of eggs (4.1.2), juveniles (4.1.13) and adults (4.1.15) into the equilibrium equation for infective stages (4.1.4) gives,

$$I^* = \sigma_1 \lambda \sigma_2 s \alpha H^* M^* (1-p(0)) / (\beta H^* + \mu_2)(\mu_1 + \sigma_1)(\mu_4 + \sigma_2)\mu_5 \qquad (4.1.16)$$

At equilibrium, equation (4.1.11) becomes

$$\beta H^*I^*-P^*(\mu_3+\mu_6)-\delta H^*M^*+M^{*2}-\alpha H^*M^*(1-p(0)) = 0 \qquad (4.1.17)$$

Substitution of equation (4.1.16) for I* in equation (4.1.17) gives a solution for H* of,

$$H^*= \frac{M^*\mu_2Y(\mu_3+\mu_6)+\delta\mu_2Y(M^*+M^{*2})+\alpha M^*\mu_2Y(1-p(0)))}{\beta XM^*(1-p(0))-YM^*\beta(\mu_3+\mu_6)-\delta\beta(M^*+M^{*2})Y-\alpha M^*\beta(1-p(0))Y} \qquad (4.1.18)$$

where $X = \sigma_1\sigma_2\lambda\alpha s$ and $Y = (\mu_1+\sigma_1)(\mu_4+\sigma_2)\mu_5$ and for the Poisson distribution $p(0) = \exp(-M^*)$.

Of the rate parameters which appear in equation (4.1.18), all but two are constant (table 4.1.1). The two which are variable are the rate at which hosts die as a result of parasite emergence (α/host/day) and the proportion of nematodes that develop as females (s). Both these parameters vary with the mean parasite burden per host ($M_{(t)}$).

The relationship between α and $M_{(t)}$ has been shown to take the form

$$\alpha = a+bM_{(t)} \qquad (4.1.19)$$

Estimates of the slope (b) and intercept (a) of this relationship are given in table 3.2.3.

The numerical value of s may be determined in one of two ways. Firstly, a regression line may be fitted to data relating s to the mean parasite burden (see figure 2.6.1), providing a method of

estimating s similar to that used for α on the previous page. The major drawback of this approach, as discussed in section 2.6, is that the fitted relationship takes on the average degree of dispersion of the distribution of parasites within the experimental host populations. The result of this is,that the sex ratio determination of the parasite will be independent of whatever frequency distribution is assumed in the model. Such an approach is clearly unsatisfactory.

The second method is that described in section 2.6 (ii), where the probability that a host contains i parasites (defined by the parasite distribution) is multiplied by the proportion of females expected from hosts with i parasites (figure 2.6.2) and summed over the range of values of i. In practice, (at 25°C) as there is a zero expectation of female production at parasite burdens of seven or more, only values of i from one to six need be considered. The result of this is,that the value of s is determined by the form of the frequency distribution assumed in the model.

As the initial value of M* is varied, the roots of the two equations for H*, the equilibrium host population (equations 3.2.3 and 4.1.18), i.e. where the numerical solution of each equation is the same, provide an estimate of the value of M*, under the prevailing conditions.

The roots of the equations are found using an iterative technique, where an initial value of M* is set, the variables (α and s) are calculated and the values of H*, given by the two equations, are calculated and compared; the value of M* is varied until the two values of H* coincide.

Under the conditions given in table 4.1.1 , the two equations, given a wide range of starting conditions, yielded four positive roots, plus the parasite extinction condition (where M* and P* equal zero). The existance of a positive root does not necessarily

Table 4.1.1 The standard rate parameter values used in the model, estimated at 25°C.

Symbol	Description	Definition	Value	Source
μ_1	Instantaneous rate of egg mortality	/egg/day	0.0047	2.8(iii)
μ_2	Instantaneous rate of infective stage mortality	/inf. stage/day	0.2355	2.4(i)
μ_3	Instantaneous rate of parasite mortality	/parasite/day	0.0000	2.5(i)
μ_4	Instantaneous rate of juvenile mortality	/juvenile/day	0.0019	appendix v
μ_5	Instantaneous rate of adult mortality	/adult/day	0.0556	appendix v
μ_6	Instantaneous rate of host mortality	/host/day	0.0033	appendix v
σ_1	Instantaneous rate of egg hatch	/egg/day	0.0193	2.8(ii)
σ_2	Instantaneous rate of juvenile development, mating & egg development	/juvenile/day	0.0645	appendix v
σ_3	Instantaneous rate of host pupation	/host/day	0.1608	3.1
β	Instantaneous rate of infection	/inf. sta./host/day/ 7.5 litres	0.000006	2.4(iii)
δ	Instantaneous rate of parasite-induced (premature) host mortality	/parasite/host/day	0.0076	3.2
α	Instantaneous rate of emergence-induced host mortality	/host/day	variable*	appendix v
s	Proportion of nematodes that are female		variable**	2.6
λ	Birth rate	eggs/female/day	137.7778	2.8(i)
Λ	Host immigration rate	hosts/day	14.2857	3.1

* = 0.1085 + 0.0056M

** is estimated from the probability distribution of parasites per host and the known relationship between sex ratio and parasite burden per host (Ch. 2.6, figure 2.6.2).

imply that such a parasite-host equilibrium will be stable. Where stability is defined as the return of the population(s) to the same equilibrium point following pertubation. One method of testing for stability is to follow the magnitude of the parasite and host populations through time from a wide range of starting conditions. Under such circumstances, if steady state levels of M* are attained, they will represent stable population equilibria. Of the four potential equilibria (roots) shown to occur in the equation system, only one was found to be stable by this method. Assuming a Poisson parasite distribution,and using the parameter values given in table 4.1.1, this equilibrium mean parasite burden was found to be 2.93 per host. The equilibrium populations of hosts (H*) and parasites (P*) were 93.75 and 274.54 respectively. Figure (4.1.1) shows two examples of the wide variety of starting conditions tested, with the populations equilibrating at the levels given above.

The predicted value of M*, at 2.93 parasites per host, is somewhat greater than that estimated to occur in the experimental tanks, where a Poisson distribution was also assumed (table 3.2.5). This is not surprising, since the model will tend to overestimate M*. In particular, the estimated values of the egg and infective stage mortality rates were minimum values, and would be expected to be greater in the face of more natural conditions. Other factors which may contribute to the discrepancy include potential overestimates of the maturation and mating of postparasitic nematodes which, in larger, less aerobic substrate volumes would probably occur at reduced rates. Irrespective of these qualifications, the closeness of the two estimates suggests that the model gives a satisfactory performance and a realistic result.

It should be remembered that the probable frequency distribution of the parasites in the tanks was overdispersed, which would tend to produce an increase in the estimated value of M* in the tanks (table 3.2.5) and a decrease in the value of the predictions

Figure 4.1.1 The equilibrium population levels of host (H*) and parasite (P*) predicted by the model for different initial conditions.

The predictions of the basic model, where the parasites are distributed at random amongst the host population (Poisson) and the parameter values are those given in table 4.1.1.

The final equilibrium levels, in both cases, were,

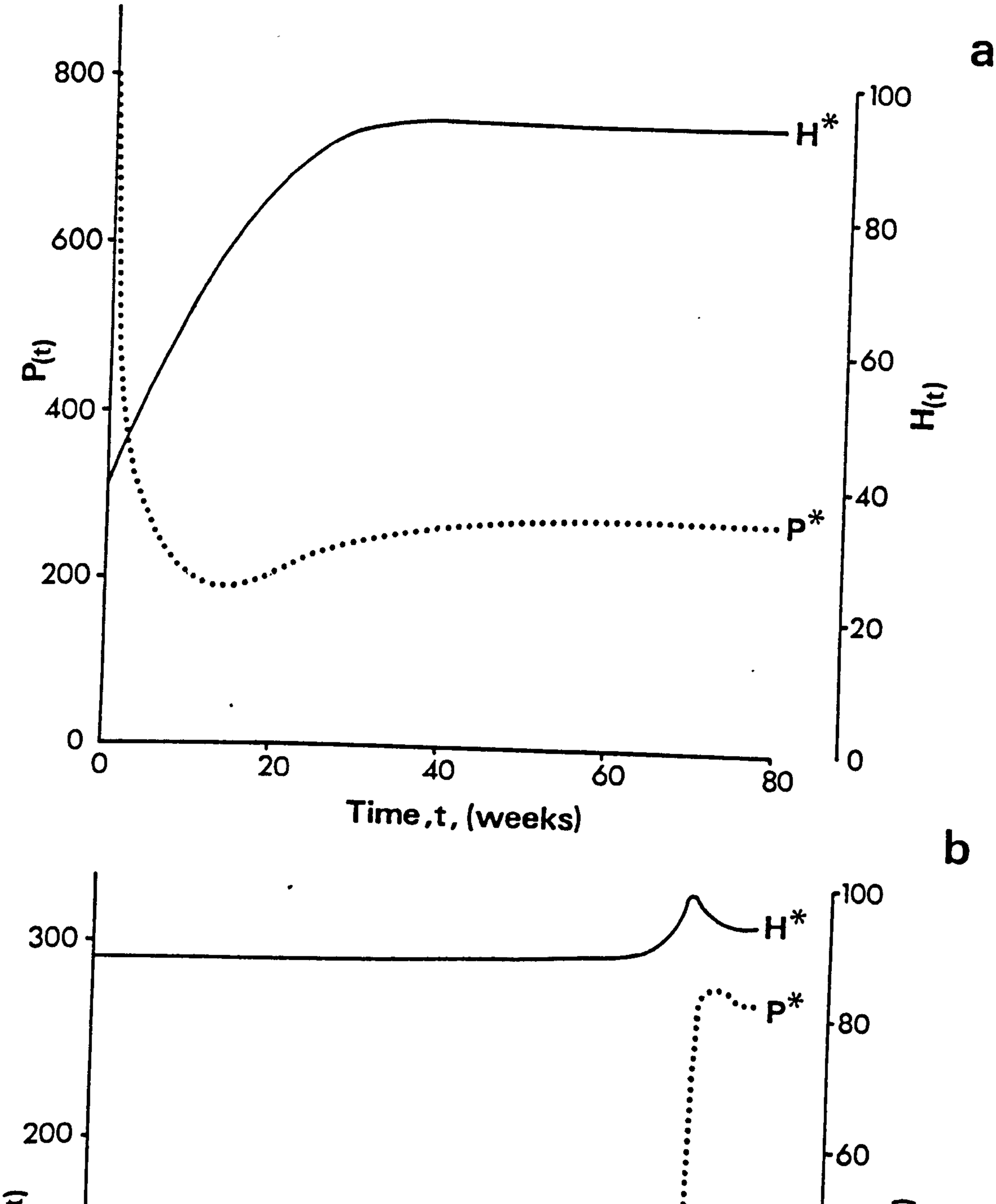
M* = 2.93 parasites/host

H* = 93.75 hosts

P* = 274.53 parasites

a Initial condition of; H (at t=zero) = 40
P (at t=zero) = 800

b Initial condition of; H (at t=zero) = 87.05
P (at t=zero) = 3.00


a
800
600
400
200
0
P(t)
0
20
40
60
80
H*
P*
100
80
60
40
20
0
H(t)
Time, t, (weeks)
b
300
200
100
0
P(t)
H*
P*
100
80
60
40
20
0
H(t)
0
10
20
30
40
50
60
Time, t, (weeks)

of the model. This will be further discussed in section 4.3.

The way in which various parameters influence the value of M* generated by the model provide a means of assessing the relative importance of each parameter in the host-parasite relationship. The precise relationship between M*, a given parameter and the magnitude of the laboratory estimates at that parameter,will provide a guide as to the required accuracy of its estimation. This exercise assists in directing future research effort towards those parameters that are of importance and/or difficult to estimate sufficiently accurately.

For a population to maintain an equilibrium, there must be at least one density-dependent factor to regulate the population level, otherwise,stochastic environmental factors will lead to massive fluctuations in the population size. Such regulation, may be produced by a number of different ecological factors, notably, predation, parasitic diseases and competition for limited resources.

For Romanomermis culicivorax three of the rate parameters show a degree of dependence upon the density of the parasite population. These are, the environmental sex determination (section 2.6), the development rate (emergence) of the parasite (section 2.5) and the parasite-induced host mortality rate (section 2.5). In addition, the transmission coefficient was shown to vary with the density of hosts (section 2.4 (v)), although this was not included in the model. The relative importance of these four factors in parasite population regulation will be discussed in section 4.2. For the present, it is sufficient that there is a density-dependent regulatory potential incorporated into the model.

The basic model is built upon a series of biological assumptions regarding the life-history of both parasite and host. That many of these assumptions are biologically unrealistic is of importance only if they detract from the accuracy of the predictions of the model. Their presence, however, should be noted. First, the host population and its dynamics are heavily constrained, the

host existing as an immigrating population of first instars and emigrating upon pupation; there being no density-dependent host-birth rate linked to the output of adult mosquitoes from the system. The advantages of this are that it: (a) simplifies the model, (b) enables the system to be investigated without the pattern introduced by a fluctuating host population and (c) allows the model predictions to be more easily tested. The major disadvantage is in translating the predictions of the simple model to a situation where the host population fluctuates.

The use of constant rates of birth, death and development for both host and parasite is a common feature of deterministic models such as this (Hassell, Lawton & Beddington, 1976; Beddington, Hassell & Lawton, 1976; Anderson & May, 1978; May & Anderson, 1978). Advantages of using what in reality are normally non-linear rates, are greatly outweighed by the increasing complexity of the model when such rates are incorporated. It can also be shown that the use of a model based on constant, average rates, can encapsulate the essential features of a relationship (see figure (2.4.1) , where the lines fitted to the data points were obtained using a constant rate of mortality, μ_2).

Of the rate parameters listed in table 4.1.1 , two are not simple rates encapsulating a single biological event, but consist of several consecutive events. The rationale of using such parameters is twofold: firstly, to make parameter estimation as simple as possible and secondly, to reduce the number of parameters in the model to increase the model's tractability. The parameters in question are the instantaneous rate of adult mortality (μ_5) and the time delay due to juvenile development (σ_2).

The rate at which adults die, μ_5, as incorporated into the model, is not that estimated in section 2.7 (iii). This is due to the requirement that each female produces the mean number of eggs reported in section 2.8 (i) during its lifetime. Given the mean egg birth rate (/female/unit time), estimated as a constant,

the easiest way of constraining the total egg output per female was to treat females which had survived long enough to produce the average number of eggs, as being exhausted. This is not unrealistic in ecological terms as females that have completed oviposition, although they do not actually die immediately, (figure 2.7.3), play no further active role in the dynamics of the parasite. That male nematodes are also subjected to this high level of mortality is of no importance, as it is assumed that there will always be sufficient males present to mate with females. This is a reasonable assumption given the typical frequency distributions of the parasites and the characteristics of the sex determination mechanism.

The second rate parameter that is not simple in form, the instantaneous rate at which juveniles develop to adults, mate and develop eggs (σ_2/juvenile/day) was estimated by summing the mean development times between the emergence of juvenile female nematodes and the onset of oviposition and then taking the inverse of the total development time to give a mean instantaneous rate. This technique is fully described in appendix V.

One further biological simplification was made. The instantaneous rate of juvenile mortality, μ_4 per juvenile per day, was estimated using the survival characteristics of female worms only. This was because of the greater relative importance of the female worm and also since, during the investigation, no juvenile males died (female survival was also high, at 98.4%).

The remainder of this section presents an examination of the relationships between the numerical values of the various parameters and the magnitude of the equilibrium mean parasite burden per host (M*) generated by the basic model (which assumes a Poisson distribution of parasites). The various parameters are classified into the following groups: mortality factors, time-delay factors, the rate of infection,and birth and immigration rates.

Mortalities.

Apart from the obvious decline in the value of M* as any of the rates of mortality are increased (figures 4.1.2 - 4.1.5), there are several other points of interest. At low to moderate mortality rates, changes in the values of these parameters result only in a slight change in the consequent value of M*; the relationship is almost linear over these ranges. In contrast, as the rate of mortality is increased, the value of M* changes very rapidly. If the mortality rate is further increased, the value of M* quickly tends to zero; the mortality-equilibrium relationship takes the form of a breakpoint (see May, 1977b). This breakpoint is the threshold between the persistence of the parasite, when M* is positive, and parasite extinction. The extinction of the parasite, in this model, allows the host to attain an equilibrium population density determined by its immigration, mortality and developmental rates, much as would be expected in the field.

In practice, the estimated values of the mortality rates are sufficiently low that large increases in their values would be required to produce a major change in the predicted value of M*. Thus, any errors in parameter estimation are probably of little importance in biasing the model's predictions.

Three factors are of significance in determining the relative importance of these mortality parameters. These are, the rate of change of M* with a given change in parameter magnitude, the likelihood that the parameter will change in the field, and the likely range of that change. Thus, for the instantaneous rates of host mortality (μ_6) and natural parasite mortality (μ_3), there is little change in M* for even quite large changes in the parameter value. In contrast, there is a marked increase in the rate of change of M* with a small change in the rate of infective stage mortality (μ_2), especially at its lower values. This suggests that μ_2 is the more important of the parameters in terms of the magnitude of M* attained. When, however, the probability and range of change in the parameter values in the field are taken into

Figure 4.1.2 The influence of mortality factors on the magnitude of M* : μ_1 and μ_2.

The predictions of the basic model. The assumed parasite distribution was Poisson.

a The relationship between the instantaneous rate of egg mortality, μ_1 (/egg/day) and M*.

b The relationship between the instantaneous rate of infective stage mortality, μ_2 (/infective stage/day) and M*.

The values of the other parameters are as given in table 4.1.1. The arrows indicate the estimated parameter values at 25°C. The shaded areas are regions of parasite extinction.

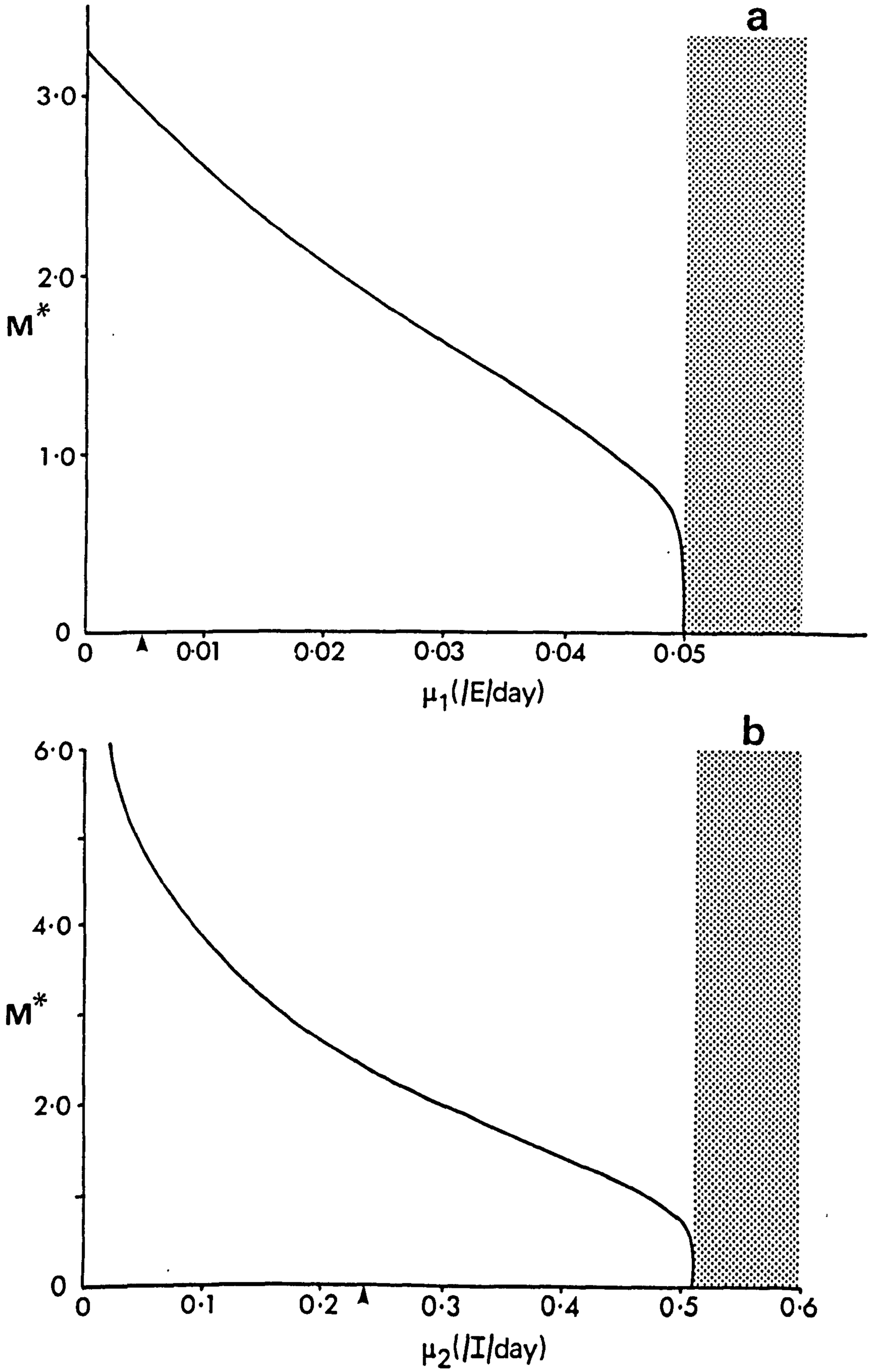

a
3·0
2·0
M*
1·0
0
0
0·01
0·02
0·03
0·04
0·05
μ1(/E/day)
b
6·0
4·0
M*
2·0
0
0
0·1
0·2
0·3
0·4
0·5
0·6
μ2(/I/day)

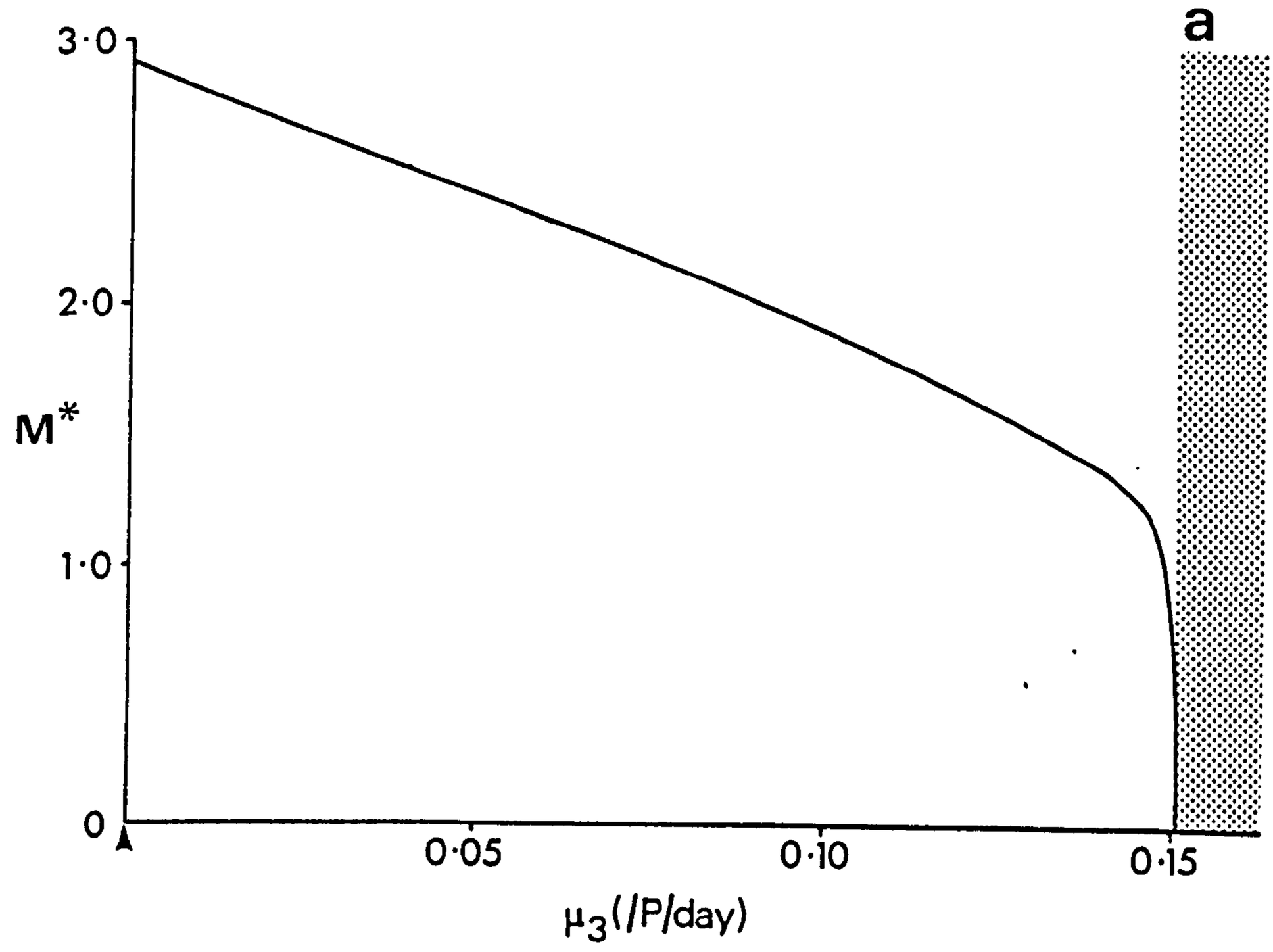
Figure 4.1.3 The influence of mortality factors on the magnitude of M* : μ_3 and μ_4.

