# POPULATION DYNAMICS OF *ICHTHYOPHTHIRIUS MULTIFILIIS*

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## ABSTRACT

*Ichthyophthirius multifiliis* is a ciliate parasite of freshwater fish species. The population dynamics of its interaction with the fish host *Poecilia latipinna* are investigated, as a laboratory model of more general host-parasite interactions. Short term experiments investigate the infection dynamics and survival of the infective stages and simple models are developed to assist in the analysis of the results. The rate of acquisition of resistance to infection, subsequent to previous exposure, and the degree of protection afforded against reinfection are examined experimentally. Experiments are described in which the death rate of the host is determined in relation to the burden of parasites harboured.

Some mechanisms generating aggregation in the distribution of parasites between hosts are investigated, and their implications at the population level are discussed. Particular attention is paid to the importance of differing host susceptibility to infection as a source of overdispersion.

Simple deterministic models are developed to describe the system, including important features of the biology of *Ichthyophthirius* identified by the experimental programme. Parasite survival of host death is found to have a strong destabilizing influence on the host parasite interaction. Acquired resistance to infection is found to stabilize the interaction, a wide range of parameter values leading to the parasite being maintained endemically at low levels within the host population. Laboratory epidemics of *Ichthyophthirius* are described, and their behaviour is discussed in relation to the predictions of the models. Finally, the likely influence of seasonal factors on the epidemiology of the parasite and future research priorities are discussed.

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# CHAPTER 1

## INTRODUCTION

The mathematical studies of the epidemiology and population dynamics of infectious disease began in 1760, with a paper by Bernoulli on the mortality induced by smallpox. Since that time, a large sophisticated literature has developed on disease epidemiology. This is mainly concerned, not unreasonably, with public health problems, and deals only indirectly with the ecology of pathogens and the role they may play in the regulation of natural populations. Although Lotka (1923) published on epidemiology before his classic work on predation and competition, ecologists tend to have concentrated their attention on predators or insect parasitoids as regulators of animal populations. With the exception of the work of Kostitzin (1934), the importance of parasites in population dynamics has been neglected until recently. Over the last decade, there has been increasing interest in the significance of parasites in the ecology of natural populations (Crofton, 1971b, Bradley, 1972, Anderson and May, 1978). This interest has perhaps culminated in the suggestion of Hamilton, (1980) that selection pressure caused by parasitism may have led to the evolution of sex.

A number of factors have probably made parasitism a less attractive subject of investigation to both experimental and theoretical ecologists than either predation or the action of insect parasitoids. The influence of a parasite on its host is,by it nature, rather more subtle than that of a predator on its prey in which the prey is killed outright, or of a parasitoid on its host, in which case there is usually a simple one to one replacement of host by parasitoid (Hassell, 1978). Parasites

are usually hidden from view, and estimation of their population size is often extremely difficult without destructive sampling of the host population. A large number of parasites have complex life histories, utilizing several intermediate hosts, and hence are difficult to deal with in laboratory culture and manipulation. The complexity of their life cycles also raises problems of mathematical description. A final factor, that should probably not be underestimated, is the rather unpleasant nature aesthetically of certain parasitological investigations.

This thesis is an integrated experimental and theoretical examination of the population dynamics of the protozoan *Ichthyophthirius multifiliis*, a parasite of freshwater fish. *Ichthyophthirius* is of considerable economic significance as a pathogen affecting commercial fish culture. The main reason for its use as an experimental organism in this study, however, is that it provides an ideal laboratory model with which to investigate some of the problems of parasite ecology.

Certain features of the biology of the organism enable the avoidance of some of the problems of investigation of parasite population dynamics mentioned above. Its life cycle is direct, involving only one host, and at laboratory temperatures, the generation time is short, being a few days as opposed to weeks. The parasite is visible externally, and the parasite burden of a host can therefore be assessed without unduly interfering with either host or parasite population.

The thesis falls into two main parts. In the first section, aspects of the biology of *Ichthyophthirius* are examined experimentally. After a review of the available literature on the biology of the organism (Chapter 2) and a brief description of experimental techniques used,

the time spent in each life cycle stage and the reproductive rate of the parasite are determined in Chapter 4. Chapters 5 and 6 examine the infectivity of tomites to naive and previously exposed hosts respectively. The complex nature of the effect of parasites on the survival of their hosts is investigated in Chapter 7, and through this, the relationship between parasite burden per host and parasite fecundity is examined. The distribution of parasites within a host population is of crucial importance to the dynamics of a host parasite interaction (Anderson and May, 1978; Bradley, 1972; Crofton 1971b). Chapter 8 examines the influence of differing host susceptibility to infection on this distribution. To conclude the experimental side of the project, the results of some laboratory epidemics of *Ichthyophthirius* are considered in Chapter 9.

The second part of the dissertation considers theoretically the consequences of the behaviour of the parasite observed in Chapters 4 - 9. Although the models developed are specifically framed around *Ichthyophthirius*, the factors they examine are common to the biology of a large number of host-parasite interactions. A general model is formulated in Chapter 10, and the significance of the time delays in the transmission of *Ichthyophthirius* is considered. Chapter 11 examines the influence of parasite survival of the death of their hosts on the stability of the interaction. In Chapter 12, the significance of acquired immunity of a host to parasites is considered, and compared with the influence of acquired immunity in models of microparasitic disease. Chapter 13 examines the influence of differences between hosts in the level of innate susceptibility to infection on a free running population, in contrast to Chapter 8, which considered the influence of varying susceptibility on parasite burdens developed after a single infection. Finally, in Chapter

14, the results of the experimental epidemics in Chapter 9 are re-examined in the light of the theoretical conclusions, and in Chapter 15, the findings of the dissertation and future research needs are discussed.

# CHAPTER 2

## BIOLOGY OF *ICHTHYOPHTHIRIUS MULTIFILIIS*

### 2.1 *Life Cycle*

*Ichthyophthirius multifiliis* (Fouquet, 1876) is a ciliate of the order Hymenostomatida (Corliss, 1979) parasitic on freshwater fish. The life cycle of the organism has been well documented. The description given below is primarily based on that of Wagner (1960).

The adult, known as a trophozoite, lives beneath the epidermis of its host, and is visible externally as a white spot, leading to the disease being commonly known as "white spot disease". Trophozoites are approximately spherical, uniformly ciliated and with a well developed cytostome. *Ichthyophthirius multifiliis* was originally described as having a horseshoe shaped macronucleus, and although variants have been described with rod shaped or spiral macronuclei there are suggestions that these may, in fact, represent different species. (Nigrelli *et al*, 1976). To date, *Ichthyophthirius multifiliis* is the only member of its genus recognized (Corliss, 1979).

After some time feeding on the host, the mature trophozoite leaves in order to reproduce. Although several trophozoites occupying a single lesion may give the impression of being products of division (Wagner, 1960), reproduction on live hosts has not been reported. Having left the host, the mature trophozoite settles on to a solid substrate, secretes a thin, gelatinous cyst wall and begins rapid mitotic division. Division has been reported in the absence of a cyst wall, or without the trophozoites attaching to a substrate (Negele, 1975), but viable tomites do not appear to be produced under such circumstances (Wagner,

1960; Bauer, 1962). If host death forces trophozoites to leave the host before reaching full maturity, most are able to successfully encyst, although there is a minimum age below which trophozoites appear to be incapable of secreting a cyst wall (MacLennan, 1937). Trophozoites leaving hosts before reaching maturity do not produce as many infective stages as do mature parasites (Wagner, 1960).

Once division is complete, the infective stages or tomites, bore through the cyst wall and are released into the environment. Tomites are ovoid in shape, ciliated and highly mobile. They are a non feeding stage, living off reserves of glycogen and proteins (Wagner, 1960). As far as is known, *Ichthyophthirius* tomites encounter hosts as a result of essentially random movements. Lom and Cerkasovova (1974) failed to detect any long range chemotactic response to either fish mucus or serum, although they believe that the observed positive phototaxis of tomites may bring them toward the water surface, a habitat favoured by certain species of fish. On locating a host, tomites attach to the epidermis and bore through it until reaching a level on or just above the basement membrane, reaching it within 24 hours at 18$^{o}$C (Hines and Spira, 1974b) where they assume a rounded shape and develop into trophozoites (Bauer, 1962).

The time required for the completion of these life cycle stages is extremely dependent on water temperature. According to both Bauer (1962) and Wagner (1960), the parasite is able to survive and reproduce in water temperatures between 3$^{o}$C and 27$^{o}$C, taking in excess of 100 days to complete the cycle at 3$^{o}$C, and less than 5 at 27$^{o}$C. The optimum temperature for reproduction is around 25$^{o}$C. The

growth rates reported in the literature are compared in Fig. 2.1 and Table 2.1. Some caution should be exercised in comparing these, as the methodologies may differ, but the striking general impression is the close similarity in rates reported over forty years from three continents. Nigrelli *et al* (1976) consider it probable that distinct physiological races or species of *Ichthyophthirius* exist, adapted to differing water temperature regimes. The constancy of the development times shown in Table 2.1 suggests that these observations relate to only one species.

## 2.2 *Pathology*

The parasite is extremely pathogenic, and is responsible for severe economic losses in the intensive conditions of commercial fish culture. The best description of the pathology and physiological effects of *Ichthyophthirius* infection is found in a series of papers by Hines and Spira (1973a, 1973b, 1974a, 1974b, 1974c). The gross appearance of an infected host is characterized by the already described white pustules and a thickened or patchy appearance of the mucus layer. In the later stages of infection, tissue necrosis is common, fins becoming frayed, scales lost and gill filaments exposed. Histological examinations reveal an increase in the number of mucous cells early in the infection, and up to a fourfold increase in the thickness of the epithelium. Examination later in the course of the infection revealed a total lack of mucous vacuoles, and loss of the epithelium down to the level of the basement membrane (Hines and Spira, 1974a).

The physiological effects of the disease on mirror carp, *Cyprinus carpio* are discussed by Hines and Spira (1974b). The primary finding

Table 2.1 *Developmental Rates of Ichthyophthirius*

(a) Time spent by trophozoites on hosts

| Temperature (°C) | Time (days) | Source |
|---|---|---|
| 2 - 4 | > 100 | Wagner |
| 5 | 70 - 80 | Wagner |
| 10 | 25 - 30 | Wagner |
| 10 | 28† | Lahav and Sarig |
| 15 | 10 - 12 | Wagner |
| 15 | 8† | Lahav and Sarig |
| 18 - 20 | 8 | Hines and Spira |
| 20 | 7 | Wagner |
| 20 | 6† | Lahav and Sarig |
| 22 | 5-6 | MacLennan (1937) |
| 25 | 5 | Wagner |
| 27 | 3.5 - 4 | MacLennan (1937) |

†"time until beginning of release from trophozoites.

(b) Time required for encystation

| Temperature (°C) | Time (days) | Source |
|---|---|---|
| 3 - 4 | 11 - 15 | Wagner |
| 5 | 7 - 8 | Wagner |
| 7 - 8 | 3 - 3.5 | Bauer |
| 10 | 2 - 2.5 | Wagner |
| 11 - 12 | 1.5 - 1.7 | Bauer |
| 15 | 1 - 1.5 | Wagner |
| 15 - 16 | 1.2 | Bauer |
| 20 | 0.75 | Wagner |
| 20 - 22 | 0.8 | Bauer |
| 24 - 25 | 0.6 | Bauer |
| 25 | 0.5 | Wagner |
| 26 - 27 | 0.45 | Bauer |
| 27 | 0.45 | MacLennan (1937) |

(Sources are given in more detail overleaf)

Table 2.1 (continued)

(c) Size of "Mature" Trophozoites

| Temperature (°C) | Diameter (mm) | Source |
|---|---|---|
| 2 - 5 | 1.0 | Wagner |
| 5 - 10 | 0.8 | Wagner |
| 10 | 0.85 | Lahav and Sarig |
| 10 - 20 | 0.6 | Wagner |
| 15 | 0.6 | Lahav and Sarig |
| 18 - 20 | 0.6 | Hines and Spira |
| 20 | 0.50 | Lahav and Sarig |
| 20 - 30 | 0.50 | Wagner |
| 22 | 0.45 | MacLennan (1942) |
| 27 | 0.3 | MacLennan (1942) |

(d) Growth Rate of Trophozoites on Hosts

| | Diameter of Trophozoites (µm) | |
|---|---|---|
| Days after infection | MacLennan (1942) (22°C) | Hines and Spira (18 - 20°C) |
| 1 | 53 | 70 |
| 2 | 71 | 84 |
| 3 | 126 | 186 |
| 4 | 203 | - |
| 5 | 316 | 317 |
| 6 | 446 | 485 |
| 7 | 512 | 586 |

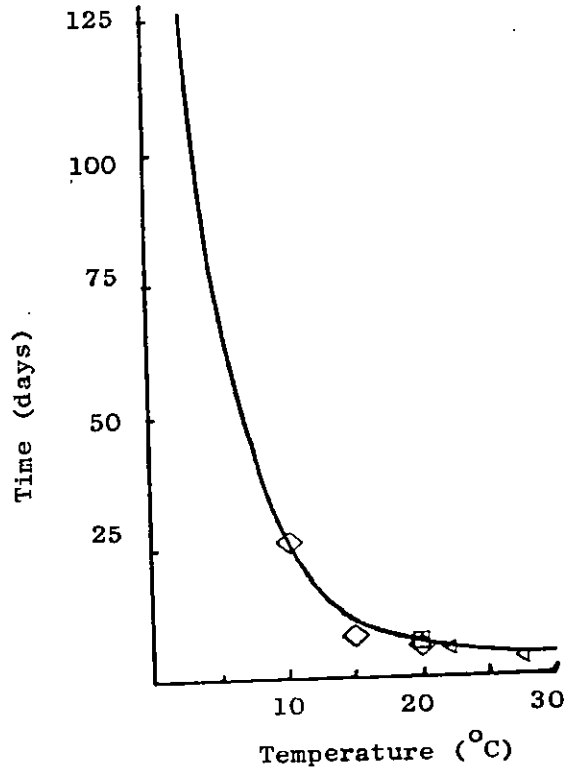
Host Species Examined

| Source | Host Species |
|---|---|
| MacLennan (1937, 1942) | 5 cm Speckled Dace *Apocope oscula carringtoni* |
| Wagner (1960) | 3 year old "carp" (40 cm), similar results for sticklebacks, gudgeon perch, guppies |
| Bauer (1962) | "carp" |
| Lahav and Sarig (1973) | 15 - 20g "carp" |
| Hines and Spira (1973a) | 250g Mirror carp *Cyprinus carpio* |

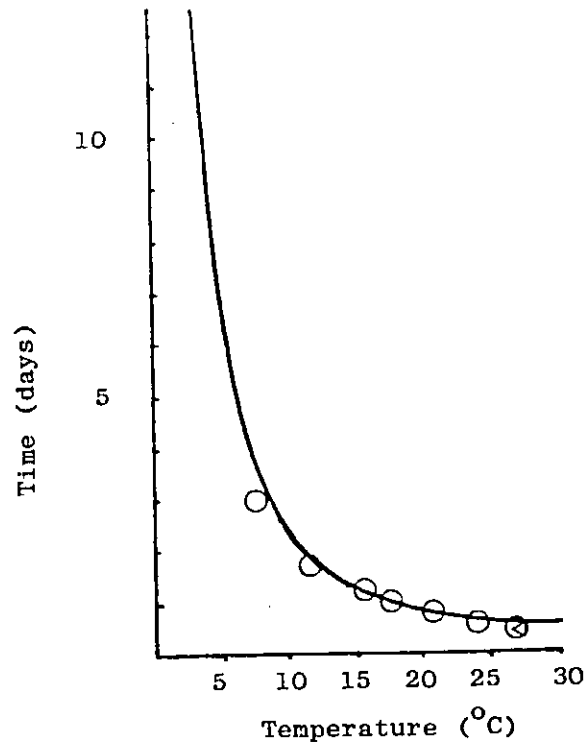
Figure 2.1 *Developmental rates of Ichthyophthirius*

Figure 2.1(a) compares times spent by trophozoites on hosts reported in the literature, and Fig. 2.1(b) compares times required for encystation. In each, the curve shown is obtained from Wagner (1960). Circles represent the observations of Bauer (1962), squares represent the observations of Hines and Spira(1973a), triangles those of MacLennan (1937) and diamonds those of Lahav and Sarig (1973).

(a)


125
100
75
50
25
Time (days)
10
20
30
Temperature (°C)

(b)


10
5
Time (days)
5
10
15
20
25
30
Temperature (°C)

was that the osmoregulatory ability of the infected carp was affected. It is suggested that this is due to changes in the mucus layer, and the physical damage caused by the parasite to the epithelium. The ability of diseased fish to tolerate low oxygen levels was also decreased. Hines and Spira suggest that this is partially caused by epithelial damage, and not solely due to the reported obstruction of the gills.

Little evidence exists in the literature concerning the number of parasites that constitute a lethal burden. Lahav and Sarig (1973), working with 15-20 g carp (species not reported), found no mortality occurring with parasite burdens of up to 300 per fish. Hines and Spira (1973a) report 100% mortality of mirror carp, *Cyprinus carpio*, weighing between 240 and 260 g with a peak infection level of 430 trophozoites per $cm^2$ of tail fin, and no mortality of carp with a peak burden of 8 $cm^{-2}$. Burdens of less than five trophozoites were found to be sufficient to kill carp fry 9-12 mm long by Komarova (1975), although older fry survived infection at this level.

Fish are capable of acquiring a degree of resistance or immunity to infection by *Ichthyophthirius*, subsequent to earlier exposure. This was first noted by Buschkiel in 1910 (Buschkiel, 1936), who observed that three successive generations of the parasite do not seem to be able to establish on the same host. Bauer (1962) was able to show that fish with a previous experience of infection developed burdens approximately one tenth of those established on hosts with no previous experience of infection, if exposed to the same density of tomites. Hines and Spira (1974c) examined the acquisition of resistance by *Cyprinus carpio* to infection by *Ichthyophthirius* in some detail. Sterile immunity was observed to develop in fish that had recovered from moderately heavy

infestations (peaking at around 100 $cm^{-2}$ of caudal fin area). This was maintained for at least eight months, in contrast to the finding of Bauer (1962) that the effectiveness of the immune response decreased after two weeks. Blood serum from fish that had recovered from infection was found to be capable of immobilizing *Ichthyophthirius* trophozoites, as were mucus washings from recovered hosts. Hines and Spira believe that the immune response acts on the parasite via the mucoid secretions of the host, as infective stages were not recovered in mucus from resistant hosts. Recently, Goven *et al* (1980) have shown that channel catfish, *Ictalurus punctatus* may be protected from *Ichthyophthirius* infection by immunization with preparations of *Ichthyophthirius* cilia, or with cilia preparations from the related *Tetrahymena pyriformis*.

## 2.3 *Epidemiology*

*Ichthyophthirius multifiliis* is extremely widespread in natural conditions, both geographically and in terms of the range of species it may attack. The parasite has been reported from freshwater fish in every part of the world, with the possible exception of the arctic (Nigrelli *et al*, 1976). Infection has been reported on a wide variety of host species. In the British Isles, for example, it has been found on the three spined stickleback *Gasterosteus aculeatus*, brown trout *Salmo trutta*, carp *Cyprinus carpio*, crucian carp *Carassius carassius*, bream *Abramis brama*, minnows *Phoxinus phoxinus*, roach *Rutilus rutilus*, tench *Tinca tinca* and pike *Esox lucius* (Kennedy, 1974).

Despite this widespread distribution, reported outbreaks of the disease amongst natural fish populations are relatively rare. When they do occur, however, epizootics may be severe. Elser (1955) describes

an epizootic amongst yellow perch *Perca flavescens* in a 3,900 acre impoundment in Maryland, U.S.A. The outbreak commenced in April, and by the end of May, Elser estimated that around 170,000 fish had been killed. A population of sticklebacks *Gasterosteus aculeatus* in a small scottish lochan (1000 $m^2$) being studied by Hopkins (1959) was nearly wiped out by an epizootic of *Ichthyophthirius*, which commenced in August. Host density during the epizootic was crudely estimated by the number of fish per net haul, and by November, the fish population had been reduced to 1% or less of its former level. Other documented outbreaks are those at Schönerlinde near Berlin amongst grass carp (Wagner, 1960) and those amongst trout in the Black Forest (Buschkeil, 1936).

Information on the distribution of the parasite amongst natural populations of fish in Europe is limited. A number of general surveys of fish parasite fauna, ranging geographically from Finland (Calenius, 1980) through England (Mishra and Chubb, 1969) and Ireland (Kane, 1966) to Bosnia and Herzogovinia (Zitnan and Cankovic, 1980) have detected *Ichthyophthirius*, but not, apparently, in large numbers. Interpretation of this information is restricted by the failure of some authors to note the level of prevalence of the parasite in the host population. Calenius (1980) recorded prevalence, finding *Ichthyophthirius* in low numbers only, on seven out of 2000 fish examined. Kane (1966) found higher levels of prevalence (30 out of 450 pike, 20 of 84 minnows, 16 of 38 carp and 30 of 80 sticklebacks). However, with the exception of the pike, he recommends caution in the interpretation of these figures, as fish were not sampled randomly. Kane also contrasts the distribution of *Ichthyophthirius*, which was found on fish at only two of over thirty sites surveyed, with the more general distribution of the

other parasites in his study. Pickering and Christie (1980) and Wooten and Smith (1980), comparing parasite faunas on wild and hatchery reared salmonids (*Salmo trutta* and *Salmo salar* respectively), detected the parasite on hatchery raised fish, but failed to find it on wild hosts in the same area.

*Ichthyophthirius* appears thus to usually be endemically present amongst wild fish populations in low numbers only, although fish populations may be subject to occasional epidemic outbreaks.

Amongst the far higher densities of fish to be found in commercial fish culture practices, *Ichthyophthirius* outbreaks are of sufficient frequency and severity to pose a considerable economic problem. Bauer (1962) reports more than ten epizootics between 1950 and 1953 in hatcheries in the Belorussian Soviet Socialist Republic (White Russia) in which between 50 and 100% of the breeding stock was killed. Severe epizootics have also been described in Israel (Lahav and Sarig, 1973) and Java (Buschkeil, 1936). It is generally believed that these outbreaks are caused by the introduction of the parasite from wild host reservoirs (Bauer, 1962, Zitnan and Cankovic, 1980). *Ichthyophthirius* is also one of the most important pathogens of aquarium fishes.

# CHAPTER 3

## GENERAL EXPERIMENTAL METHODS

### 3.1 *Source and Maintenance of Hosts and Parasites*

An aquarium variety of *Poecilia latipinna* (Lesueur, 1821), the black mollie, was utilized as the host species throughout these experiments. Fish were obtained from a commercial supplier (Queensborough Fish Farm, Wraysbury, Berkshire). Fish sold commercially as "black mollies" may be black strains of several related species native to the south eastern U.S.A. and central America (*Poecilia sphenops, P. latipinna* and *P. velifera*) or hybrids between them (Schiotz, 1972). The species identification may be determined by the number of dorsal fin rays. Fourteen were found to be present in fish used in these experiments, and on this basis, they were identified as *Poecilia latipinna*.

Fish were fed ad libitum with *Aquarian* tropical fish flakes (Thomas's, Halifax) and maintained in aerated, filtered water at 20°C. Hosts used in experiments ranged in size from 35-45 mm, measured from snout to caudal peduncle. Cultures of *Ichthyophthirius multifiliis* were collected from a variety of fish from Queensborough Fish Farm, the most reliable source species being goldfish *Carassius auratus*. It is probable that the infection was brought in with these fish from Singapore. The disease was maintained by serial transmission on black mollies, and the culture replenished periodically by fresh introductions of *Ichthyophthirius*. Stock infection tanks held ten litres of aerated, but unfiltered water at 20°C, and had a layer of coarse gravel on the bottom. It was found necessary to treat tap water with a commercial "conditioner" (Aquasafe, Tetrawerke Gmbh) to remove chlorine. High

levels of infection were found to be best maintained by commencing a culture with one heavily infected fish and ten susceptible hosts. After two days, the heavily infected host was removed and the water changed. A week later, large numbers of now mature trophozoites could be obtained from the remaining fish. One or more of the fish were used to repeat the process, ensuring a constant supply of parasites. Hosts were treated with commercially available remedies to control pathogens other than *Ichthyophthirius* (few such treatments affect *Ichthyophthirius* trophozoites on the host). However, such treatments were used only when necessary.

### 3.2 *Methods of Obtaining Tomites and of Experimental Infection*

Mature trophozoites were obtained by confining heavily infected fish, eight to nine days after infection, in a small body of conditioned water so that parasites were dislodged by the agitated movements of their hosts. The fish were then removed, and the trophozoites incubated at 20°C in diffuse lighting for 24 hours, by which time large numbers of tomites had been produced. The suspension containing tomites was then decanted, and the concentration of infective stages determined by counting the number present in nine 0.1 ml samples drawn from the suspension after agitation. To facilitate counting, tomites were immobilized with a drop of formalin. All experiments reported here were commenced between 24 and 28 hours after the trophozoites had been removed from the fish.

Infection experiments, except when explicitly stated to the contrary, were undertaken using the following procedure. Hosts were placed individually in clear plastic containers holding 500 ml of conditioned water at 20°C, and the required amount of tomite suspension added. They

were then maintained in diffuse lighting at 20°C. Parasite burden was assessed four or five days after infection by complete counting, under a stereomicroscope, of all parasites visible on the fish. Fish were first anaesthetized using ethyl-m-aminobenzoate (Sigma) at a concentration of 1:10,000 in tap water.

### 3.3 *Numerical Methods*

The statistical analysis in the early part of this thesis (Chapters 4 - 7) was carried out using a Hewlett Packard 9845 desktop microcomputer and associated Hewlett Packard software. The analysis in the remainder of the thesis required more numerical power and (with the exception of analysis for Fig. 12.17), the Imperial College computer system was used. Routines available in the Numerical Algorithms Group library were utilized extensively. In particular, the numerical solutions of systems of differential equations in Chapters 10 - 14 were obtained using a Runge-Kutta-Merson method that adjusted the step length to maintain an accuracy of approximately one part in 1000.

# CHAPTER 4

## THE REPRODUCTIVE RATE

### 4.1 *Introduction*

A number of estimates of the life expectancies of each of the life cycle stages of *Ichthyophthirius* may be found in the literature (Table 2.1). It is not clear, however, whether these represent means or maxima, and evidence concerning the form of the survivorship functions and the number of infective stages produced per cyst is limited. The experiments described in this section are intended to estimate the survivorship functions of the three life cycle stages of *Ichthyophthirius* when infecting black mollies under controlled laboratory conditions. Together with an estimate of the number of tomites produced per cyst, these data enable the determination of the maximum reproductive rate of the parasite under these conditions.