The predictions of the basic model. The assumed parasite distribution was Poisson.

a The relationship between the instantaneous rate of natural parasite mortality, μ_3 (/parasite/day) and M*.

b The relationship between the instantaneous rate of postparasitic juvenile nematode mortality, μ_4 (/juvenile/day) and M*.

The values of the other parameters are as given in table 4.1.1. The arrows indicate the estimated parameter values at 25°C. The shaded areas are regions of parasite extinction.


a
3·0
2·0
1·0
0
M*
0·05
0·10
0·15
μ3(/P/day)

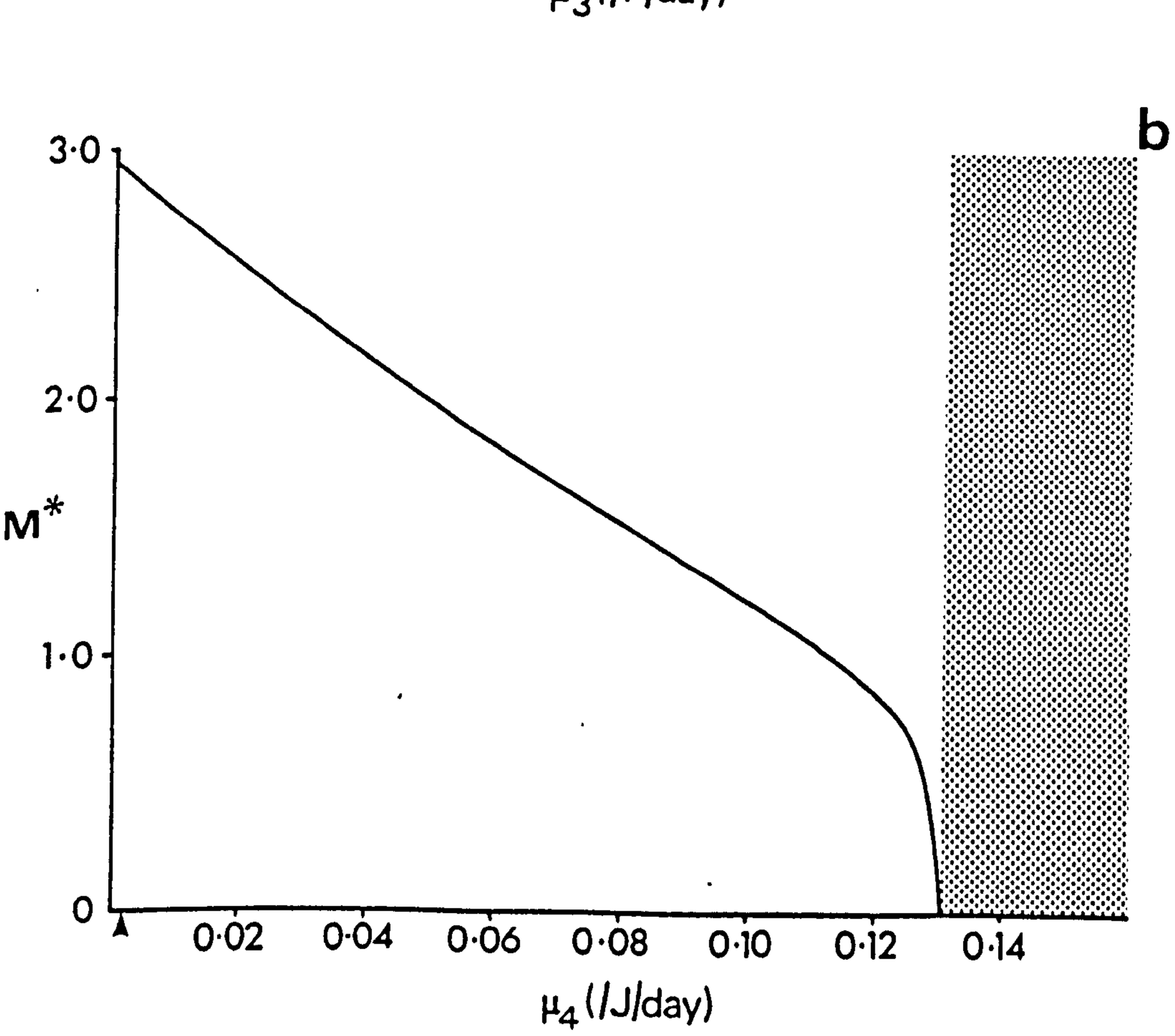

b
3·0
2·0
1·0
0
M*
0·02
0·04
0·06
0·08
0·10
0·12
0·14
μ4(/J/day)

Figure 4.1.4 The influence of mortality factors on the magnitude of M* : μ_5 and μ_6.

The predictions of the basic model. The assumed parasite distribution was Poisson.

a The relationship between the instantaneous rate of adult mortality, μ_5 (/adult/day) and M*.

b The relationship between the instantaneous rate of host mortality, μ_6 (/host/day) and M*.

The values of the other parameters are as given in table 4.1.1. The arrows indicate the estimated parameter values at 25°C. The shaded areas are regions of parasite extinction.

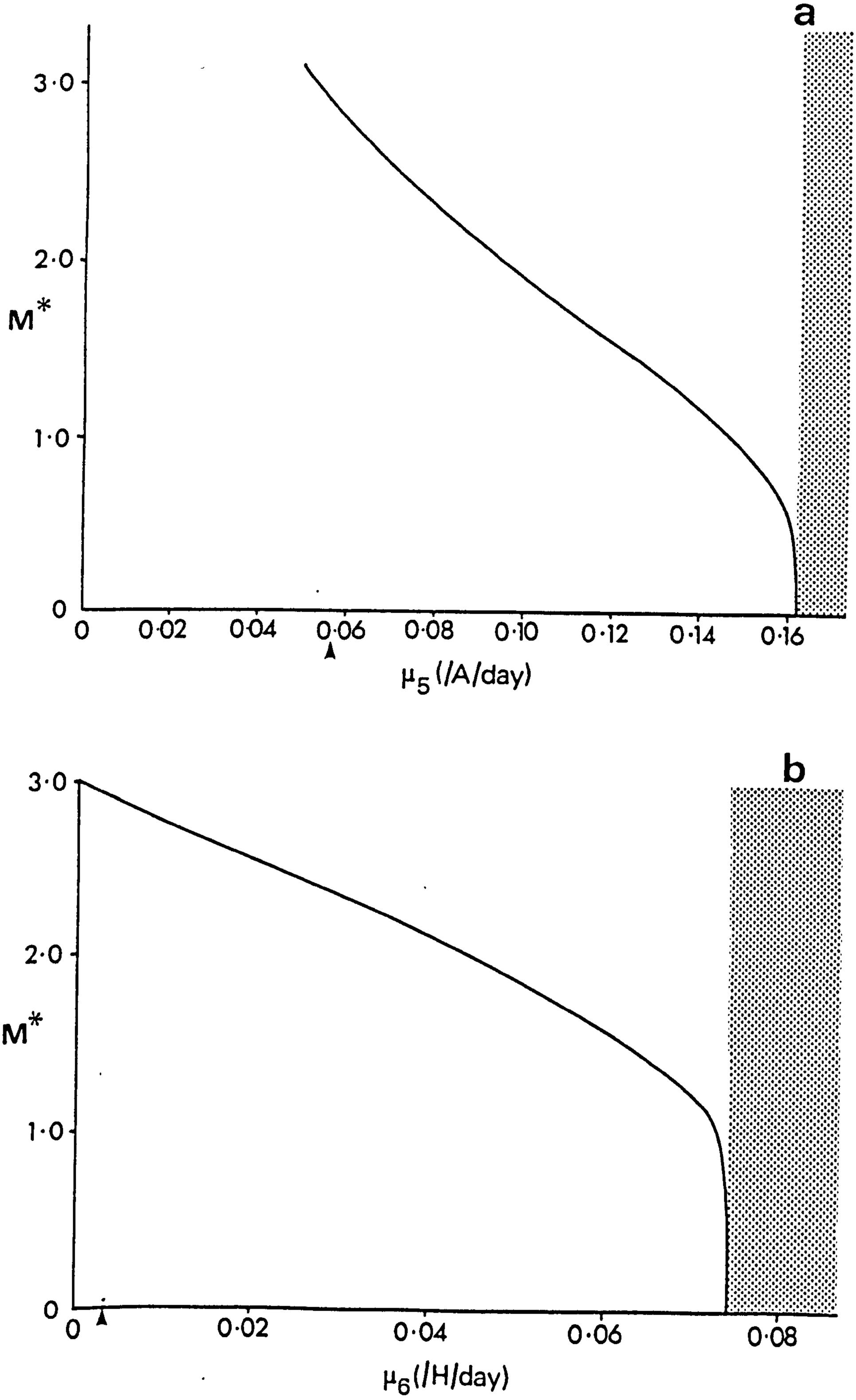
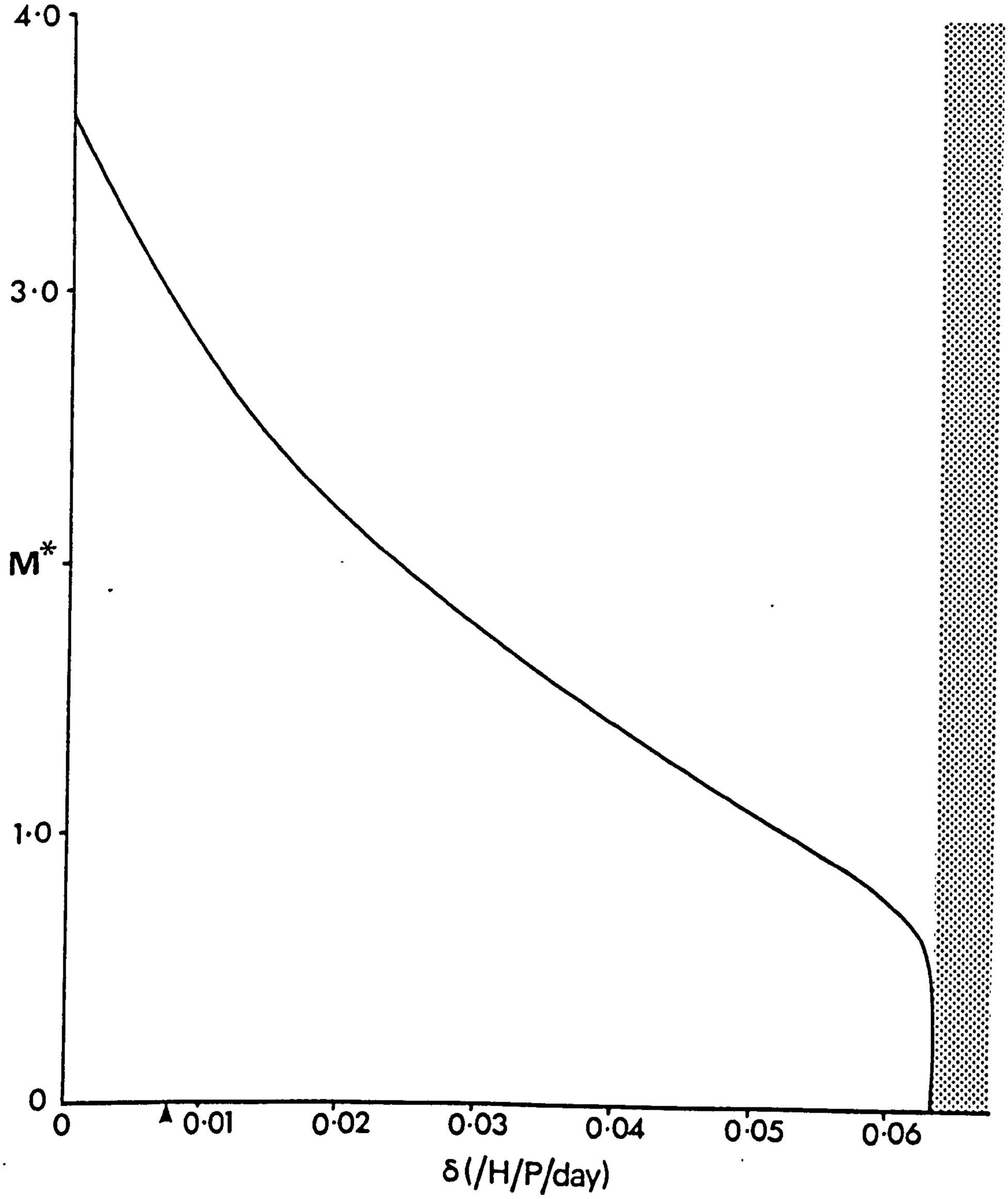

a
3·0
2·0
1·0
0
M*
0
0·02
0·04
0·06
0·08
0·10
0·12
0·14
0·16
μ5 (/A/day)
b
3·0
2·0
1·0
0
M*
0
0·02
0·04
0·06
0·08
μ6(/H/day)

Figure 4.1.5 The influence of mortality factors on the magnitude of M* : δ.

The relationship between the instantaneous rate of parasite-induced (premature) host mortality, δ (/parasite/host/day) and M*.

The predictions of the basic model. The assumed parasite distribution was Poisson.

The values of the other parameters are as given in table 4.1.1. The arrow indicates the estimate of δ at 25°C. The shaded area is a region of parasite extinction.


4·0
3·0
M*
1·0
0
0
0·01
0·02
0·03
0·04
0·05
0·06
δ(/H/P/day)

account, a different picture emerges. The estimated rate of host mortality is very low compared with that seen from field studies (Service, 1968; Lakhani & Service, 1974; Service 1977), thus, μ_6 may, in reality, have a value which under these conditions, may produce a value of M* much nearer the breakpoint (figure 4.1.4b). The value of μ_2 was also estimated under ideal conditions and is thus unlikely to be lower in the field, where adverse conditions and predation would tend to give higher values. It becomes apparent that μ_2 is unlikely to enter the region where large values of M* are generated (figure (4.1.2b)) except when there is a fall in the temperature, which will also affect most of the other parameter values.

Time delays.

The rate at which the eggs of R. culicivorax hatch, and thus input new individuals into the infective stage pool, has already been shown to be one of the major time delays in the life-cycle of the nematode (section 2.9). Time delays have been shown to destabilize models of this type (Maynard Smith, 1974; May & Anderson, 1978), tending to produce oscillatory behaviour in the parasite population and thus also in that of the host. Increasing time delays within a life-cycle will also tend to reduce the population level attained, by increasing the period during which a (constant) mortality rate can operate.

The effect of varying the instantaneous rate of egg hatch (σ_1) is that, as the time delay is increased (smaller values of σ_1), the value of M* generated by the model declines at an ever increasing rate (figure 4.1.6a). Initially, the alteration in the rate of change in M* is minimal, giving way to a region of σ_1 where M* changes very rapidly, leading to an extinction threshold.

The estimated rate of egg hatch falls into the region where the value of M* changes rapidly with small changes in the parameter value, indicating that accurate estimation of σ_1 is

Figure 4.1.6 The influence of time delays on the magnitude of M* : σ_1, σ_2 and σ_3.

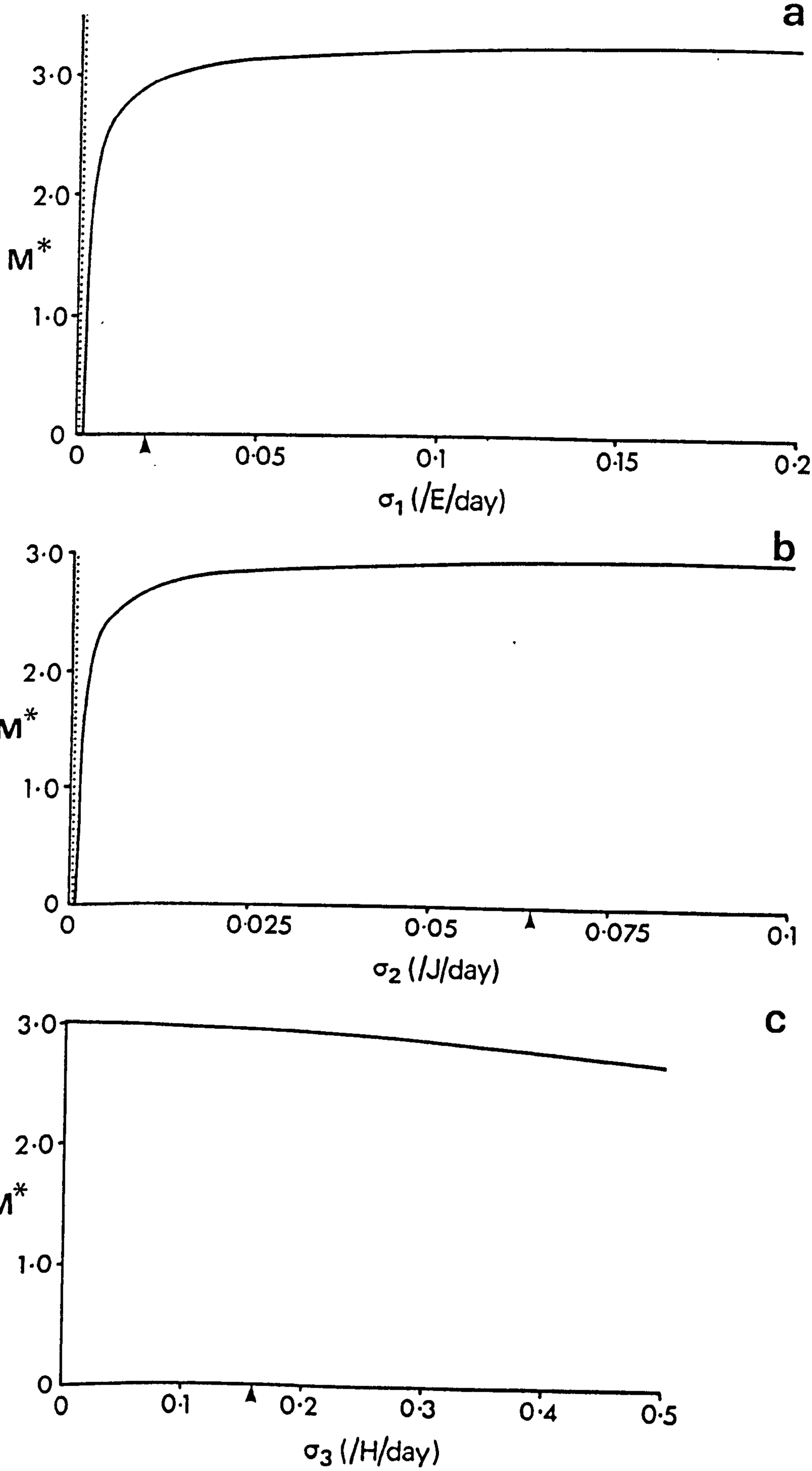
The predictions of the basic model. The assumed parasite distribution was Poisson.

a The relationship between the instantaneous rate of egg hatching, σ_1 (/egg/day) and M*.

b The relationship between the instantaneous rate of juvenile development, moulting, mating and egg development, σ_2 (/juvenile/day) and M*.

c The relationship between the instantaneous rate of host pupation, σ_3 (/host/day) and M*.

The values of the other parameters are as given in table 4.1.1. The arrows indicate the estimated parameter values at 25°C. The shaded areas are regions of parasite extinction.


a
3·0
2·0
1·0
0
M*
0
0·05
0·1
0·15
0·2
σ1 (/E/day)
b
3·0
2·0
1·0
0
M*
0
0·025
0·05
0·075
0·1
σ2 (/J/day)
c
3·0
2·0
1·0
0
M*
0
0·1
0·2
0·3
0·4
0·5
σ3 (/H/day)

essential. This may be balanced against the magnitide of the mean time to hatching ($1/\sigma_1$), where a halving of σ_1 requires a doubling of the fifty-one day mean time to hatch (section 2.8), which is already considerable. In this context, it is of relevance to note that the extinction threshold, under the prevailing conditions, occurs with a very small value of σ_1 corresponding to a mean development time of approximately 1½ years.

The relationship between the rate at which juveniles develop to adults, moult, mate and develop eggs (σ_2), and the value of M*, is very similar to that shown to occur between σ_1 and M* (cf. figures 4.1.6a and b). The same arguments apply as have been demonstrated for σ_1, with the exception of the need for highly accurate parameter estimation; the estimated value of σ_2 occurs well up on the plateau of the M*-σ_2 relationship (figure 4.1.6b). The value of σ_2 estimated was, however, maximal, with the delay between moulting and mate-finding assumed to be effectively zero, indicating a minimum time for mating (appendix V). The field value of σ_2 would, therefore, be expected to be significantly lower, due to the time required for mate-finding, although it may be presumed that this also occurs whilst the nematodes are still juveniles.