### 4.2 *Methods*

#### 4.2.1. *Trophozoite survival*

Twenty-five fish were placed individually in containers holding 150 ml of conditioned water at 20°C, and exposed to five concentrations of infective stages. Hosts were maintained in the infective solution for a time exceeding the lifespan of the tomites. Each concentration of infective stages was replicated five times. The fish were anaesthetized with ethyl-m-amino benzoate (Sigma) and the parasite burden assessed by direct counting five, seven, eight, nine and ten days after the initial infection

#### 4.2.2. *Time required for encystation*

Trophozoites were removed from the fish eight or nine days after

infection, using the procedure described in Section 3.2. Eighty were then measured with an eyepiece graticule and transferred individually to 0.25 ml tissue culture wells (Cooke microtitre) filled with conditioned water. They were then incubated at 20°C in diffuse lighting and examined every two hours for the presence of tomites. Tomites are not released instantaneously from cysts: excystation was taken to be complete when a substantial number of tomites (> 50) could be detected in the well.

4.2.3. *Number of infective stages produced per cyst*

Hosts were infected with heavy *Ichthyophthirius* burdens by placing them in containers holding suspensions of freshly hatched tomites obtained by the method described in Section 3.2. Each day after infection, the hosts were placed in a small volume of conditioned water so that trophozoites were dislodged by the movements of the hosts. The container was then briefly agitated and then allowed to settle for five minutes, by which time the majority of trophozoites had attached themselves to the bottom of the container. Working in order of proximity to the centre of the container, trophozoites were measured with an eyepiece graticule and then individually pipetted into 0.25 ml tissue culture wells containing conditioned water. The above method aims to produce a random sample of the trophozoites dislodged from the fish. After 24 hours, the number of tomites present in wells in which division had been successful was determined by pipetting out the entire contents of the well onto a gridded slide, and counting the number of tomites present after immobilizing them with a drop of formalin. It was not always possible to count the number of tomites produced by every trophozoite

that had divided within a reasonable time, so to minimize bias, well counts were chosen in the same order in which the trophozoites had been placed in the wells.

4.2.4. *Tomite survival*

Mature trophozoites were obtained by the method described in Section 3.2 and incubated at 20°C in diffuse lighting for 24 hours. The suspension containing tomites was then decanted, and the concentration of infective stages determined by counting, after agitation, the number present in nine 0.1 ml samples drawn from the suspension. To facilitate counting, tomites were immobilized with a drop of formalin, but before this, samples were examined beneath a stereomicroscope to ensure that all tomites visible were active. If necessary, motionless tomites were disturbed with a fine wire. This counting procedure was repeated at intervals until live tomites could no longer be detected. Four times during the experiment, the infectivity of the remaining tomites was assessed by individually exposing five hosts to 2 ml of infective solution.

4.3 *Results*

4.3.1. *Trophozoite survival*

Figure 4.1 shows the proportion of the trophozoites present at day five that are still present on hosts, plotted against the time since infection. Survival data of this form can often be well described empirically by assuming that the death rate increases exponentially with time (Anderson and Whitfield, 1975) so that

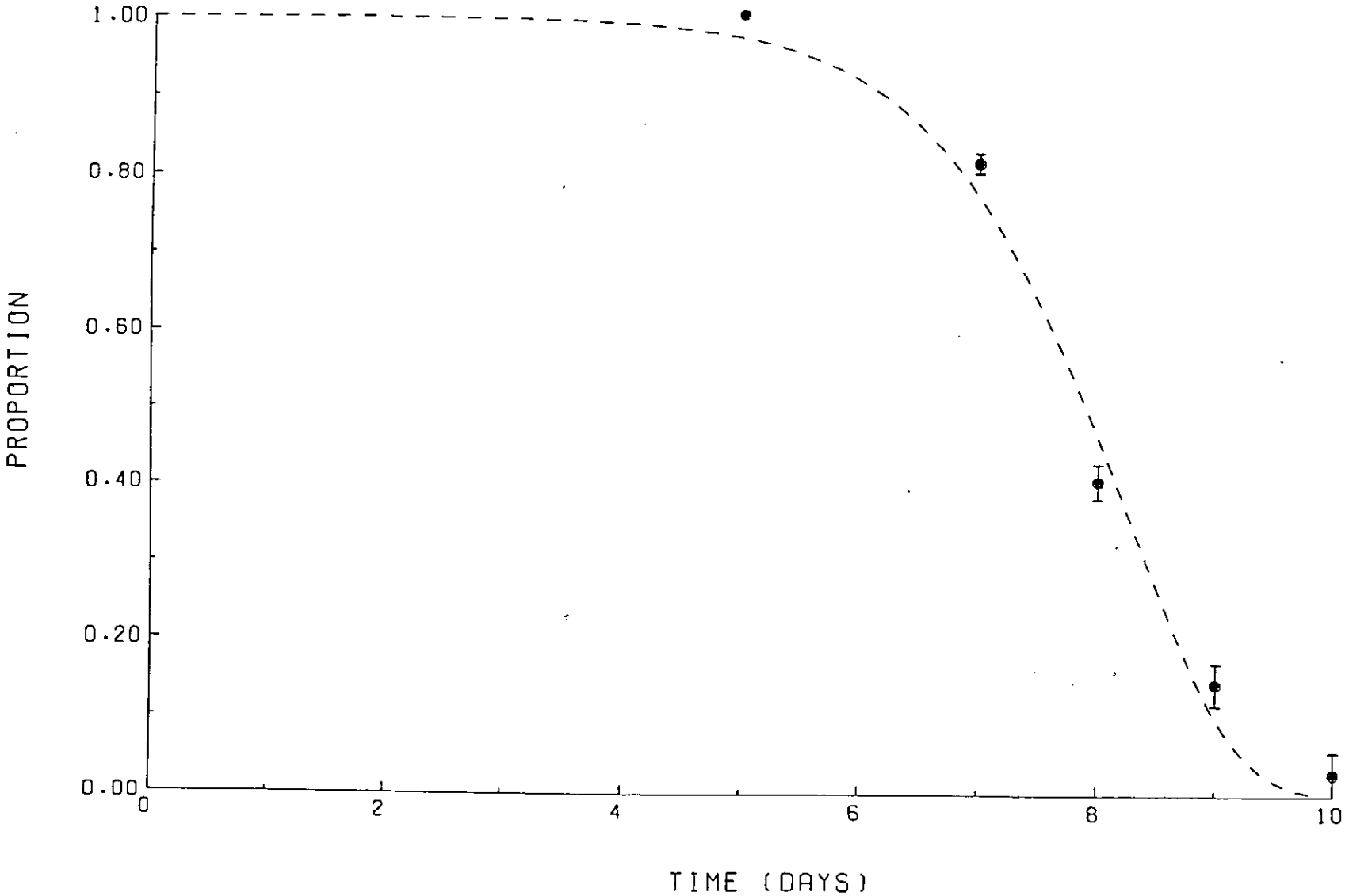
$$dP(x)/dx = -a \exp(bx)\, P(x) \qquad (4.1)$$

where $x$ is the age in days of a cohort of trophozoites, $P(x)$ is the

Figure 4.1 *Survival of Trophozoites*

The horizontal axis shows the time since infection of the hosts in days. The proportion of those trophozoites on fish at day 5 still present is shown on the vertical axis. The error bars shown are 95% confidence limits for the overall proportion of trophozoites surviving. Also shown on the figure is the solution of eqn. (4.2) with parameters estimated by a non linear least squares procedure. The estimated values of $a$ and $b$ are:
$a = 1.44 \times 10^{-4}$ per day and $b = 1.084$ per day.
The mean lifespan of trophozoites, as calculated by eqn. (4.3) is 7.7 days. The results are based on 4592 trophozoites on 25 hosts (the range of parasite burdens can be seen in Fig. 4.2)

The confidence limits may underestimate the true error in the experiment, as they do not allow for the heterogeneity in trophozoite survivorship between hosts.


1.00
0.80
0.60
0.40
0.20
0.00
PROPORTION
0
2
4
6
8
10
TIME (DAYS)

size of the cohort at age $x$ and $a$ and $b$ are constants. Equation (4.1) may be integrated to find $l(x)$, the proportion of the cohort surviving to age $x$:

$$l(x) = \exp\left[a/b \quad [1 - \exp(bx)]\right] \tag{4.2}$$

The line shown on Figure 4.1 is a solution of eqn. (4.2) with the parameters $a$ and $b$ estimated from the experimental data by means of a non linear least squares procedure (Appendix 1). Equation 4.2 may be used to estimate the mean time, $l_o$, spent by trophozoites on hosts,

$$l_o = \int_0^\infty l(x)\, dx \tag{4.3}$$

Using the values of $a$ and $b$ found from the non linear least squares procedure, $l_o$ was estimated as 7.7 days.

No evidence of density dependence in the time spent on hosts was found in this experiment. Figure 4.2 shows the proportion of parasites present on hosts at day five remaining at day eight, plotted against the size of the original burden. A negative correlation would be expected if higher burdens resulted in trophozoites leaving the host earlier, but no such relationship is evident. There is, however, considerable variability in the proportions of parasites surviving to day eight between individual hosts. A $\chi^2$ test of homogeneity of the numbers of parasites remaining on hosts yielded a highly significant value of $\chi^2 = 439$ with 24 degrees of freedom.

These differences may be caused by differing levels of activity

Figure 4.2 *Proportion of trophozoites remaining on hosts at day 8*

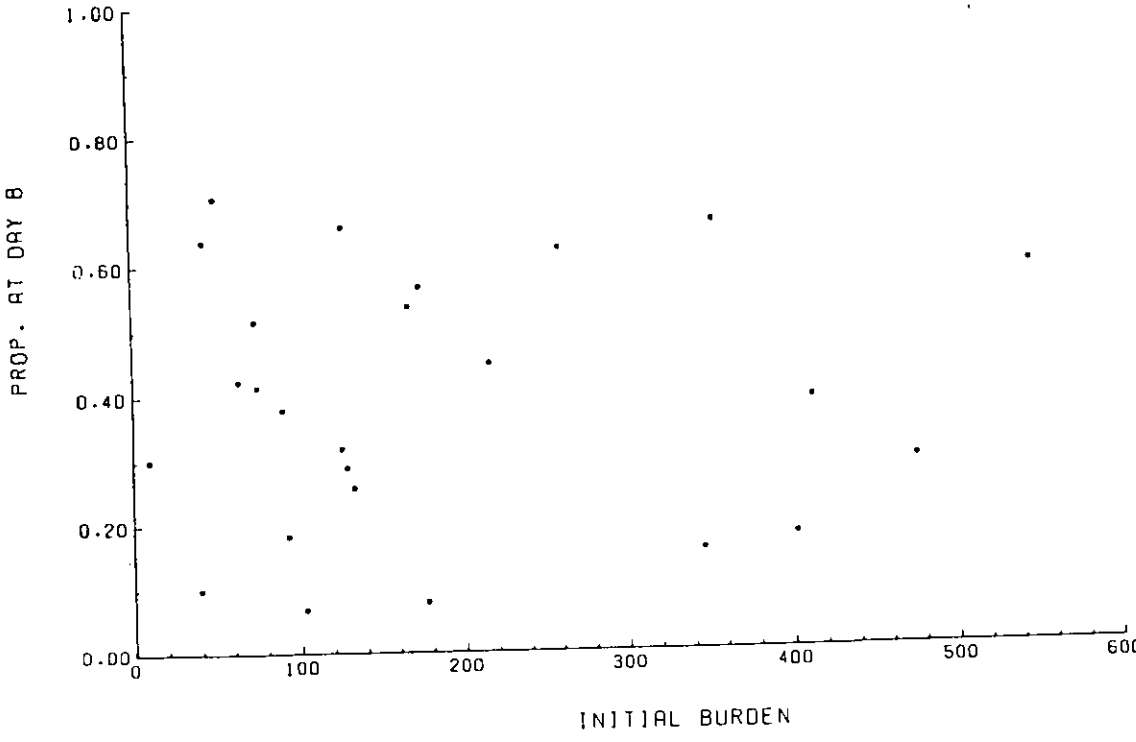
The parasite burden at day 5 (horizontal axis) is shown plotted against the proportion of that burden remaining on hosts 8 days after infection (vertical axis)

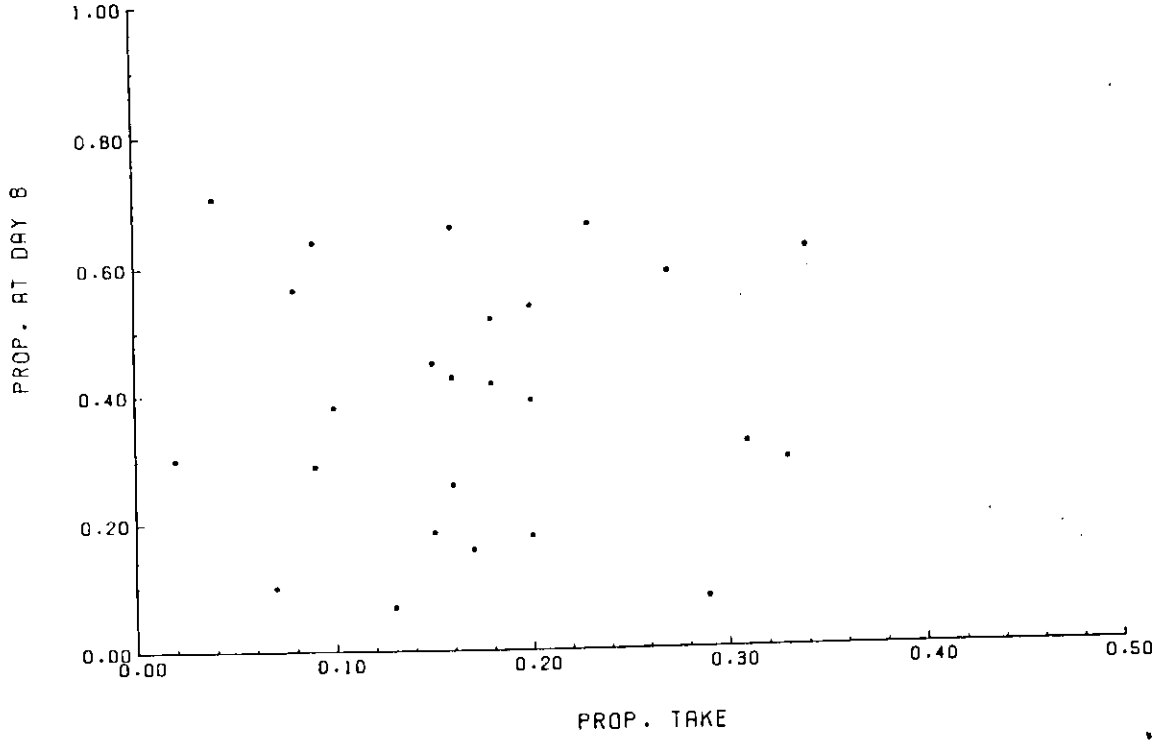
($r^2$ = 0.0015 with 23 degrees of freedom)

Figure 4.3 *Trophozoite establishment and survival*

The proportion of the tomites to which hosts were exposed that were counted as trophozoites at day 5 (the "proportion take", horizontal axis) is shown plotted against the proportion of trophozoites established at day 5 remaining to day 8 (vertical axis)

($r^2 = 8.6 \times 10^{-8}$, 23 df).


1.00
0.80
0.60
0.40
0.20
0.00
PROP. AT DAY 8
0
100
200
300
400
500
600
INITIAL BURDEN


1.00
0.80
0.60
0.40
0.20
0.00
PROP. AT DAY 8
0.00
0.10
0.20
0.30
0.40
0.50
PROP. TAKE

of the fish. Active hosts may dislodge their trophozoites earlier than those that are less active. They may also be caused by differences in the efficiency of host defence mechanisms to parasitic invasion. The differences may be genetic in origin, or caused by differing history of exposure to the disease. If such differences do exist, however, their influence on parasite longevity does not seem to be related to the degree of resistance to the initial infection. Figure 4.3, for example, shows the proportion of tomites added that were initially detected as trophozoites, plotted against the proportion of trophozoites established that are still present at day 8. No relationship is evident. A third possibility is that inaccuracies in counting may be responsible for some of the differences observed.

4.3.2. *Time required for encystation*

The proportion of viable cysts yet to release tomites is shown related to time since removal from the host in Fig. 4.4. The curve shown is a solution of eqn. (4.2) with parameters $a$ and $b$ estimated by a non linear least squares procedure. A majority of cysts are found to produce tomites between 19 and 25 hours after removal from the host. The time by which half the viable cysts have released tomites is estimated at approximately 21 hours.

Figure 4.5 shows the results of an analysis of variance comparing the diameter of trophozoites requiring differing amounts of time to produce infective stages. Trophozoites requiring more than 21 hours are larger than those requiring either less than this time, or failing to produce tomites at all. As 85% of excystation occurs between 19 and 24 hours, this effect is unlikely to be of great significance to

Figure 4.4 *Time required for encystation.*

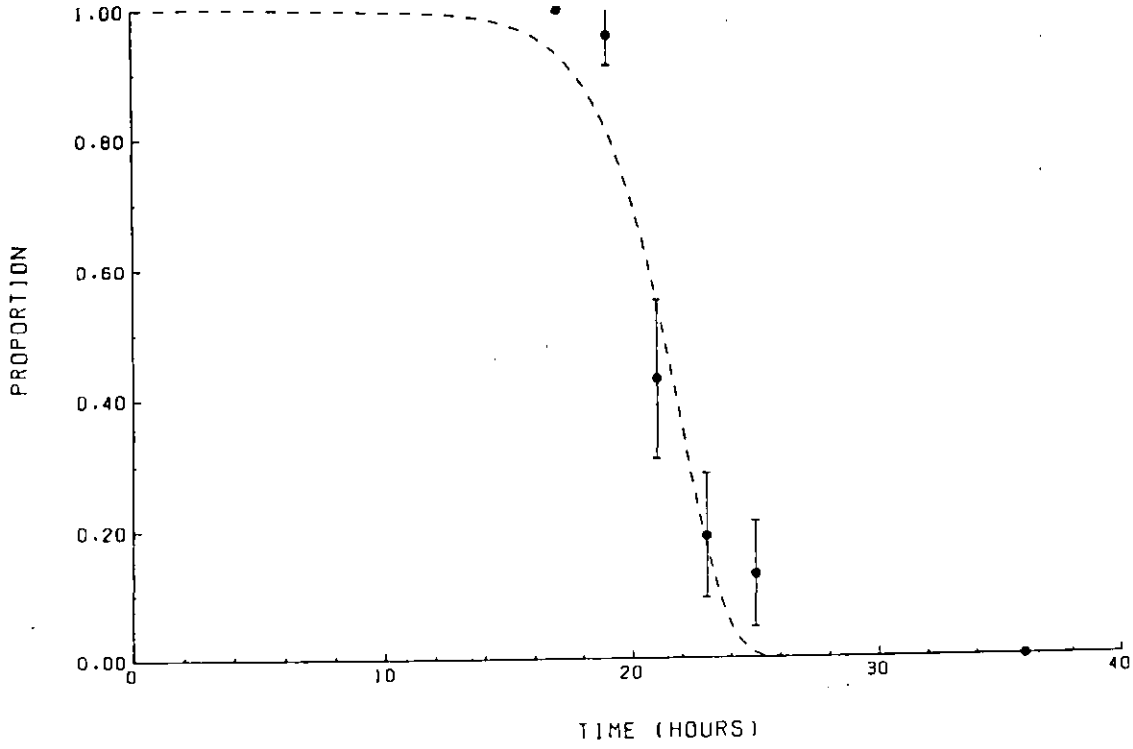
The proportion of viable cysts yet to release tomites (vertical axis) is shown related to the time in hours since the release of the trophozoites from the host. 95% confidence limits for the proportions are shown on the figure. The curve shown is the solution of eqn. (4.2) with parameters $a$ and $b$ estimated by a non linear least squares procedure. Estimated values of $a$ and $b$ are: $a = 4.9 \times 10^{-6}$ per hour, $b = 0.5277$ per hour. The data presented are based on the 67 viable cysts of 80 examined.

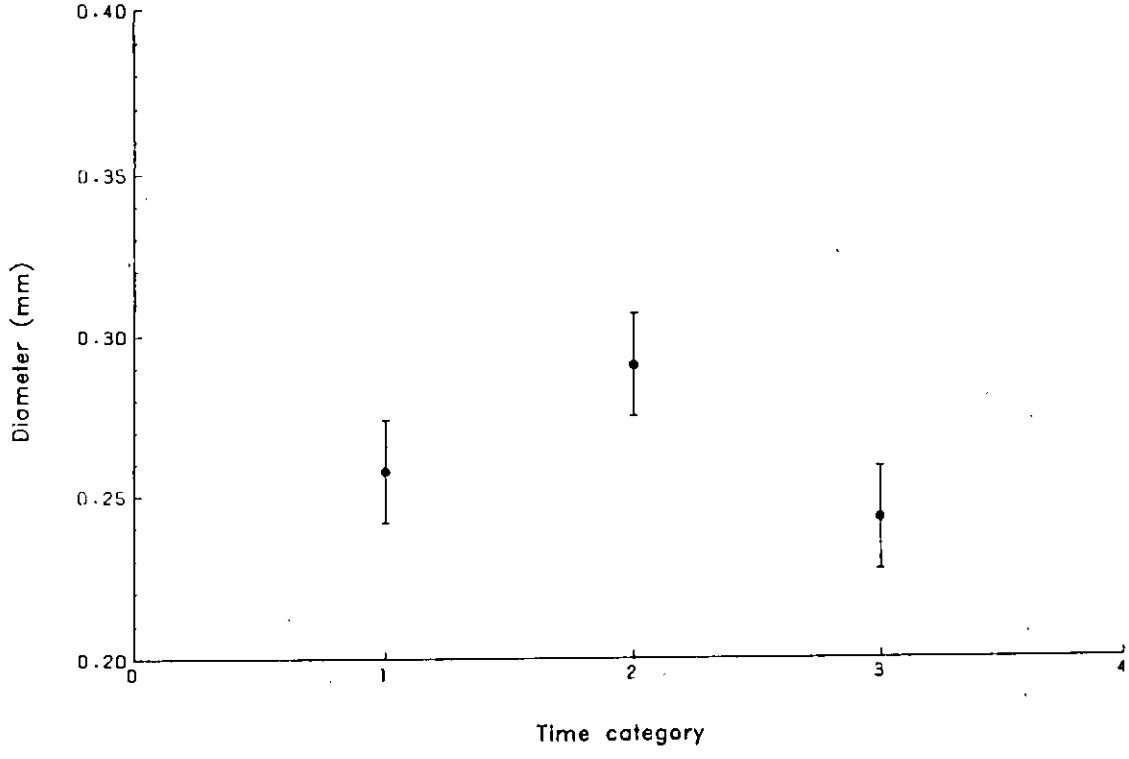
Figure 4.5 *Trophozoite size and time required for encystation.*

The figure presents the results of an analysis of variance comparing trophozoite diameter and the time elapsed before trophozoites are produced. Trophozoite diameter in mm is shown on the vertical axis. The level numbers on the horizontal axis represent the time elapsed until tomites appear and correspond to: < 21 hours (1); > 21 hours (2) and unsuccessful encystation (3). The bars represent half the least significant difference in either direction.

Analysis of Variance

| Source | S.S. | df | M.S. | F | P |
|---|---|---|---|---|---|
| Total | 0.229 | 79 | | | |
| Time | 0.027 | 2 | 0.0137 | 5.21 | < 0.01 |
| Error | 0.202 | 77 | 0.0026 | | |


1.00
0.80
0.60
0.40
0.20
0.00
PROPORTION
0
10
20
30
40
TIME (HOURS)


0.40
0.35
0.30
0.25
0.20
Diameter (mm)
0
1
2
3
4
Time category

the population dynamics of the system.

4.3.3. *Number of tomites produced per cyst*

No trophozoites were found to be detached from hosts earlier than four days after the initial infection. The relationship between the diameter of trophozoites and time since infection is shown in Figure 4.6.

The growth rate, as measured by increase in diameter, is considerably smaller than that found by Hines and Spira (1973a) at a similar temperature (see Table 2.1), but it should be noted that Hines and Spira's results are biased upward, as they did not count parasites less than 0.1 mm in diameter after day 4.

Table 4.1 shows the number of trophozoites of different ages that successfully produced tomites. The overall proportion of cysts producing tomites is only 0.49, but this result should be interpreted with a degree of caution. It is probable that a number of trophozoites may have been damaged or ruptured during pipetting. No trend in proportion of cysts producing tomites with age is evident, and the age related values do not depart significantly from the total proportion producing tomites over all ages ($\chi^2 = 9.25$, df = 5,n.s). The number of tomites produced per cyst is shown related to time since infection in Figure 4.7. These results are well described empirically by a relation of the form:

$$n(x) = ax^b \qquad (4.4)$$

where $x$ is the time in days since infection, and $n(x)$ is the number of tomites produced from a trophozoite leaving the host at that time. The parameters $a$ and $b$ are constants.

Table 4.1 *The Number of Trophozoites Producing Tomites*

| Age of trophozoite (days) | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|
| Number producing tomites | 28 | 20 | 19 | 26 | 13 | 8 |
| Number failing to produce tomites | 19 | 27 | 17 | 18 | 27 | 9 |
| Total | 47 | 47 | 46 | 46 | 40 | 17 |

Figure 4.6 *Trophozoite Growth*

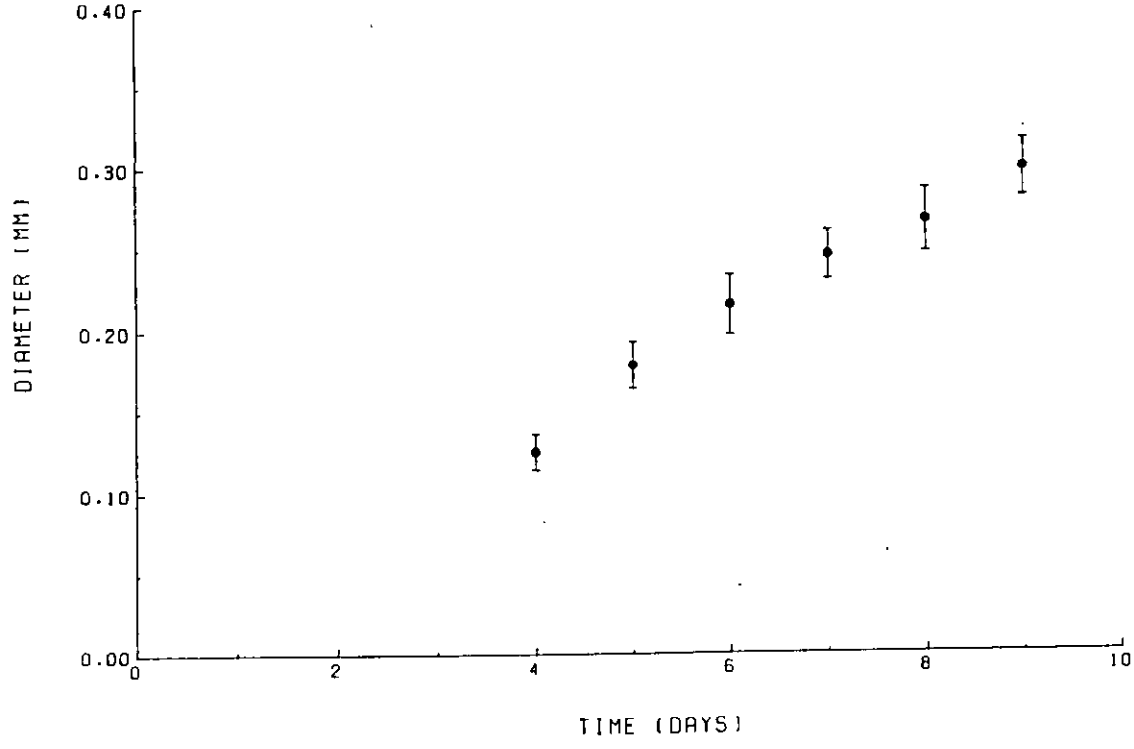
The mean diameter of trophozoites (in mm) is shown plotted against the time since infection in days. The bars represent 95% confidence intervals for the means.

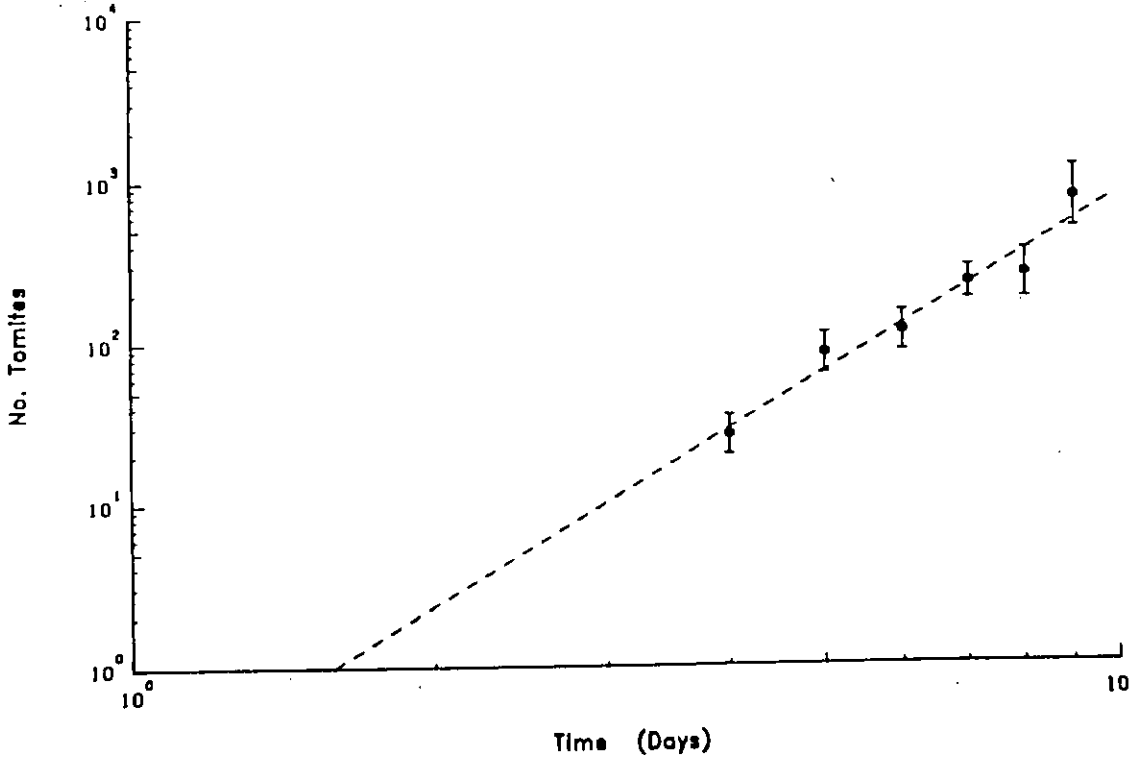
Figure 4.7 *Number of tomites per cyst.*

The number of tomites per cyst (vertical axis) is shown plotted against time since infection, on double log axes. The circles represent the geometric mean of the number of tomites per cyst, and the bars are 95% confidence intervals for this mean, obtained from the transformed data. The line shown is the solution of eqn. (4.4) with $b$ and $\log_{10}(a)$ obtained by linear least squares regression of $\log_{10}[n(x)]$ on $\log_{10}(x)$. Details of the regression are given below.

| Coefficient | Estimated value ± se | t |
|---|---|---|
| $\log_{10}(a)$ | -0.725 ± 0.176 | - 4.13 |
| $b$ | 3.633 ± 0.226 | 16.1 |

107 degrees of freedom.


0.40
0.30
0.20
0.10
0.00
DIAMETER (MM)
0
2
4
6
8
10
TIME (DAYS)


No. Tomites
Time (Days)

4.3.4. *Mean number of tomites per cyst*

Over a short time period $\Delta x$, $l(x)-l(x+\Delta x)$ trophozoites leave the host, producing

$$n(x) \quad [\ l(x) - \quad l(x+\Delta t)]$$

tomites. In the limit as $\Delta x$ tends to 0, the rate of tomite production is:

$$-n(x)\ \mathrm{d}\ l(x)/\mathrm{d}x \tag{4.5}$$

Hence the mean number of infective stages produced is

$$M = -\int_0^\infty [\mathrm{d}\ l(x)/\mathrm{d}x][n(x)]\ \mathrm{d}x \tag{4.6}$$

Using the expression found for $l(x)$ in Section 4.3.1. (eqn. 4.2) and eqn. (4.4)this was calculated to be 344.24.

4.3.5. *Net reproductive rate*

The maximum reproductive rate, $r_o$, of an age structured population is given by the Euler equation (Roughgarden, 1979):

$$1 = \int_0^\infty \exp(-r_o x)\ l(x)m(x)\ \mathrm{d}x \tag{4.7}$$

where $x$ is the age of the organisms, $l(x)$ the proportion surviving to time $x$, and $m(x)$ the age specific fecundity (the mean number of births to an individual of age $x$). In reality, the population is unlikely to grow at a rate as high as $r_o$, as this rate will only be

realized in the absence of density dependent constraints, and if unconstrained population growth has occurred for long enough for the population to attain a stable age distribution (Roughgarden, 1979). Nevertheless, $r_o$ is a valuable measure of the maximum reproductive potential of the population and will be required in the mathematical models of *Ichthyophthirius* dynamics in Chapter 14. Equation (4.7) requires some modification before it can be applied to *Ichthyophthirius*. Trophozoites reproduce only when they leave the host, when they produce $n(x)$ tomites. In a short period $\Delta x$, the proportion of trophozoites leaving the host is $l(x) - l(x+\Delta x)$, each of which is assumed to produce $n(x)$ tomites. Hence, the rate of production of tomites per trophozoite present on the host at time $x$ is

$$n(x) \quad [l(x) - l(x+\Delta x)] \; / \; [l(x)\Delta x] \qquad (4.8)$$

This expression is equivalent to the $m(x)$ of eqn. (4.7). Letting $\Delta x$ approach 0, the modified version of eqn. (4.7) is:

$$1 = \int_0^\infty -[d\, l(x)/dx]\; n(x) \quad \exp(-r_o x)\; dx \qquad (4.9)$$

Two further complications must be dealt with before $r_o$ can be estimated. All tomites do not successfully infect a host and form a trophozoite. The size of this proportion is dependent on host density, but experiments described in Section 5 indicate that it will saturate at high host densities to around 10% of the number of tomites present. There is also a time delay of approximately one day caused by the time required for encystation. This may be included in eqn. (4.9) by using $\exp(-r_o[x+1])$ instead of $\exp(-r_o x)$. All trophozoites

do not successfully encyst, but as this proportion appears to be variable, and it is the maximum reproductive rate which is of interest here, all encystations are assumed successful. The following equation was therefore used to estimate $r_o$

$$1 = - \int_0^\infty \exp(-r_o[x+1]) \left[ d\, l(x)/dx \right] n(x)\, s\, dx \qquad (4.10)$$

where $l(x)$ is obtained from eqn. (4.2), $n(x)$ is obtained from eqn. (4.4) and $s$, the proportion of tomites successfully forming trophozoites, is set at 0.1.

The value of $r_o$ was estimated to be 0.392, using an iterative procedure and numerical integration. This rate may be compared with that obtained by assuming that all trophozoites leave the host at exactly the mean time spent on hosts found in Section 4.3.1, and that all produce the mean number of tomites found in Section 4.3.4. after one day. In this case,

$$1 = \exp(r_o[7.7+1]).(344) \qquad (4.11)$$

Here $r_o$ is found to be 0.407. This rate is slightly higher than the estimate obtained from eqn.(4.10.) In the more exact expression (eqn. 4.10), the higher number of trophozoites leaving hosts later than the mean time is discounted by the increased time they take to produce tomites.

4.3.6. *Tomite survival*

The number of tomites present in the suspension remained approximately constant for twenty hours, after which the rate of mortality rose rapidly (Fig. 4.8). Dead tomites were not detected: it

appears likely that they are rapidly broken down after death. The data are well described by the curve shown in Figure 4.8, which is a logged version of eqn. (4.2).

$$\log(T(x)) = \log(T_o) + a/b\ (1 - \exp(bx)) \qquad (4.12)$$

where $T(x)$ is the number of tomites of age $x$ present, $T_o$ is the number of tomites of age 0 and $a$ and $b$ are constants. The parameters $T_o$, $a$ and $b$ were estimated by means of a non linear least squares procedure (Appendix 1).

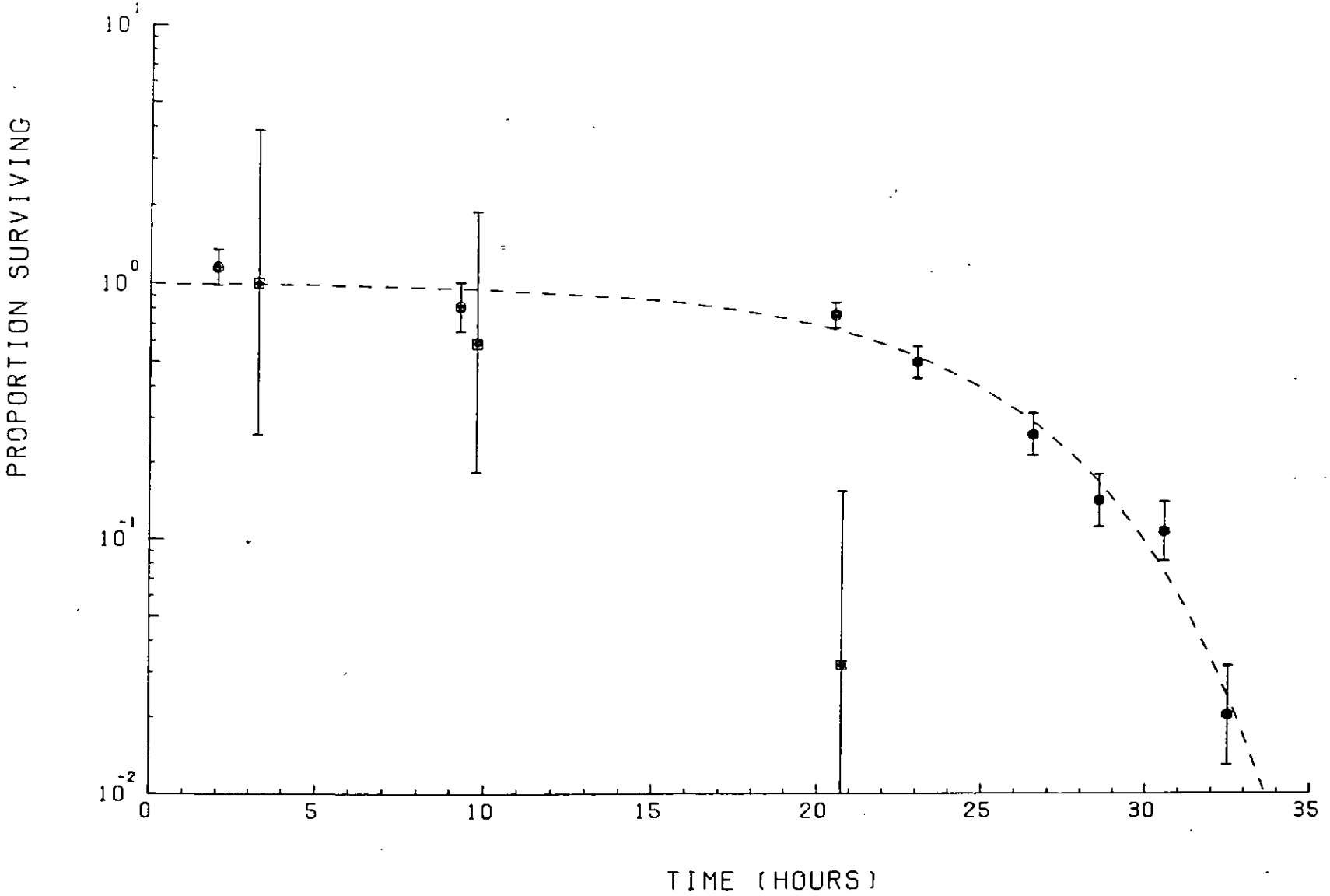
Survival curves of the general form of that shown in Figure 4.8 in which the death rate increases exponentially with age have been observed for a wide variety of non feeding infective stages (Anderson and Whitfield, 1975). The parameters $a$ and $b$ determined from the non linear least squares procedure may be used in conjunction with eqn. (4.3) to estimate the mean life expectancy of the tomites, which was estimated to be 22.5 hours. It should be realized, however, that this estimate is approximate, as it is not possible to precisely determine the time at which tomites were produced. The results in Section 4.3.3. indicate that tomites are released over a relatively short period prior to 24 hours after the removal of trophozoites from the host.

The infectivity of the tomite suspension, as measured by the burden on hosts infected at various times during the course of the experiment, appears to decline faster than the number of tomites present (Fig. 4.8). This indicates that the ability of surviving tomites to successfully infect hosts decreases with age.

Figure 4.8 *Tomite survival and age dependent infectivity*

The proportion of tomites surviving of those originally present (vertical axis) is shown plotted against the time since release of tomites from cysts. A log transformation has been performed, which acts to stabilize the variance at different times. The circles represent the mean proportion of tomites surviving, which is recorded with 95% confidence limits based on the transformed data. The curve shown is the solution of eqn. (4.12) with parameters $a$, $b$ and $\log_{10}(T_o)$ estimated by a non linear least squares procedure. It is possible for the first data point to exceed unity because $T_o$, the number of tomites originally present, was determined as a parameter of the model. Estimated parameter values are: $\log_{10}(T_o) = 1.87$ ($\equiv$ 74 tomites per 0.1 ml), $a = 0.00166$ per hour, $b = 0.1858$ per hour.

Also shown on the figure, represented by squares, is the infectivity of the tomites in the suspension. This is defined as the number of trophozoites established after exposure to tomites of a given age, relative to the mean number established after exposure to tomites of age 3.75 hours (the variable is log transformed and 95% confidence intervals are shown). When hosts were exposed to tomites of age 26 hours or older, no successful infections were recorded. (This point cannot be plotted on the logged axes). The geometric mean number of trophozoites established using tomites of age 3.75 hours was 260, which is 15% of the number of tomites to which the hosts were exposed.


PROPORTION SURVIVING
$10^{1}$
$10^{0}$
$10^{-1}$
$10^{-2}$
0
5
10
15
20
25
30
35
TIME (HOURS)

## 4.4 *Conclusions and Discussion*

The estimates made in this section of the time spent at 20°C in each of the three life cycle stages are in close agreement with those reported by other authors, working with a variety of host species (Table 2.1). No evidence was found that parasite density affects the time spent by trophozoites on the host, although the range of parasite densities used did not approach the level at which significant host mortality occurs.

Trophozoite survival could not be monitored from the time of infection, because of the difficulty in counting very small trophozoites. It is possible that a considerable number of trophozoites are overcome by the host defences in the early stages of infection. For the purposes of this analysis, they are included with tomites that encounter a host, but fail to establish upon it. Later in the infection, no distinction is drawn between trophozoites that have left the host to encyst, and those that might have been killed by the host defences. No evidence was seen of trophozoites being resorbed by the host.

It was not possible to determine when cysts that failed to produce tomites actually died, and the curve showing encystation time therefore refers only to those cysts that proved to be viable by eventually releasing tomites.

Each of the three curves describing the proportion of parasites originally present still in the life cycle stage under consideration is well fitted by assuming that the death or emigration rate increases exponentially with time. This assumption was first used by Gompertz in 1825 (Benjamin and Pollard, 1980) to describe the increasing rate

of mortality of humans with age. It should be thought of as a convenient two parameter method of generating a sigmoid curve, rather than suggesting any particular underlying biological mechanism.

Estimation of confidence intervals for the parameters of these survivorship functions is an extremely difficult statistical problem, and hence only point estimates are given.

# CHAPTER 5

# TOMITE INFECTIVITY

## 5.1 *Introduction*

The infection process is of fundamental importance in the population dynamics of any parasite. *Ichthyophthirius multifiliis*, which is unable to reproduce on its host, and has a short life expectancy in each of its life history stages, must have a high infectivity if it is to persist within fish populations. Having encountered a host, a tomite must attach to its surface and successfully penetrate the mucus layer and the epidermis in order to establish as a trophozoite. It is at this stage and immediately afterwards, that one might expect the parasite to be most vulnerable to the host's specific and non-specific defence mechanisms.

In this chapter, the infectivity of tomites to previously unexposed black mollies is investigated in a series of linked experiments. Possible sources of density dependence are examined, and an estimate is made of the proportion of tomites infecting a host that are able to form trophozoites.

## 5.2 *Experimental Methods*

Tomites for experimental infections were obtained by the methods described in Section 3.2. Trophozoite burdens were assessed by complete counting of parasites on hosts four or five days after the initial infection. A number of experiments were carried out on parasite infectivity. The methods used for each are described below.

5.2.1. *Varying the number of infective stages*

Fish were placed individually in containers holding 150 ml of conditioned water at 20°C and exposed to five concentrations of infective stages. Hosts were maintained in the infective suspension for a time exceeding the maximum lifespan of the tomites. Five replicates were used at each concentration. Parasite burden was assessed five days after the initial exposure.

5.2.2. *Varying the number of hosts*

Differing numbers of hosts, ranging from one to fifteen, were placed in tanks holding 10 l of conditioned water at 20°C. Each host density was replicated at least twice.

Approximately 1150 tomites were added to each tank

The fish were maintained in this infective solution until the parasite burden was assessed four days after the initial exposure.

5.2.3. *Varying the concentration of infective stages*

Fish were placed individually in containers holding between 250 ml and 10 l of conditioned water at 20°C. The same amount of a tomite suspension was added to each container, thus varying the concentration of tomites present, whilst maintaining a constant overall number of infective stages. Hosts were removed five days later and the parasite burden assessed. Each treatment was replicated five times.

5.2.4. *Varying the duration of exposure time*

Hosts were exposed individually to the same amount of tomite suspension (containing approximately 1070 tomites) at 20°C for six different times, ranging from ½ hour to 6 hours, in two water volumes, 250 ml and 500 ml. Each combination of exposure time and water volume was replicated five times. At the end of their exposure period, fish

were removed and placed in 500 ml of conditioned water, in which they were kept until the parasite burden was assessed four days later.

5.2.5. *Distribution of parasites on the surface of the host.*

This experiment follows a different pattern from the others reported in this section. One heavily infected host was added as a "seed" to a tank holding 10 l of conditioned water, together with twenty-five naive hosts. Seven days later, ten of the previously uninfected hosts were removed and the parasite density at eight points on their surface was assessed by counting the number of trophozoites visible within a square of 0.08 $cm^2$ area, measured by an eyepiece graticule. The following positions were examined : right pectoral fin, left and right opercula; left and right sides of the tailfin, immediately behind the peduncle; either side of the flank, immediately below the dorsal fin; and the top of the head, between the eyes.

5.3 *A Model to Describe the Infection Process*

The following simple model, modified from Anderson (1978) will be used to assist in the analysis of experimental results.

Suppose that initially there are $T_o$ tomites and $N$ hosts present in a tank. No tomites are added during the course of the experiment and infective stages are removed either by dying or infecting a host. Suppose also that each host has a "susceptibility" to infection, $s$, between 0 and 1, such that only the proportion $s$ of tomites infecting this host develops to form detectable trophozoites. The remainder are assumed to be overcome by host defences (either nonspecific or immunological). Initially, both the death rate of the tomites and their

infectivity are taken as constant. In a short time period, $\Delta t$, the probability that an individual infective stage will die is assumed to be $\mu\Delta t$, where $\mu$ is the instantaneous death rate of the tomites (defined per capita, per unit time). The probability that one will infect a host is assumed to be $\beta N\Delta t$, where $\beta$ is the instantaneous infection rate of the tomites (defined per host, per tomite, per unit of time). This expression is based on the assumption that infection occurs as the result of an encounter between randomly distributed hosts and infective stages, and the value of $\beta$ should therefore be inversely proportional to the water volume in which the experiment is being carried out.

Letting $T(t)$ and $P(t)$ be the number of tomites and trophozoites present at time $t$ and letting $\bar{s}$ be the mean susceptibility of the host population, these assumptions lead to the following differential equations to describe temporal changes in the two variables;

$$dT(t)/dt = -\mu T(t) - \beta N T(t) \qquad (5.1)$$

$$dP(t)/dt = \beta N \bar{s} P(t) \qquad (5.2)$$

The solution of eqn. (5.1) and eqn. (5.2) for the number of parasites present on hosts by time $t$ is:

$$P(t) = NT_o\bar{s}/\left[(\mu/\beta) + N\right]\left\{1 - \exp(-\left[\mu + \beta N\right] t)\right\} \qquad (5.3)$$

If the exposure time, $t$, is long compared with the lifespan of the infective stages ($\mu t$ large), the number of parasites established, $P(t \to \infty)$ is simply:

$$P(t \to \infty) = NT_o\bar{s} / \left[(\mu/\beta) + N\right] \qquad (5.4)$$

5.4 *Results*

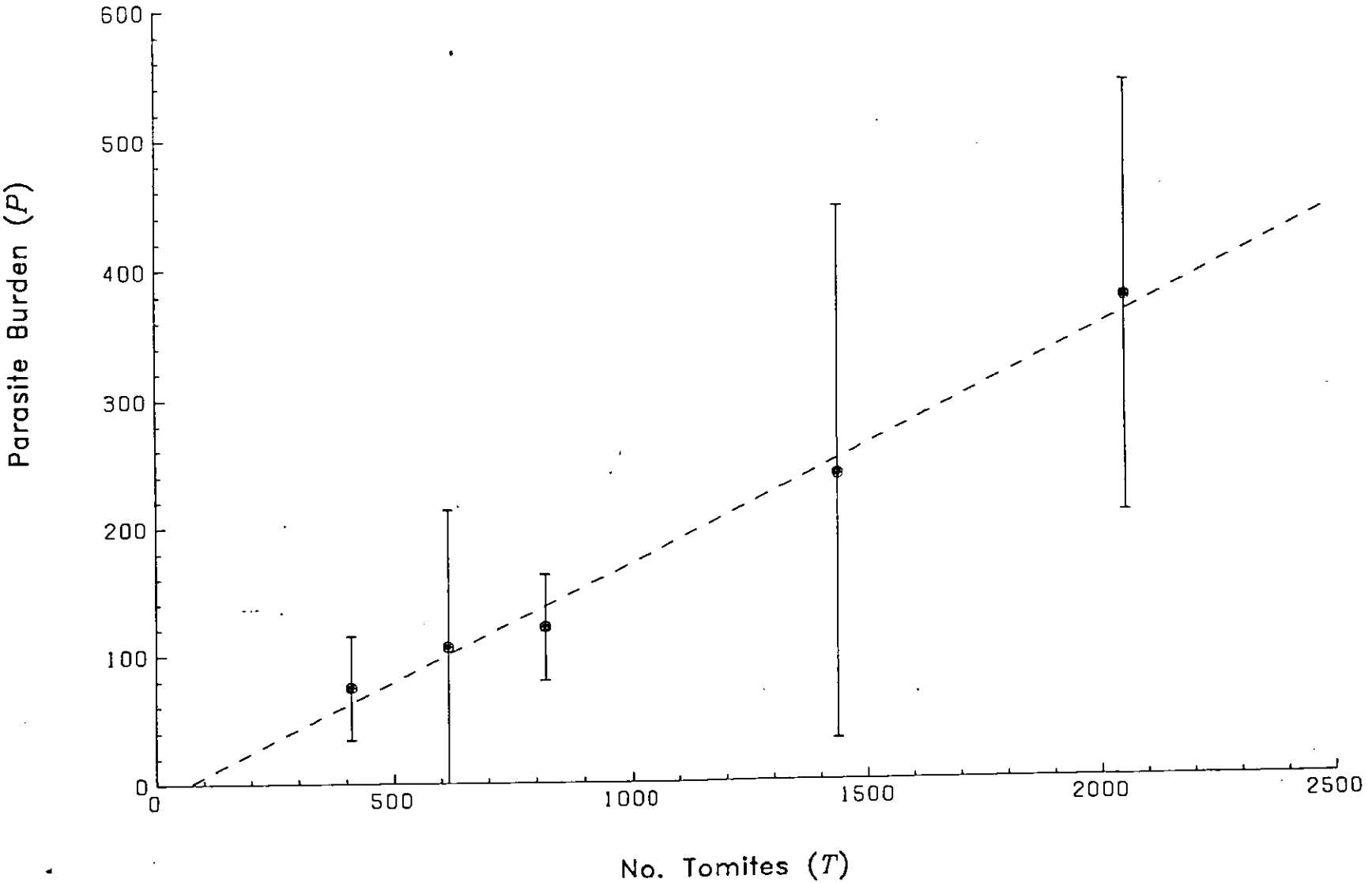
The number of trophozoites established on hosts subjected to differing numbers of tomites was found to be linearly dependent on the number of tomites added to the infection arena (Fig. 5.1). Although there is considerable variability in the burden established from the same infective dose, there is no evidence of an infection threshold nor of nonlinearity over this range of parasite burdens. The gradient of the line of best fit is 0.18: slightly less than one fifth of all tomites are found as adult trophozoites on the host. This linear relationship may exist for two quite separate reasons. It may be that only a proportion of tomites locate a host before they die, or that only a proportion of the tomites that infect a host survive to form detectable trophozoites. In the forms of the model defined in Section 5.3, the gradient of the best fit line in Figure 5.1 is:

$$\bar{s} \; / \; \left[\mu/\beta + 1\right]$$

The relative importance of the two terms, the susceptibility of the hosts to the infective stages, $\bar{s}$, and the death rate divided by the infectivity, $\mu/\beta$, to the infection dynamics may be determined by subjecting differing numbers of hosts to the same number of infective stages in water bodies of equal size (Section 5.2.2.). If essentially all tomites locate a host before death, the hosts should divide the number of tomites between them. Provided host location is the limiting factor, mean parasite burden should be approximately independent of

Figure 5.1 *Trophozoite establishment*

The relationship is shown between the number of parasites established on a single host ($P$) and the number of tomites to which it was exposed ($T$). The circles represent the observed mean number of trophozoites established, and the bars are 95% confidence intervals for these means.
The line shown is the linear least squares regression line of $P$ on $T$ : $P = -12.6 + 0.184T$ (se of intercept = 40.4, se of coefficient = 0.0311, df = 28).