In the absence of further information, the rate at which the host develops, σ_3 per host per day, might be expected to be an important parameter in determining the level of parasitism. However, figure (4.1.6c) shows this to be an erroneous supposition; variation in σ_3 has little influence upon the predicted value of M*. This is probably due to several factors but will be principally determined by the efficiency of transmission. In this case, the density of infective stages (given the level of M* attained), will be relatively high; reduced levels of M* will not necessarily result in lower numbers of female nematodes, due to the environmental sex determination (see figure 2.6.5). Thus, with high densities of infective stages there would be an associated

high probability of infection of hosts early in their lives. There would, however, be more complex changes, due to the extended life-span of infected hosts and also, the net parasite-induced host mortality rate (δP).

Changes in σ_3 will mostly result from changes in ambient temperature, which, as previously stated, will result in other parameter changes. The value of σ_3 will also be determined by the species of host and such factors as food availability and host crowding.

The rate of infection.

As discussed in section 2.4 (ii), the rate of infection is one of the most difficult of all parameters to estimate, due to the complexity of the transmission process and the potential for many rate limiting factors to affect it. That the estimate of the transmission coefficient, β, should fall so close to the parasite extinction zone (figure 4.1.7) provides some cause for concern. However, the value of β is a minimum estimate for these conditions (section 2.4 (iii)).

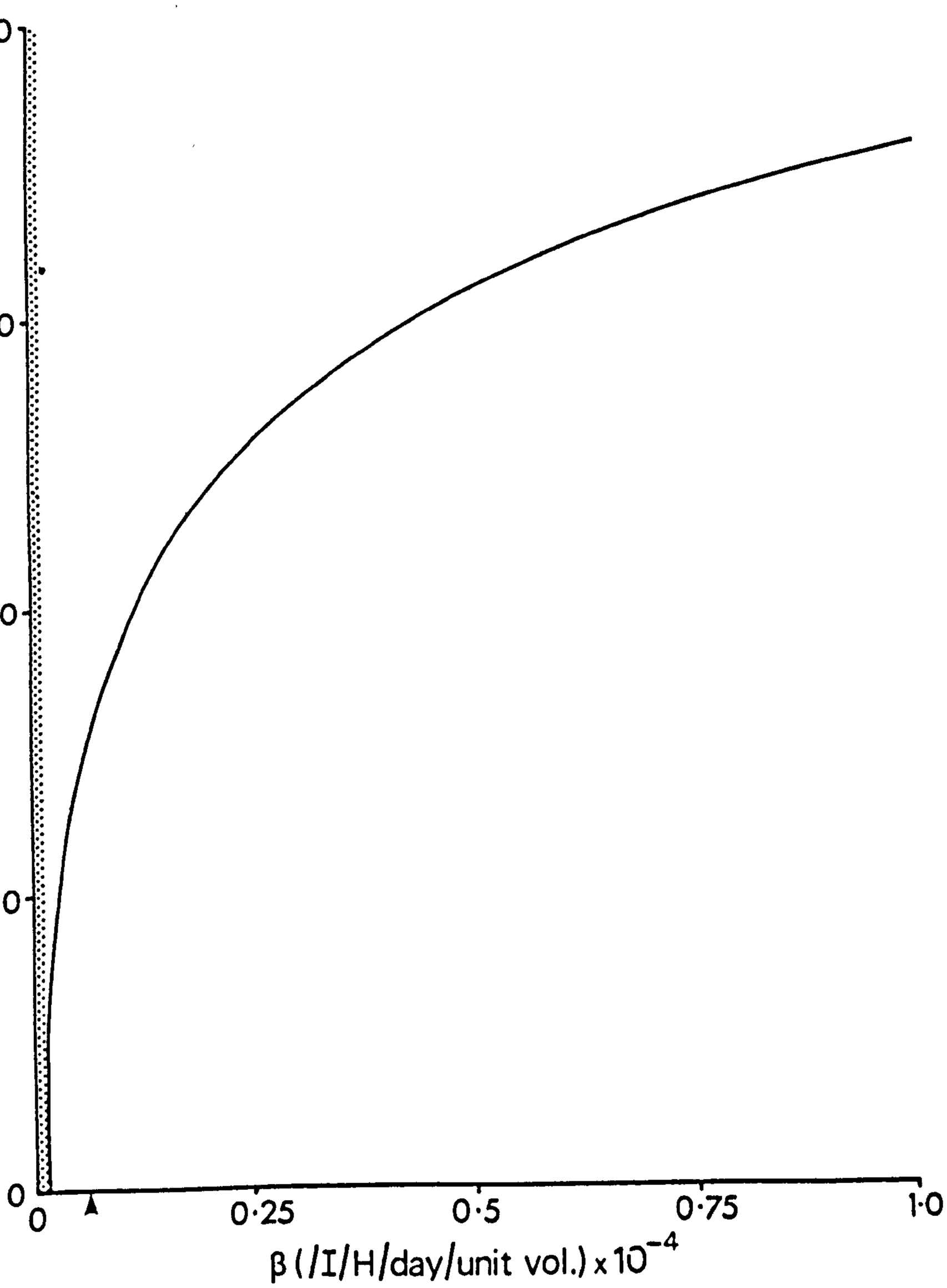
Clearly, the rate of infection is of major importance. In particular, an increase in β would cause a marked increase in M^*, up to values not attainable by altering other parameters in isolation. Factors which affect the magnitude of β will, therefore, be of major importance in determining the size of the parasite population, and thus the degree of depression of the host population.

It is apparent, that factors which affect the efficiency of transmission must be fully investigated. This is true, not only with respect to the dynamics of the parasite population, but also in terms of the biological control potential of the nematode. The latter point applies irrespective of whether the mode of application is a 'classical' single introduction to depress the host populaion, or an inundative release as a biological insecti-

Figure 4.1.7 The influence of the instantaneous rate of infection, β (/host/infective stage/7.5 litres/day) on M*.

The predictions of the basic model. The assumed parasite distribution was Poisson.

The values of the other parameters are as given in table 4.1.1. The arrow indicates the estimated value of β at 25°C. The shaded area is a region of parasite extinction.


8·0
6·0
4·0
2·0
0
M*
0
0·25
0·5
0·75
1·0
β (/I/H/day/unit vol.) x 10⁻⁴

cide. Both these strategies are dependent upon the successful infection of hosts, a process largely controlled by the magnitude of β.

Birth and immigration rates.

In the model, the only true birth rate is that of the parasite, when the eggs are released by the females. As may be seen in figure (4.1.8a), this rate may exert a significant influence upon the level of M* attained. The existence of a large extinction zone reflects the degree of uncertainty of an egg surviving to form the next generation of adults; principal losses occur during the period spent as an infective stage.

The biology of egg production in this parasite is somewhat unusual in that the eggs are produced from stored nutrients by non-feeding adults (section 2.8). Thus, there is a maximum number of eggs which can be produced by any given female, principally determined by her size and age at mating. An increase in the number of eggs produced could occur by an increase in the mean size of females or in their metabolic efficiency, changes only likely to occur on an evolutionary time scale. Clearly, there is little scope for change in the birth rate (except with temperature), although the mean egg output per female may well be reduced by a high female mortality rate,such that females died before completion of oviposition. Reduction in egg output may also be caused by a long pre-mating time delay, during which the female will use resources which might otherwise be directed into egg production. A reduction in mean egg output per female might be expected at high values of M*, due to an increased proportion of smaller females coming from multiply infected hosts. There is little reason to suppose that the mean birth rate would decline, except due to adverse environmental conditions.

It may thus be seen, that although the per capita birth rate of eggs per unit time may have an influence upon the value of M*,

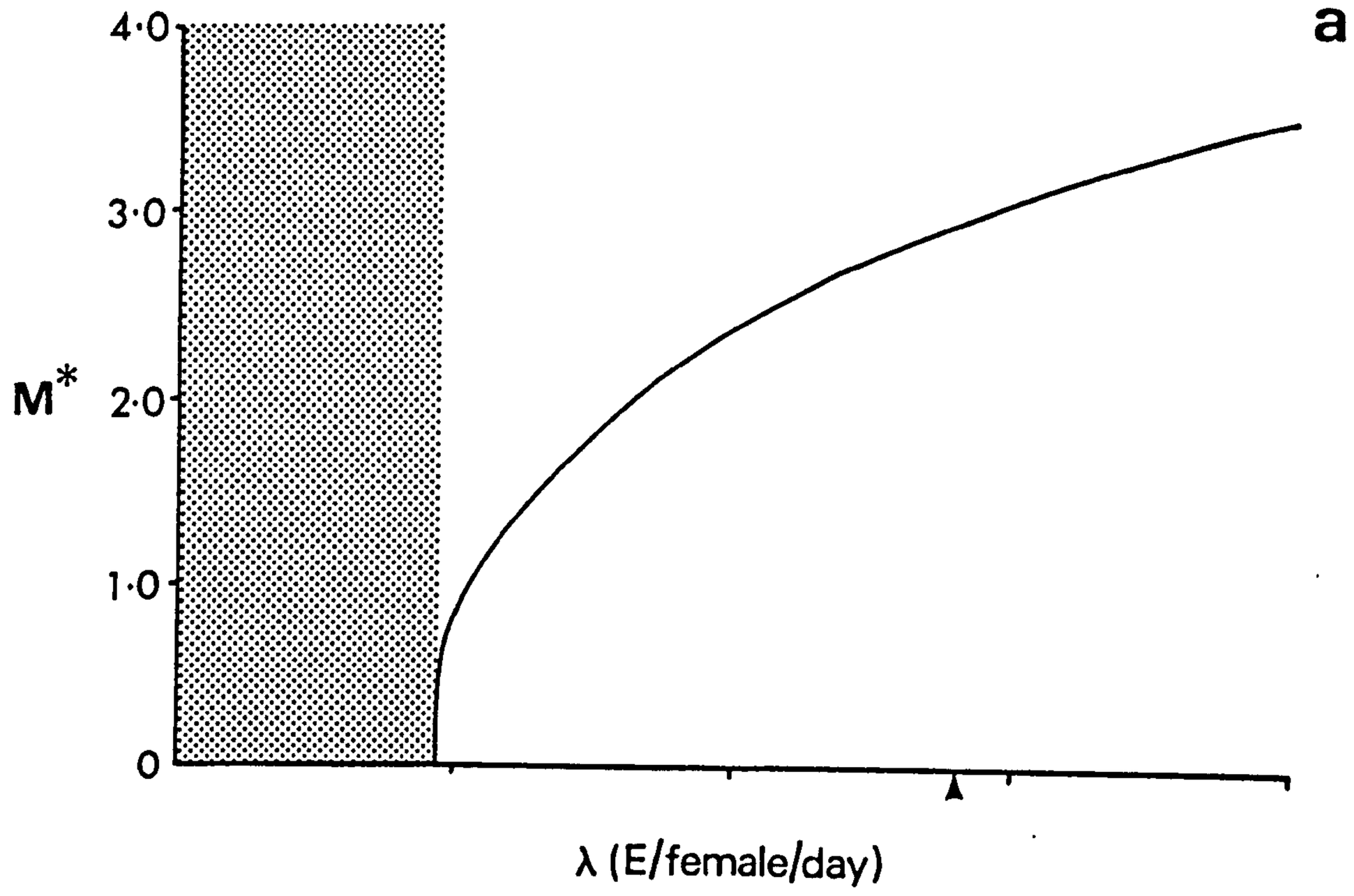
Figure 4.1.8 The influence of input factors on the magnitude of M* : λ and Λ .

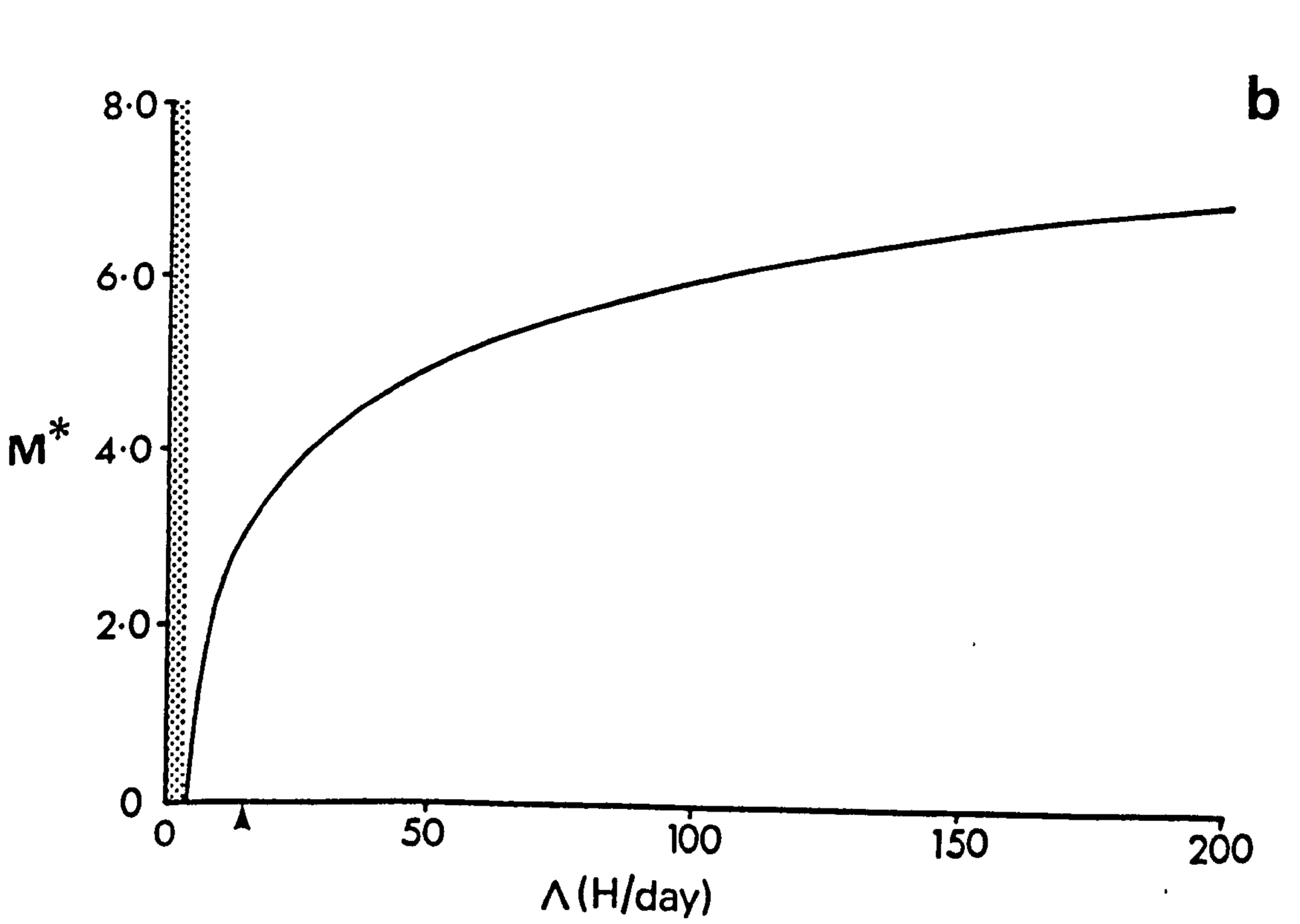
The predictions of the basic model. The assumed parasite distribution was Poisson.

a The relationship between the birth rate of nematode eggs, λ (eggs/female/day) and M*.

b The relationship between the host immigration rate, Λ (hosts/day) and M*.

The values of the other parameters are as given in table 4.1.1. The arrows indicate the estimated parameter values at 25°C. The shaded areas are regions of parasite extinction.


a
4·0
3·0
2·0
1·0
0
M*
λ (E/female/day)


b
8·0
6·0
4·0
2·0
0
M*
0
50
100
150
200
Λ (H/day)

as depicted in figure (4.1.8a), the effect in the field is unlikely to be of major importance.

The immigration rate of hosts, Λ (per day), although essentially an artificial process, because of the lack of feedback between the rate of immigration and the emergence of adult mosquitoes (as would occur with a true birth process), may be viewed as the birth process of a population of hosts at equilibrium. A field interpretation might be the input of eggs into a (relatively) isolated breeding site from a much larger reservoir population of mosquitoes.

The rate at which new hosts enter the environment is, clearly, of importance to the parasite and greatly affects the level of M^* attained (figure 4.1.8b). The increase in the level of M^* with an increase in the rate of host immigration is due to the proportionally greater net rate of infection, as defined in equation (4.1.11). This is based upon the assumption of direct proportionality between the host density and the number of parasites acquired in a given time (section 2.4 (ii)), an assumption which is probably unjustified in the light of the results presented in section 2.4 (v). The eventual plateau seen in the value of M^* with increasing Λ is as a result of the density-dependent processes, acting upon the parasite population (see section 4.2).

One further point of note relates to the extinction zone. If the rate of host immigration (or birth) falls below the threshold for parasite persistence, then the parasite will go extinct and the host population attains a level undepressed by the parasite (section 3.1). This has profound implications for the use of the parasite in an integrated control programme, in conjunction with other pathogens, insecticides or insect growth regulators. The depression of the host to a level such that the input of new hosts is below this threshold, would result in the loss of the parasite from the system (Hominick & Tingley, 1982).

4.2 DENSITY-DEPENDENT REGULATION OF THE PARASITE POPULATION

If a population is to attain a state of (dynamic) equilibrium, there is a requirement for some form of density-dependent (i.e. negative feedback) regulation to act upon it. Generally, the precise nature of the regulator is immaterial and may take many forms; competition for limited resources, increased risk of infection, the attraction of predators to areas of high prey density and, particularly in the case of parasites, density-dependent increases in parasite-induced host mortality and parasite-induced host immune responses.

The mode of action of the regulatory process may take one (or a combination) of three forms: an increase in the mortality rate of the population, a decrease in the birth rate or an increase in the level of dispersal. Examples of these three forms of regulation may be found both in the ecological literature (Bakker, 1961; Varley et al., 1973; Boag, McCourt, Herzog & Alway, 1979; Dixon, 1979), and the parasitological literature (Randolph, 1975; Kennedy, 1975; Anderson & May, 1978; Khalil, 1979; Burn, 1980).

In considering the possible ways in which populations of the mermithid R. culicivorax may be regulated, several potential regulators have been identified. Each of these potential regulators relies upon the effect of a given process occurring at an increasing rate as the parasite population density increases. The developmental rate of the nematode was found to vary with the mean parasite burden per host (section 2.5), as were the parasite-induced premature host mortality (sections 2.5 and 3.2) and the sex ratio of the parasite population (section 2.6). In addition, the rate of the instantaneous rate of infection, β, (/host/infection stage/unit vol./unit time) was shown to vary with the density of hosts in the environment (section 2.4 (iv)). The last of these four was not investigated further, due to the unavailability of suitable (i.e. long exposure time) estimates of β for differing host densities.

The development rate of the parasitic stage of R. culicivorax

was shown to increase with an increasing mean parasite burden per host. This is likely to act as a positive feedback, by shortening the mean development time and promoting an increase in the mean parasite burden. However, two factors act to mitigate this effect. First, the change in the development rate is very small and thus affects the parasite development time only marginally (figure 2.5.5). Second, proportionally more males than females will experience an increase in the development rate, since it is these that predominate at the higher individual parasite burdens.

Density-dependent parasite-induced premature host mortality, where each parasite is assumed to exert a constant level of pathogenicity (which is cumulative as the parasite burden increases), has the potential to regulate the parasite population. The regulation will appear as a reduction in the mean parasite burden per host and a reduction in the degree of parasite dispersion amongst the hosts. Although there will be a reduction in the mean parasite burdens of the current generation, the effect of preferentially eliminating hosts with high parasite burdens will cause the majority of parasites that die to be male. Thus, the long term regulation of the parasite population may not be as marked as might otherwise be expected.

The environmental sex determination has already been shown to be a regulatory process of major importance (section 2.6). It may also be seen, that the environmental sex determination operates to a greater extent than the parasite-induced premature host mortality at lower mean parasite burdens. It is, therefore, probable that this method of sex determination will form the major density-dependent, regulatory control upon the parasite population density.

The basic model was altered to accommodate each of the density-dependent processes in turn. The other parameters were made constant. Using the daily host immigration rate, Λ, (hosts/day) as the independent variable, the effect of the different regimes was assessed.

With no density-dependent constraint upon it, the parasite population density (measured as the equilibrium mean parasite burden per host, M*) increased linearly with an increasing host immigration rate (figure 4.2.1a). This represents unregulated population growth. It may be contrasted with the situation when all the density-dependent constraints are operative (figure 4.1.8b).

Where the parasite emergence rate, (measured in terms of the emergence-induced host mortality rate, α /host/day , and the statistical distribution of the parasites: see section 3.2), was the only variable influenced by the density of the parasite population, there was growth in the parasite population in an unregulated manner, identical to that seen in figure (4.2.1a).

Figure (4.2.1b) depicts the situation when the parasite-induced premature host mortality acts in isolation. There is an obvious regulation of the parasite population, although the magnitude of the equilibrium mean parasite burdens obtained are very large. The reason why the regulatory effect of this variable is so limited, is twofold. Firstly, the basic level is set by the value of δ, the pathogenicity of the parasite to the host. Secondly, the relationship between the parasite burden and the parasite-induced host mortality rate has been assumed to be linear. The effect, if this relationship is assumed to be non-linear, is explored in section 4.4. The degree of regulation attributable to the pathogenicity of the parasite does, however, fall far short of that required to maintain the mean parasite levels seen in figure (4.1.8b). This remains true even when the (constant) sex ratio is highly biased in favour of male nematodes (figure 4.2.1b).

Finally, when the effect of density-dependent environmental sex determination is modelled, in the absence of the previous density-dependent constraints, the parasite population may be seen to be regulated to a level such that the values of M* are slightly greater than those predicted by the basic model (cf. figures 4.2.2 and 4.1.8b). This is a clear indication that the environmental

Figure 4.2.1 The influence of density-dependent regulation upon the relationship between the host immigration rate, Λ (hosts/day) and M*.

a The predictions of the model given no density-dependent regulation.

s = proportion of parasites that are female.
α = 0.1253/host/day
δ = 0.0000/host/parasite/day

The shaded area is a region of parasite extinction.

b The predictions of the model where the parasite-induced premature host mortality is the only density-dependent constraint upon the parasite population, assuming host mortality varies linearly with parasite burden.

s = proportion of parasites that are female.
α = 0.1253/host/day
δ = 0.0076/host/parasite/day

The values of the other parameters are as given in table 4.1.1.

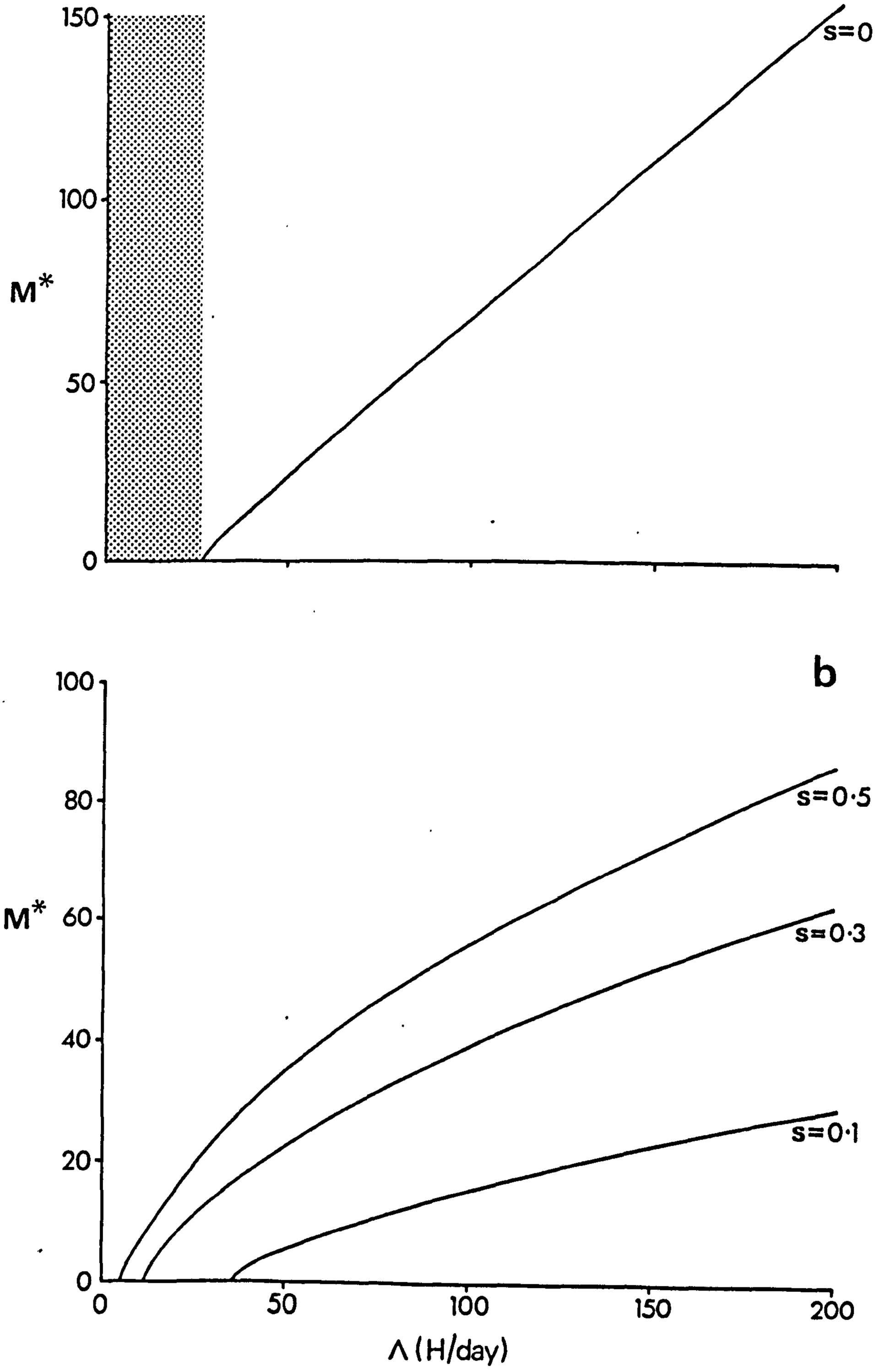

a
150
100
50
0
M*
s=0·1
b
100
80
60
40
20
0
M*
s=0·5
s=0·3
s=0·1
0
50
100
150
200
Λ (H/day)

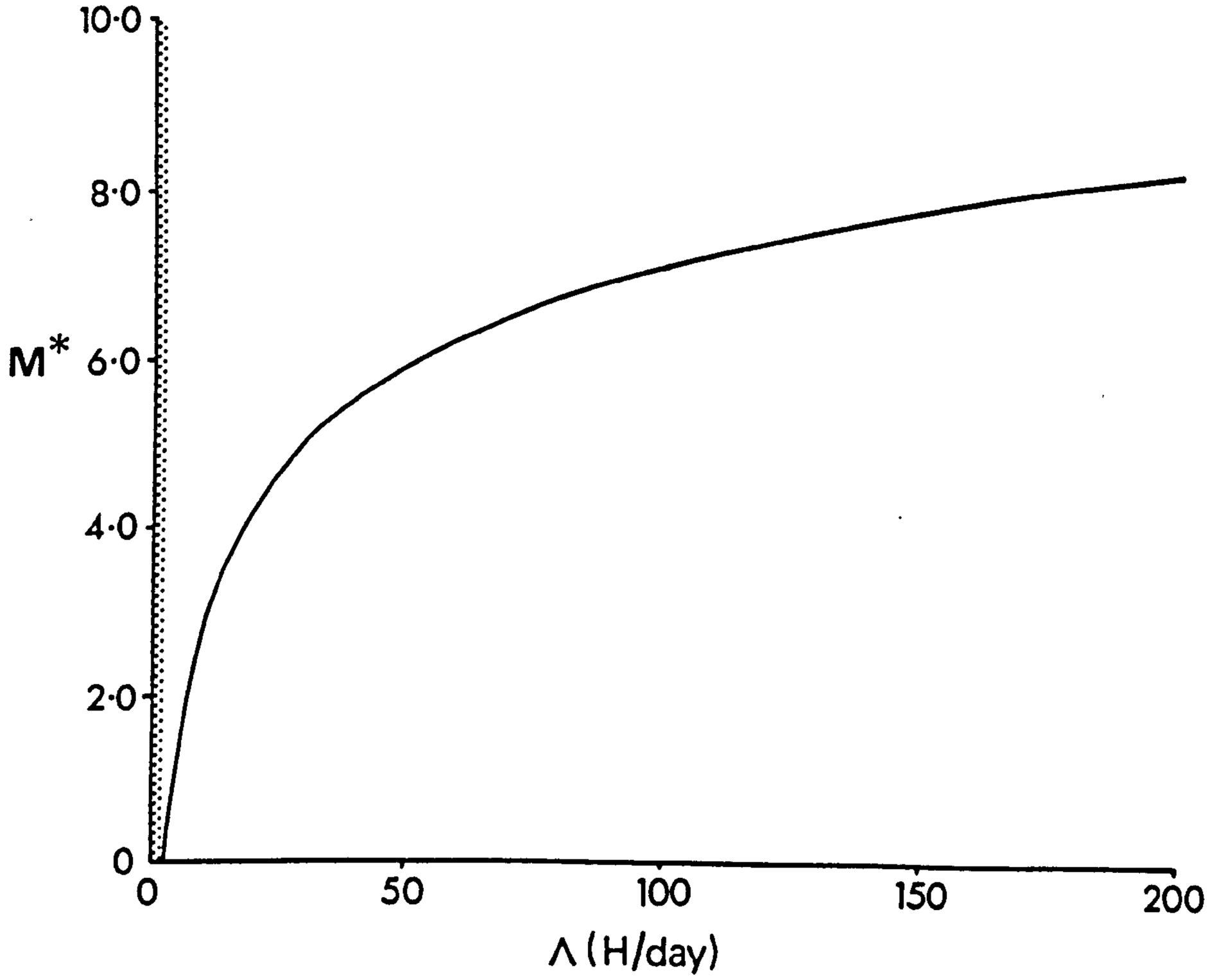
Figure 4.2.2 The influence of density-dependent environmental sex determination upon the relationship between the host immigration rate, Λ (hosts/day) and M*.

The predictions of the model where the environmental sex determination is the only density-dependent constraint upon the parasite population.

α = 0.1253/host/day

δ = 0.0000/host/parasite/day

The values of the other parameters are as given in table 4.1.1. The shaded area is a region of parasite extinction.


10·0
8·0
M*
6·0
4·0
2·0
0
0
50
100
150
200
Λ (H/day)

sex determination, shown by this parasite, is the major density-dependent regulatory control of parasite numbers. The difference in the levels of M*, seen in figures (4.2.2) and (4.1.8b), is attributable to the effect of the parasite-induced premature host mortality rate, acting in a density-dependent way, to reduce the parasite population still further.

There is one further parameter which is likely to show a degree of density-dependence; the rate of mating, where, the rate at which females are mated, would be expected to increase with increasing adult density. The form of the relationship would be expected to tend to be an asymptote, or to be sigmoidal; the plateau occurs due to the maximal rate of mating described in section (2.7(iv)). Lack of data prevents further exploration of this phenomenon, but it is unlikely to be of importance except when there is a very small adult population.

4.3 THE EFFECT OF THE PARASITE DISTRIBUTION

Overdispersion in the distribution of parasites within populations of hosts is now generally accepted to be the norm. Examples of overdispersed distributions, frequently described by the negative binomial distribution, have been found in all the parasite groups examined. These include the digenea (Pennycuick, 1971; Anderson, Whitfield & Dobson, 1978; Scott, McLaughlin & Rau, 1979); the nematodes (Li & Hsü, 1951; Welch, 1960; Phelps & Defoliart, 1964; Petersen et al., 1968; Tsai & Grundmann, 1969; Homimick & Welch, 1971; Galloway & Brust, 1976); the cestoda (Rau, 1979; Keymer & Anderson, 1979); the acanthocephala (Pennycuick, 1971; Crofton, 1971a); the crustacea (Boxshall, 1974) and the arachnida (Randolph, 1975).

The reasons for, and consequences of, overdispersion in the statistical distribution of parasites amongst hosts are manifold. Factors which tend to generate or promote overdispersion in parasite distributions include the spatial aggregation of infective stages within the environment, heterogeneity in the susceptibility of the host to infection and in the infectivity of the infective stages, and also numerous wavesof random infection in a host population. This area has recently been reviewed by Anderson & Gordon (1982).

The influence a particular distribution has upon the dynamics and ecology of the parasite population, and also upon the stability of the host-parasite interaction, has been shown to be profound (Crofton, 1971b; May, 1977a; Anderson,1978a & b, 1979a; Anderson & May, 1978; Anderson & Gordon, 1982). The principal effect of overdispersion is to stabilise the host-parasite interaction, with a tendency also to reduce the domain of parameter space where parasite persistence can occur. Reduction in the level of parasite-induced host population depression may also occur as the degree of overdispersion increases (see section 3.2).

The predictions of the basic model so far presented, have been based upon the assumption of a Poisson (random) distribution of parasites within the host population. Although random distributions have been shown to occur occasionally (Galloway & Brust, 1976), the majority of both laboratory and field data show mermithids to be overdispersed amongst their hosts (see earlier references and section 2.4 (ii)). A good empirical fit to the overdispersed frequency distributions seen,is given, in most instances, by the negative binomial model, which is commonly used to model parasite frequency distributions (Pennycuick, 1971; Crofton, 1971a).

The effect of the degree of overdispersion (as measured by the parameter k of the negative binomial distribution) upon the equilibrium mean parasite burden per host (M*), was investigated for two of the principal rate parameters. Those parameters which proved most difficult to estimate were used as the independent variables; the transmission coefficient, β, (/preparasite/host/unit volume/day) and the index of parasite pathogenicity to the host, δ, (/parasite/host/day). The assumption of a relationship of direct proportionality between the parasite burden and the rate of parasite-induced premature host mortality was maintained. Another major assumption was that the distribution of parasites, once set, was constant, irrespective of the value of the mean parasite burden.

The change in the assumed distribution of the parasites from Poisson to negative binomial required slight alteration to the model. The p(0) term of the statistical distribution (the zero frequency class), which appears in equations (3.2.3) and (4.1.18), becomes,

$$(1 + M/k)^{-k} \qquad (4.3.1)$$

where k is the dimensionless parameter of the negative binomial

distribution. In addition, the expectation of the sum $\Sigma i^2.p(i)$ which appears in equation (4.1.8) becomes $M^2_{(t)}(k+1)/k+M_{(t)}$ (Anderson & May, 1978).

The predictions of the model when the parasites are distributed in a negative binomial form are presented in figure (4.3.1a and b). There are two main features associated with an increase in the level of parasite aggregation. Firstly, there is, under otherwise constant conditions, a decline in the equilibrium mean parasite burden per host, M*. Secondly, the domain of parameter space that enables parasite persistence to occur is diminished. The observed reduction in M* is the sum of two processes: the increase in the level of aggregation results in the density-dependent sex determination becoming severe at lower values of M* and so leads to a reduced reproductive output by the parasite population; this is associated with an increase in parasite mortality due to the pathogenicity of the parasite to the host.

The reduction in the parameter space that allows parasite persistence, seen in figure (4.3.1), is dependent upon the same processes as the reduction in M*. However, the effect will be to delimit the range of the level of overdispersion at which the parasite can occur. High degrees of overdispersion ($k \leq 0.1$) either fail to allow parasite persistence or permit such a small parasite population that extinction becomes highly probable. It may be predicted, therefore, that such levels of parasite aggregation will seldom be seen in the field. Table 2.6.1 shows field estimates of k which vary between 0.47 and 3.96, which supports this prediction of the model.

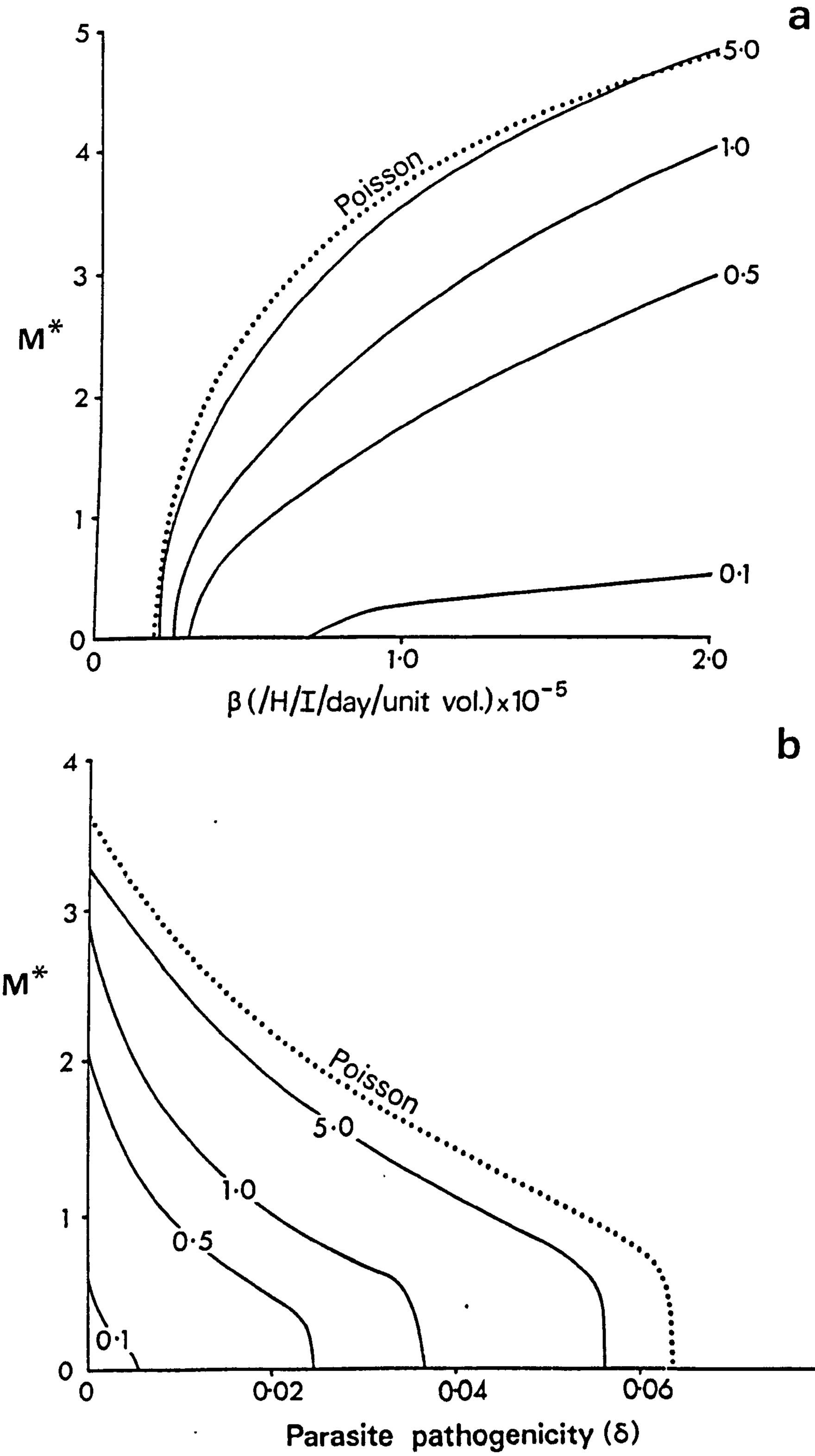
Figure 4.3.1 The influence of the statistical distribution of the parasites upon M*.

The dotted lines are for the Poisson distribution and the solid lines are for the negative binomial distribution given fixed values of k.

a The relationship between the instantaneous rate of infection, β (/host/infective stage/7.5 litres/ day) and M* for a range of degrees of parasite aggregation.

b The relationship between parasite pathogenicity, (δ) and M* for a range of degrees of parasite aggregation.

The values of the other parameters are as given in table 4.1.1.


a
5
4
3
2
1
0
M*
Poisson
5·0
1·0
0·5
0·1
0
1·0
2·0
β (/H/I/day/unit vol.) x 10⁻⁵
b
4
3
2
1
0
M*
Poisson
5·0
1·0
0·5
0·1
0
0·02
0·04
0·06
Parasite pathogenicity (δ)

4.4 NON-LINEAR PARASITE-INDUCED HOST MORTALITY

The predictions of the magnitude of M* by the model in the previous sections have, in part, stemmed from the assumption that the relationship between host mortality and parasite burden is linear, i.e. δi. This assumption was based upon experimental evidence presented in sections (2.5) and (3.2). However, it has been shown that some species of parasite, induce host mortalities in a manner more severe than linear. These non-linear relationships often conform to a power law (see Anderson & May, 1978; Anderson, 1979). As few mermithids have been studied to show the relationship between the parasite burden and the host mortality rate, it is entirely possible that non-linear relationships of this type may occur. Pending such investigations, the effect of non-linearity in parasite-induced premature host mortality, will be explored for the C. p. fatigans - R. culicivorax association.

If it is assumed that the host mortality rate varies as the square of the parasite burden (i.e. δi^2), then the expectation of the sum in equation (3.2.1) becomes $M_{(t)}+M^2_{(t)}$ (Anderson & May, 1978). Thus, the per capita rate of host mortality is altered from δM^* to $\delta(M^*+M^{*2})$ for the Poisson distribution and to $\delta(M^*+M^{*2}(k+1)/k)$ for the negative binomial distribution.

The change from a linear to a power relationship between parasite burden and host mortality also results in a change in the net rate of parasite mortality, a change attributable to the increased mortality of heavily infected hosts. Equation (4.1.8) becomes,

$$\delta H_{(t)} \sum_{i=0}^{i=\infty} i^3 . p(i) \qquad 4.4.1$$

where the expectation of the sum (as given by Anderson & May, 1978) is $M_{(t)}{}^3 + 3M_{(t)}{}^2 + M_{(t)}$ for the Poisson distribution and

$M_{(t)}^{3}(k+1)(k+2)/k^2 + 3M_{(t)}^{2}(k+1)/k + M_{(t)}$ for the negative binomial distribution.

As in the previous section, two independent variables were assessed for their effect upon M* for this model: β, the transmission coefficient and δ, the pathogenicity of the parasite to the host. Figure (4.4.1a) depicts the predicted values of M* given a range of values of β and different degrees of parasite aggregation. The form of the relationship between M* and the degree of aggregation takes the form now familiar: decreasing M* with increasing aggregation. The domain of parameter space within which parasite persistence may occur is also substantially reduced as a result of the non-linear parasite-induced premature host mortality (cf. figures 4.3.1a and 4.4.1a). This agrees with results presented by Anderson & May (1978) and Anderson (1978b, 1979a and 1980) for more generalised models. It may also be assumed, that the smaller domain of parameter space is associated with an increased stability to perturbation, as seen in the more generalised models.

The same observations may be made with respect to the relationship between δ and the magnitude of M* (figure 4.4.1b). As δ, the measure of parasite pathogenicity, is the parasite rate parameter most directly involved with the induction of premature host mortalities, the reduction in the parameter space within which the parasite can persist is very marked in this case.

The effects upon the parasite population and its dynamics by the occurrence of non-linear parasite-induced host mortality will, in general, be the same as those seen for increasing levels of overdispersion; lower levels of overdispersion, smaller parasite populations and a greater probability of chance extinctions. In terms of the potential of the nematode as a control agent of mosquitoes, the severe density-dependent constraint that this places upon the parasite will tend to enable the host to evade any significant depression in its population density (see Anderson, 1980).

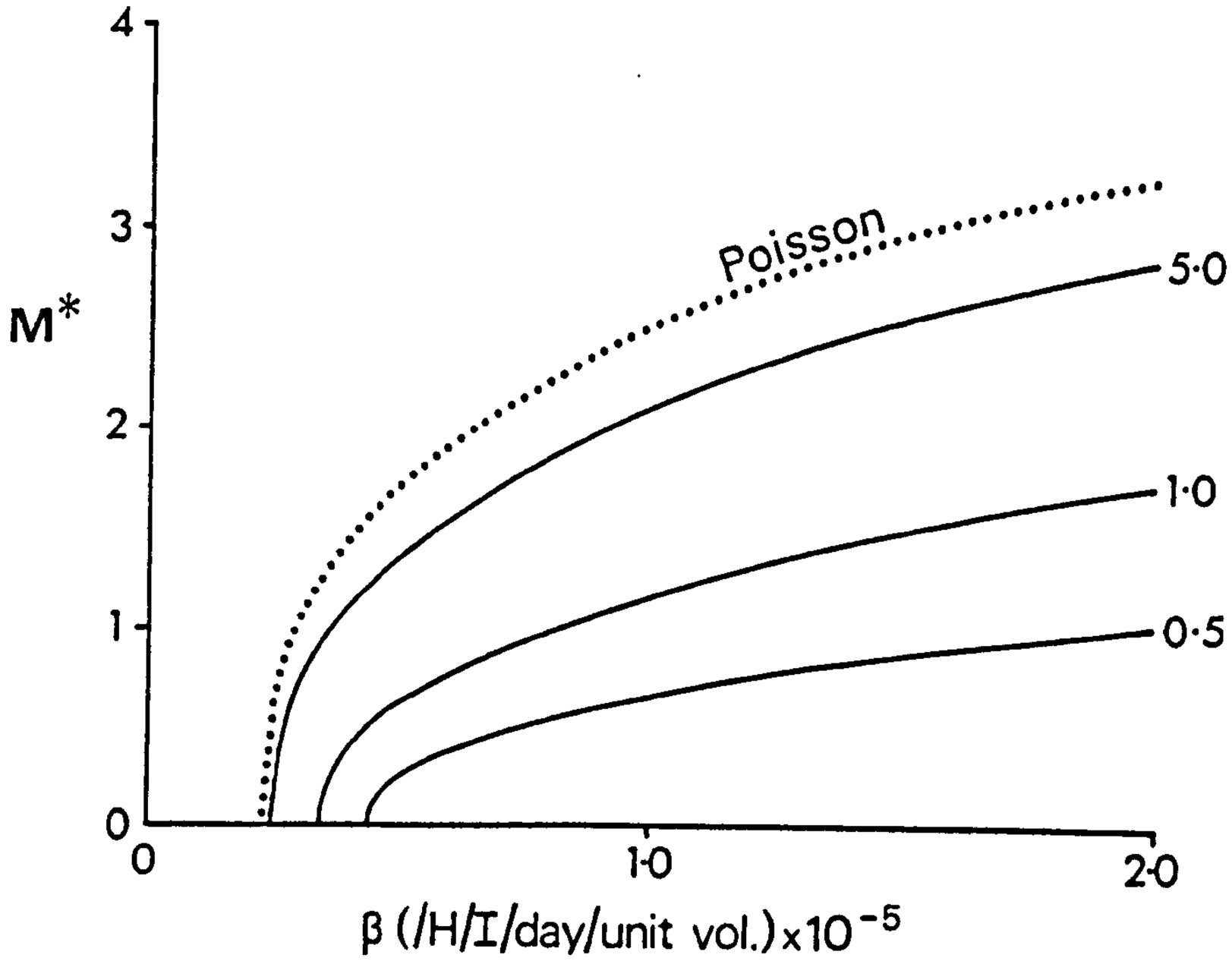
Figure 4.4.1 The influence of non-linear parasite-induced (premature) host mortality on M*.