Parasite Burden (P)
600
500
400
300
200
100
0
0
500
1000
1500
2000
2500
No. Tomites (T)

host density. Equation (5.4) predicts that the burden, $M$, per host is:

$$M = T_o \, s \; / \; [\mu/\beta + N] \qquad (5.5)$$

Figure 5.2 shows the results of experiment (5.3.2.) with the inverse of parasite burden per host plotted against host density. The relationship between inverse of burden per host and host density is indeed linear ($F = 12.7$, d.f. = 1,76 $p < 0.001$ for regression; $F = 0.2$, d.f. = 3,73 N.S., lack of fit test). The intercept does not differ significantly from 0 ($t = 0.81$, $n = 76$, N.S.). It therefore appears that in water bodies of up to 10 l, essentially all tomites will locate a host before death.

The results of the tomite survival experiment (Section 4.3.6) indicate that the assumption of a constant tomite death rate made in the model of Section 5.3 is an oversimplification. It is possible to modify the model to include an age dependent death rate of the form defined in eqn. (4.2), so that eqn. (5.1) becomes:

$$\mathrm{d}\, T(t)/\mathrm{d}t = -a \exp(bt)\, T\,(t) - \beta N T(t) \qquad (5.6)$$

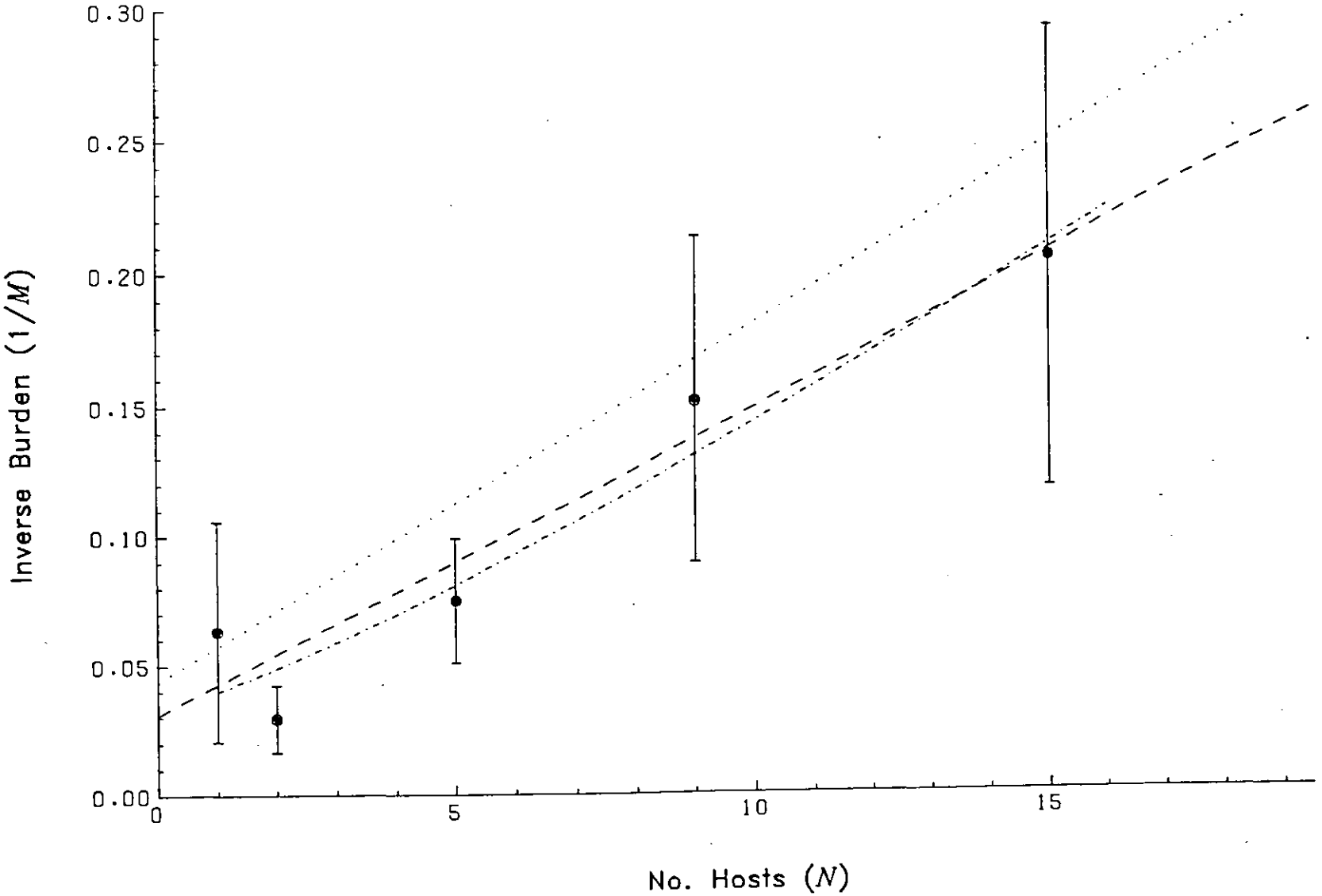
where $a$ and $b$ are constants, and the other parameters and variables are as defined in Section 5.3. Equation (5.2)remains unchanged. The time dependent solution of eqn. (5.2) and eqn. (5.6) is of the form:

$$P(t) = \beta T_o N \bar{s} \int_0^t \exp\left[a/b(1-\exp[b\tau]) - \beta N \tau\right] \mathrm{d}\tau \qquad (5.7)$$

For any particular set of parameter values, this equation can be solved by numerical means (It is not possible to solve eqn. (5.7) analytically).

Figure 5.2 *The effect of host density on trophozoite establishment.*

The relationship is shown between the inverse of parasite burden per host ($1/M$) and number of hosts in a tank. Each tank contained approximately 1150 tomites. The circles represent the observed means of inverse parasite burden, with 95% confidence limits shown. The evenly dashed line represents the linear least squares regression of $1/M$ on $N$ using the experimental data ($1/M = 0.0307 + 0.0119N$; se of intercept = 0.0378; se of coefficient = 0.0036, 76 df). The dotted line is the prediction of the simple model, eqn. (5.4). The solution of the complex model, eqn. (5.7) is denoted by the unevenly dashed line.


0.30
0.25
0.20
0.15
0.10
0.05
0.00
Inverse Burden (1/M)
0
5
10
15
No. Hosts (N)

Values of $1/\left[ P(t \to \infty)/N \right]$ for a range of $N$ were calculated from eqn. (5.7) using estimates of $a$ and $b$ obtained in Section 4.3.6. and values of $\beta$ and $\bar{s}$ obtained from the results of experiment 5.2.4. These are shown on Fig. 5.2. (The integration was performed between 0 and 40 hours). Figure 5.2 also displays the inverse burden per host ($1/M$) predicted by the original model (eqn. 5.4) using $\mu$ calculated as the inverse of tomite life expectancy from Section 4.3.6. and $\beta$ and $\bar{s}$ again obtained from experiment 5.2.4. It can be seen that both lines are very similar and close to the best fit experimental line. In this case, the admittedly more biologically realistic model with time dependent tomite survival has little extra predictive power when compared with the simple model in which $\mu$ is assumed to be constant.

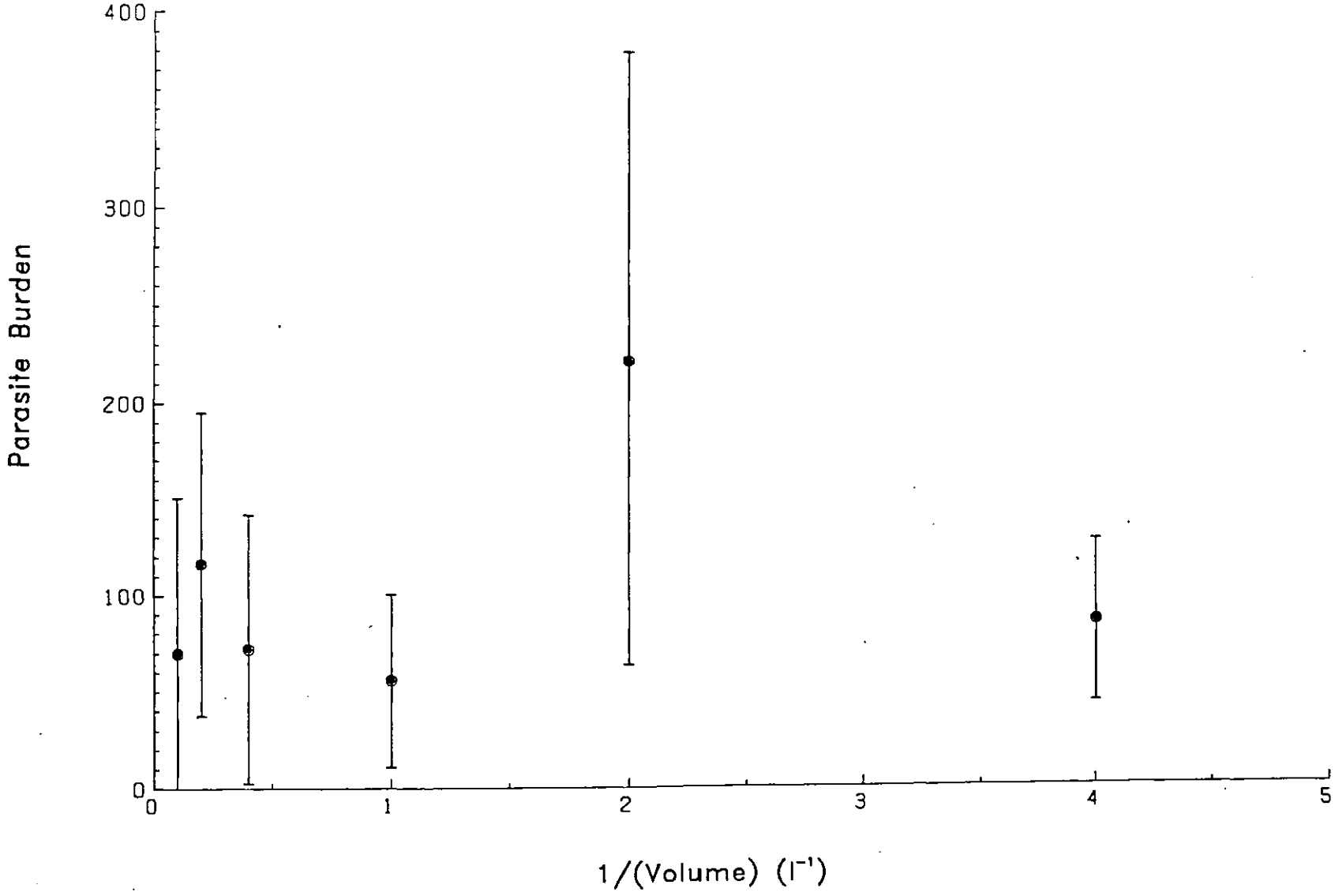
A further result that lends support to the contention that most tomites will locate a host before death in water bodies of less than 10 l is given by experiment 5.2.3. If host location is the main determinant of parasite establishment, there should be a positive linear relationship between the inverse of water volume and mean parasite burden when the volume of the infective arena is varied but the number of infective stages is held constant. Figure 5.3 shows that no such relationship was found to exist for water volumes of up to 10 l.

The rate of infection, $\beta$, can be directly measured using the results of experiment 5.2.4. If it is assumed that over the six hours of the experiment, tomite death may be neglected, and that the instantaneous infection rate is inversely proportional to the volume of the experimental container, eqn. (5.3) becomes:

Figure 5.3 *Effect of water volume on trophozoite establishment*

The relationship is shown between the number of parasites established on a single fish and the inverse of the water volume in which the hosts were exposed to approximately 810 tomites. The circles represent mean burden per host, with 95% confidence limits shown.

($r^2$ = 0.02 df = 22.)


Parasite Burden
400
300
200
100
0
0
1
2
3
4
5
1/(Volume) (l⁻¹)

$$P(t) = T_o s \left[ 1 - \exp \left[ -\beta t/V \right] \right] \qquad (5.8)$$

where $V$ is the volume of the water body in litres, $t$, the duration of exposure in hours, and $\beta$ is the infection rate, which has units 1 $hr^{-1}$.

At each exposure time, the number of parasites established on hosts in 500 ml of water was around half that established in 250 ml of water, confirming the hypothesis that a volume dependent infection rate is being measured. A two way analysis of variance performed on the logged data gave a 95% confidence interval for the overall ratio of burden in 250 ml to burden in 500 ml of (1.85 - 3.28).

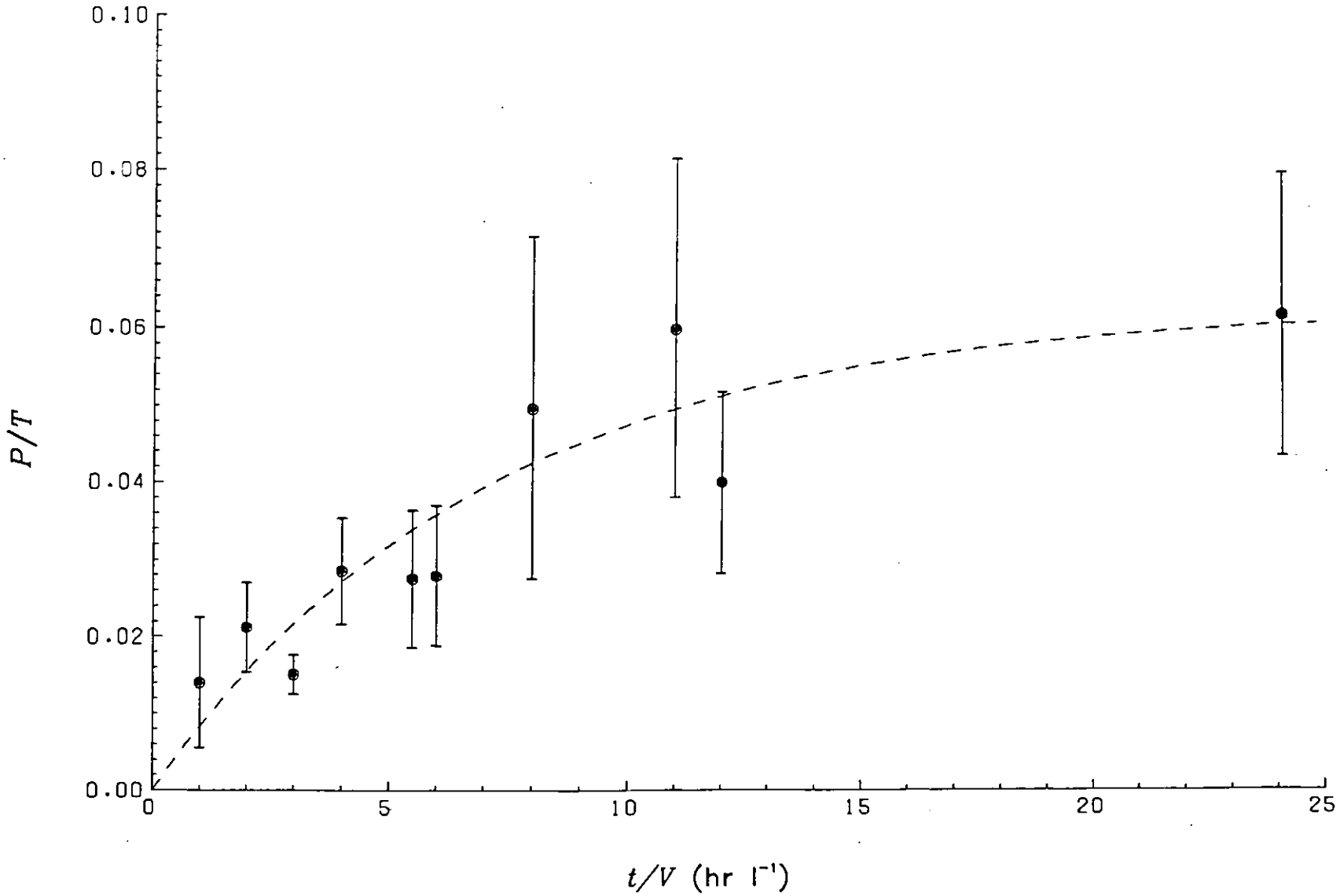
A solution of eqn. (5.8) with parameters $\bar{s}$ and $\beta$ chosen by a non linear least squares procedure is shown, together with the observed data, in Fig. 5.4. Approximate confidence limits for parameters $\bar{s}$ and $\beta$ are given in the legend of the figure. These should be interpreted with a considerable amount of caution, as they are based on the assumption that over this range, the function may be approximated by the linear terms of a Taylor series. A better way to consider the suitability of the model is to compare the non linear model with a simple linear regression. A linear regression of parasite burden on exposure time per unit volume is highly significant ($F = 14.2$; d.f. = 1,58; $p < 0.001$) but the intercept is significantly greater than zero ($t = 3.44$, d.f. = 58, $p < 0.001$). To be biologically realistic, the relation must pass through the origin (with an exposure time of zero, no parasites can establish). A linear model is therefore inadequate to describe the observed relationship. Furthermore, the residual sum of squares of the unconstrained two parameter linear model is greater (51084) than that of the two parameter non linear model (49772).

Figure 5.4 *Time dependent infectivity*

The relationship is shown between number of parasites established per tomite added to the exposure arena ($P/T$) and the time of exposure to tomites (defined per unit of volume). The circles represent means, and the bars represent their standard errors.
The curve shown is a solution of eqn. (5.8) with parameters $\bar{s}$ and $\beta$ estimated by a non linear least squares procedure.
The estimated values of $\bar{s}$ and $\beta$, together with linear confidence limits, are given below.

| Parameter | Estimated | 90% confidence limits | | | |
|---|---|---|---|---|---|
| | Value | One at a time | | Simultaneous | |
| | | Lower | Upper | Lower | Upper |
| $\bar{s}$ | 0.063 | 0.040 | 0.086 | 0.033 | 0.093 |
| $\beta$ | 0.141 | 0.040 | 0.241 | 0.009 | 0.272 |

(90% confidence limits are given, as the 95% linear confidence limits for $\beta$ allow negative values, which are biologically unrealistic).


0.10
0.08
0.06
0.04
0.02
0.00
P/T
0
5
10
15
20
25
t/V (hr l⁻¹)

By combining the estimate of $\beta$ with the life expectancy of a tomite (Section 4.3.6.), the saturation term $\mu/\beta$ is estimated at $0.32\ l^{-1}$

5.4.1. *Distribution of parasites over the surface of hosts.*

Results of a two way analysis of variance on the logarithm of the number of trophozoites per 0.08 $cm^2$ grid are shown in Figure 5.5. The sites at which parasite density was measured divide into two clear groups. The five sites on the surface of the fish itself have approximately equal mean parasite densities, whereas the density of trophozoites on the three sites on the fins is approximately twice as great.

The uniformity of trophozoite density at different sites on the body (excluding fins) contrasts with the observations of Hines and Spira (1973a), who found considerably larger numbers of *Ichthyophthirius* on the dorsal surface of mirror carp *Cyprinus carpio* than on either ventral or lateral surfaces. Wagner (1960) also asserts that *Ichthyophthirius* infections are more dense on the dorsal surface. His observation that fins are more heavily infected than the body is,however, consistent with the results reported here. The apparent uniformity of *Ichthyophthirius* distribution on black mollies found in this study is convenient, in that it permits overall parasite density to be estimated from the density at any site on the body of the fish.

5.5 *Discussion and Conclusions*

The infection of black mollies by *Ichthyophthirius* tomites can adequately be described by an extremely simple population model. The linear relationship between the number of infective stages to which a host

Figure 5.5 *Trophozoite densities at different points on the fish surface*

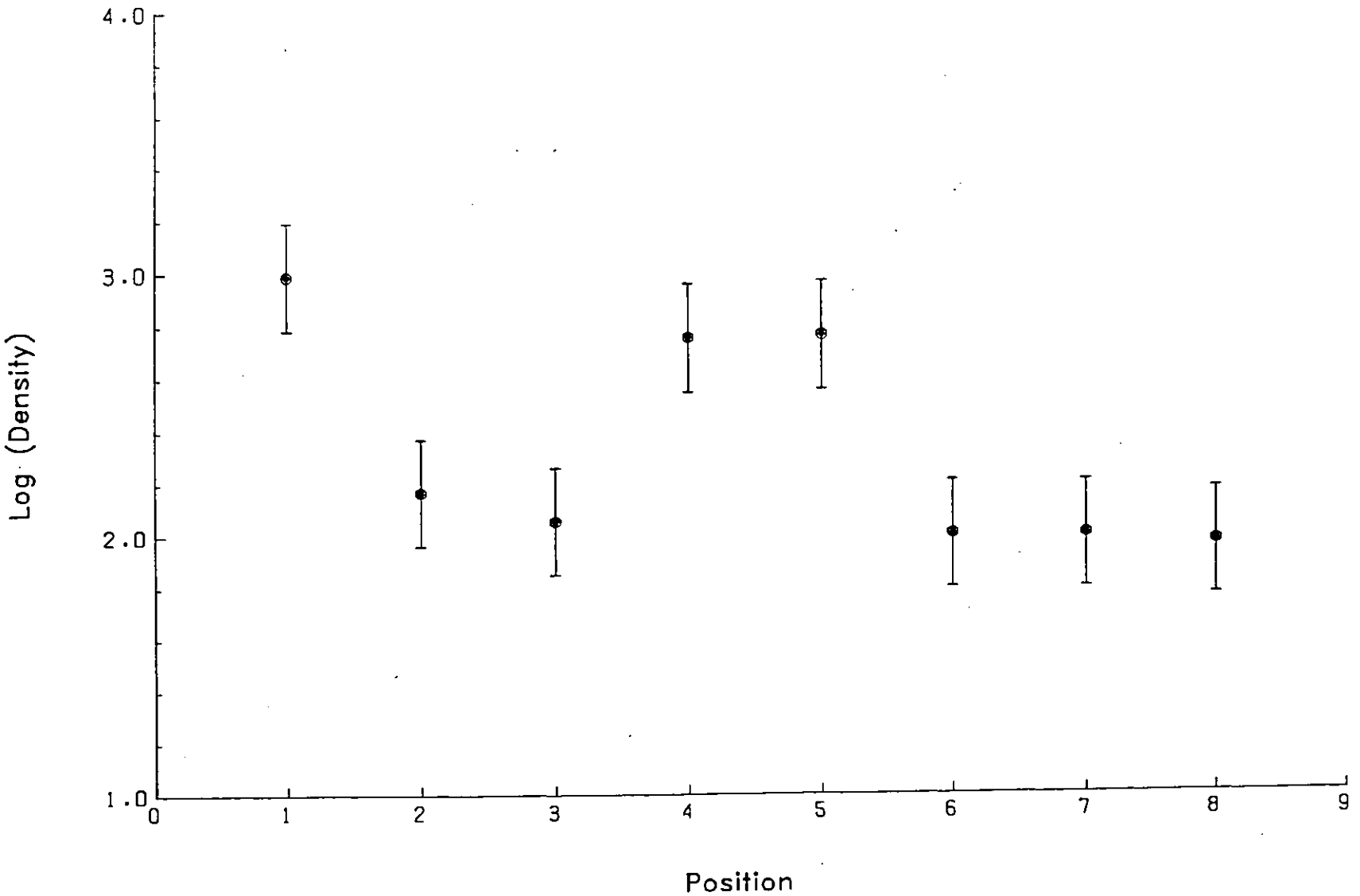
The mean logarithm of parasite density is shown related to the position on the host. The bars shown represent half the least significant difference in each direction. Various positions on the host are shown along the horizontal axis as follows:

1. Right pectoral fin
2. Flank right side
3. " left side
4. Tail right side
5. " left side
6. Operculum right side
7. " left side
8. Top of head

The values on the vertical axis are natural logarithms of the number of trophozoites per 0.08 $cm^2$ of fish surface

Analysis of Variance

| Source | df | S.S. | M.S. | F | P |
|---|---|---|---|---|---|
| Total | 79 | 32.512 | 0.411 | | |
| Position | 7 | 12.426 | 1.775 | 8.37 | <0.001 |
| Fish | 9 | 6.740 | 0.748 | 3.53 | <0.01 |
| Error | 63 | 13.345 | 0.212 | | |


4.0
3.0
2.0
1.0
Log (Density)
0
1
2
3
4
5
6
7
8
9
Position

is exposed and the resulting burden implies the absence of density dependent constraints on parasite establishment. A linear relationship was also found for the digenean *Transversotrema patialense* (Anderson, Whitfield and Dobson, 1978a) and miracidia of *Echinostoma lindoense* infecting snails (Anderson, 1978). Infection, in this case, appears to be a rather simpler process than predation, with its complications of interference and handling time that may produce non linear functional responses (Hassell, 1978). The searching behaviour of *Ichthyophthirius* tomites is apparently simple. Although Lom and Cerkasovova (1974) found that tomites will attach preferentially to agar blocks containing fish serum, they were unable to detect any evidence of a long range chemotactic response to either fish mucus or serum.

A number of possible sources of density dependence in parasite establishment can be postulated. A host has only a finite capacity to harbour infective stages and hence space will ultimately limit parasite burden (Anderson, 1978). *Ichthyophthirius* tomites, however, are so small (30 μm) that for any but the smallest fish, this capacity would be extremely large. Hosts may respond either behaviourally or physiologically in a fashion that limits parasite establishment after an initial contact with the parasite. Fish are capable of acquiring a degree of resistance to *Ichthyophthirius* subsequent to exposure, but this resistance does not appear to develop rapidly enough to cause any non linearity in parasite establishment from a single infection (see Chapter 6). If hosts are irritated by the process of infection, they may move from areas of high infective stage density (Marshall, 1981). For this to act as a density dependent constraint on parasite establishment, infective stages must be aggregated in their spatial distribution. It is unlikely, even if this process is a feature of

*Ichthyophthirius* infection, that experiments in small containers would detect such effects. Density dependence may also be introduced if infection occurs as a result of predation (Anderson *et al* 1978b,Keymer and Anderson, 1979). Although the mechanism by which tomites locate the host is not known, predation by the fish does not appear to be involved.

The most significant result of the experiments described in this chapter is the importance of the susceptibility of the hosts to infection. In the conditions under which the experiments were carried out, essentially all tomites were able to locate a host before death, but only a proportion were able to successfully form trophozoites. A partial explanation of the failure of all tomites to produce trophozoites may be that only a proportion of the tomites counted are viable. However, the degree of heterogeneity observed in parasite burdens established on hosts exposed to the same concentration of infective stages indicates that differences in host susceptibility to infection are also of importance. Tomites may well differ in their ability to infect hosts, due to such factors as nutritional status or the size of the parent trophozoite. These variations in tomite infectivity will effect the mean parasite burden established, but would not result in the distribution of parasites amongst hosts being overdispersed, unless the differences make tomites more likely to attack particular hosts. Anderson (1978) suggests that a similar degree of heterogeneity in parasite burdens generated by miracidia infecting snails may be generated by variation in the attractiveness of snails to miracidia, and Anderson, Whitfield and Dobson (1978) suggest heterogeneity in burdens of cercariae of *Transversotrema patialense* establishing on the fish host *Brachydanio rerio* may result from differing activity patterns

of the fish affecting the ability of cercariae to locate and attach to the host. Given that all tomites appear to be able to locate a host within their lifespan, it is unlikely that such mechanisms are responsible for the high degree of heterogeneity observed in these experiments. It is probable that this heterogeneity is generated by the efficiency of the hosts' defences against infection (both specific and non specific) varying between fish, resulting in differential survival on the host. Such heterogeneity may be genetically based or may result from differing past experiences of infection by pathogens. Variability in the susceptibility of the hosts to infection is of fundamental importance to the dynamics of the system, and is considered further in Chapter 8.

# CHAPTER 6

## ACQUIRED RESISTANCE TO INFECTION

### 6.1 *Introduction*

A number of authors have found that fish may acquire a degree of resistance to infection by *Ichthyophthirius* after exposure to the pathogen. (Buschkeil, 1936; Bauer, 1962; Hines and Spira, 1974c; Goven *et al* 1980). The conclusions reached, however, vary as to whether protection is complete and long lasting (Hines and Spira, 1974c) or partial and of fairly short duration (Bauer, 1962). This chapter describes a series of experiments designed to investigate the following problems concerning the acquired resistance of black mollies to *Ichthyophthirius* infection. These are as follows:

(i) time taken to develop resistance;

(ii) degree of protection against infection afforded;

(iii) length of the refractory period;

(iv) dependence of the previous three factors on the level of the initial infection;

(v) the effect of acquired resistance on trophozoites, once on the host.

### 6.2 *Experimental Methods*

#### 6.2.1. *Time required to develop resistance (I)*

Forty-five uninfected hosts were divided into three groups of fifteen. Each group was subjected to infection, the parasite burden was assessed, and the fish within each group ranked according to the severity of infection. Host sex was also recorded. These infected hosts were then assigned systematically in rotation to three groups,

A, B and C. Each group thus consisted of three subgroups of five hosts with similar infective burdens, and differences in parasite load between groups A, B and C were as small as possible.

Once assessment of burden was complete, each host was isolated and maintained in a 500 ml container at 20°C. The containers were scrubbed and the water replaced daily with tap water in order to prevent self reinfection. Each fish was fed daily, uneaten food being removed after approximately two hours.

Seven days after the initial infection, group A was subjected to reinfection, together with five control fish. The fish used in the controls were obtained from the same batch provided by the suppliers as the previously infected hosts. The parasite burden established from this second infection was assessed four days afterwards. Groups B and C were similarly reinfected ten and fifteen days after the initial infection. The numbers of tomites used for each reinfection experiment were kept approximately constant.

### 6.2.2. *Time required to develop resistance II*

A broadly similar experimental procedure to that used in the first experiment was employed. Seventy fish were divided into two groups of thirty-five. Within each group, hosts were infected with the same number of infective stages and individually maintained in 500 ml containers. The infective dose to which one group was subjected was ten times that used for the other. Parasite burden was assessed four days after the initial infection, and fish in each group ranked according to the severity of infection. They were then assigned in turn to five groups of fourteen hosts each, consisting of seven with a

light infection, and seven with a heavier parasite.burden. The order in which these groups were to be reinfected was randomized. Until hosts were to be reinfected, they were isolated and maintained in 500 ml containers at 20°C. The water was replaced with tap water and the containers scrubbed daily to prevent self reinfection. Fish were fed daily and uneaten food removed after two to three hours.

Reinfection of the first group, together with seven controls from the same batch of fish was attempted four days after the initial infection. The other groups were reinfected, together with controls, ten, fourteen, twenty and forty-two days after the initial infection.

6.2.3. *Effect of previous exposure on time spent by trophozoites on hosts*

Twenty fish were infected with the same number of tomites. Parasite burden was assessed six days after the initial infection. As before, until reinfection was attempted, the fish were maintained individually at 20°C in 500 ml containers, with daily changes of tap water to prevent self reinfection. Twelve days after this initial infection, the hosts were subjected to a second infection, as were twenty controls. The previously infected fish were given five times the infective dose of the controls in order to establish absolute numbers of parasites on each host that were comparable. Three days after this infection, the burden on all hosts was counted, and recounted daily until ten days after infection.

6.2.4. *Effect of starvation on resistance to infection*

Twenty fish obtained together from the supplier were divided into two groups of ten, and placed in 10 l tanks containing aerated, conditioned water. One group was not fed for 24 days, and the other was fed

*ad libitum* with "Aquarian" tropical fish food. After this time, the fish were placed individually in 500 ml containers of conditioned water, and all fish subjected to the same number of infective stages.

## 6.3 *Results*

### 6.3.1. *Time required to develop resistance (I)*

The same method of analysis was used for experiments I and II. All trophozoite counts were transformed using the following equation:

$$Z = \ln (P + 1) \qquad (6.1)$$

where $P$ is the parasite burden and $Z$ is the transformed count. As variability is primarily generated by differences between hosts in the proportion of tomites forming trophozoites (see Chapter 8), the error expected is multiplicative and a log transformation is required to produce the additive error assumed in analysis of variance. A log transformation also enables conclusions about the ratio of parasite burden on controls to that on resistant fish to be drawn.

Data were analysed using a nested analysis of variance, which assumes that each point may be represented as

$$Z_{ijk} = \mu + \alpha_i + \beta_{ij} + \varepsilon_{ijk} \qquad (6.2)$$

where $Z_{ijk}$ is the transformed value of the $k$th replicate reinfected at time $i$ with previous burden level $j$, $\alpha_i$ is the effect on $Z$ of being reinfected at time $i$, and $\beta_{ij}$ the effect on $Z$, when reinfected at time $i$, of having previous burden level $j$. $\varepsilon_{ijk}$ represents the error associated with each observation. The important quantities for

the determination of the rate of acquisition of resistance are the terms $\beta_{ij}$.

The results of this analysis are given in Table 6.2 (Table 6.1 shows the influence of host sex on the parasite burden established after the initial infection). Two hosts in the third reinfection were found to have reinfected themselves, and were omitted from the analysis, the data values being replaced by the means of the other replicates in the cell.

There is a highly significant difference between overall parasite burden at different times since the initial infection, but this is not necessarily of interest, as it may merely represent differences in the infectivity of the tomites used at each time.

The significant value of F for $\beta$ indicates that the previous level of infection does indeed affect the parasite burden established, and this result therefore provides evidence of a significant host response (possibly immunological in nature) to reinfection. To identify which values are responsible for this difference, the estimated values of $\beta_{ij}$ for each combination of time since infection and previous parasite burden are plotted in Figure 6.1, together with bars representing half the least significant difference in either direction. If the bars do not overlap, there is evidence of a difference between the means with a 95% significance level. Unfortunately, the high degree of variability found in this experiment makes the result not entirely clear cut. With 95% confidence, only the difference between the control at day 14 and the reinfection burden on hosts with the highest previous burden is clearly significant. The significance of other differences is borderline. With 90% confidence, it appears that the

Table 6.1 *The Influence of Host Sex on Parasite Burden*

| Level of infection | Mean Burdens ♂ | ♀ | Number of fish ♂ | ♀ | t |
|---|---|---|---|---|---|
| Low | 12.71 | 11.13 | 7 | 8 | 0.38 |
| Medium | 41.37 | 61.32 | 6 | 9 | 0.92 |
| High | 169 | 175 | 4 | 11 | 0.12 |

Table 6.2 *Results of a Nested Analysis of Variance on Data of Experiment I*

| Source | df | S.S. | M.S. | F | P |
|---|---|---|---|---|---|
| Total | 59 | 50.42 | 0.855 | | |
| Time (α) | 2 | 27.96 | 13.979 | 47.12 | 0.001 |
| Previous exposure (β) | 9 | 8.23 | 0.914 | 3.08 | 0.01 |
| Error | 48 | 14.24 | 0.297 | | |

Table 6.3 *Results of a Nested Analysis of Variance on Data of Experiment II*

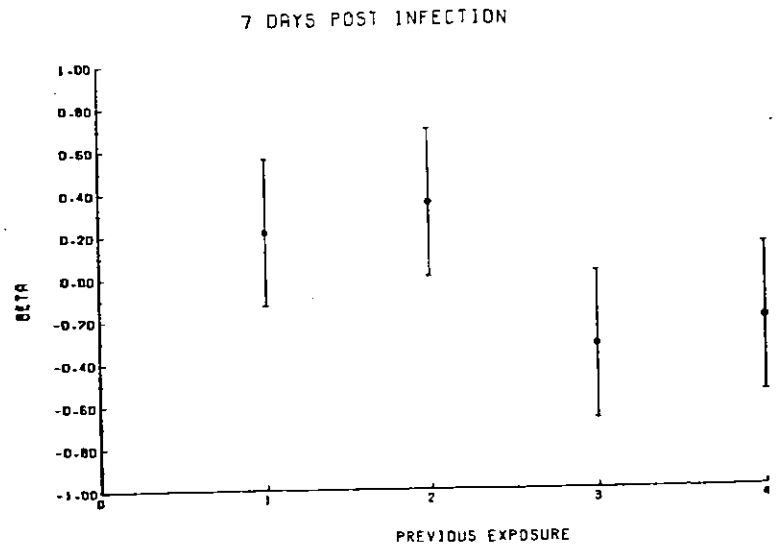
| Source | df | S.S. | M.S. | F | P |
|---|---|---|---|---|---|
| Total | 104 | 393.12 | 3.780 | | |
| Time (α) | 4 | 193.46 | 48.366 | 29.3 | 0.001 |
| Previous exposure (β) | 10 | 51.28 | 5.128 | 3.11 | 0.005 |
| Error | 90 | 148.39 | 1.649 | | |

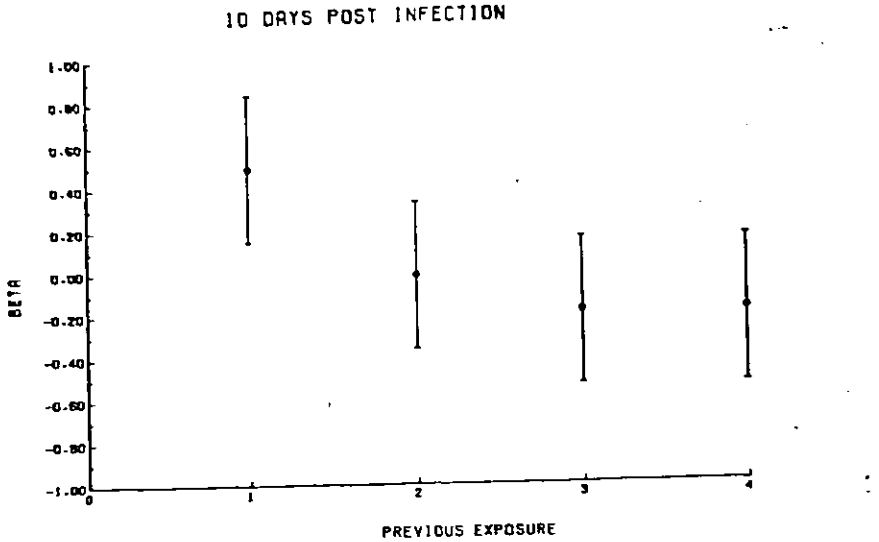
Figure 6.1 *Time required to develop resistance to infection (I)*

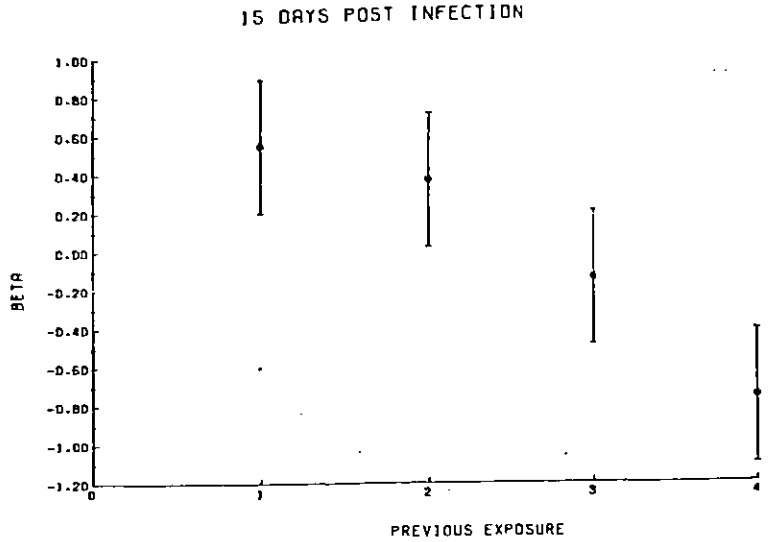
The figure shows the results of a nested analysis of variance, using the model described by eqn. (6.2). Results of reinfections at each time since the initial infection are shown on a separate graph. On each, the level of previous exposure to infection is represented on the horizontal axis in the following way:

| Level | Mean parasite burden after initial infection | |
|---|---|---|
| 1 | 0 | (Control) |
| 2 | 12 | (light) |
| 3 | 53 | (moderate) |
| 4 | 173 | (heavy) |

The vertical axis shows the estimated value of $\beta$ ( defined in eqn. (6.2)). Low values of $\beta$ relative to the control are evidence of acquired resistance to infection. The bars shown represent half the least significant difference in each direction. If they do not overlap, the values differ with 95% confidence.


7 DAYS POST INFECTION
BETA
1.00
0.80
0.60
0.40
0.20
0.00
-0.20
-0.40
-0.60
-0.80
-1.00
0
1
2
3
4
PREVIOUS EXPOSURE


10 DAYS POST INFECTION
BETA
1.00
0.80
0.60
0.40
0.20
0.00
-0.20
-0.40
-0.60
-0.80
-1.00
0
1
2
3
4
PREVIOUS EXPOSURE


15 DAYS POST INFECTION
BETA
1.00
0.80
0.60
0.40
0.20
0.00
-0.20
-0.40
-0.60
-0.80
-1.00
-1.20
0
1
2
3
4
PREVIOUS EXPOSURE

burden established on hosts with intermediate previous exposure differs from the control at day 7. By day ten, hosts with intermediate and high burdens differ with 90% confidence from the control, and by day 14, the differences are similar but more marked. Due to the low levels of significance, however, conclusions drawn from this experiment can only be tentative.

### 6.3.2. *Time required to develop resistance II*

The results of the second reinfection experiment lend more weight to the conclusion that hosts are able to mount an effective response to reinvasion. Replication was greater, and the size of the error term consequently reduced. Once again, a nested analysis of variance was applied to the logged data, the results of which are shown in Table 6.3. Four observations were excluded and replaced by the means for their cells because of either death of the host or self reinfection. These four records came from separate cells.

In this case there is very strong evidence ($p < 0.005$) that the level of previous exposure affects the parasite burden established. Figure 6.2 displays estimated values of $\beta$, together with 95% least significance bars, for each time reinfection was attempted. Four days after the initial infection, there is no evidence of development of acquired resistance. After 10 days, there is an indication that hosts which previously had a high parasite burden are less susceptible to infection than controls, but this is only significant with 90% confidence. There is clear evidence that both categories of experienced hosts are resistant to infection 14 days after the initial infection, but there does not appear to be any difference in susceptibility between previously exposed groups. The difference between the logged

burden on controls and the resistant hosts is 1.87, indicating that previously exposed hosts are of the order of 6.5 times less susceptible to infection than the controls. A more accurate estimate of the degree of resistance acquired is obtained in the next section. Twenty days after the initial infection, the hosts with high previous experience of *Ichthyophthirius* infection are more resistant than the controls, but the fish with only light previous experience appear to be in the process of losing resistance. This indication is confirmed with the results 42 days after the initial infection, when the previously heavily infected hosts are still resistant, but those with only light infection experience appear to be as susceptible as the controls to *Ichthyophthirius*.

### 6.3.3. *Effect of previous exposure on time spent on hosts*

Figure 6.3 compares the survivorship of trophozoites on hosts with previous history of infection, with survivorship on control hosts. There appears to be a tendency for trophozoites to remain longer on controls. The significance of this observation was tested by comparing the proportion of trophozoites remaining on hosts in each group at day six. This proportion was calculated for each host, and the difference between control and resistant groups was found to be highly significant using a Mann-Whitney U test ($U = 66.5$, $p < 0.001$). Trophozoites do therefore remain for a longer time on naive hosts than experienced hosts, but as median survival times differ by only one day, the effect is of less importance to the dynamics of the disease than the effects of previous infection experience on parasite establishment.

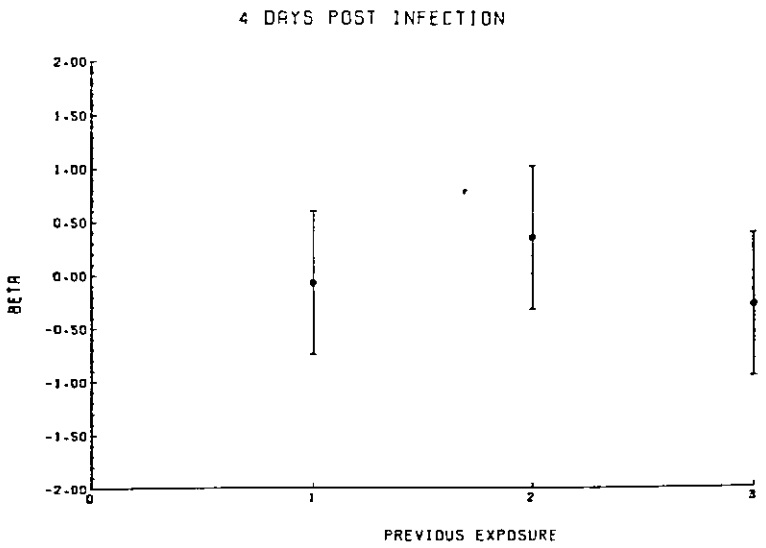
Analysis of the counts three days after infection reveals further information about the immune response. Although the means of logged

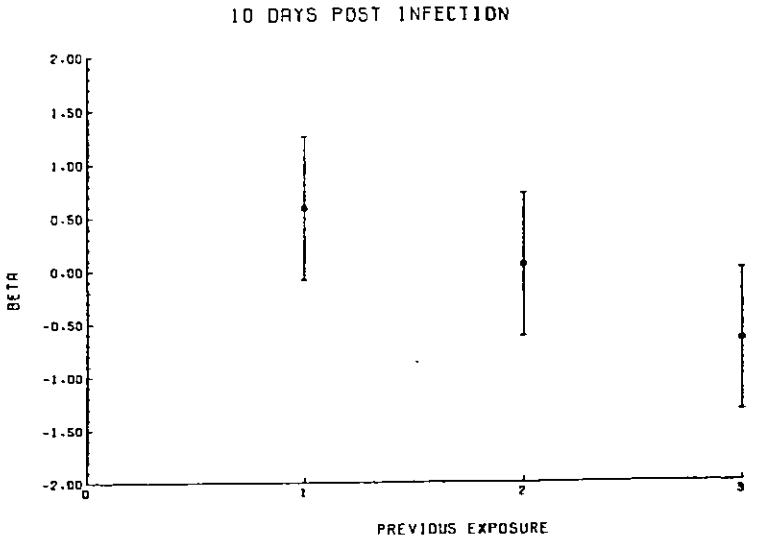
Figure 6.2 *Time required to develop resistance to infection (II)*

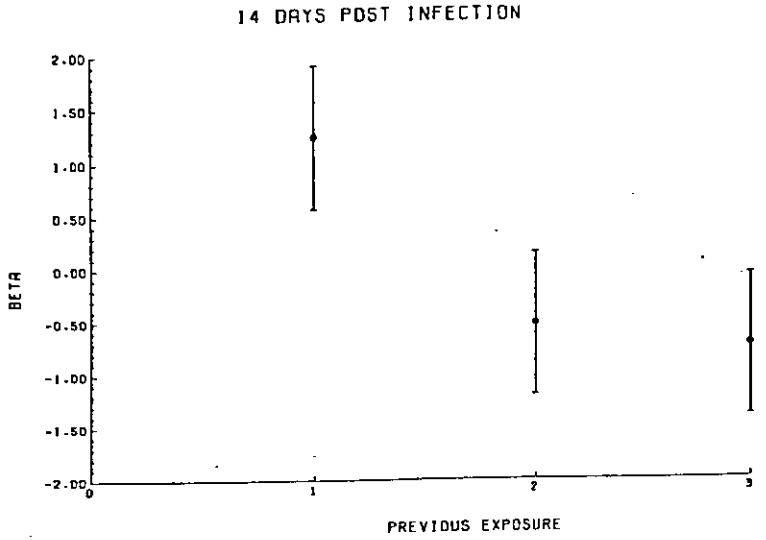
The figure shows the results of a nested analysis of variance, using the model defined by eqn.(6.2.) Results of reinfections at each time since the initial infection are shown a separate graph. On each, the level of previous exposure to infection is represented on the horizontal axis in the following way:

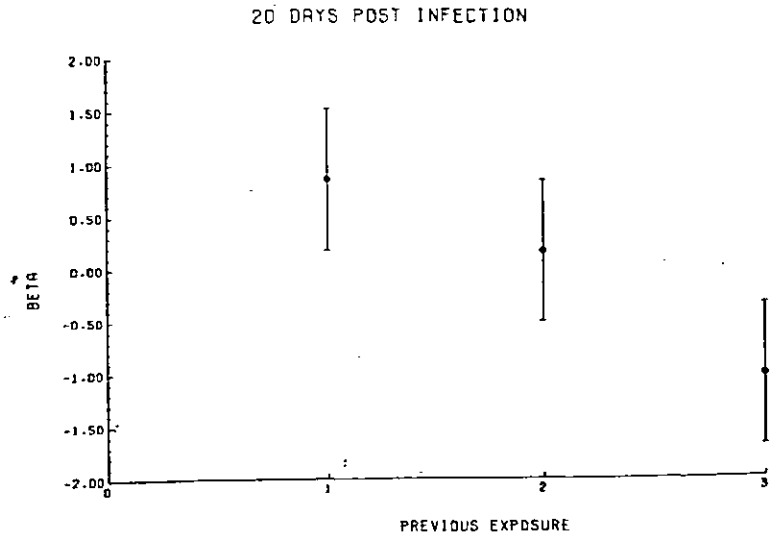
| Level | Mean parasite burden after initial infection | |
|---|---|---|
| 1 | 0 | (Control) |
| 2 | 18.8 | (light) |
| 3 | 151.0 | (heavy) |

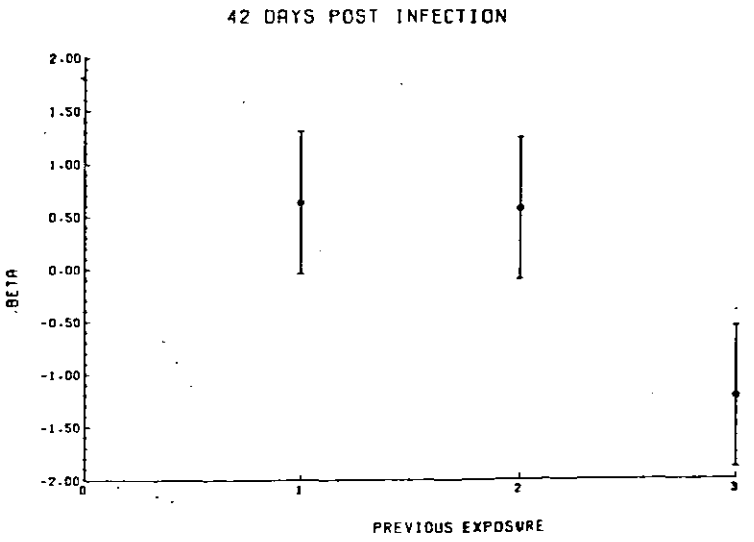
The vertical axis shows the estimated value of $\beta$ (defined in eqn. 6.2). Low values of $\beta$ relative to the control are evidence of acquired resistance to infection. The bars shown represent half the least significant difference in either direction. If they do not overlap, the values differ with 95% confidence.


4 DAYS POST INFECTION
BETA
2.00
1.50
1.00
0.50
0.00
-0.50
-1.00
-1.50
-2.00
0
1
2
3
PREVIOUS EXPOSURE


10 DAYS POST INFECTION
BETA
2.00
1.50
1.00
0.50
0.00
-0.50
-1.00
-1.50
-2.00
0
1
2
3
PREVIOUS EXPOSURE


14 DAYS POST INFECTION
BETA
2.00
1.50
1.00
0.50
0.00
-0.50
-1.00
-1.50
-2.00
0
1
2
3
PREVIOUS EXPOSURE


20 DAYS POST INFECTION
BETA
2.00
1.50
1.00
0.50
0.00
-0.50
-1.00
-1.50
-2.00
0
1
2
3
PREVIOUS EXPOSURE


42 DAYS POST INFECTION
BETA
2.00
1.50
1.00
0.50
0.00
-0.50
-1.00
-1.50
-2.00
0
1
2
3
PREVIOUS EXPOSURE

number of parasites per host are very similar between controls and resistant fish (4.07 for control fish, 4.15 for resistant fish) the variance for previously exposed hosts is very much greater than that for the controls ($F = 7.05$; $df = 19,19$; $p < 0.001$). Fish appear to differ considerably in their ability to acquire resistance to infection. These differences do not appear to be related to the actual parasite burden established in the first infection. Figure 6.4 shows no evidence of correlation between log burden established in the initial infection, and log burden established in the second infection.

The ratio of susceptibility of exposed fish to susceptibility of naive fish was estimated to be 0.22, using the logged data from day three and recalling that controls were given one fifth of the infective dose of experienced hosts. Allowing for the large difference in variance, a 95% confidence interval for this ratio was found to be 0.10 to 0.48. The level of resistance found is similar to that observed in the previous experiment.

### 6.3.4. *Effect of starvation on resistance to infection*

The mean of the natural logarithm of parasite burdens established on hosts that had been deprived of food for 24 days was found to be 2.09, compared with 1.8 for the controls. This difference is not significant ($t = 0.74$, 18 df).