The dotted lines are for the Poisson distribution and the solid lines are for the negative binomial distribution given fixed values of k. Host mortality was assumed to vary as the square of the parasite burden (see text).

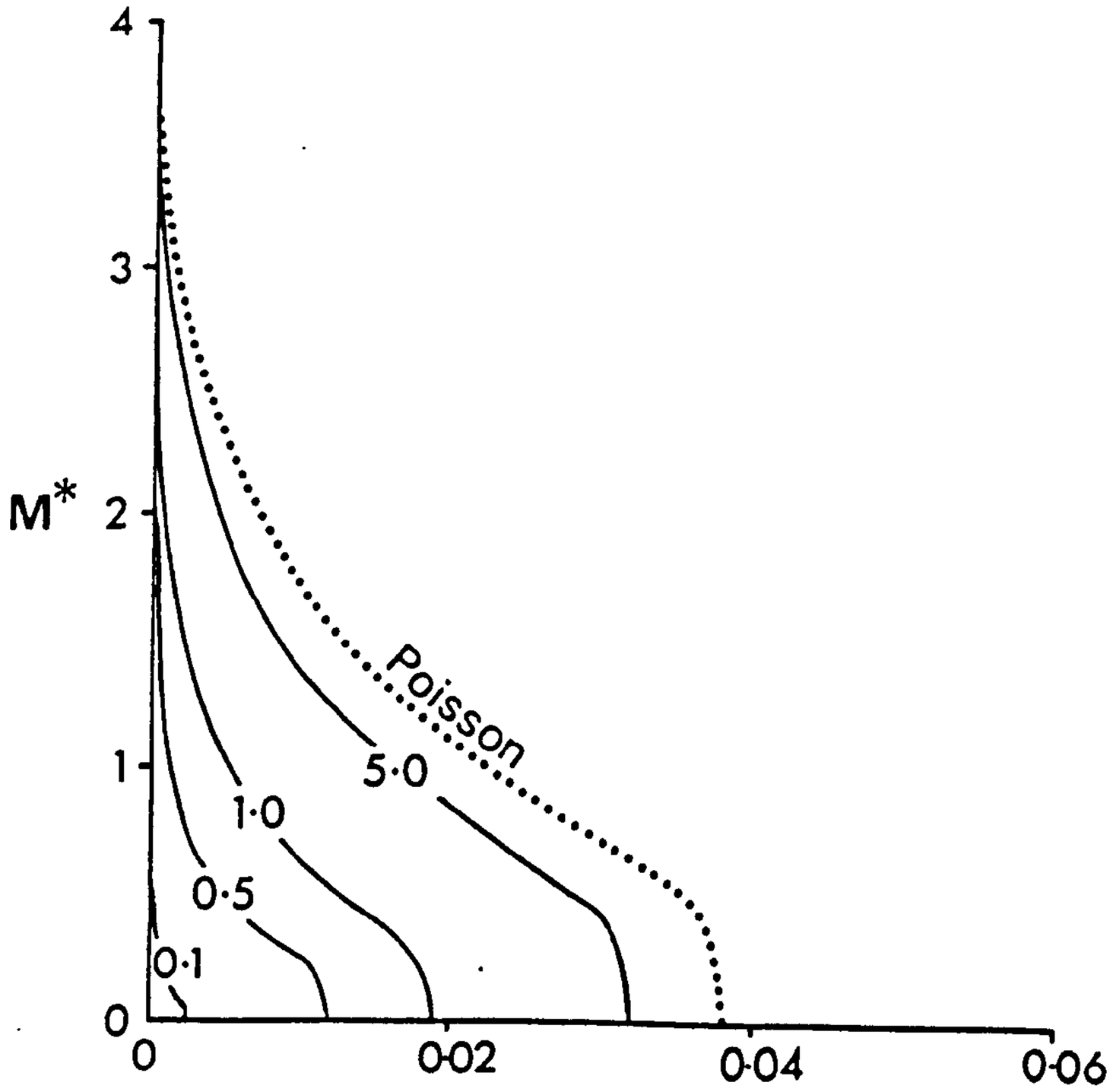
a The relationship between the instantaneous rate of infection, β (/host/infective stage/7.5 litres/day) and M* for a range of parasite aggregation.

b The relationship between parasite pathogenicity, (δ) and M* for a range of parasite aggregation.

a


4
3
2
1
0
M*
Poisson
5·0
1·0
0·5
0
1·0
2·0
β (/H/I/day/unit vol.)x10⁻⁵

b


4
3
2
1
0
M*
Poisson
5·0
1·0
0·5
0·1
0
0·02
0·04
0·06
Parasite pathogenicity (δ)

The effects of the non-linear relationship between host mortality and parasite burden upon M* (depicted in figure (4.2.1))are in addition to the density-dependent constraints imposed by the environmental sex determination. If the sex ratio of the parasite is made constant, together with the rate of parasite emergence-induced host mortality (α/host/day) (as in section 4.2),the regulatory effect of the non-linear parasite-induced premature host mortality can be assessed. A comparison of figures (4.4.2) and (4.2.1b) show a substantially improved regulatory performance of this mortality factor when the non-linearity is present. This is associated with only a slight reduction in the parameter space permitting parasite persistence. This illustrates the small effect the change in mortality has upon the host population.

Figure 4.4.2 The influence of non-linear parasite-induced (premature) host mortality on M* when there are no other density-dependent constraints.

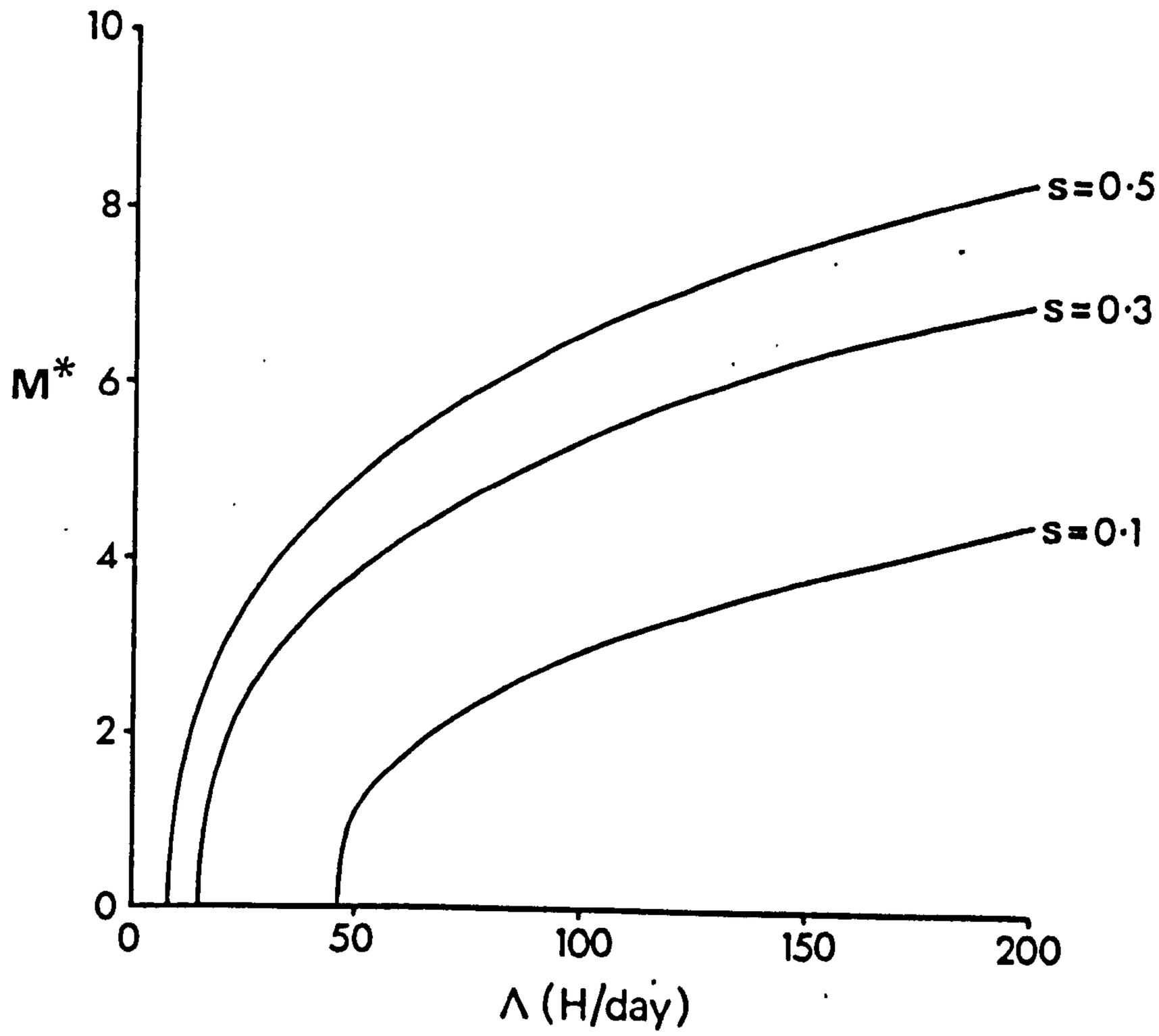
The relationship between the host immigration rate, Λ (hosts/day) and M*. The distribution of parasites was Poisson. Host mortality was assumed to vary as the square of the parasite burden.

s = proportion of parasites that are female.

α = 0.1253/host/day

δ = 0.0076/host/parasite/day

The values of the other parameters are as given in table 4.1.1.


10
8
6
4
2
0
M*
0
50
100
150
200
Λ (H/day)
s=0·5
s=0·3
s=0·1

CHAPTER 5 : THE USE OF NEMATODES AS AGENTS OF BIOLOGICAL CONTROL, WITH EMPHASIS UPON MOSQUITO CONTROL.

5 THE USE OF NEMATODES AS AGENTS OF BIOLOGICAL CONTROL, WITH EMPHASIS UPON MOSQUITO CONTROL.

The control of pest species has long occupied the thoughts of men, irrespective of whether the pests are agricultural, veterinary or agents of human disease. Successful methods of control have largely been found by trial and error over long periods of time, with the success of particular methods usually measured subjectively. This trend has recently been challenged, by the use of a more scientific methodology, both in the development of control measures (Croll, Anderson,Gyorkos & Ghadirian, 1982) and in the evaluation of success (Morris & Meek, 1980). The quantification of the measurement of success has principally been restricted to areas of direct economic interest, particularly in the agricultural and veterinary fields (Pantelouris, 1965). In this context, it is of interest to note,that the vast majority of chemical pesticides have been found by trial and error methods.

The methods of controlling unwanted organisms (pests) currently in use are many, but principal amongst them is the use of chemicals, both of artificial and natural origin. The chemicals function by exerting a toxic effect upon the target organisms, an effect frequently eliminated by the pests as they develop 'resistance' to the pesticide. The problem of resistance, allied with the adverse effects of pesticides upon non-target organisms (see Carson, 1962; Graham, 1970) have resulted in a growing interest in alternative methods of control. These include the resurrection and design of management techniques (e.g. crop rotation, inter-cropping systems, the removal of animals to uncontaminated pasture and reduction in water contact for diseases such as schistosomiasis) and the use of predators and parasites to suppress pest abundance.

The recent resurgence in the investigation of methods of biological control has, to a great extent, been funded by the World Health Organization (WHO), principally aimed at medically important vectors of human disease (see Arata, Chapman, Cupello, Davidson, Laird, Margalit and Roberts, 1978).

At this point it is necessary to distinguish between the methods of use of biological control agents. There are two basic modes of use, the first is the single introduction, where the parasite or predator is introduced and becomes established within the environment. There is a resultant reduction in the abundance of the target organism which is sustained; the use of myxomatosis to control rabbits in Australia is a classic example (Waithman, 1979). The reduction in the abundance of the pest will usually occur to a level below a nuisance, economic or, in the case of vectors, a transmission threshold (see Bedford, 1980). Failure to achieve sufficient suppression usually results in the abandonment of control attempts using that organism. Occasionally pest extinction will result following a biological control introduction, usually leading to the loss of the control agent as well. It may be necessary to repeatedly re-introduce a control agent, particularly in seasonal or periodically destroyed habitats (e.g. in glass houses and agricultural systems).

The second mode of use of biological control agents is as biological pesticides, where an innudative release results in the temporary extinction of the pest with little likelihood of the control agent becoming established (see Undeen and Colbo, 1980; Petersen, Chapman, Willis & Fukada, 1978).

The possibility of controlling mosquitoes (and other biting Diptera with aquatic larvae) with biological agents, has been suspected for some time, hinging upon either predation (Hinman, 1934; Laird, 1956) or use of parasites (Christophers, 1952; Laird, 1959). Much of the published literature is anecdotal in form, relating to predators observed consuming mosquito larvae (James, 1961; 1965; Spielman & Sullivan, 1974; Gerberg & Visser, 1978; Levy and Miller, 1979) or that larvae were found parasitized by a given agent at a particular site (Coluzzi & Rioux, 1962; Novak, 1967; Henry, 1981). There is, however, a growing data base, produced by controlled experiments and field studies, of the effectiveness of various predators and pathogens as control agents of mosquitoes. Examples of the investigation of predators include

turbellarians (George, 1978, 1979; Case & Washino, 1979) and fish such as Gambusia and *Poecilia* spp. (see appendix IV), whilst for parasites, Linley and Nielsen (1968a & b) report viral transmission amongst mosquitoes and Undeen and Colbo (1980) have shown the effectiveness of Bacillus thuringiensis var israelensis against simuliid flies. A major review of the use of parasites as biological control agents has recently been published and covers the use of viruses, bacteria, protozoa, trematodes, nematodes, fungi and insect parasitoids, as well as an assessment of the hazards associated with the use of microbial insecticides and an examination of the theoretical basis of the use of pathogens to control pests (Anderson & Canning, 1982).

The body of literature on nematodes as biological control agents is large and growing rapidly, but the subject suffers greatly from the highly confused state of the taxonomy of many of these organisms (see Curran, 1982). This problem has arisen primarily, due to the use of juveniles to identify species, problems associated with the lack of hard parts in nematodes and the 'plasticity' of their morphology.

It has apparently been generally accepted that any insect parasitic nematode has the 'potential' to be used as a biological control agent of the natural host or other insects, with little consideration of the biology of either host or parasite: an assumption, in the light of recent work, that was far too optimistic (Hominick & Tingley, 1982).

If the use of the nematode is to be as a biological insecticide, then only two aspects of the biology are of importance: (1) a life-cycle and physiology that allows mass production, preferably by in vitro culture and (2) the process of infection. For a sustained depression in host abundance far more information is required, and it must relate to the population biology of the host as well as that of the control agent.

There have been many reviews published within the last twenty years which document those groups and species of insect parasitic

nematodes that may be of use in controlling insect populations. Notable examples published recently, include the major work by Poinar (1979) which gives an in depth coverage of nine insect-parasitic nematode families and the more compact work by Petersen (1982). A series of papers in the Journal of Nematology has provided an interesting contrast to the more usual reviews, covering a wide variety of the important aspects of nematology as applied to biological control. This includes an assessment of the potential of mermithid and steinernematid nematodes (Gaugler, 1981; Molloy, 1981; Platzer, 1981; Nickle, 1981), parasite physiology (Gordon, 1981; Gordon et al., 1981), in vitro culture (Finney, 1981) and commercial development (Petersen & Cupello, 1981).

A feature of the majority of reviews is the strong element stressing the 'potential' of the nematodes as control agents, frequently with little supportive data. The lack of supportive data is principally due to the lack of success many of these nematodes have shown in depressing host populations in the field (see Poinar, 1979). There are, however, a few noteworthy exceptions, one of the earliest was the use of the Neotylenchid, Deladenus siricidicola, to control the woodwasp Sirex noctilo in Australia (Bedding & Akhurst, 1974). The nematode was introduced and led to a significant and sustained depression in the woodwasp population, resulting in the cessation of the problem of damaged trees. It is of interest, and probably of some importance in this case, that D. siricidicola is not an obligate parasite of woodwasps, but can survive by feeding upon a fungus, which is itself symbiotically associated with the wasp (Bedding, 1972).

One of the other nematode families which is currently thought of as a source of biological control agents, is the Steinernematidae. It is from within this family that another of the successful control agents may be found, this time used as a biological insecticide. The nematode Neoaplectana bibionis, was successfully used to suppress currant borers (Synanthedon tipuliformis) on blackcurrant cuttings (Bedding & Miller, 1981). The Steinernematidae and Heterorhabditidae are the two families that are likely to contain

the most successful of future nematode biological control agents. This is because: (1) their life-cycles show no obvious severe density-dependent regulatory constraints that will inhibit insect control, (2) the hosts die rapidly, due to the septicaemia caused by the symbiotic bacterial associates of the nematodes, (3) their large reproductive output, due to the completion of more than one generation within the host cadaver (thus each host will tend to produce the maximum number of nematodes that can develop upon the available nutrient), and (4) the nematodes from both families can be cultured in vast numbers in vitro on artificial media (see Poinar, 1979). It is relevant to examine some of the successes achieved by these nematodes; Poinar (1979) lists thirty-three field trials using N. carpocapsae against a wide variety of insects. Significant mortality was recorded in only about one third of the trials, but reduced pest damage was more frequently observed. Given suitable hosts and conditions compatable with their life-cycle and moisture requirements it is clear that, at least some of these nematodes may have a role to play in the control of certain insect pests.

The Mermithidae, the family within which R. culicivorax is placed, has formed a major area of study in looking for control agents. A principal reason for this, is that the hosts within which many mermithids develop are medically important vectors, including mosquitoes and simuliids (see Poinar, 1979) and also tse-tse flies (Poinar et al., 1981; Hominick, Croft and Kuzoe, 1982). In natural mermithid infections the prevalence can vary greatly, both between sites (Colbo & Porter, 1980) and with the season (Petersen & Willis, 1971; Ebsary & Bennett, 1975; Curran, 1982). The general trend is for low to moderate levels of prevalence (0-30%) to predominate, with occasional high levels the product of the chance coincidence of favourable factors.

Successful establishment of mermithids in the field, a pre-requisite for their use as long term biological control agents, has been successfully achieved on several occasions (Woodward, 1978; Nickle, 1979; Brown Westerdahl, Washino & Platzer, 1982).

It has, however, been shown that such establishment is unlikely to effect more than a marginal reduction in the adult mosquito population, despite even the relatively high levels of prevalence obtained (see sect. 2.6 & 3.2). Two further points are relevant: (1) that the levels of prevalence recorded were subject to overestimation (due to the extended life-span of infected hosts, as seen in figure (3.2.3a)) and, (2) in the establishment obtained by Woodward (1978), prevalence levels were higher in the cooler months, an observation that is in some respects counter-intuitive, but supported by the increased female bias in the sex ratio seen at lower temperatures (section 2.6(iii)).

Attempts to use R. culicivorax as an innundative, biological insecticide have also been made. Petersen and co-workers mass reared R. culicivorax in vivo and introduced the infective stages into mosquito breeding sites in El Salvador by spraying (Petersen, Willis & Chapman, 1978; Petersen, Chapman, Willis & Fukada, 1978; Willis, Chapman & Petersen, 1980). The results were of mixed success, with an initial 39% of Anopheles albimanus larvae infected. This was improved to 86% by altering the time of application of the infective stages, an indication of the requirement of biological control agents to have all factors in their favour. The introduction of infective nematodes led to a 94% reduction in the density of mosquito larvae(estimated by dipping) following the seven-week treatment period, but there was a clear indication of increasing larval density in the weeks following the cessation of nematode application.

Some success was also achieved by Levy & Miller (1977b and c) when they applied infective R. culicivorax to mosquito breeding sites in ditches, potholes and sewage settling tanks.

For nematodes such as R. culicivorax to form part of the arsenal for use against insect pests, it is a requirement that they be relatively insensitive to chemical pesticides used in the environment. This is particularly true if the intention is to use the nematode as part of an integrated control programme, a concept

whereby a range of control strategies are combined together (see Axtell, 1979; Olson, 1979; Steelman, 1979; Womeldorf, 1979; Bird, 1980; Bird & Thomason, 1980). It has been shown that R. culicivorax is tolerant of many of the insecticides and growth regulators currently in use against mosquitoes (Mitchell et al., 1974; Finney, Gordon, Condon & Rusted, 1977; Levy & Miller, 1977d).

Despite these successes there are distinct problems associated with the use of R. culicivorax as a biological control agent. Most important, is the lack of success in attempts to culture the worm in vitro (Finney, 1981; Chapman & Finney, 1982). This limits production to in vivo methods which are labour-intensive, expensive and have a restricted output. This, associated with problems in the shipment of eggs, and the size of the commercial market have been largely responsible for the failure of attempts to produce the parasite commercially (Petersen & Cupello, 1981: Petersen, 1982; Chapman & Finney, 1982; Service, 1983).

Another problem which may not be resolved, is the effect of predation upon the nematodes, which may occur at any stage during the life-cycle (Platzer and MacKenzie-Graham, 1978, 1980; see also appendix IV).

Given sufficient predator densities, such as might be expected in many mosquito breeding sites, predation may severely reduce any tendency of the parasite to depress the host population.

A factor frequently unconsidered in the assessment of parasites as biological control agents, is the possibility of resistance to the parasite being developed by the host. This does not normally happen (except upon an evolutionary time scale) because the selection pressure exerted by the parasite will frequently be small, and less than other ecological pressures; the parasite is also subjected to evolutionary pressures. However, under the high selective pressure, produced by constantly high parasite prevalence maintained by a control programme, resistance may develop. Immune responses

against R. culicivorax have been recorded in several species of mosquito, where melanization processes were involved in killing the nematodes once penetration had occurred (see Poinar, Hess & Petersen, 1979). Of greater potential importance, was the development of reduced susceptibility by C. p. quinquefasciatus when subjected to strong selection pressure exerted by R. culicivorax (Petersen, 1978a). The reduction in susceptibility, recorded as a decrease in prevalence, was not associated with an immune response and was presumably the result of a behavioural change in the host. Given continuously high levels of exposure, such a change in the field would rapidly eliminate the effect of the parasite in depressing the mosquito population.