## 6.4 *Conclusions and Discussion*

Black mollies are able to acquire a degree of resistance to infection by *Ichthyophthirius* in approximately ten days after an initial infection. There is some evidence that resistance may be acquired more rapidly by heavily infected hosts than by lightly

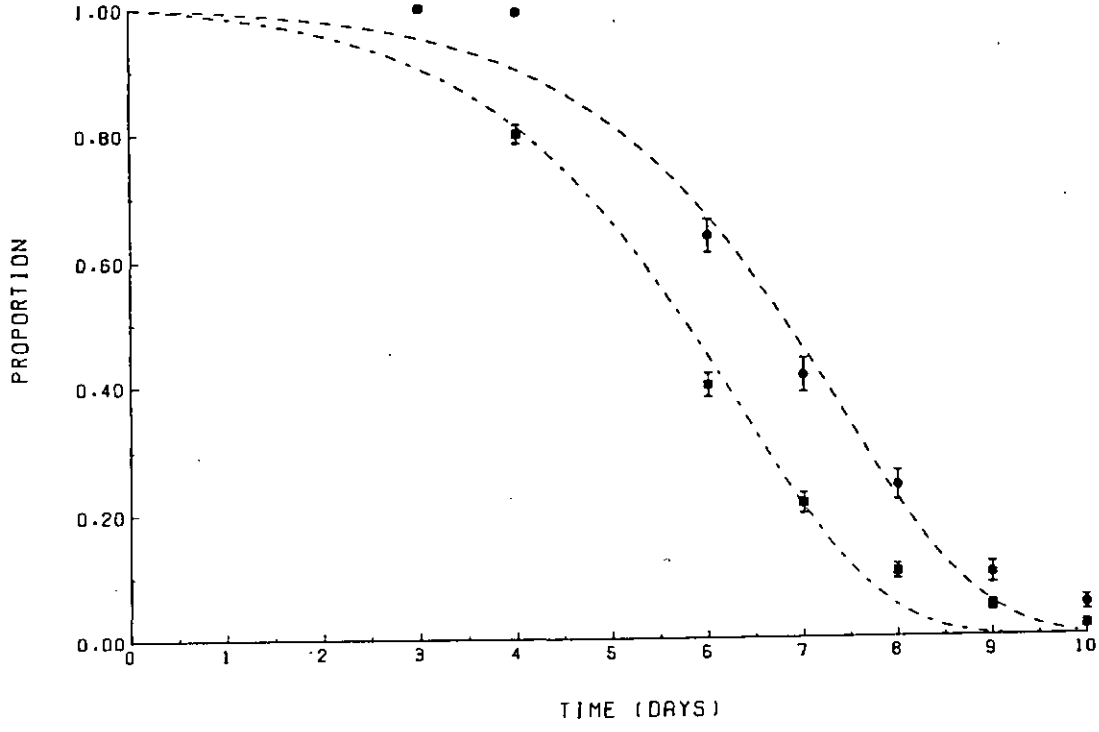
Figure 6.3 *Survival of trophozoites on control and previously exposed hosts*

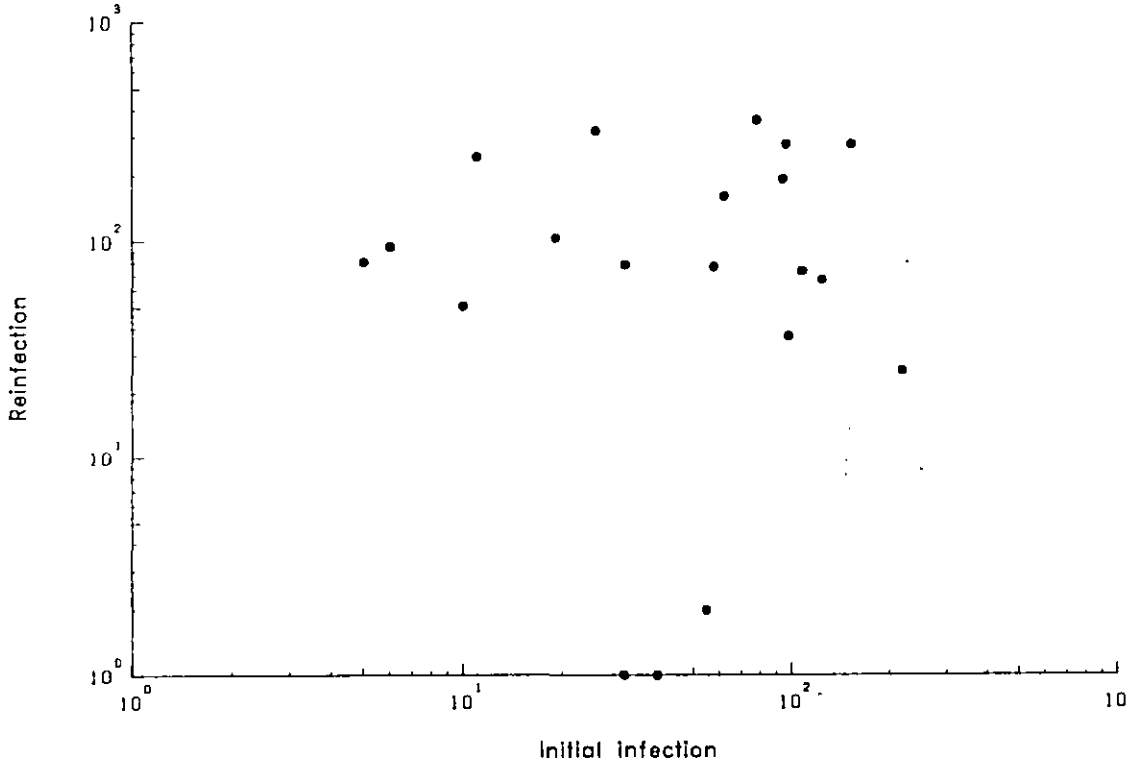
The time since infection is shown on the horizontal axis. The proportion of those trophozoites established on day 3 still remaining on hosts is shown on the vertical axis. The circles represent data points for control hosts, and the squares represent data points for hosts with previous history of infection. The bars shown are 95% confidence intervals for the overall proportion of trophozoites in each category surviving. The equally dashed line is eqn. (4.2) fitted to the control data using a non linear least squares procedure, and the unequally dashed line represents the same equation fitted to the data of the previously infected hosts. The geometric mean of the burden established on experienced hosts in the initial infection was 44.

Figure 6.4 *The relationship between the number of parasites established in a second infection, and number established in an initial infection*

The number of trophozoites established in the second infection is plotted on the vertical axis, and the number established in the first infection is shown on the horizontal axis. Double log axes are used.

($r^2$ = -0.05, 18 df, based on logged data).


1.00
0.80
0.60
0.40
0.20
0.00
PROPORTION
0
1
2
3
4
5
6
7
8
9
10
TIME (DAYS)


$10^3$
$10^2$
$10^1$
$10^0$
Reinfection
$10^0$
$10^1$
$10^2$
$10^3$
Initial infection

infected hosts. Resistant fish appear to be approximately one fifth as susceptible to infection as previously unexposed fish, and the degree of protection against infection does not appear to depend on the level of the initial exposure. It appears that resistance is more rapidly lost by hosts with only a mild experience of infection. This resistance is true post invasive immunity, rather than premunition (in which parasites currently on a host protect it from further attack) because, more than ten days after infection, no parasites from the initial cohort remain on the host. Those trophozoites that succeed in establishing on resistant hosts do not remain on the host for as long as do trophozoites on naive fish.

These conclusions are more closely in agreement with those of Bauer (1962) than with those of Hines and Spira (1974c). In Bauer's experiments, which were similar in principle to those described in this chapter, previously infected fish were found to develop parasite burdens one tenth of those established on control fish, in contrast to the nearly solid resistance reported by Hines and Spira. These differences may result from the experimental methods used in each study. Hines and Spira exposed fish to infection until they recovered, which corresponds to a number of generations of parasites. The hosts were therefore exposed until nearly complete resistance was established. In the experiments reported in this chapter, and in Bauer's experiments, the fish were subjected to one infection only. Hines and Spira report maintenance of high levels of resistance for eight months or more, but this is for fish continually exposed to *Ichthyophthirius*.

The experiments of this chapter do not attempt to examine the

mechanism responsible for the acquired resistance. Hines and Spira (1974c) were able to demonstrate that serum from resistant fish was able to immobilize *Ichthyophthirius* trophozoites. They suggest, however, that the resistance actually operates at the level of the mucus surrounding the fish, as no tomites were recovered from mucus scrapings from immune fish. They were also able to detect qualitative differences electrophoretically between mucus from resistant and control fish. Goven *et al* (1980) were able to demonstrate resistance to *Ichthyophthirius* infection after inoculation with preparations of *Ichthyophthirius* cilia or with cilia from the related *Tetrahymena pyriformis*. It therefore appears that the acquired resistance observed is immunological in nature.

The most interesting aspect of these results, from a population dynamic point of view, is that very low parasite burdens, relative to the burden necessary to produce significant host mortality, are sufficient to confer considerable resistance to reinfection. The importance of this to the dynamics of the parasite is discussed in Chapter 12. Similar acquisition of significant resistance after only light exposure to a parasite is reported by Weinmann (1958) with regard to infection of laboratory mice *Mus musculus* by the cestode *Hymenolepis nana* and by Wassom *et al*(1973)concerning infection of the deer mouse *Peromyscus maniculatus* by *Hymenolepis citelli*. Both these studies also reported faster rates of acquisition of resistance following heavy initial infections.

# CHAPTER 7

## PARASITE INDUCED MORTALITY

### 7.1 *Introduction*

As one of the major parasites affecting commercial fish culture, the high potential pathogenicity of *Ichthyophthirius* is well known (Bauer, 1962). Despite the detailed examination of the pathology of the disease by Hines and Spira (1974a, 1974b, 1974c), there is little available information on the relationship between parasite burden and the rate of host mortality. The experiment described in this chapter examines this problem in quantitative terms.

### 7.2 *Experimental Methods*

Fish were infected with high parasite burdens by either placing them in highly infective tanks for twenty-four hours or by directly infecting them with large numbers of tomites. Two days after the commencement of the initial infection, the trophozoite density on the host was assessed by counting the number visible within two 0.16 $cm^2$ grids positioned one on each side of the body just in front of the caudal peduncle. This particular position was chosen as a plane surface on which small trophozoites are readily visible. Given the uniformity of trophozoite distribution over the surface of the host found in Section 5.4, these counts should be representative of the overall parasite density. After assessment of the parasite burden, hosts were placed individually in 500 ml containers of tap water and maintained at 20$^{o}$C in diffuse lighting. Each day, survival was checked, and the remaining fish fed. The water was changed regularly to restrict self reinfection. Monitoring of survival was continued for ten days

after the initial infection, by the end of which time the initial infecting cohort of parasites has left the fish (Section 4.3.1).

## 7.3 *Age Dependent Parasite Induced Mortality*

Raw results obtained are in the form of a table of number of days survived against number of trophozoites per 0.16 $cm^2$ square (see Appendix 2). Analysis of data of this form is difficult. Days survived cannot simply be regressed against parasite density, because of the considerable number of hosts surviving more than ten days. A second difficulty is that it was not possible to count burden earlier than two days after the initial infection. This is not a problem over a greater part of the range of trophozoite densities examined, but a number of attempts to investigate very high densities failed because the fish died before their parasite burden could be counted. The few results obtained at very high densities (in excess of 300 trophozoites per square) represent only the tails of the distribution, and must be assessed with caution.

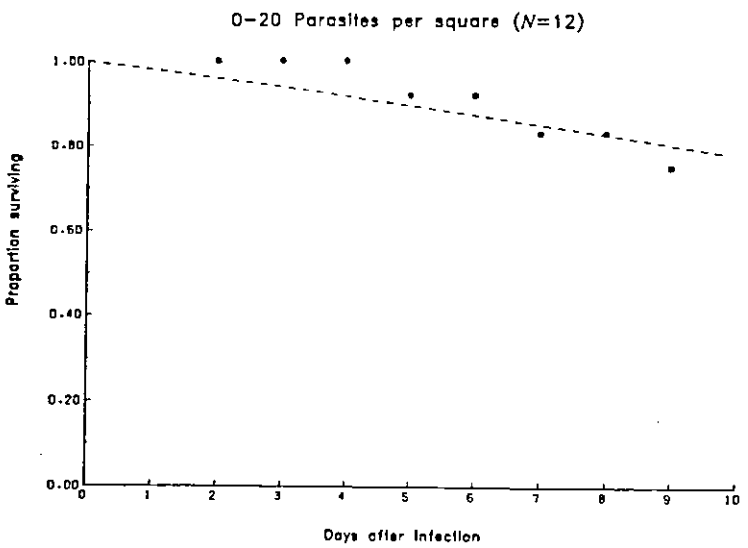
A satisfactory way to deal with these data is to group fish according to the severity of infection, and to calculate, for each day, the proportion of each group surviving. Figure 7.1 presents the results of this analysis.

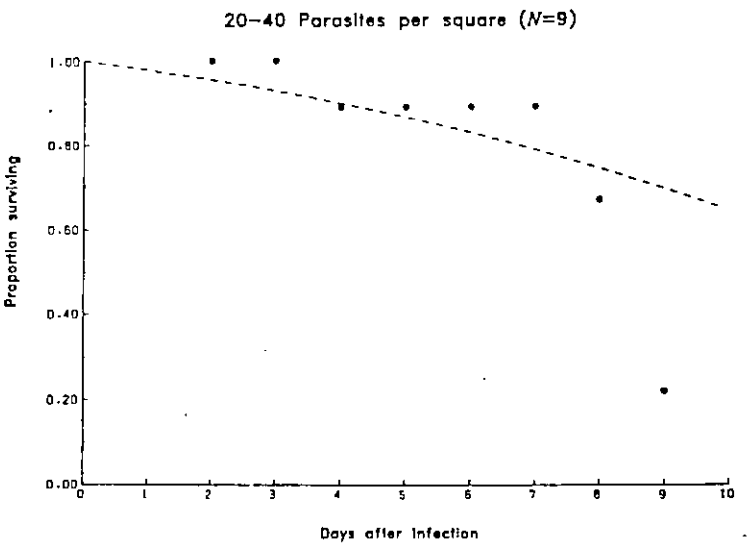
Some form of age dependent survival curve is clearly required to fit these data. Over the range of the experiment, it appears that a function of the following form may adequately describe the observed data:

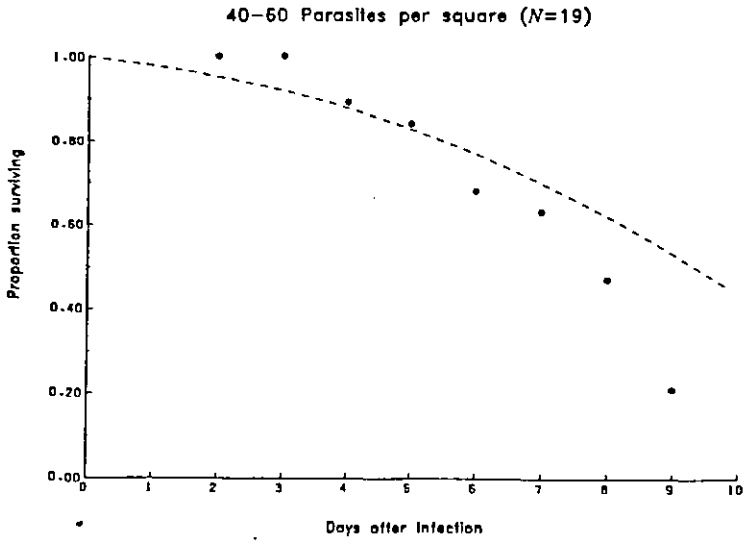
$$dN/dt = -N\, a(P)\exp\,[b(P)t] \qquad (7.1)$$

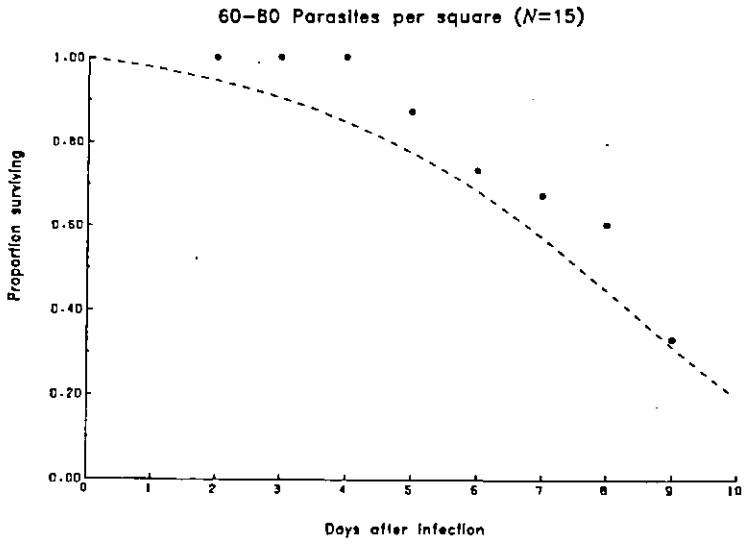
Figure 7.1 *The age dependent parasite induced death rate*

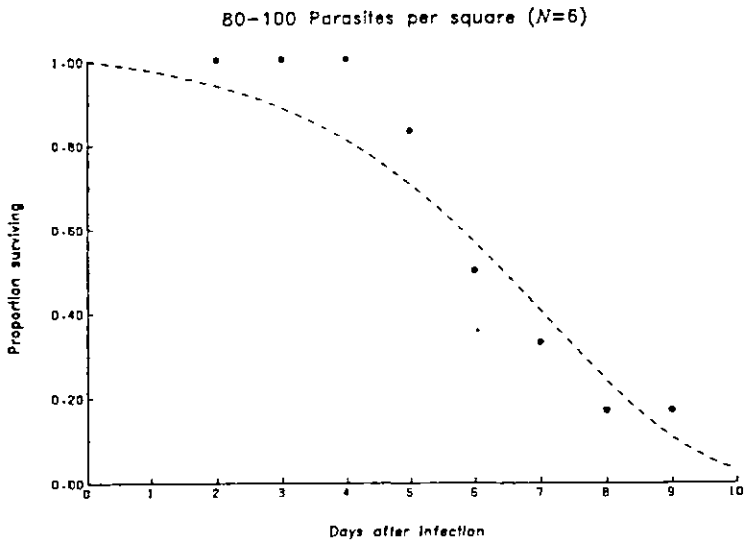
A series of graphs are presented, each grouping results from fish with parasite densities (per 0.16 $cm^2$ of body surface) over the range given in the graph heading. The horizontal axis of each graph shows the time in days since infection, and the vertical axis shows the proportion of the hosts originally present surviving. The solid circles represent observed data values. The number of hosts originally present, $N$, is shown in the heading of each graph. The dashed lines shown are a solution of eqn. (7.2) with parameters $a$ and $b$ estimated from all the data points together, using a non linear curve fitting routine. (The estimated values of $a$ and $b$ are : $a$ = 0.195 per day, $b$ = 0.00485 per parasite, per day.)

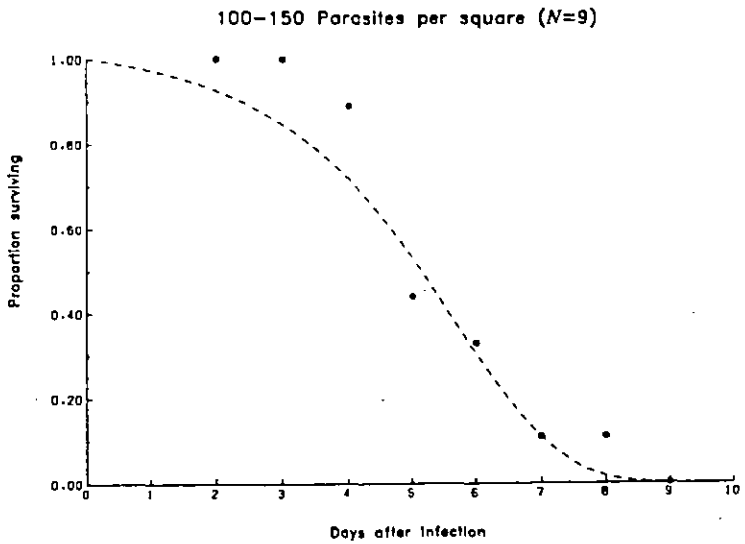

0–20 Parasites per square (N=12)
Proportion surviving
1.00
0.80
0.60
0.40
0.20
0.00
0 1 2 3 4 5 6 7 8 9 10
Days after infection

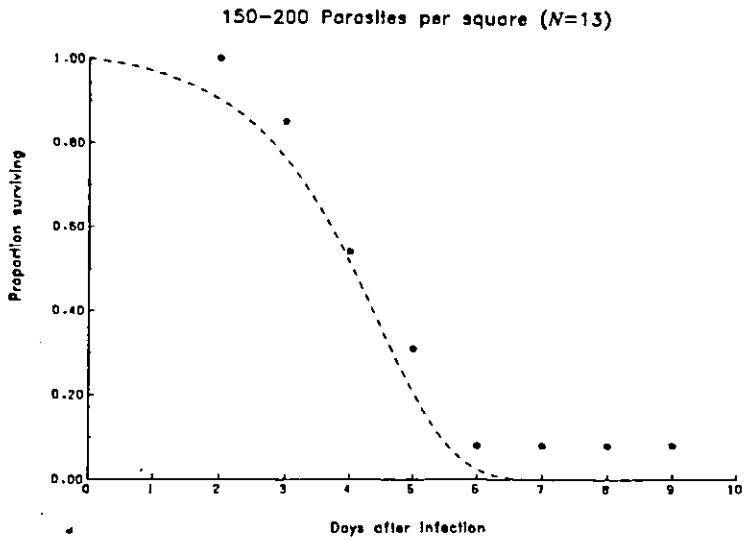

20–40 Parasites per square (N=9)
Proportion surviving
1.00
0.80
0.60
0.40
0.20
0.00
0 1 2 3 4 5 6 7 8 9 10
Days after infection


40–60 Parasites per square (N=19)
Proportion surviving
1.00
0.80
0.60
0.40
0.20
0.00
0 1 2 3 4 5 6 7 8 9 10
Days after infection


60–80 Parasites per square (N=15)
Proportion surviving
1.00
0.80
0.60
0.40
0.20
0.00
0 1 2 3 4 5 6 7 8 9 10
Days after infection


80–100 Parasites per square (N=6)
Proportion surviving
1.00
0.80
0.60
0.40
0.20
0.00
0 1 2 3 4 5 6 7 8 9 10
Days after infection


100–150 Parasites per square (N=9)
Proportion surviving
1.00
0.80
0.60
0.40
0.20
0.00
0 1 2 3 4 5 6 7 8 9 10
Days after infection


150–200 Parasites per square (N=13)
Proportion surviving
1.00
0.80
0.60
0.40
0.20
0.00
0 1 2 3 4 5 6 7 8 9 10
Days after infection

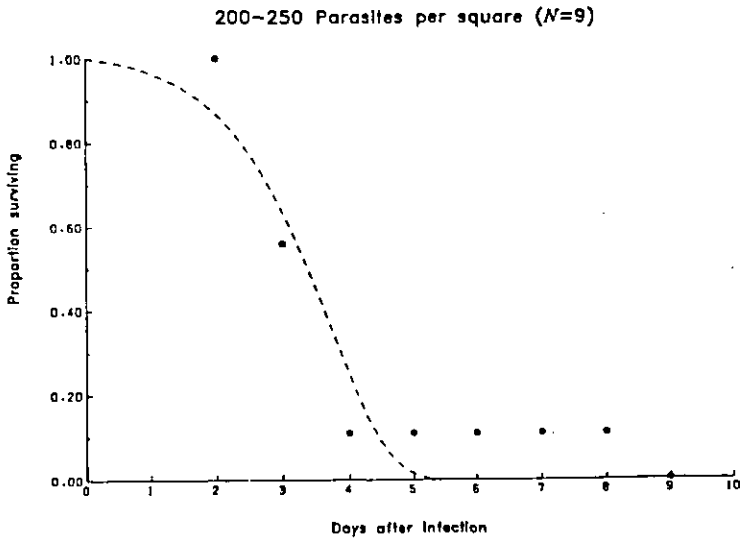
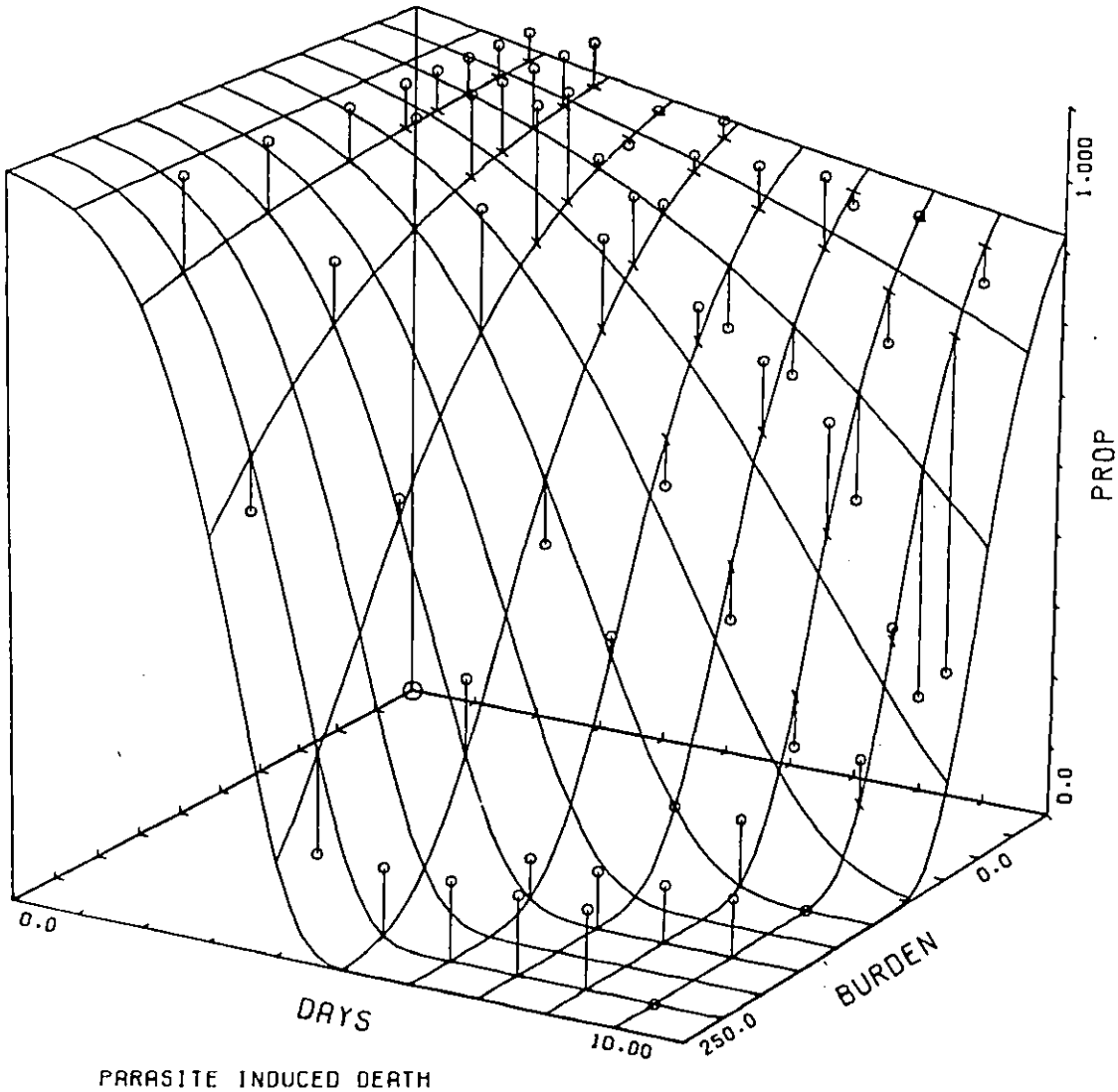

200–250 Parasites per square (N=9)
Proportion surviving
1.00
0.80
0.60
0.40
0.20
0.00
0 1 2 3 4 5 6 7 8 9 10
Days after infection

Figure 7.2 *An alternative presentation of the results shown in Figure 7.1.*

The solution of eqn. (7.2) is shown as a surface, and the data points are shown as "lollipops" above or below the fitted surface. The vertical axis shows the proportion of hosts surviving, and the two horizontal axes show the time in days since infection, and the parasite burden in numbers per 0.16 $cm^2$ square.