In conclusion, therefore, there is a high probability of some insect parasitic nematodes becoming successful biological control agents. Romanomermis culicivorax is, however, unlikely to be one of these unless the in vitro culture barrier can be overcome, and then, given the results of this study (sections 2.9, 3.2 and Ch. 4) only as a biological insecticide, a conclusion arrived at by Hominick & Tingley (1982)and Service (1983).

GENERAL DISCUSSION

GENERAL DISCUSSION

The dynamics of populations of animals are necessarily complex, the animals having to cope with constantly changing environmental and demographic parameters. The dynamics of parasite populations are, therefore, more complex still, having, in addition, a highly intimate relationship with the population of another organism: the host.

It has been shown that the dynamics of microparasitic organisms (pathogens) may be determined by factors such as acquired immunity and host population levels (Bailey, 1975; Anderson & May, 1979b). As immunity to infection does not apparently occur in this particular host-parasite association, regulation of the parasite population growth must be due to some other processes. Those processes which may contribute to the regulation of the parasite population are listed below.

1) Parasite establishment.

Three factors which have the potential to limit the establishment of parasites within the host population have been identified. All three would tend to reduce the parasite population were they to occur.

a) Time dependent infectivity: It was shown (section 2.4(ii)) that increasing the exposure duration does not necessarily lead to increased levels of the mean parasite burden. The observed situation strongly suggested the cause to be an abrupt change in the rate of infection after a fairly brief exposure duration. As the reasons behind this unexpected relationship are unknown, further investigation is required to elucidate the situation.

b) Increasing host density: This has been shown to lead to a reduction in the per capita rate at which parasites become established (section 2.4(v)). As the tendency is for a decline in the mean parasite burden per host as host density rises, the result will be to destabilise the host-parasite interaction. It is, however, not known to what extent this occurs in the field or how important it is.

c) Resistance: Described by Petersen (1975), where the host population exhibits a reduced level of susceptibility to infection

following prolonged, high risk, exposure to infection. Although it has been shown that this is not due to any observable host immunity, the process is very poorly understood. This particular aspect of the host-parasite association should be futher explored for several reasons: there is little evidence for behavioural avoidance of infection by potential hosts and this may prove a fruitful system to investigate from this point of view. Secondly, it would be of use to quantify the amount of selective pressure required to elicit this response from the host,and also to maintain the response. The use would come in the increase in the ability to be predict host resistance to biological control agents. The evaluation of any evolutionary response in the infectivity of the parasite may also be possible.

2) Density-dependent environmental sex determination.

The dependence of the sex ratio of the nematode upon the mean parasite burden and statistical distribution of the parasites is the major regulatory constraint that acts upon the parasite. This was shown both by inspection in section 2.6 and by the use of the model described in chapter 4. The severity of this constraint upon the parasite population, given the typical range of aggregation shown by the parasite, will clearly limit the range of mean parasite burdens that may occur. Stochastic effects, that may produce very high or low mean parasite burdens, will be opposed by this, and any other density-dependent regulatory factors. It is the occurence of this very strong density-dependent control upon the rate of parasite growth, which, above all else, suggests that this parasite will be unable to control its hosts population density in a regulated manner. In population terms, the environmental sex determination shown by this nematode may be viewed as a density-dependent control of the fecundity of the nematode population.

3) Parasite-induced host mortalities.

The adverse effects that parasites exert upon their hosts frequently manifest themselves as parasite-induced mortalities. In this association, the death of the host is obligatory, a developmental necessity of the parasite. Thus, a distinction must be drawn between

the mortality of hosts that results in the release of viable nematodes and that which results in the death of the parasites aswell. The relationship between the premature host mortality and the parasite burden was shown to one of direct proportionality, and as such,would be a less severe constraint upon parasite population growth than would a non-linear (increasing) density-dependent relationship. The regulatory effect of the parasite-induced mortality is dependent upon the pathogenicity of the parasite to the host. The estimate of pathogenicity for this parasite in this host was relatively low (0.0076/parasite/host/day), suggesting that the regulatory effect of this aspect of the relationship would be small. This conclusion was borne out when the effect on density-dependent parasite-induced host mortality was modelled. Stressed hosts, however, suffer far greater effects due to parasitism (Petersen, 1972). Thus, the effects of even linear density-dependent parasite-induced host mortality in the field may play a more important role than that suggested here. It should be borne in mind that any increase in parasite mortality thus caused will still affect the male nematodes preferentially.

Aggregation of the parasites within the host population is an important factor in the regulation of the parasite population and may determine whether or not the parasite regulates the host population. The level of aggregation in the parasite distribution is instrumental in determining the net rates of parasite-induced host mortality and parasite mortality. It is also one of the major determinants, along with the mean parasite burden, of the sex ratio of the parasite population. For a given mean parasite burden, the degree to which parasites are clumped within the host population also defines the proportion of the hosts that will be infected, and that will die as a result (ie. the immediate impact upon the host population). Clearly, increasing levels of overdispersion will reduce the impact of the parasite upon the host, and thus, will decrease the probability of parasite regulated host population growth occuring.

An important factor, shown to occur in this association by

the model, is the existence of a host density threshold, below which the parasite cannot maintain itself. This is an important concept, especially in the area of disease transmission, and further investigation is called for. Practical exploration of this threshold in this association is comparitively easy, the experimental design described in chapter 3 being ideally suited for the purpose.

Mathematical models of populations are useful tools which can be used to investigate aspects of the dynamics of populations which are not necessarily obvious and that may be difficult or impossible to investigate experimentally. The model of this association is a very simple example, based upon the formulation described by Anderson and May (1978) and May and Anderson (1978). The exclusion of certain biological features from the model, such as the relationship between the rate of parasite transmission and the density of hosts, does not invalidate the predictions of the model. Changes in the quantitative levels of the predictions may result from incorporating such factors, but it is unlikely that the qualitative form of the predictions will change substantially.

The simplicity of the model, in lacking a true host birth rate, for example, was decided upon so that the experimental investigation reported in chapter 3 could be modelled more precisely. The expansion of the model to incorporate the host birth process would, however, enable a better understanding of the potential of the parasite to regulate host population growth and would, therefore, be desirable. Further expansion of the model to enable the assessment of stochasticity, of both demographic and environmental origin, would also be worthwile.

In conclusion, therefore, this study has described some of the dynamics of the host-parasite association and determined which of the many rate determining parameters are the more important. An assessment of the biological control potential of the parasite strongly suggests that the parasite will be unable to regulate populations of mosquitoes. This conclusion does not, however, preclude the use of the nematode as a biological insecticide, provided problems associated with its mass production can be overcome.

SUMMARY

(1) The volumetric estimation of nematode numbers in liquid suspension was found to be highly accurate.

(2) Survival of infective preparasites of R. culicivorax was found to be both age and temperature dependent, mean expected life-span varying between 8.9 to 1.1 days in a linear manner over the temperature range 10^{o} - 35^{o}C.

(3) A linear relationship was shown to occur between the density of infective stages and the level of parasitism, measured as the mean parasite burden per host. An estimate of the instantaneous rate of infection of 0.0193/host/infective stage/3 ml/hour was obtained. Increasing infective stage density resulted in increasing aggregation of the parasites within the hosts.

(4) Increasing exposure duration (of hosts to infective nematodes) was demonstrated to produce a maximum mean parasite burden, shown to be due to a decline in the rate of infection. An estimate of the mean constant rate of infection for long exposure times was made (0.000664/host/infective stage/3 ml/hour).

(5) The age of the preparasitic nematodes was confirmed to be of importance in their infectiveness, infectivity declining exponentially with increasing age.

(6) Increases in host density resulted in a decline in the rate of infection, a factor that may limit parasite populations in dense host populations.

(7) Susceptibility of the host was shown to vary with age, the third instar being the most susceptible and the fourth instar being very insusceptible.

(8) The volume of the infection arena was shown to have no effect upon either the mean parasite burden or the distribution of parasites amongst the hosts.

(9) It proved impossible to assess natural parasite mortalities, but these were assumed to be of no significance (if they occurred). Parasite mortalities also occur as a result of the premature death of infected hosts, both when the hosts die of natural causes and due to the presence of the parasite. The natural host mortality rate was estimated at 0.0033/host/day, at 25°C., parasites dying at a rate proportional to the mean parasite burden per host. Parasite-induced premature host mortalities were shown to vary linearly with the mean parasite burden per host. The rate at which parasites die,due to their presence killing the hosts,was shown to be dependent upon the parasite burden and statistical distribution of the parasites within the host population. The rate of parasite-induced host mortality was estimated at 0.0076/host/parasite/day.

(10) The rates of parasite and host development were shown to be dependent upon temperature, the relationship in both cases was linear. Developmental zero temperatures for host and parasite were estimated at 8.0°C. and 12.0°C. respectively. The development rate of the parasite was also shown to be dependent upon the parasite burden; higher parasite burdens resulted in shorter development times. Male nematodes, therefore, emerged on average prior to females.

(11) The sex of the nematodes was confirmed to be environmentally determined. The sex of parasites was shown to be dependent upon the number of parasites within individual hosts: the greater the parasite burden the greater the proportion of male nematodes formed. At 25°C., no female parasites developed in hosts

containing more than six parasites. This resulted in increases in both the mean parasite burden per host and the degree of parasite aggregation within hosts causing a decline in the proportion of female nematodes in the population. It was suggested that this density-dependent relationship would regulate parasite numbers.

(12) Low temperature was shown to increase the proportion of females that developed in hosts with a given parasite burden.

(13) Under laboratory conditions, postparasitic juvenile survival was found to be almost 100%.

(14) The mean time from emergence to moulting of the juvenile nematodes was estimated at 7.79 days for males and 8.56 days for females.

(15) Adult male and female survival was shown to be age-dependent. The mean expected life-spans were estimated at 185.7 days for males, 334.6 days for unmated (non-ovipositing) females and 66.9 days for mated (ovipositing) females. Significantly, the mean expected life-span of ovipositing females is longer than the time taken by females to complete oviposition.

(16) Males were demonstrated to be polygamous. The mean time to mating was dependent upon the sex ratio, with a maximum rate of mating of 0.5 females/male/day.

(17) The average time between mating and the initiation of oviposition was estimated at 4.39 days.

(18) Extreme difficulty was experienced in maintaining ovipositing females without a solid (i.e. gravel) substrate. The mean time for oviposition was estimated as 6.59 days, from data presented by Petersen (1975). From this same data the mean rate of oviposition was estimated at 137.78 eggs/female/day over an eighteen day period.

(19) The eggs of R. culicivorax were demonstrated to hatch spontaneously over a fourteen week period: the mean time to hatch was 51.93 days. This proved to be the largest component of the mean generation time of the nematode.

(20) Egg survival was measured at 80.4%.

(21) Comparisons of the estimates of the mean generation times of the parasite (82.7 days) and host (44.7 days: Gomez et al., 1977), and of their relative reproductive outputs (2480/ parasite eggs/ ♀ ; Petersen, 1975 and 507.4 hosts/eggs/♀ ; Gomez et al., 1977) suggested that the parasite would not be able to regulate host population growth.

(22) A simple mathematical model, using laboratory estimated rate parameters, was shown to mimic the average number of emergent adult mosquitoes from an experimental system.

(23) The presence of the parasite led to a reduction of between 27% and 50% in the average number of emergent adult mosquitoes from an experimental system. Significant numbers of emergent mosquitoes contained parasites. Estimates of the mean parasite burden/host of the mosquito larvae under these conditions (obtained from the model) varied between 0.57 and 0.96, (assuming a Poisson parasite distribution) and between 0.96 and 2.24 when a negative binomial distribution ($k = 0.5$) was assumed.

(24) Using a simple mathematical model of the parasite and host populations (of the form described by Anderson & May (1978), and May & Anderson (1978)), the laboratory estimates of the parameters were used to assess the probable importance of various processes in the biology of both parasite and host. Emphasis was placed upon the level of the mean parasite burden per host attained.

(25) Various factors which act in a density-dependent manner to regulate the parasite population were examined. Of these the environmental sex determination was shown to be most important.

(26) The statistical distribution of the parasite was shown, by use of the model, to be a major determinant in the level of mean parasite burden attained and also in the domain of parameter space which permitted parasite persistence.

(27) The effect of more severe than linear parasite-induced host mortality was also shown to be able to regulate the parasite population.

(28) In experiments, no evidence of prey preference or prey switching was shown by guppies (L. reticulatus) when presented with infected and uninfected fourth instar mosquito larvae as the prey choices.

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APPENDIX I

TABLE A.1

Table A.1. Volumetric estimation technique.

Trial	Nematodes/0.2 ml aliquot	Sample size	Mean/ 0.2 ml	Variance	Chi-squared statistic	Distribution	Significance level
1 High Density	35, 45, 45, 33, 38, 35, 36, 41	8	38.50	21.70	3.95	Random	0.9>p>0.5
2 Medium Density	13, 20, 11, 16, 23, 15, 12, 12, 20, 17, 14, 17, 27, 20, 14, 23, 12, 13, 19, 13, 15, 16, 23, 20, 14, 19, 13, 16, 14, 16, 13, 10, 10, 18, 14, 16, 17, 16, 14, 16, 22, 16, 15, 18, 17, 15, 11, 14, 17, 19, 21, 18, 15, 19, 20, 18, 17, 22, 19, 11, 11, 22, 14, 16, 21, 16, 23, 18, 18, 16, 12, 11, 18	73	16.45	13.36	58.46	Random	0.9>p>0.5
3 Low Density	11, 8, 5, 7, 7, 8, 12, 5, 9, 9, 7, 6	12	7.83	4.70	6.60	Random	0.9>p>0.5

Table A.1 (cont'd)

	Size of aliquot ml	Expected mean/ aliquot size	Nematodes/aliquot	Sample size	Observed mean/ aliquot	95% C.L.
1	0.156	30.00	31, 34, 32, 24, 25, 32, 37, 25	8	30.00	±4.58
	0.470	90.00	82, 84, 84, 92, 96. 91	6	88.17	±9.86
2	0.171	14.10	8, 11, 14, 17, 14, 13, 19, 16, 15, 16, 12, 19, 12, 16, 12, 10, 11, 10, 12, 11, 16, 17, 11, 10, 13, 15, 17, 15, 14, 14	30	13.67	±1.06
	0.343	28.20	19, 27, 33, 36, 30, 26, 23, 25, 36, 33, 17, 33, 30, 22, 29, 28, 32, 27, 32, 20, 30, 13, 23, 18, 25, 23, 20, 17, 36, 24, 19	31	26.00	±2.31
	0.365	30.00	25, 39, 31, 38, 31, 29, 31, 21, 41, 32, 26, 25, 22, 29, 25, 31, 36, 29, 24, 42, 27, 36, 32, 24, 39, 23, 37, 26, 30, 37, 34, 23, 21, 35, 30, 20, 32, 26, 32, 39	40	30.25	±1.94
3	0.766	30.00	29, 32, 23, 30, 27, 32, 28, 19, 33, 29, 40, 25, 32, 35, 25, 41	16	30.00	±2.92
	2.298	90.00	92, 90, 86, 97, 95, 96	6	91.00	±10.01

APPENDIX II

TABLES A.2.4.1 - A.2.8.4

Table A.2.4.1

The proportion of preparasitic nematodes remaining alive at time t, $P_{(t)}$, for six temperatures. Cohort size = 100; time, t, in hours.

a (10°C)		b (15°C)		c (20°C)	
t	$P_{(t)}$	t	$P_{(t)}$	t	$P_{(t)}$
0	1.000	0	1.000	0	1.000
30	1.000	45	0.990	24	0.970
60	0.944	72	0.948	48	0.881
108	0.789	96	0.804	96	0.624
168	0.649	117	0.650	120	0.436
228	0.505	141	0.438	144	0.297
288	0.299	165	0.284	168	0.158
336	0.196	189	0.170	192	0.059
384	0.103	213	0.072	216	0.020
432	0.010	237	0.026	240	0.000
456	0.000	258	0.010		
		264	0.000		

d (25°C)		e (30°C)		f (35°C)	
t	$P_{(t)}$	t	$P_{(t)}$	t	$P_{(t)}$
0	1.000	0	1.000	0	1.000
26	1.000	5	0.990	4	0.990
44	0.990	27.5	0.898	16	0.760
50	0.980	35	0.816	20	0.692
68	0.929	50	0.612	24	0.490
92	0.626	55	0.485	28	0.346
98	0.505	75	0.233	40	0.067
116	0.293	80	0.165	44	0.029
		95	0.078	48	0.010
		100	0.019	52	0.000
		105	0.010		
		120	0.000		

Table A.2.4.2

Estimates of the age-dependent instantaneous rate of preparasite mortality, $\mu_{(t)}$ per preparasite per hour, from equation (2.4.1) for six temperatures. Time, t, in hours.

a (10°C)		b (15°C)		c (20°C)	
t	$\mu_{(t)}$	t	$\mu_{(t)}$	t	$\mu_{(t)}$
45	0.001921	58.5	0.001606	12	0.001269
84	0.003737	84	0.006865	36	0.004010
138	0.003514	106.5	0.010125	61	0.004096
198	0.003922	129	0.016448	85	0.010837
258	0.008735	153	0.018052	108	0.014938
312	0.008799	177	0.021382	132	0.015996
360	0.013404	201	0.035797	156	0.026297
408	0.048586	225	0.042440	180	0.041044
		247.5	0.045501	204	0.045075
d (25°C)		**e (30°C)**		**f (35°C)**	
t	$\mu_{(t)}$	t	$\mu_{(t)}$	t	$u_{(t)}$
35	0.000564	2.5	0.002010	2	0.002513
47	0.001709	16.25	0.004335	10	0.022032
59	0.002940	31.25	0.012767	18	0.023433
71	0.011239	42.5	0.019179	22	0.086295
83	0.018176	52.5	0.046517	26	0.086992
95	0.035846	65	0.036656	34	0.136812
107	0.030274	77.5	0.069019	42	0.209349
119	0.099118	87.5	0.049949	46	0.266178
131	0.077016	97.5	0.282454		
143	0.115525	102.5	0.588888		

Table A.2.4.3

The relationship between temperature and the mean life expectancy of the preparasitic nematodes.

Temperature (°C)	Mean life expectancy (hours)
10	213.1
15	133.7
20	117.3
25	101.9
30	56.1
35	26.8

Table A.2.4.4

The relationship between initial infective stage density and the mean parasite burden per host for two exposure times (t), two and four hours. The arena volume was 3 ml.

	Initial infective stage density/arena	Mean parasite burden/host ± 95% C.L.	Sample size (no. of hosts)
a. t=2	6	0.083 ± 0.066	72
	15	0.479 ± 0.195	71
	30	1.406 ± 0.486	96
	45	1.917 ± 0.462	48
	60	2.383 ± 0.724	94
	75	2.722 ± 0.754	72
	90	4.451 ± 0.927	122
b. t=4	6	0.268 ± 0.133	71
	15	1.070 ± 0.295	71
	30	2.019 ± 0.254	216
	45	2.792 ± 0.523	48
	60	3.438 ± 0.633	96
	75	4.250 ± 0.836	48
	90	6.563 ± 1.407	96

Table A.2.4.5

a. The relationship between the initial infective stage density and the percentage of exposed hosts which became infected during a four hour exposure period.

b. The relationship between the initial infective stage density and the variance-to-mean ratio of the number of parasites per host acquired during a four hour exposure period.

The arena volume was 3 ml.

Initial infective stage density per arena	a % hosts infected	b variance-to-mean ratio
6	21.1	1.172
15	63.4	1.451

30	84.3	1.788
45	95.8	1.158
60	92.7	2.828
75	100.0	1.957
90	90.1	7.316

Table A.2.4.6

The frequency distributions of parasites per host generated at different initial infective stage densities with a four hour exposure period. The arena volume was 3 ml.

Frequency class	Initial infective stage density per arena						
	6	15	30	45	60	75	90
	Frequency						
0	56	26	34	2	7		9
1	11	27	70	12	24	6	20
2	4	12	57	9	17	10	12
3		3	19	10	14	6	11
4		1	18	8	9	9	5
5			5	3	7	5	2
6		2	5	2	4	3	2
7			1	1	4	4	3
8			5	1	1	1	2
9			1		2	1	2
10					2	1	1
11					2		
12			1		2	1	3
13							6
14						1	2
15					1		3
16							2
17							1
18							2
19							1
20							2
21							2
22							1
25							1
26							1

Table A.2.4.7

The relationship between the duration of exposure and the mean parasite burden per host with an initial infective stage density of 10/ml.

Duration of exposure (hours)	Mean parasite burden /host ± 95% C.L.	Sample size (No. of hosts)
0.5	0.152 ± 0.091	145
1	0.512 ± 0.102	213
2	1.406 ± 0.486	96
4	2.019 ± 0.254	216
6	1.729 ± 0.220	144
8	1.989 ± 0.436	96
10	1.871 ± 0.330	70

Table A.2.4.9

a. The relationship between the mean parasite burden per host and the age of the infective stages.

b. The relationship between the percentage of exposed hosts that became infected and the age of the infective stages.

The initial infective stage density was 10/ml.

Infective stage age (hours)	a. Mean parasite burden /host ± 95% C.L.	b. % infected	Sample size (No. of hosts)
1	2.019 ± 0.254	84.3	216
24	2.036 ± 0.201	80.5	384
48	2.435 ± 0.384	85.1	131
72	0.715 ± 0.162	48.2	137
96	0.133 ± 0.072	13.3	90
120	0.198 ± 0.081	19.8	96
144	0.107 ± 0.059	10.7	121
168	0.100 ± 0.223	10.0	10

Table A.2.4.10

The frequency distributions of parasites generated by preparasites of increasing age. The initial preparasite density was 10/ml.

Frequency class	Preparasite age (hours)							
	1	24	48	72	96	120	144	168
	Frequency							
0	34	75	18	71	78	77	108	9
1	70	134	31	45	12	19	13	1
2	57	59	31	14				
3	19	43	21	4				
4	18	22	16	2				
5	5	22	5	1				
6	5	12	4					
7	1	8	2					
8	5	6	1					
9	1	3						
12	1		1					
15			1					

Table A.2.4.11

The relationship between the variance-to-mean ratio of the number of parasites per host and the infective stage age. The initial infective stage density was 10/ml. See table A.2.4.9 for mean values.

Infective stage age (hours)	variance-to-mean ratio	sample size (no. of hosts)
1	1.788	216
24	1.946	384
48	2.023	131
72	1.274	137
96	0.880	90
120	0.808	96
144	0.907	121
168	1.000	10

Table A.2.4.12

a. The relationship between the mean parasite burden per host and the age of spontaneously active infective stages.

b. The relationship between the percentage of exposed hosts that became infected and the age of spontaneously active infective stages.

The initial infective stage density was 10/ml.