PARASITE INDUCED DEATH

where $N$ is the number of hosts originally present, $P$ is the initial parasite density per host, $t$ is the time since infection, and $a(P)$ and $b(P)$ are functions of $P$.

Two possibilities were examined:

$$l(x) = \exp\ (a/bP\ [1- \exp\ (bPt)\ ]\ ) \qquad (7.2)$$

in which $b(P)$ is assumed to be a linear function of $t$, but $a$ is constant, and

$$l(x) = \exp\ (aP/b\ [1- \exp(b\ t)\ ]) \qquad (7.3)$$

is which $a(P)$ is a linear function of $P$ and $b$ is constant. For both these functions, a non linear curve fitting procedure was used to find values of $a$ and $b$ that minimized the residual sum of squares.(Appendix 1). The value of $P$ at the midpoint of each time interval was used. A value of 1.02 for the residual sum of squares was obtained for eqn. (7.3) compared with 0.80 for eqn. (7.2). The data therefore are better described by eqn. (7.2), which is shown fitted to the data of Figure 7.1. This model is also more satisfactory in that it predicts a constant, disease independent death rate of 0.02, which is in the region observed for uninfected mollies. Equation (7.3) predicts no disease independent death whatsoever. Figure (7.2) shows eqn. (7.2) plotted as a surface.

It must be realized that the functional form of dependence of death rate on infective burden is almost certainly considerably more complicated than eqn(7.2). The rate will, in fact, depend on the number of trophozoites currently on the host, their size, and the number that have left the host already. The data are not sufficiently com-

prehensive to justify attempts to fit a more complex relation. Equation (7.2), with only two free parameters, provides an adequate fit over the range of the observations.

## 7.4 *An Averaged Death Rate*

Although the age dependent survivorship function is evidently complex, there is a simple relationship between median survival times of hosts with similar burdens and the level of the infection. Figure 7.3 relates median survival time (the time since infection by which half the hosts have died) to the initial trophozoite density. These medians were calculated by simple linear interpolation from the data presented in Figure 7.1. The inverse of the median survival time appears to be linearly dependent on the initial parasite burden. Furthermore, the inverse median survival time predicted by eqn. (7.2) shows approximately the same relationship (Figure 7.3). This linear relationship is expected from the simplest conceivable form of parasite induced mortality, in which the death rate is constant with time, and directly proportional to the burden. In this case;

$$1/t_{\frac{1}{2}} = b/\ln 2 + \alpha P/\ln 2 \qquad (7.4)$$

where $1/t_{\frac{1}{2}}$ is the inverse of the median survival time, $b$ is the disease independent death rate and $\alpha$ is the per parasite increment in the disease induced host death rate. Ignoring the complications of age dependence, $\alpha$ may be estimated from the gradient of the line in Figure 7.3. This estimate is of more value when expressed in terms of the total parasite burden rather than parasite density. The surface area of the black mollies used in these experiments was estimated with the aid of the stylized drawing of a fish in Figure 7.4, the measurements

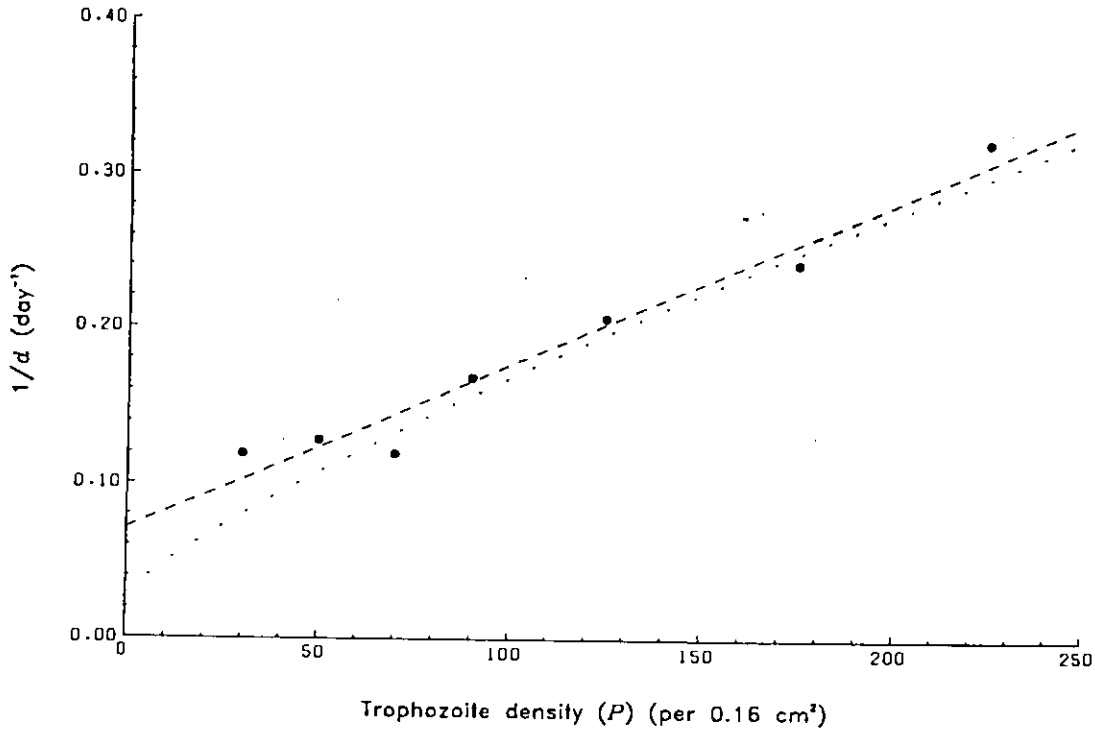
Figure 7.3 *An averaged death rate*

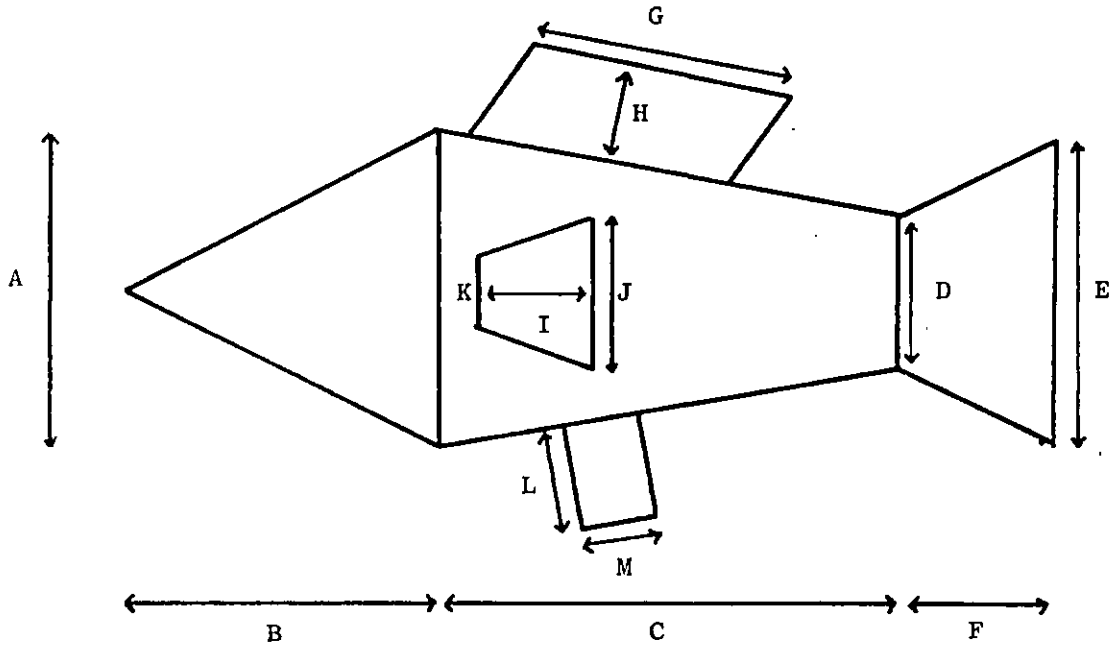
The relationship is shown between Trophozoite density ($P$) per 0.16 $cm^2$ of host surface area, and the inverse of the median survival time (in days) of infected hosts ($1/d$). The solid dots represent experimental observations, and the closely spaced dashed line represents a linear least squares regression on the experimental data ($1/d = a + bP$; $a$ = 0.0712, se = 0.0119, $b$ = 0.00104, se = 0.00009, 5 df). The widely spaced dashed line represents the inverse of the median survival time predicted by eqn. (7.2).

Figure 7.4 *The surface area of a black mollie*

The figure shows the stylized drawing used to estimate the surface area of a black mollie. Means of measurements from eighteen fish are given below.

| Measurement | Mean (cm) |
|---|---|
| A | 1.24 |
| B | 1.79 |
| C | 2.20 |
| D | 0.67 |
| E | 1.07 |
| F | 0.65 |
| G | 0.99 |
| H | 0.37 |
| I | 0.71 |
| J | 0.42 |
| K | 0.25 |
| L | 0.58 |
| M | 0.24 |


0.40
0.30
0.20
0.10
0.00
1/d (day⁻¹)
0
50
100
150
200
250
Trophozoite density (P) (per 0.16 cm²)


G
H
A
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F

shown being obtained as means from eighteen fish. This is obviously only a crude approximation to the area of a highly complex three dimensional surface, but gives an estimate accurate to at least an order of magnitude. The body area was thus estimated as 6.4 $cm^2$, and fin area as 2.6 $cm^2$. Using the observation of Section 5.4 that trophozoite density is similar on all parts of the host body surface, but approximately double on the fins, trophozoite numbers per 0.16 $cm^2$ area may be converted to total burden by multiplying by 73.

The increment in host death rate, $\alpha$, per parasite, per day is estimated to be in the region of $1 \times 10^{-5}$.

## 7.5 *Parasite Survival of Host Death*

*Ichthyophthirius* trophozoites are not killed by the death of their host (Section 2.1). It was not possible to directly count the number of tomites produced per trophozoite on a dead fish, however, because putrifaction of the corpse of the host prevented encystation in the small amounts of water necessary to produce measurable tomite densities. There is no reason to suppose that trophozoites of a particular age leaving fish because of host death produce fewer tomites than trophozoites leaving the host for any other reason. If this assumption is correct, it is possible to estimate the density dependent decline in fecundity of trophozoites resulting from parasite induced death.

The following assumptions are made:

(i) Parasites on the host have a density independent survival rate of the form of equation (4.2)

$$l(t) = \exp\left[(a/b)(1 - \exp(bx))\right] \qquad (7.5)$$

The results of Section 4.3.1. indicate that this is a reasonable assumption over the range of parasite burdens examined in that experiment. At very high parasite densities, there may be trophozoite losses due to large scale sloughing of the epidermis, and with it the parasites beneath it, but as such sloughing rapidly results in the death of the host, it can be dealt with as part of the density dependence due to parasite induced death.

(ii) The survival of infected hosts may be described by an equation of the form of eqn. (7.2). The proportion of hosts with an initial burden, $P$, surviving to a time, $x$, since infection, is therefore given by a function $L(x,P)$, where

$$L(x,P) = \exp(a'/[b'P] \ (1 - \exp(b'Px))) \tag{7.6}$$

where $a'$ and $b'$ are constants.

(iii) The number of tomites produced from a trophozoite leaving the host at age, $x$ for whatever reason is described by equation (4.4):

$$n(x) = cx^{d} \tag{7.7}$$

$N(t)$, the number of infective stages produced per trophozoite on a host dying at time $t$ after infection has two components:

(a) Tomites produced from trophozoites that have already left the host by time $t$. This quantity is given by:

$$\int_{o}^{t} -n(x) \ \frac{dl(x)}{dx} \ dx \tag{7.8}$$

( b) Tomites produced from trophozoites forced to leave the host at time $t$ due to host death. This component is given by the product

$l(t)$. $n(t)$, where $n(t)$ is obtained from eqn. (7.7) evaluated at time $t$. Hence

$$N(t) = \int_0^t -n(x) \frac{d\, l(x)}{dx} dx + n(t)\, l(t) \qquad (7.9)$$

The rate at which hosts die is

$$- \frac{\partial L(t,P)}{\partial t} \qquad (7.10)$$

and the expected number of offspring per trophozoite at an initial density $P$ is

$$O(P) = \int_0^\infty -N(t) \frac{\partial l(t,P)}{\partial t} dt \qquad (7.11)$$

which can be simplified by integrating by parts to

$$O(P) = \int_0^\infty l(t). \quad L(t,P) \frac{d\, n(t)}{dt} \quad dt \qquad (7.12)$$

Numerical solutions of eqn. (7.12) using parameter values obtained from Sections 4.3.1, 4.3.3. and 7.3 are shown over a range of values for P in Figure 7.5.

A variety of simple functions have been used to model density dependence in single species populations (Bellows, 1981). One of the most general, derived by Maynard Smith and Slatkin (1973), is

$$f(N) = (1 + (v.N)^w)^{-1} \qquad (7.13)$$

where $N$ is the population size and $f(N)$ is the fecundity relative to the fecundity at population sizes approaching zero. The dashed line

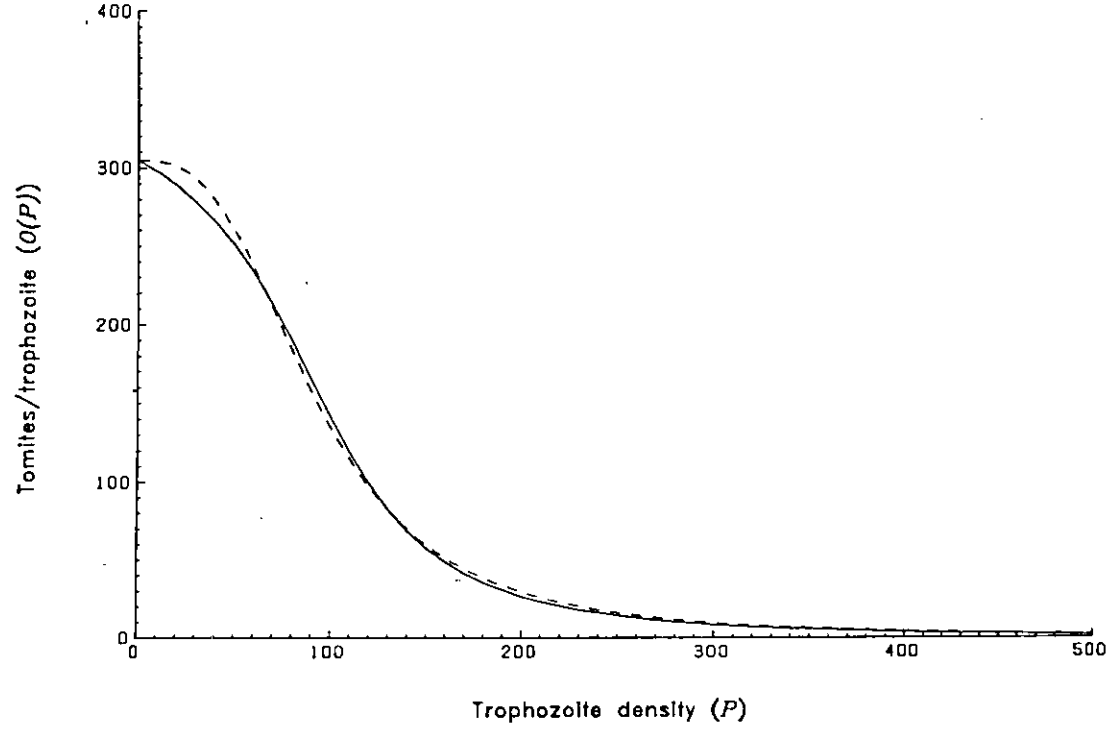
Figure 7.5 *The relationship between trophozoite density and tomite production per trophozoite*

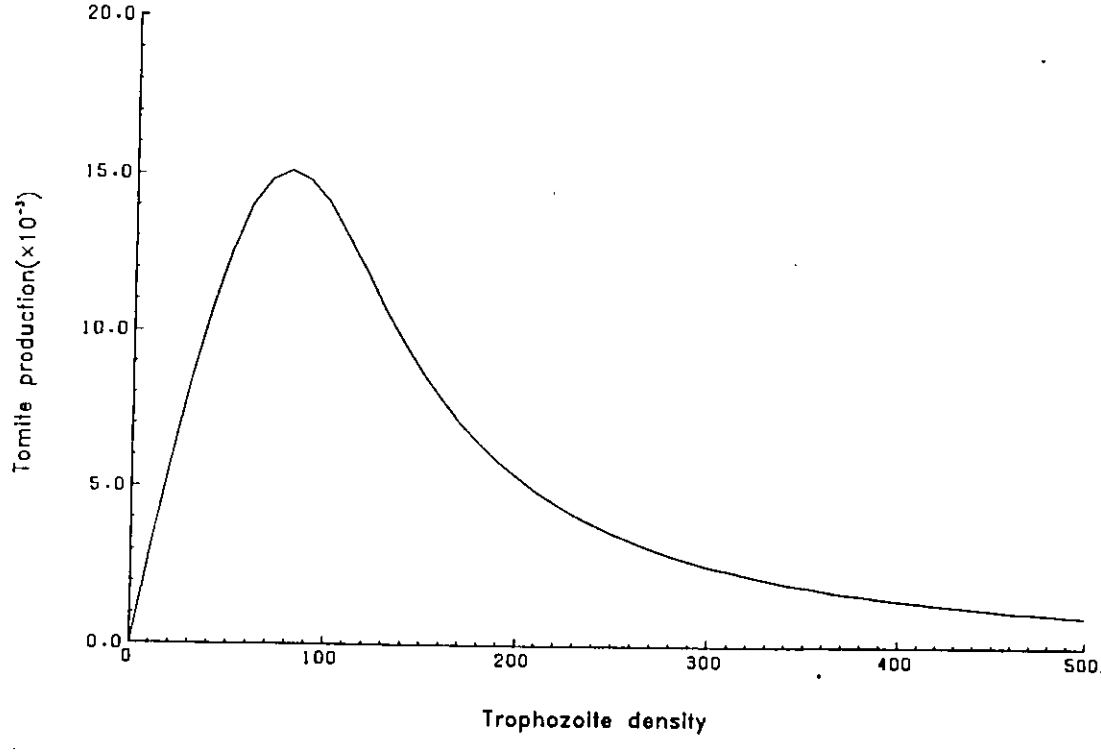
Trophozoite density is expressed as the number of parasites per $0.16cm^2$ of surface area of the host. The solid line represents the results of numerical integrations of eqn. (7.12), and the dashed line is a solution of eqn. (7.13), fitted (using a non-linear least squares procedure) to fifty equally spaced points generated from eqn. (7.12).

The estimated values of $v$ and $w$ are: $v = 0.0109$, $w = 2.87$.

Figure 7.6 *Density dependence in total tomite production*

The tomite production from a fish surface area of 0.16 $cm^2$ (predicted from eqn. (7.12)) is related to the trophozoite density in that area.


400
300
200
100
0
0
100
200
300
400
500
Tomites/trophozoite (O(P))
Trophozoite density (P)


20.0
15.0
10.0
5.0
0.0
0
100
200
300
400
500.
Tomite production(×10⁻³)
Trophozoite density

in Figure 7.5 represents a solution of eqn. (7.13) with $v$ and $w$ chosen by a non linear curve fitting procedure using 50 equally spaced points generated from equation (7.12). Equation (7.13) provides a very satisfactory approximation to eqn. (7.12).

The nature of the intra specific competitive interaction determines the value of the parameter $w$ of the model (Bellows, 1981). High values of $w$ occur if the competition is a "scramble", in which the overall number of progeny decreases at high population levels. If perfect contest competition exists, in which the total number of progeny reaches a saturation level at high host densities, $w$ equals 1. The high value of $w$ obtained here indicates scramble competition, and the relationship between the total number of tomites produced and trophozoite density is clearly humped (Fig. 7.6). Such a relationship is destabilizing, and may lead to stable limit cycles, if the intrinsic rate of increase of the population is great enough. The function in Figure 7.6 cannot, however, be directly translated into a recruitment curve: it represents only the relationship for a single host between the total number of tomites produced and trophozoite density. The proportion of these tomites able to form the next generation of trophozoites is clearly dependent on the host density, which may be a dynamic variable. This problem is returned to in Chapter 14.

## 7.6 *Discussion*

The parasite induced mortality considered in this chapter is what might be termed the death rate of hosts from acute *Ichthyophthirius* infection, as it is the short term effect of the parasite on the survival of its host. When free of infection, the death rate of a fish

that has been infected with *Ichthyophthirius* might be expected to be considerably greater than that of a host that has never been infected. *Ichthyophthirius* infection results in damage to the epithelium and gills, and these wounds are liable to be infected by a variety of other pathogen species, particularly fungi of the genus *Saprolegnia* (Richards and Pickering, 1978). As such pathogens do not normally attack healthy fish, mortality due to these secondary infections should properly be considered a component of the mortality caused by *Ichthyophthirius*. It is probable that the death rate resulting from a second infection of *Ichthyophthirius* on a host already debilitated by an initial infection will exceed the rate of mortality resulting from a single infection.

Although the inverse of the median life expectancy of hosts was found to be approximately linearly dependent on the parasite burden, it is unlikely that this relationship holds at low infective burdens. If the regression line shown in Fig. 7.3 is extrapolated back to a parasite burden of zero, the disease free instantaneous rate of mortality is predicted to be 0.1. This is too high to be plausible. Mortality at this rate would leave only 35% of uninfected hosts alive after ten days. Such a high rate of host death was not observed in any of the experiments described in Chapters 5 and 6. The rate of parasite induced mortality must therefore initially rise more steeply with increasing parasite burden than predicted by the regression presented in Fig. 7.3.

Despite the differences in experimental design and host species, it is valuable to compare the results obtained in this chapter with

the observations of Hines and Spira (1973a), which were discussed in Chapter 2. Hines and Spira, working with 250 g mirror carp *Cyprinus carpio*, report no mortality resulting from infections that peaked at 11 trophozoites per $cm^2$ of dorsal fin, and 100% mortality resulting from infections peaking at 430 per $cm^2$ of dorsal fin area (this latter figure is equivalent to 68 per 0.16 $cm^2$ of dorsal fin area). Even allowing for the density of trophozoites on the fins being higher than that on the body surface, Fig. 7.1 shows that few black mollies survived more than 10 days with infections of this intensity. Infections of around 10 per $cm^2$ of fin area produced very little mortality of black mollies.

Anderson and May (1978) reviewed a number of studies of the relationship between the parasite burden of a host and its instantaneous death rate. In several cases the relationship appears to be linear, although in others the rate of mortality increases exponentially with parasite burden. Lanciani (1979) investigated the influence of parasitic larvae of water mites on nymphs of the hemipteran *Hydrometra australis* and on larvae of the mosquito *Anopheles crucians*. In both cases, the instantaneous death rate increased linearly with increasing parasite burden. *Ichthyophthirius* appears to be unusual in that the function relating rate of mortality to parasite burden is apparently convex rather than linear or concave.

Parasites may also act to decrease the fecundity of their hosts (May and Anderson, 1978). No direct evidence on this was available in this study, as the black mollies did not readily reproduce under experimental conditions. It appears that *Ichthyophthirius* may have

a considerably greater effect on fry than on adult fish. Komorova (1975) found burdens of three to five trophozoites sufficient to kill carp by less than 15 mm in length. High fry mortality due to parasitism has a very similar effect in population dynamic terms to parasite induced reduction of the fecundity of adult fish.

# CHAPTER 8

## THE DISTRIBUTION OF PARASITES WITHIN THE HOST POPULATION

### 8.1 *Introduction*

In the models of Anderson and May (1978), the death rate of hosts due to parasite induced mortality is dependent on the distribution of parasites amongst hosts. The nature of this distribution is of considerable importance, as it contributes in the determination of both the stability of the systems and the numerical levels of host and parasite abundance. Most parasites are distributed in an aggregated or overdispersed manner amongst hosts, whether in natural conditions (Crofton, 1971a;Pennycuick, 1971; Anderson and May, 1978) or whether the hosts have been infected under uniform laboratory conditions (Keymer and Anderson, 1979; Anderson, Whitfield and Dobson,1978a; Anderson, Whitfield, Dobson and Keymer, 1978b). Many of these distributions are well described empirically by the negative binomial distribution.

The negative binomial distribution was originally derived as the probability distribution for the number of binomial trials necessary to produce exactly $k$ successes (Feller, 1968). This has little biological application, but the same mathematical form may be produced by a broad range of processes, many based on plausible biological assumptions (e.g. Pielou, 1969; Boswell and Patil, 1973). The distribution can be described by two parameters, the mean, $\mu$, and a dispersion parameter, $k$. The value of the parameter $k$ varies inversely with the degree of aggregation or overdispersion of the distribution. If $k$ is large and positive, the distribution approaches a Poisson

form (variance = mean) while when $k$ is small and positive, the distribution is highly aggregated (variance >> mean). The parameter $k$ is therefore a convenient measure of the overdispersion of a distribution, independent of its mean, although its use for this purpose has recently been criticized (Taylor, Woiwod and Perry, 1979).