Infective stage age (hours)	a Mean parasite burden/host ± 95% C.L.	b % infected	Sample size (no. of hosts)
1	2.019 ± 0.254	84.3	216
24	2.036 ± 0.201	80.5	384
48	2.225 ± 0.221	88.0	275
72	2.125 ± 0.252	83.9	192
96	1.922 ± 0.352	66.1	192
120	1.109 ± 0.176	60.4	192
144	0.819 ± 0.122	54.3	221
168	1.112 ± 0.204	59.1	170

Table A.2.4.13

The relationship between the variance-to-mean ratio of the number of parasites per host and the age of spontaneously active infective stages. The initial infective stage density was 10/ml. See table A.2.4.12 for mean values.

Infective stage age (hours)	Variance-to-mean ratio	Sample size (no. of hosts)
1	1.788	216
24	1.946	384
48	1.561	275
72	1.653	192
96	3.192	192
120	1.381	192
144	1.037	221
168	1.707	170

Table A.2.4.14

a. The relationship between the mean parasite burden per host and the host density.

b. The relationship between the transmission coefficient, β (/host/infective stage/3 ml/hour), estimated using equation (2.4.19) and the host density.

The initial infective stage density was 10/ml with a four hour exposure duration.

Host density /arena	a Mean parasite burden/host ± 95% C.L.	b β	Sample size (No. of hosts)
1	2.019 ± 0.254	0.0174	216
2	1.540 ± 0.382	0.0135	100
3	1.240 ± 0.202	0.0110	192
4	1.130 ± 0.285	0.0102	100
5	0.890 ± 0.255	0.0080	100
8	0.427 ± 0.169	0.0038	96
10	0.210 ± 0.133	0.0018	100
20	0.200 ± 0.123	0.0018	100

Table A.2.4.15

a. The relationship between the variance-to-mean ratio of the number of parasites per host and the host density.

b. The relationship between the average number of parasites established per arena, $P_{(t)}$ and the host density.

The initial infective stage density was 10/ml with a four hour exposure duration. See table A.2.4.14 for mean values.

Host density /arena	a Variance-to-mean ratio	b $P_{(t)}$	Sample size (No. of hosts)
1	1.788	2.019	216
2	2.419	3.080	100
3	1.633	3.720	192
4	1.835	4.520	100
5	1.858	4.450	100
8	1.614	3.426	96
10	2.143	2.100	100
20	1.920	4.000	100

Table A.2.4.16

The relationship between the mean parasite burden per host and the host age (instar). The initial infective stage density was 10/ml with a four hour exposure duration.

Host instar	Mean parasite burden/host ± 95% C.L.	Sample size (No. of hosts)
1	2.019 ± 0.254	216
2	2.317 ± 0.284	120
3	3.483 ± 0.786	94
4	0.575 ± 0.228	106

Table A.2.4.17

The relationship between the mean parasite burden per host and the volume of the arena. The initial infective stage density was 10/ml with a four hour exposure duration.

Arena volume (ml)	Mean parasite burden/host ± 95% C.L.	Sample size (No. of hosts)
3	2.019 ± 0.254	216
12	1.930 ± 0.764	86
24	2.020 ± 0.503	49
75	2.333 ± 0.693	21

Table A.2.4.18

The relationship between the variance-to-mean ratio of the number of parasites per host and the arena volume, with an initial infective stage density of 10/ml and a four hour exposure. See table A.2.4.17 for mean values.

Arena volume (ml)	Variance-to-mean ratio	Sample size (No. of hosts)
3	1.788	216
12	6.555	86
24	1.516	49
75	2.457	21

Table A.2.5.1

The relationship between the variance-to-mean ratio of the number of parasites per host and the age of the infection for four different initial mean values.

Time post-infection (days)	Variance-to-mean ratio 4.033+	2.700+	1.867+	1.033+
0	1.667	0.898	2.369	4.971
1	1.782	0.898	1.553	1.448
2	1.237	1.055	1.857	1.030
3	1.280	0.524	1.382	1.310
4	1.791	0.546	1.144	1.379
5	1.180	0.830	1.807	3.000
6	1.927	0.820	1.492	1.176
7-11	1.606*	0.642*	-	-

* value at emergence
+ initial mean parasite burden/host

Table A.2.5.2

The relationship between the proportion of exposed hosts that died and the mean number of parasites per host, at 25°C.

Mean number of parasites/host	Proportion of hosts dying	Sample size (No. of hosts)
0	0.04	100
0	0.06	50
0	0.02	49
0.031	0.01	98
0.130	0.06	49
0.692	0.07	300
1.473	0.13	150
2.295	0.19	150
2.874	0.13	100
8.941	0.24	67

Table A.2.5.3

The relationship between the proportion of exposed hosts that died and the mean parasite burden per host at 15, 20 and 30°C.

	Mean number of parasites/host	Proportion of hosts dying	Sample size (No. of hosts)
a. 15°C	0	0.04	100
	0.405	0.26	50
	0.457	0.28	50
	0.906	0.36	50
	1.139	0.28	50
	1.909	0.12	25
	2.550	0.20	25
	3.034	0.08	25
	3.636	0.08	24
	3.905	0.07	148
	4.561	0.12	215
	4.928	0.17	151
b. 20°C	0	0.00	25
	0	0.00	25
	0	0.04	25
	0	0.04	25
	0.471	0.49	100
	1.167	0.28	25
	1.368	0.24	25
	2.177	0.32	25
	2.250	0.08	26
	5.260	0.21	200
c. 30°C	0	0.02	50
	0	0.04	50
	0.689	0.17	54
	1.000	0.12	25
	1.200	0.20	50
	1.213	0.06	50
	1.458	0.02	49
	2.444	0.28	25
	3.021	0.02	49
	3.553	0.06	50
	5.578	0.10	50
	7.359	0.22	50

Table A.2.5.4

The relationship between the proportion of parasites emerging from hosts and the age of the infection.

Time post-infection (days)	Proportion of parasites emerging	
	Males	Females
6	0.012	-
7	0.635	0.080
8	0.346	0.339
9	0.008	0.515

10	-	0.059
11	-	0.007

with a sample size of 665 male, 425 female nematodes and 623 hosts, giving a mean parasite burden/host of 1.750.

Table A.2.5.5

The relationship between the mean time to parasite emergence and the mean parasite burden per host, at four temperatures.

	Mean parasite burden/host	Mean time to emergence (days)	Sample size (No. of parasites)
a. 15°C	0.405	25.20	15
	0.457	24.56	16
	0.906	24.86	29
	1.139	24.49	41
	1.909	24.88	42
	2.550	23.45	51
	3.044	23.44	70
	3.636	21.66	80
	3.905	23.77	535
	4.561	23.57	862
	4.928	22.95	616
b. 20°C	0.471	13.33	24
	1.167	14.24	21
	1.368	13.31	26
	2.177	15.43	37
	2.250	13.64	54
	5.260	12.64	831
c. 25°C	0.031	9.00	3
	0.130	8.83	6
	0.291	9.04	104
	0.406	8.43	65
	0.692	8.48	193
	0.858	8.34	350
	1.473	7.63	193
	1.934	7.50	675
	2.295	7.28	280
	2.874	7.43	250
	3.259	6.42	189
	8.941	6.13	456

d. 30°C	0.689	6.065	31
	1.000	6.273	22
	1.200	6.167	48
	1.213	5.895	57
	1.458	6.171	70
	2.444	5.886	44
	3.021	5.524	145
	3.553	5.611	167
	5.578	5.151	251
	7.359	5.240	287

Table A.2.5.6

The relationships between the mean development time and the temperature and the mean development rate (per capita per day) and the temperature, of both theparasitic nematode (a) and the host (b).

	Temperature (°C)	Mean time to develop (days)	Mean rate of development (/capita/day)	Sample size
a. The parasite	15	27.04	0.0378	98
	20	15.33	0.0620	39
	25	9.25	0.1009	222
	30	6.67	0.1735	99
b. The host	15	14.51	0.0699	92
	20	9.33	0.1047	98
	25	6.22	0.1493	199
	30	5.28	0.2084	97

N.B. The development time of hosts is from a mean age of 20 hours post-hatching to pupation.

The development time of parasites is for single females per host.

Table A.2.6.1

The relationship between the proportion of parasites that developed as females and the mean parasite burden per host at 25°C.

Mean parasite burden/host	Proportion developing as females	Sample size (No. of parasites)
0.031	1.000	3
0.130	1.000	6
0.291	0.817	104
0.692	0.547	193
1.473	0.269	193
2.295	0.146	280
2.874	0.224	250
3.259	0.212	189
8.941	0.009	456

Table A.2.6.2

The influence of parasite burden upon the sex ratio of the parasite at 25°C.

Parasite burden/host	Proportion of parasites developing as females	Sample size (No. of parasites)
1	0.958	330
2	0.669	272
3	0.346	327
4	0.141	248
5	0.045	155
6	0.033	120
≥7	0.000	597

Table A.2.6.6

The relationships between the mean parasite burden per host and (a) the variance-to-mean ratio of the mean number of parasites per host and (b) the parameter k, of the negative binomial distribution.

Mean parasite burden/host	Variance-to-mean ratio	k	Sample size (No. of hosts)
0.291	1.289	0.888	357
0.405	1.159	3.478	37
0.457	1.588	0.781	35
0.471	1.900	0.554	51
0.689	1.308	2.580	45
0.692	1.828	0.918	279
0.906	0.880	-	32
1.000	1.143	11.710	22
1.139	0.961	-	36
1.167	1.034	-	18
1.200	0.906	-	40
1.213	1.109	14.987	47
1.368	0.910	-	19
1.458	0.582	-	48
1.473	6.509	0.309	131
1.909	1.093	-	22
2.176	1.506	5.123	17
2.208	0.944	-	24
2.295	5.320	0.464	122
2.444	0.636	-	18
2.550	1.299	-	20
2.851	6.058	0.640	87
2.938	1.150	-	48
3.043	1.000	-	23
3.375	1.892	4.476	56
3.550	2.127	4.042	47
3.636	2.319	2.741	22
3.869	1.797	6.303	137
4.577	2.248	5.339	189
5.259	1.689	9.793	158
5.578	1.503	11.652	45
7.359	1.398	18.892	39
8.941	3.473	4.934	51

Table A.2.6.7

The relationship between the proportion of parasites that developed as females and the mean parasite burden per host at three temperatures.

	Mean parasite burden/host	Proportion developing as females	Sample size (No. of parasites)
a. 15°C	0.405	0.867	15
	0.457	0.813	16
	0.906	0.862	29
	1.139	0.805	41
	1.909	0.691	42
	2.550	0.275	51
	3.044	0.500	70
	3.636	0.263	80
	3.905	0.368	535
	4.561	0.284	862
	4.928	0.274	616
b. 20°C	0.471	0.583	24
	1.167	0.667	21
	1.368	0.462	26
	2.177	0.622	37
	2.250	0.444	54
	5.260	0.069	831
c. 30°C	0.689	0.613	31
	1.000	0.591	22
	1.200	0.688	48
	1.213	0.719	57
	1.458	0.657	70
	2.444	0.455	44
	3.021	0.200	145
	3.553	0.222	167
	5.578	0.056	251
	7.359	0.028	287

Table A.2.6.9

The influence of parasite burden upon the sex ratio of the parasite at three temperatures.

	Parasite burden/host	Proportion of parasites developing as females	Sample size (No. of parasites)
a. 15°C	1	0.971	102
	2	0.859	206
	3	0.652	342
	4	0.398	412
	5	0.246	285

	6	0.178	258
	7	0.064	140
	8	0.039	104
	9	0.017	117
	≥10	0.000	386
b. 20°C	1	0.974	38
	2	0.643	56
	3	0.299	147
	4	0.104	96
	5	0.065	185
	6	0.022	138
	7	0.014	140
	≥8	0.000	193
c. 30°C	1	0.980	100
	2	0.699	136
	3	0.246	171
	4	0.102	128
	5	0.044	90
	6	0.050	120
	7	0.018	56
	≥8	0.000	317

Table A.2.6.11

The relationship between the temperature and the ratio of the mean per capita development rate of single female parasites to the mean per capita development rate of hosts*

Temperature (°C)	$\bar{x}$ parasite development rate (/capita/day) / $\bar{x}$ host development rate (/capita/day)
15	0.5408
20	0.5922
25	0.6758
30	0.8325

* when the developmental period of the host was from a mean age of 20 hours post-hatching to pupation.

Table A.2.7.1

The relationship between the proportion of postparasitic juvenile nematodes moulting and the time post emergence.

Time post-emergence (day)	a. Proportion moulted		b. Cumulative proportion moulted	
	♂	♀	♂	♀
6	0.038	0.032	0.038	0.032
7	0.358	0.252	0.396	0.286
8	0.415	0.270	0.811	0.556
9	0.151	0.206	0.962	0.762
10	0.038	0.111	1.000	0.873
11	-	0.063	-	0.937
12	-	0.063	-	1.000

Sample size of males was 54 and females, 63.

Table A.2.7.2

The relationship between unmated adult age and the proportion of adults surviving.

Time post-moulting (days)	a. Proportion of males alive	b. Proportion of females alive
0	1.000	1.000
28	0.988	1.000
63	0.950	0.975
91	0.925	0.963
161	0.488	0.863
189	0.463	0.850
217	0.300	0.813
252	0.200	0.738
273	0.125	0.650
301	0.075	0.625
329	0.025	0.550
357	0.013	-
385	0.000	-
420		0.313
455		0.188
490		0.113
525		0.063
560		0.025
595		0.025
630		0.000

with male and female sample sizes of 80 each.

Table A.2.7.3

The relationship between the age of mated adult female nematodes and the proportion surviving. The sample size was 37.

Time post-mating (days)	Proportion of adult females surviving
0	1.000
23	1.000
26	0.973
38	0.973
40	0.919
42	0.919
44	0.865
46	0.865
48	0.838
50	0.757
54	0.757
58	0.676
60	0.595
62	0.541
64	0.432
66	0.378
68	0.378
70	0.324
74	0.270
78	0.243
82	0.216
86	0.183
94	0.162
100	0.135
106	0.081
112	0.054
128	0.054
147	0.054
154	0.027
161	0.000

Table A.2.7.4

a. The relationship between the proportion of ovipositing females and the sex ratio of the adult nematodes.

b. The relationship between the mean number of egg laying females and the sex ratio of the adult nematodes.

No. of females /male	a. Proportion of females that oviposited	b. Mean number of egg laying females/male	Sample size (No. of replicates)
1	0.800	0.80	30
2	0.719	1.44	16
3	0.455	1.36	11
5	0.329	1.64	14
9	0.222	2.00	3
10	0.150	1.50	8

Table A.2.7.5

The relationship between, (a) the proportion of females showing initial oviposition, (b) the cumulative proportion of females showing initial oviposition and the time post-mating. Sample size of 45 females.

Time post-mating (days)	Proportion of females showing initial oviposition	Cumulative proportion of females showing initial oviposition
0.5	0	0
1.5	0	0
2.5	0.156	0.156
3.5	0.267	0.422
4.5	0.289	0.711
5.5	0.178	0.889
6.5	0.044	0.934
7.5	0.067	1.000

Table A.2.8.1

The relationship between the time post-mating and (a) the proportion and (b) the cumulative proportion of eggs laid. The sample size was one female, laying 2381 eggs.

Time (days)	Proportion of eggs laid	Cumulative proportion of eggs laid
1	0.004	0.004
2	0.039	0.043
3	0.038	0.081
4	0.073	0.154
5	0.105	0.259
6	0.094	0.353
7	0.102	0.455
8	0.103	0.558
9	0.104	0.662
10	0.094	0.756
11	0.071	0.827
12	0.069	0.896
13	0.044	0.940
14	0.023	0.963
15	0.035	0.998
16	0.004	1.002

Table A.2.8.3

a. The frequency histogram of the daily (post-ovipositional) proportion of eggs that hatched.

b. The relationship between the post-ovipositional age of eggs and the cumulative proportion hatched.

The sample size was 967 eggs.

Time (days)	a. Proportion hatched/day
12.5 - 13.5	0.0136
13.5 - 14.5	0.0137
14.5 - 16.5	0.0101
16.5 - 18.5	0.0056
18.5 - 21.5	0.0044
21.5 - 23.5	0.0070
23.5 - 25.5	0.0033
25.5 - 28.5	0.0042
28.5 - 31.5	0.0059
31.5 - 34.5	0.0067
34.5 - 37.5	0.0088
37.5 - 39.5	0.0112
39.5 - 42.5	0.0117
42.5 - 45.5	0.0138
45.5 - 47.5	0.0175
47.5 - 49.5	0.0200
49.5 - 54.5	0.0333
54.5 - 57.5	0.0174
57.5 - 60.5	0.0153
60.5 - 63.5	0.0448
63.5 - 67.5	0.0232
67.5 - 69.5	0.0121
69.5 - 72.5	0.0064
72.5 - 75.5	0.0084
75.5 - 78.5	0.0106
78.5 - 81.5	0.0075
81.5 - 84.5	0.0057
84.5 - 87.5	0.0041
87.5 - 95.5	0.0004
95.5 - 99.5	0.0003

Time (days)	b. Cumulative proportion hatched
0	0
5.7	0
11.5	0
13.6	0.029
14.3	0.039
16.6	0.062
18.6	0.073
21.4	0.086
23.5	0.100
25.7	0.108
28.6	0.120
31.4	0.137
34.7	0.158
37.5	0.183
39.7	0.208
42.7	0.243
45.6	0.283
47.5	0.315
49.6	0.359
54.4	0.517
57.6	0.573
60.5	0.617
63.5	0.752
67.4	0.843
69.5	0.868
72.5	0.887
75.5	0.912
78.5	0.944
81.6	0.967
84.5	0.984
87.5	0.996
95.6	0.999
99.6	1.000

Table A.2.8.4

The frequency histogram of the proportion of eggs dying per day (post-oviposition).

The sample size was 92 eggs.

Time (days)	Proportion of fatalities/day
0 - 3	0.0531
3 - 5	0.1417
5 - 8	0.0182
8 - 11	0.0188
11 - 14.8	0
14.8 - 16	0.0415
16 - 18	0.0276
18 - 60	0
60 - 63	0.0185
63 - 67	0.0419
67 - 72	0
72 - 75	0.0186
75 - 84	0
84 - 95	0.0051

APPENDIX III

TABLES A.3.1.1. - A.3.2.1

Table A.3.1.1

The number of adult C. p. fatigans that emerged, on a weekly basis from an aquarium with a weekly imput of 100 first instar larvae.

Time (weeks)	Number of emergent instars	Time (weeks)	Number of emergent adults
1	91	17	94
2	86	18	94
3	94	19	74
4	94	20	64
5	83	21	92
6	93	22	96
7	98	23	92
8	96	24	89
9	97	25	76
10	97	26	96
11	90	27	99
12	97	28	97
13	90	29	94
14	86	30	98
15	71	31	96
16	82		

Table A.3.2.1

The number of adult C. p. fatigans that emerged on a weekly basis from aquaria with a weekly imput of 100 first instar larvae. Each aquarium was seeded with a known number of postparasitic nematodes on week $t_{(-3)}$;

a) 4 ♂ and 2 ♀ nematodes; b) 20 ♂ and 10 ♀ nematodes;
c) 60 ♂ and 30 ♀ nematodes; d) 200 ♂ and 100 ♀ nematodes

Time (weeks)	Number of emerged adults			
	a	b	c	d
1	11	2	0	0
2	43	0	0	0
3	80	65	1	0
4	100	86	35	0
5	82	43	77	17
6	90	47	86	19
7	93	77	91	64
8	69	70	85	60
9	25	86	94	88
10	8	93	80	97
11	62	70	93	85

12	88	82	80	89
13	93	57	89	89
14	86	47	62	91
15	88	31	76	79
16	57	47	79	91
17	18	66	77	58
18	31	72	73	18
19	70	70	64	5
20	86	87	26	5
21	57	61	11	1
22	64	66	5	38
23	60	76	11	57
24	58	64	2	79
25	28	72	22	89
26	81	92	59	86
27	86	87	15	95
28	85	28	0	73
29	75	56	1	29
30	87	84	2	40
31	71	92	25	12

APPENDIX IV

PREDATION OF INFECTED AND UNINFECTED MOSQUITO LARVAE

APPENDIX IV: THE PREDATION OF INFECTED AND UNINFECTED MOSQUITO LARVAE BY THE GUPPY (*POECILIA RETICULATA*).

An effect many parasites have upon infected hosts, is to reduce their 'fitness', where fitness is defined in terms of the magnitude or growth rate of a population. The parasite-induced reduction in fitness may manifest itself as a reduction in one, or a combination,of the following; survival, birth rate, inter and/or intraspecific competitive ability and the ability to avoid secondary infections and/or predation (Park, 1948; van Dobben, 1952; Herting & Witt, 1967; Seidenberg, 1973; Vaughan & Coble, 1975; Lanciani, 1975, 1979; Bethel & Holmes, 1976; Lester, 1976; Lanciani & Boyt, 1977; Rau & Caron, 1979; Camp & Huizinga, 1979; Uznanski & Nickol, 1980; Wilson & Denison, 1980). Given such a body of literature, covering a wide variety of host-parasite associations,there is a distinct possibility that the mermithid R. culicivorax may exert a similar influence upon infected mosquito larvae.

The experiments described in this appendix were specifically aimed at investigating the effect of infection upon the ability of the host to avoid predation. The importance of this, hinges upon the potential use of the nematode as a biological control agent of mosquitoes in areas where natural or introduced predators occur.

There are many potential predators of mosquito larvae, including turbellarians (Case & Washino, 1979), coelenterates (Levy & Miller, 1979; Sollers-Riedel, 1979) and many aquatic insects (Laird, 1956). However, fish, such as Gambusia spp. and Poecilia spp., have been shown to be major predators of mosquito larvae and have been used in attempts to control populations of mosquito larvae (Hoy, O'Berg & Kauffman, 1971; Hoy & Reed, 1971; Kurihara, 1973; Kurihara & Sasa, 1973; Kurihara, Hata & Sasa, 1973; Kurihara, Sasa, Miyamoto & Sato, 1973).

Clearly, if such predators were to preferentially avoid or

select infected mosquito larvae, the impact of predation upon the dynamics of the parasite population could be substantial, with implications for the use of the nematode in biological or integrated control programmes.

In an attempt to demonstrate whether infection affects the predation of mosquito larvae, an experimental investigation was conducted. The predator was the guppy, *Poecilia reticulata*, and the prey was the larvae of C. p. fatigans.

A one litre crystalizing dish, filled with aged water from an established aquarium, was used as the predation arena. This was placed inside a larger, water filled container, to eliminate internal reflections within the arena. Into each arena was placed one adult female guppy. Each fish had an overall length of between 3 cm to 4.5 cm and had been starved for 24 hours prior to experimentation. The fish were allowed to acclimate within the arena for five-to-ten minutes before a known number of fourth instar prey were introduced. The exposure time of the prey to the predator was five minutes, at the end of which,those prey remaining were removed and counted.

To provide base line data on the predation of larvae of C. p. fatigans by the guppy, fish were exposed to a range of densities of prey. The number of prey consumed at each prey density was recorded; there were at least thirty replicates at each density. The mean numbers of prey consumed (± 95% C.L.) are portrayed in figure (A.4.1a). The form of the functional response (the relationship between the prey density and the number of prey eaten) is a simple asymptotic curve; a classic type II response (Holling, 1965).

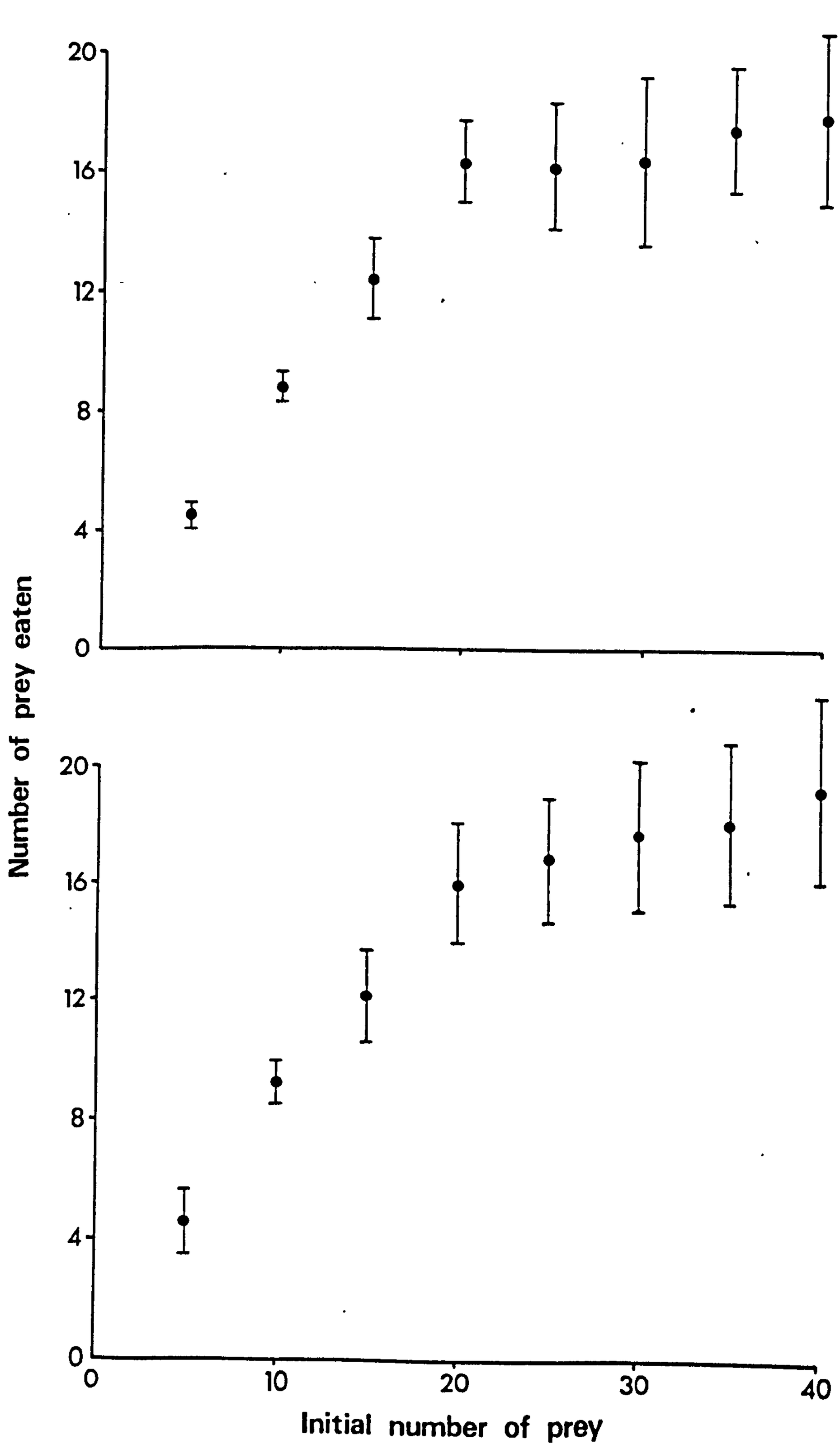
To assess whether there was any preference (or avoidance) of infected mosquito larvae by the fish, the above experiment was repeated using infected fourth instar prey. The prey were infected en masse as first instars and raised as usual (section 2.3 (ii)).

Figure A.4.1 The functional response of a predator to larval mosquito density.

a Uninfected fourth instar C.p.fatigans. The points are observed data (± 95% confidence limits).

b Infected fourth instar C.p. fatigans. The points are observed data (± 95% confidence limits).

The arena volume was 1 litre, the exposure duration was 5 minutes and the predator (female *Poecilia reticulata*) density was one/arena.


a
b
Number of prey eaten
0
4
8
12
16
20
0
10
20
30
40
Initial number of prey

When the mosquito larvae had developed to fourth instars, those which appeared (by visual inspection) to be infected, were removed from the culture containers. From each population of infected hosts thus selected, a sample of thirty was removed and dissected to assess the prevalence and intensity of infection.

The functional response of fish to infected larvae is, again, type II in form (figure A.4.1b). The data for both experiments (table A.4.1) shows that there was no significant difference between the number of infected and uninfected prey consumed at any of the prey densities.

Table A.4.1

The mean number of infected and uninfected mosquito larvae consumed in five minutes by single female guppies (*P. reticulata*).

	Uninfected prey		Infected prey	
Initial prey density/arena	n	Mean number eaten (± 95% C.L.)	n	Mean number eaten (± 95% C.L.)
5	30	4.43±0.42	30	4.57±1.04
10	30	8.80±0.56	30	9.20±0.74
15	30	12.37±1.33	30	12.13±1.54
20	30	16.33±1.35	30	15.97±2.04
25	35	16.14±2.10	30	16.83±2.15
30	30	16.37±2.82	30	17.70±2.52
35	30	17.43±2.14	30	18.13±2.72
40	30	17.80±2.87	30	19.23±3.17

n = number of replicates

A lack of prey preference, by the predator, when the prey are presented in this way, does not preclude the possibility of prey selection or prey switching (see Murdoch & Oaten, 1975; Murdoch, Avery & Smyth, 1975) when the prey occur in a mixed population.

To test whether prey selection or prey switching occurs as the relative densities of infected and uninfected larvae are varied in the prey population, a second experiment was conducted. Infected and uninfected prey were presented in three ratios at a total density of 28 prey per arena. The ratios were 1:1, 3:1 and 1:3 infected to uninfected larvae. Otherwise, the same conditions were employed, with the number of infected and uninfected larvae that survived recorded after each presentation to a predator. The results are presented as the mean number of each prey type eaten, for 31 replicates, (together with the expected number for each ratio) in table A.4.2. The expected number eaten is that for the null hypothesis where there is no prey preference or switching by the predator.

Table A.4.2

The mean numbers of infected (I) and uninfected (U) prey eaten by single female guppies, (*P. reticulata*), when presented at different ratios.

Ratio of I to U.	Observed mean number eaten		Expected mean number eaten		χ^2	significance
	I	U	I	U		
1:1	9.10	9.35	9.23	9.23	8.209	ns
3:1	12.94	4.55	13.11	4.37	15.341	ns
1:3	4.39	14.32	4.68	14.03	13.431	ns

ns = not significant at the 5% level.

The average prevalence and intensity of infection in those prey assumed to be infected (for both this and the earlier experiment) were 87.2% and 1.39 parasites per host respectively.

It may be seen from table A.4.2 that the mean numbers of prey consumed at the three ratios were very similar and within the expected range for the density of prey (see figure A.4.1). It may also be seen that there was no difference in the observed and

expected number of prey consumed (χ^2 test). This strongly suggests that there is no prey preference or switching by the predator.

It may thus be assumed, that, under these conditions, the effect of predation by fish upon the parasite population, will be a proportional reduction in the population, dependent upon the mean parasite burden and the densities of both hosts and predators. Some caution should be excercised in extrapolating from these results to the field. It is possible that, given a more complex arena with refuges, differences in the appearance and behaviour of infected, compared with uninfected larvae may elicit a different response from the predator. It has been shown, that, apart from the obvious colour change in infected mosquitoes (mostly an increase in transparency enabling the white parasite to be seen), infection results in greatly reduced movement (Welch, 1960); a change in behaviour likely to reduce the level of predation; predators finding moving prey more attractive.

APPENDIX V

ESTIMATION OF RATE PARAMETERS

APPENDIX V: ESTIMATION OF RATE PARAMETERS

Table 4.1.1 gives a list of the rate parameters used in the model, including a definition and value of each, estimated at 25°C. This appendix contains the data and/or methods used in the estimation of parameters which are not estimated in the main body of the thesis.

The instantaneous rate of juvenile mortality (μ_4/juvenile/day).

This parameter is estimated from data relating to female nematodes only (see section 2.7 (i)).

$$\mu_4 = \left(\frac{\sigma}{p}\right) - \sigma \qquad \text{(see equation 2.8.3)}$$

where σ is the development (moulting) rate of female nematodes, (the inverse of the mean time to moulting: 8.56 days) and p is the proportion that survived to moult (63/64 = 0.9844).

Thus, at 25°C, μ_4 = 0.0019/juvenile/day.

The instantaneous rate of adult mortality (μ_5/adult/day).

On average, each female must lay 2480 eggs during its life-time, at a rate of 137.78 eggs/female/day (see section 2.8 (ii)). This constrains the rate of mortality as it appears in the model (section 4.1). For females to comply with the above restrictions, their mean expected life-span must be 18 days (18 x 137.78 = 2480). Thus, at 25°C, μ_5, the instantaneous rate of adult mortality (the inverse of the mean expected life-span) is 0.0556/adult/day.

The instantaneous rate of natural host mortality (μ_6/host/day).

The values of μ_6 that appear in table 3.2.4 were estimated

from the survival and development characteristics of groups of 16 to 24 hour old mosquito larvae (table A.5.1).

Table A.5.1

Data required for the estimation of the natural host mortality rate, μ_6 (/host/day).

Temperature (°C)	n	p	$1/\sigma_3$	σ_3	μ_6
15	96	.9583	14.51	0.0689	0.0030
20	100	.9800	9.33	0.1072	0.0022
25	199	.9799	6.22	0.1608	0.0033
30	50	.9600	5.28	0.1894	0.0079

where n is the sample size (number of larvae), p is the proportion surviving to pupation, $1/\sigma_3$ is the mean development time to pupation (in days) and σ_3 is the instantaneous rate of development per host per day. The proportion surviving, p, was estimated from the survival of larvae in a number of replicate groups of about 25 larvae (summed as n).

Given equation (2.8.3),

$$\mu_6 = (\sigma_3/p)-\sigma_3$$

Thus, at 25°C, μ_6 = 0.0033/host/day.

The instantaneous development rate from emergence of the parasites to initial oviposition (σ_2/juvenile/day).

This parameter was estimated by taking the inverse of the mean time between emergence and initial oviposition. At 25°C, the mean time to : (1) moulting of female nematodes was 8.56 days

(section 2.7 (ii)), (2) mating was 2.56 days (assuming a 1:0.75 male:female sex ratio; table 2.7.1), and (3) initial oviposition was 4.39 days (section 2.7 (v)). The sum of these development times is 15.51 days, giving an estimate for σ_2 of 0.0645/juvenile/day.

<u>The instantaneous rate of emergence-induced host mortality (α/host/day).</u>

As α varies with the mean parasite burden per host (M), (see section 3.2), the values of α were calculated for observed values of M. The inverse of the mean development times seen in table A.2.5.5 produced estimates of α for a range of values of M. The linear regression of α on M gave the slope (b) and intercept (a) of the relationship, where

$$\alpha = a+bM.$$

Estimates of a and b appear in table 3.2.3.

USE OF MERMITHID NEMATODES TO CONTROL INSECT VECTORS OF HUMAN DISEASE

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Mermithid nematodes are frequently reported as parasites of medically important insects (see D.W. Roberts and M.A. Strand, editors, Bull. WHO 55, Supp.1, 419 pp., 1977). Because they may infect a substantial proportion of the larval stages of a population, and because they are lethal, mermithids are thought to act as natural biological control agents. Numerous studies and several recent reviews support this notion (J.J. Petersen, 1981 Autumn Symposium of the British Society for Parasitology, to be published in Parasitology, 1982; Symposium in J. Nematol. 13, 239-290, 1981; G.O. Poinar, Jr., CRC Press, Boca Raton, Florida, 277 pp, 1979). Indeed, it seems intuitively obvious that lethal parasites depress their host populations from levels that would be achieved in their absence. However, ecological studies have shown that natural mortalities of immature stages of mosquitoes may be 95% or more and yet sufficient numbers of adults still emerge to constitute a problem (M.W. Service, Parasitology 82, 123-124, 1981). Thus, the fundamental question for mermithid-vector interactions is, "How is a depression in numbers of immature stages related to transmission of disease by the adult stages of the vector?" The answer lies in long-term epidemiological studies on the effects of mermithids in reducing the Annual Biting Rate or the Annual Transmission Potential Rate. These do not yet exist. This is unfortunate, because the answer will affect our concept of the utilization of mermithids for biological control of vectors. At present, they are thought to have potential for inundative (i.e. periodic mass releases) as well as the more classical inoculative (i.e. single introduction) biological control procedures. In the absence of any answers, we propose to analyze the question from a theoretical viewpoint.

WHICH VECTORS ARE AMENABLE TO CONTROL BY MERMITHIDS?

Mermithids offer potential for mosquito control because they have adapted to the life cycle of their host, are host specific, kill their hosts, produce high levels of infection, have a high reproductive potential, are easily disseminated having the potential for establishment and recycling, and offer no threat to non-target organisms or the environment (J.J. Petersen, Proc. First Int. Coll. Invert. Pathol., pp. 236-240, 1976). Only one species has been developed to the point where this potential can be realized. Largely through the efforts of Petersen, an *in vivo* system for producing masses of *Romanomermis culicivorax* was developed and permitted field trials to test its effectiveness against culicids. The trials involved use of inundative releases of masses of preparasites to achieve immediate control and/or inoculative releases of postparasitic stages to allow the parasite to establish in a host habitat. Results were variable and could never be predicted, but in some cases over 90% of mosquito larvae sampled were parasitized and sometimes the nematodes became established to provide continuous but lower levels of parasitism. Petersen (*op. cit.*, 1982) and E.G. Platzer (J. Nematol. 13, 257-262, 1981) have recently reviewed these trials.

Mermithids are also leading candidates for eventual use in controlling simuliids (D.P. Molloy, J. Nematol. 13, 250-256, 1981). However, *in vivo* laboratory culture of mermithids in blackflies is not yet possible, so the large numbers of parasites required for field trials cannot be produced. Thus, only one realistic field trial with mermithid preparasites against blackflies has been attempted.

Mortality of early instars was over 70% near the site of release but large numbers of preparasites were required, making the cost prohibitive (D.P. Molloy and H. Jamnback, Mos. News 37, 104-108, 1977). In the mid to late 1970's, attempts were made to use R. culicivorax against blackflies, particularly S. damnosum. In view of the close adaptation of mermithids to their hosts, it is not surprising that a species adapted to parasitize mosquitoes failed to control blackflies.

Interactions between other medically-important insects and mermithids are less well known than those mentioned above. Reports are limited to records of occurrence, sometimes to species descriptions, and occasionally to anecdotal accounts of the bionomics of the parasites. For example, an immature mermithid was recorded from Glossina palpalis in 1910 (R.T. Leiper, Proc. Zool. Soc. Lond. 79, 147). Subsequently, there have been several records of mermithids from tsetse flies, but only last year was a species description produced (G.O. Poinar, Jr. et. al., Can. J. Zool. 59, 858-861, 1981). Little is known about the bionomics of the parasites. Prevalence is always low (2-5%) and more male (12.3%) than female (2.1%) G. palpalis were infected (W.M. Hominick and S.L. Croft, Can. J. Zool., in prep.). The life cycle of the parasite is still unknown. Therefore, at present the use of mermithids to control vectors is limited to practical aspects of mosquito control and possibilities for blackfly control.

DYNAMICS OF THE INTERACTION BETWEEN MERMITHIDS AND VECTOR POPULATIONS

Natural regulation of vector populations is extremely complex. When one additional biotic factor such as mermithid nematodes is introduced to the system, it is difficult to separate its effects from all the other factors that are acting. Furthermore, assessment of the impact of mermithids in field studies is complicated by limitations of sampling techniques, movement of hosts and parasites in and/or out of sampling areas, changes in water levels caused by precipitation and desiccation, and variability of the comparison controls. Thus, whether introductions are successful or not, reasons for success or failure are difficult to ascertain because of the complexity of the system. For these reasons, workers frequently resort to controlled laboratory experiments to investigate the effects of mermithids on vector populations. Another technique is also available. Mathematical models can be used to quantify the dynamics of the interaction between parasite and host populations. This approach is especially useful if material available for study is limited. Predictions from the model can then be compared with observations recorded in the literature or experiments can be devised to test hypotheses that are formulated. In this way, key variables can be assessed in the absence of uncontrolled or complicating factors. R.M. Anderson (In, Population Dynamics, Edited by, R.M. Anderson, B.D. Turner and L.R. Taylor, Blackwell, Oxford, pp. 245-281, 1979; J. Theor. Biol. 82, 283-311, 1980) used simple population models to identify the biological attributes of direct life cycle macroparasites which determine the degree to which they can depress the population of their host from disease-free equilibrium levels. These theoretical predictions were related to the use of pathogens as biological control agents. The following discussion assesses some of the predictions when they are applied to mermithids parasitic in mosquitoes and blackflies.

1) Pathogenicity of the parasites.

It is usually assumed that highly pathogenic organisms will be the most effective in depressing the growth of their host population. This notion is not supported by the model, which predicts that maximum depression will be achieved by parasites with low to moderate pathogenicity. High pathogenicity is desirable only if it is coupled with high transmission efficiency. However, highly pathogenic species such as mermithids, with a low transmission efficiency, will be characterized by infections in a small proportion of the hosts and with a low number of parasites per host. This prediction is supported by facts, for

most natural mermithid infestations exist at low to moderate levels in the host population. Thus, 3-15% of simuliids may be infested (D.P. Molloy, J. Nematol. 13, 250-256, 1981) and results of J.J. Petersen and O.R. Willis (Mos. News 35, 526-532, 1975) showed that the prevalence of R. culicivorax in mosquitoes after the nematodes had been released and recycled was usually 0-25%. The model also predicts that highly pathogenic species will cause little depression in the host population and may fail to persist in a stable association with the host. That is, they are likely to cause their own extinction but not that of their host. Again, results of Petersen and Willis (op. cit., 1975) support this prediction. They speculated that sites which failed to exhibit recycling had such high levels of infection (84-97%) after the initial release of the parasites that establishment may have failed because of the early mortality of multiply-infected hosts.

2) Density-dependent constraints on parasite reproduction

Regulation of populations to stable equilibria is almost universally caused by one or more density-dependent constraints upon the growth of the populations (R.M. Anderson and R.M. May, J. Anim. Ecol. 47, 219-247, 1978). For mermithids, the most important density-dependent constraint is environmental sex determination. As parasite burdens increase, a decreasing proportion of the nematodes differentiate as females, so reducing the input into the next generation. Hence, suppression of the host population is decreased and the parasite could even become extinct. A characteristic of mermithids is their patchy geographical distribution, which is usually attributed to their limited powers of dispersal. Perhaps their environmental sex determination and their high pathogenicity also contribute to their discontinuous distribution.

3) Distribution of parasite numbers per host

Maximum depression of host numbers is achieved by maximizing the parasite population i.e. by achieving the maximum equilibrium mean parasite burden per host (M*). For any given value of M*, the degree of depression of the host population is dependent upon the statistical distribution of the parasites within the host population (see Anderson, 1980). Random patterns lead to maximum depression. As the parasites become more aggregated, a greater proportion is harboured in fewer and fewer hosts. These hosts are more likely to die before the parasites attain maturity. Furthermore, for mermithids, high parasite burdens give rise mainly to males. The net effect is that the parasite population decreases in size and the degree of suppression of the host population also decreases. Thus, for biological control, the most desirable parasite is one that shows low levels of overdispersion. However, the limited data available for mermithids show that their distribution varies from Poisson (random) to highly overdispersed, with a tendency for moderate overdispersion to predominate.

4) Reproduction of parasites and their hosts

Hosts with high reproductive potentials can offset losses to pathogens so that the impact of a parasite is closely correlated with the reproductive potential of the host. If the host potential is high, parasites cause a small proportion of deaths while most hosts die because of severe levels of competition for finite resources. The parasite may only be important in such cases if its reproductive potential is several orders of magnitude greater than that of the host. Reproductive potentials depend on fecundity and generation times. Mean egg production of mermithids is in the order of several thousand (e.g. R. culicivorax produces a mean of 2480 eggs per female (J.J. Petersen, J.Nematol. 7, 211-214, 1975)) while their mosquito hosts produce 500 or more (e.g. C.p. fatigans produces 507 eggs per female (C. Gomez et. al., J. Med. Entomol.

were randomly distributed within the host population (i.e. that they followed a Poisson distribution) and that the only density-dependent constraint operating on the parasites was environmental sex determination. For specified conditions of host density (defined by immigration, death and emmigration rates) the model predicted a value of M* = 1.05 parasites per host. Since this prediction is in good, and perhaps fortuitous, agreement with parasite burdens reported in the field and also with long-term free-running laboratory experiments, the model appears to be an acceptable simulation of this mermithid-mosquito interaction.

With the model, it is relatively simple to relate the percent of hosts infected to a given mean parasite burden. Thus, to obtain a 50% depression in the equilibrium mean host population (H*), M* would have to be maintained at 1.3 parasites per host; for an 80% depression, M* = 5.6; and, for a 90% depression, M* = 12.7 parasites per host. However, as discussed earlier, mermithids are usually overdispersed and not random in their distribution in the population of hosts. As the degree of overdispersion increases, there is a concomitant reduction in the degree of depression of the host population for any given M*. Consequently, levels of M* higher than those given above would have to be maintained to achieve the same levels of control. Such high parasite burdens are unrealistic.

CONCLUSIONS

Predictions from a general mathematical model for the influence of parasitic infections on the dynamics of host population growth and from a specific model for mermithid infections in mosquitoes lead to the conclusion that natural infestations of mermithids can cause at most only moderate depressions in larval populations of vectors. The occasional epizootics that do occur would be too localized and infrequent to have a significant effect on adult populations, and hence disease transmission. Therefore, mermithids will probably not be useful in biological control programs aimed at providing effective long-term control with a single introduction of the parasite. Others have reached the same conclusion from practical experience rather than theoretical considerations (e.g. D.P. Molloy and H. Jamnback, op.cit., 1977; B. Mondet, op.cit., 1981). Mermithids may also prove unsatisfactory for certain integrated control programs because vector populations may be depressed below levels adequate to maintain the parasites. The type of program best suited for mermithids is one that utilizes periodic inundative releases of the parasites. The possibility of their recycling, even at low levels, would be an asset, for subsequent releases would then require fewer parasites. Thus, there is no doubt that mermithids offer promise for the biological control of vectors, but their limitations must be appreciated before they can be used rationally.

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