## 8.2 *Experimental Methods*

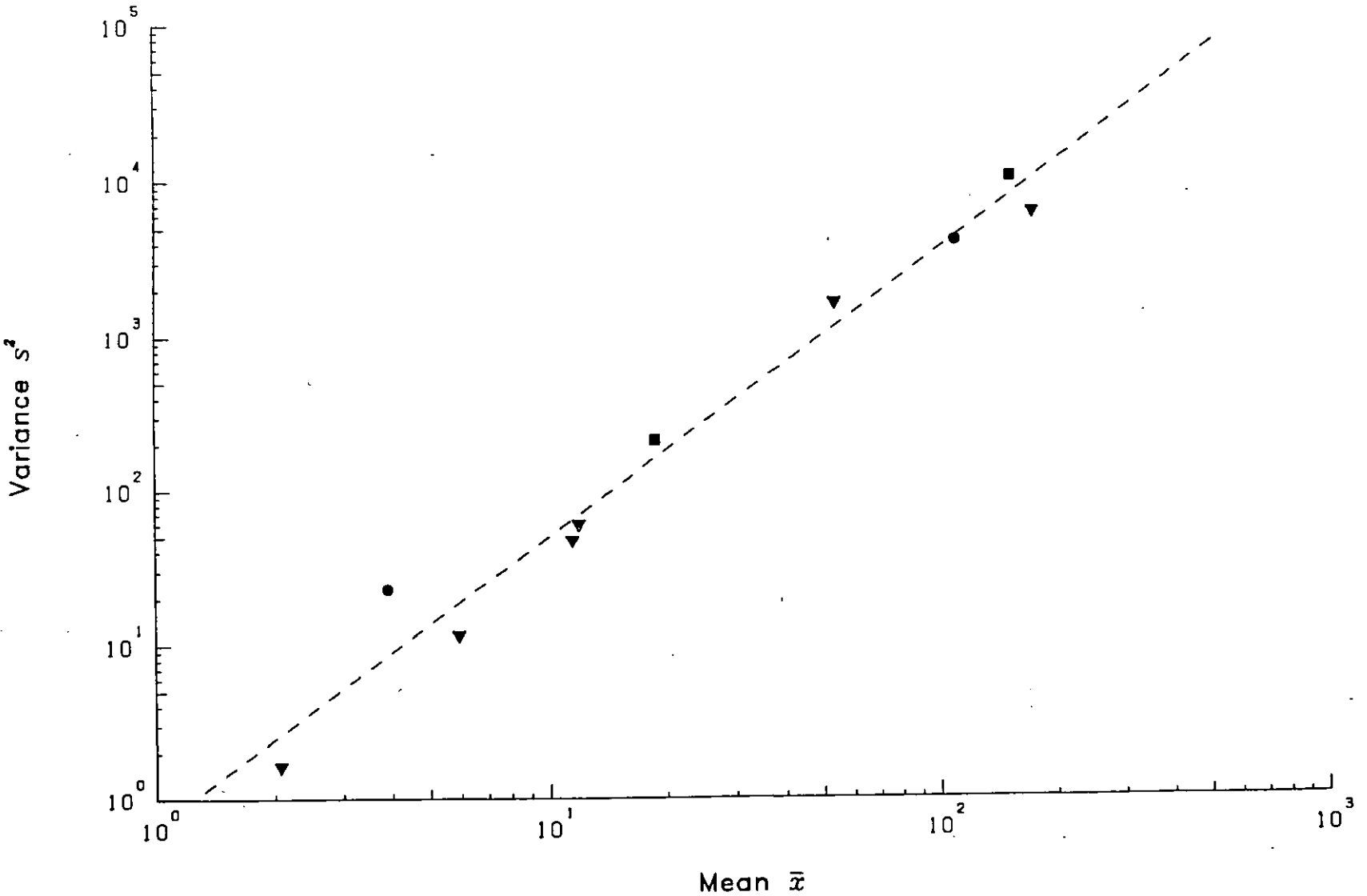
The results analysed in this section were obtained during the course of a number of the experiments described in previous sections. In each of these, a number of hosts were simultaneously infected with the same concentration of tomites in 500 ml of conditioned water and kept individually until the parasite burden was assessed four or five days after the initial infection. The experiments were not all carried out at the same time, and are therefore obtained from a variety of different batches of fish and tomite suspensions. Fish numbers used in the experiments ranged from 15 to 35.

## 8.3 *Results*

The variance of the parasite burden established in each experiment is compared with the mean parasite burden in Figure 8.1. In all but one case, the variance is greater than the mean, indicating overdispersion. Note also that the variance is approximately proportional to the square of the mean burden per host. In these controlled conditions, overdispersion can be generated by two factors: variability in the number of infective stages encountering each host, or variability in the proportion of infective stages successfully forming trophozoites. As was discussed in Chapter 5, essentially all tomites appear able to locate a host before death, and this heterogeneity is therefore unlikely

Figure 8.1 *Mean and variance of parasite burden per host*

The relationship is shown between the mean parasite burden per host ($\bar{x}$, horizontal axis) and the variance in parasite burden per host ($s^2$, vertical axis) for ten separate infection experiments. Double log axis are used. The line shown represents the linear least squares regression of $\log_{10}(s^2)$ on $\log_{10}(\bar{x})$: $\log_{10}(s^2) = a + b \log_{10}(\bar{x})$. ($a$ = -0.174, se = 0.158; $b$ = 1.88, se = 0.11; 8df)
Solid circles represent observations based on 30 replicates, solid squares, observations based on 35 replicates, and triangle observations based on 15 replicates.


Variance $s^2$
$10^5$
$10^4$
$10^3$
$10^2$
$10^1$
$10^0$
$10^0$
$10^1$
$10^2$
$10^3$
Mean $\bar{x}$

to be generated by variability in the number of tomites encountering each host. It is probable that the heterogeneity is generated by variability in the proportion of tomites infecting a host that are able to form trophozoites (in the language of the model of Section 5.3, variability in $s$). Such variability could be due to innate genetic factors, differing previous experience of infection, or physiological status.

If the observed heterogeneity is generated by differing host susceptibilities to infection, a simple relationship can be predicted between the variance of parasite burden per host and its mean. Suppose that each host has a susceptibility, $s$, to infection, such that the probability of a tomite encountering that host surviving to form a trophozoite is $s$. If the number of infective stages encountering each host follows a Poisson distribution (a reasonable assumption under these controlled experimental conditions), it is shown in Appendix 3 that the expected number of parasites establishing per host, E($P$), is given by

$$E(P) = T\bar{s} \tag{8.1}$$

and the variance in burdens between hosts, var ($P$) is given by

$$var(P) = T^2 \, var(s) + T\bar{s} \tag{8.2}$$

Here $T$ is the mean number of tomites infecting each host, $\bar{s}$ is the mean susceptibility of the host population, and var($s$) is the variance in susceptibility between hosts.

If the distribution of parasites on hosts can be considered to be negative binomial in form, the parameter, $k$, which describes the degree of overdispersion can be obtained from eqns. (8.1) and (8.2) where

$$k = (\bar{s})^2 / \text{var}(s) \tag{8.3}$$

The parameter $k$ should therefore be independent of the mean parasite burden.

The data were further analysed by a method described by Bliss and Owen (1958) which tests whether a common $k$ can be used to describe a series of distributions. The procedure is based on the moment estimate of $k$.

$$\hat{k} = (\bar{x})^2 / s^2 - \bar{x}) \tag{8.4}$$

where $\hat{k}$ is the estimated value of $k$ and $\bar{x}$ and $s^2$ are the sample mean and variance. The inverse of $k$, which has better statistical properties then $k$, is estimated as

$$y' = s^2 - \bar{x} \tag{8.5}$$

divided by

$$x' = (\bar{x})^2 - s^2/N \tag{8.6}$$

where $N$ is the size of the sample. The inverse of a common $k$ describing a series of distributions is estimated as the gradient of a

regression of $y'$ on $x'$, constrained to pass through the origin, and weighted inversely according to the expected variance at each point. Two tests of significance are produced: a statistic with a $\chi^2$ distribution which tests for the homogeneity of the distributions with respect to $k$, and an analysis of variance which, if significant, indicates a trend in $k$ with the mean. It is important to note that the test does not examine whether the individual samples follow negative binomial distributions. It is a test of whether, given that each sample does follow a negative binomial distribution, a constant $k$ is appropriate to describe all of them.

The relationship between $y'$ and $x'$ calculated from the data of Fig. 8.1 is displayed in Fig. 8.2. No extreme outlying data points are evident, but the significant value of $\chi^2$ indicates that a common $k$ does not describe all the distributions. The inverse of $k$, estimated at $y'/x'$, is shown related to the mean parasite burden of each sample in Fig. 8.3. No trend in $1/k$ with the mean is evident, and the analysis of variance does not detect any indication of systematic change in $1/k$ with mean burden.

Despite attempts to standardize the experimental conditions, there is a great deal of variability between experiments in the mean proportion of tomites forming trophozoites. (Table 8.1). This may be due to the mean susceptibility of hosts varying between batches of fish, or may be due to differing tomite viability between experiments. Given the variability in $\bar{s}$ between experiments, the degree of constancy in $\text{var}(s)/(\bar{s})^2$ is surprising.

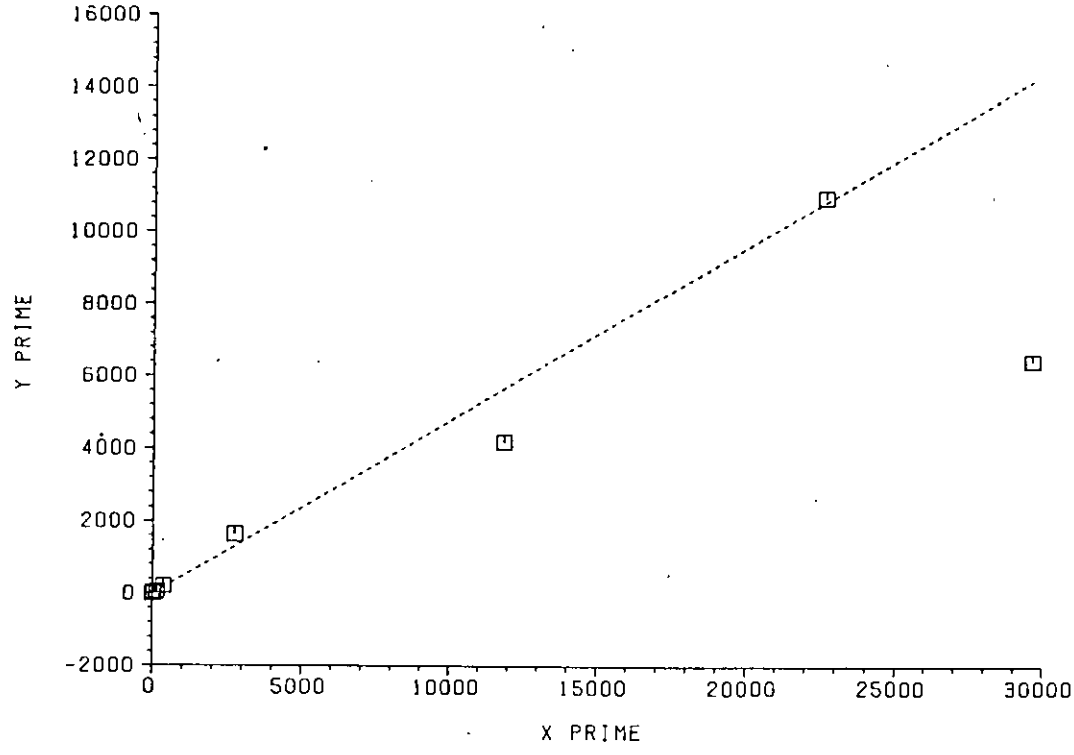
The number of observations in each sample is too small to satisfactorily examine whether the individual samples follow negative binomial

Figure 8.2 *The relationship between $y'$ and $x'$, using data of Fig. 8.1.*

The horizontal axis represents $x' = (\bar{x})^2 - s^2/N$, and the vertical axis represents $y' = s^2 - (\bar{x})^2$. The dashed line shown is the weighted least squares regression of $y'$ on $x'$, calculated following the method of Bliss and Owen (1958). The test indicates that a single $k$ is inappropriate to describe all the distributions of parasites on hosts ($\chi^2 = 19.2$, 8df, $p < 0.05$).

Figure 8.3 *The relationship between 1/k and mean parasite burden*

The estimated value of $1/k$ (vertical axis) for each distribution of parasites on hosts is shown related to the mean parasite burden for each distribution (horizontal axis). The dashed line represents the constant $1/k$ of 0.474 determined by the weighted regression of $y'$ on $x'$. No evidence of a significant trend in $1/k$ with mean burden is found. ($F = 0.036$; 1, 7 df).


16000
14000
12000
10000
8000
6000
4000
2000
0
-2000
Y PRIME
0
5000
10000
15000
20000
25000
30000
X PRIME

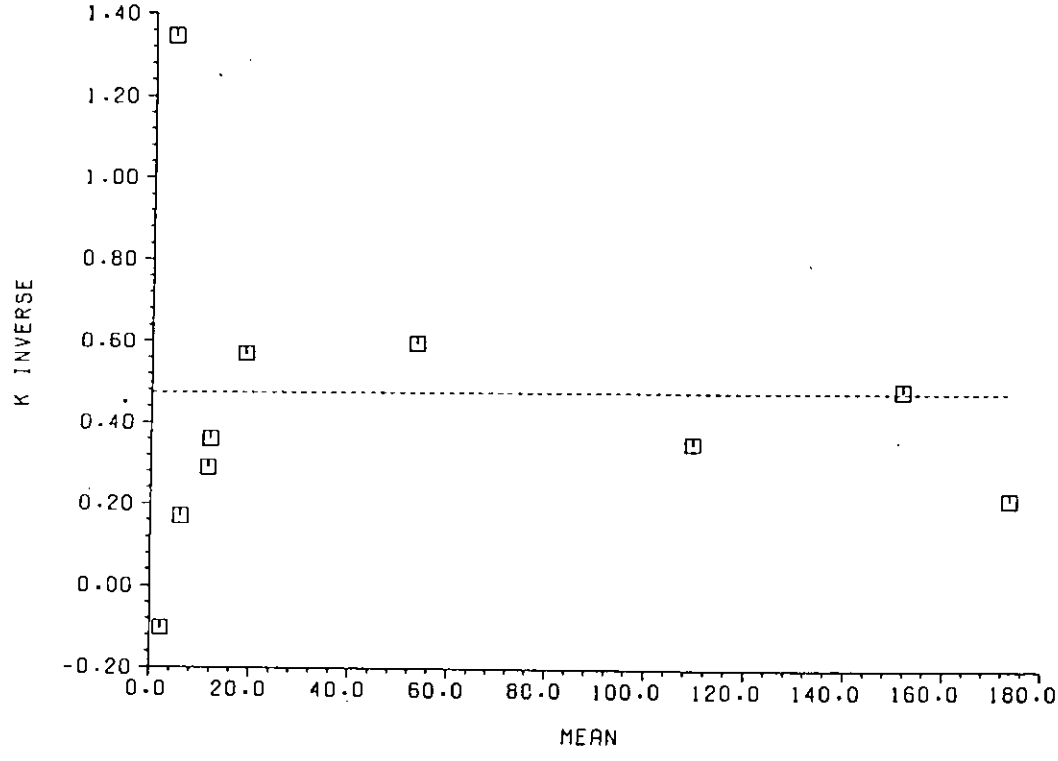

1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
-0.20
K INVERSE
0.0
20.0
40.0
60.0
80.0
100.0
120.0
140.0
160.0
180.0
MEAN

Table 8.1 *Variability in Susceptibility to Infection.*

| Sample size | Mean parasite burden | Mean proportion of tomites forming trophozoites |
|---|---|---|
| 35 | 151.7 | 0.069 |
| 35 | 18.7 | 0.085 |
| 29 | 3.9 | 0.002 |
| 28 | 109.6 | 0.359 |
| 15 | 2.01 | 0.030 |
| 15 | 5.93 | 0.044 |
| 15 | 11.4 | 0.034 |
| 15 | 11.9 | 0.114 |
| 15 | 53.4 | 0.128 |
| 15 | 173.4 | 0.173 |

distributions. Figure 8.4 shows frequency distributions of the number of parasites per host for the four largest samples used in the analysis described above. Overlayed on each is the distribution expected if the sample followed a negative binomial with $k$ = 2.1. One sample, with a mean of 18.7, appears to show a significant departure from the expected distribution, with too many high parasite burdens, but the other three distributions appear to be satisfactorily described by the negative binomial distribution. The next section explores the expected distribution of parasites on hosts by means of a simple probability model.

## 8.4 *The Expected Distribution of Parasites on Hosts*

In the previous section, a simple relationship between the mean and variance of parasite burden per host is predicted, assuming that the heterogeneity in burden per host is generated solely by differing host susceptibility to infection. The actual form of the probability distribution of parasites on hosts is given by:

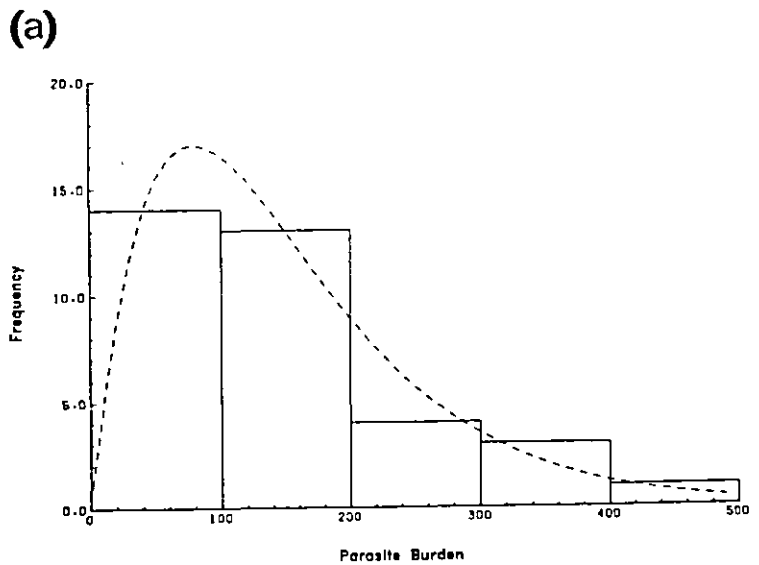
$$P(P = p) = \int_0^1 [f(s)\ \exp(-Ts)(Ts)^p]/\ p!\ ds \qquad (8.7)$$

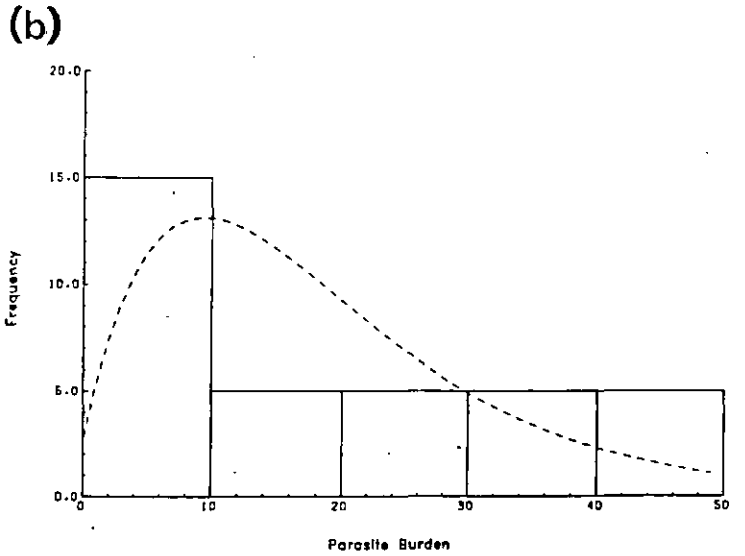
where $P(P = p)$ is the probability of a host harbouring exactly $P$ parasites, $T$ is the mean number of infective stages encountering each host, and $f(s)$ is the probability density function of the susceptibility, $s$ (see Appendix 3). The distribution $\{P(P = p)\}$ is exactly negative binomial if $s$ follows a gamma distribution and the integration is performed between 0 and infinity rather than 0 and 1 (Moran, 1953). A logical difficulty with this assumption is that certain hosts will have more parasites establishing than tomites infecting them

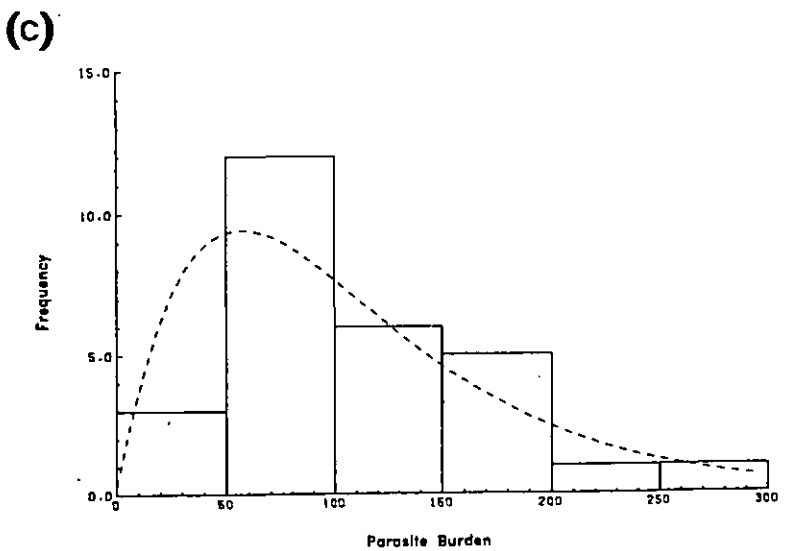
Figure 8.4 *Distributions of parasites on hosts*

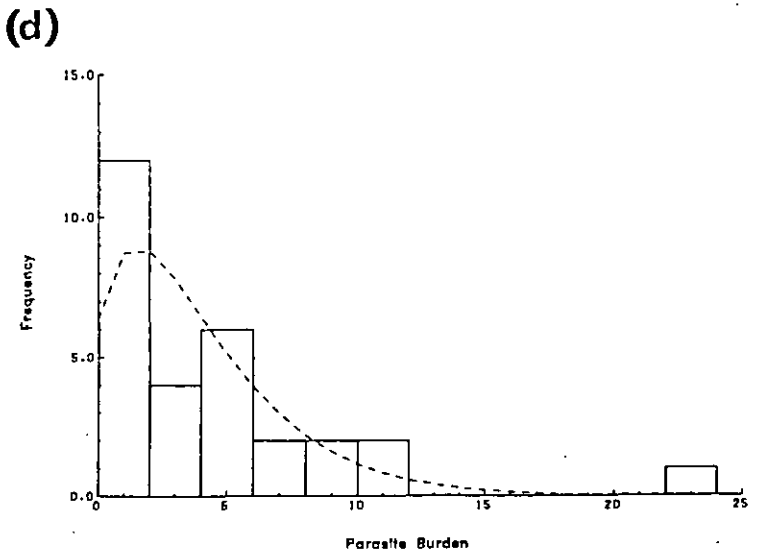
Frequency distributions of the number of parasites per host are shown for the four largest samples used in the preceeding analysis. Negative binomial distributions with the same mean as the observed samples and the common value of $k$, 2.1 (determined in the preceeding analysis), are shown as a dashed curve overlayed on each distribution. Details of the distributions are given below. The $\chi^2$ values compare the observed distributions with the negative binomial distributions shown. The tails of each distribution were amalgamated so that the expected frequency in any category was greater than 5.

| Sample | Size of sample | Mean | $\chi^2$ | d.f. | P |
|---|---|---|---|---|---|
| (a) | 35 | 151.7 | 0.22 | 1 | N.S. |
| (b) | 35 | 18.78 | 8.13 | 2 | <0.05 |
| (c) | 28 | 109.2 | 2.82 | 2 | N.S. |
| (d) | 29 | 3.90 | 4.87 | 2 | N.S. |


(a)
20.0
15.0
10.0
5.0
0.0
Frequency
0
100
200
300
400
500
Parasite Burden


(b)
20.0
15.0
10.0
5.0
0.0
Frequency
0
10
20
30
40
50
Parasite Burden


(c)
15.0
10.0
5.0
0.0
Frequency
0
50
100
150
200
250
300
Parasite Burden


(d)
15.0
10.0
5.0
0.0
Frequency
0
5
10
15
20
25
Parasite Burden

($s > 1$). A more plausible form of the distribution of $s$ is a beta distribution of the first kind, (Moran, 1968) which is a continuous function defined between 0 and 1 with a probability density function, $B(s)$ :

$$B(s) = \Gamma(l + m)/[\Gamma(l)\Gamma(m)]\ s^{l-1}(1-s)^{m-1} \qquad (8.8)$$

where $l$ and $m$ are parameters describing the distribution, and $\Gamma$ denotes the gamma function (the mean of the distribution is $l/(l + m)$, and the variance is $l(l + m)^{-2}(l + m + 1)^{-1}$). If eqn. (8.8) is substituted into eqn. (8.7), the resulting integral is not analytically soluble. Numerical solutions of eqn. (8.7) with $f(s)$ of the form of eqn. (8.8) are shown in Fig. 8.5. These are compared with negative binomial distributions, with the same mean and $k$ obtained from substituting the mean and variance of the beta distribution into eqn. (8.3). The solutions of eqn (8.7) would, for practical purposes, be indistinguishable from negative binomial distributions.

This simple model therefore predicts that if heterogeneity is generated solely by differences in susceptibility to infection between hosts, the distribution of parasites on hosts should be approximately negative binomial in form, with the parameter $k$ dependent on the susceptibility of the hosts, and independent of the mean number of infective stages to which hosts are exposed. This provides some justification for the assumption made by Crofton(1971b) and Anderson and May (1978) that the parasite distribution may be described by a negative binomial distribution with constant $k$.

In free running infections, however, the distribution would be

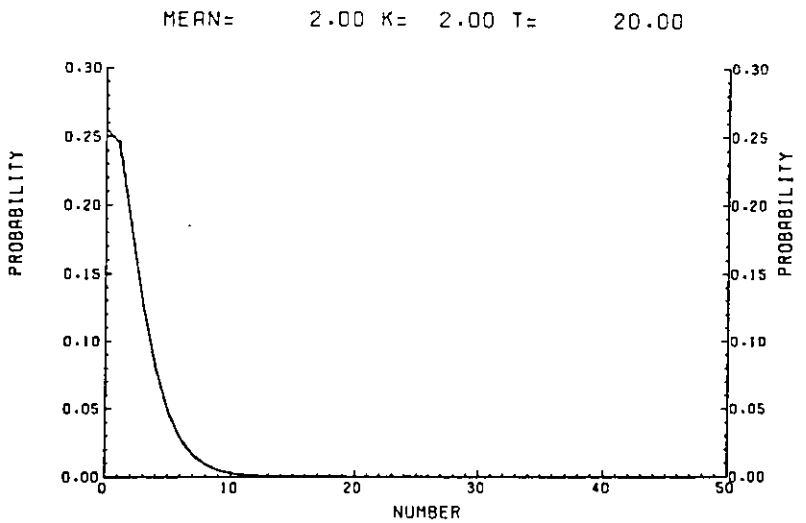
Figure 8.5 *Numerical solutions of equation (8.7) compared with negative binomial distributions.*

The solutions of eqn. (8.7) are shown as solid lines, and the negative binomial distributions are shown as dashed lines. On each graph, the number of parasites per host is shown on the horizontal axis, and the vertical axis shows the probability of that number of parasites occurring, given the probability model under consideration. Negative binomial distributions with $k$ = 0.5 and $k$ = 2.0 are shown, with means ranging from 2 to 100.

The parameters $l$ and $m$ with which the solutions of eqn. (8.7) were calculated were chosen to satisfy two criteria:

(i) The mean of the beta distribution, $l/(l+m)$ was set at 0.1, which is approximately the mean susceptibility of black mollies to *Ichthyophthirius* ($T$, the total number of infective stages, is hence 10 times the mean)

(ii) $(\bar{s})^2/var(s)$ was set equal to the $k$ of the negative binomial with which the solution of eqn. (8.7) was being compared. Hence: $(l/m)\ (l+m+1) = k$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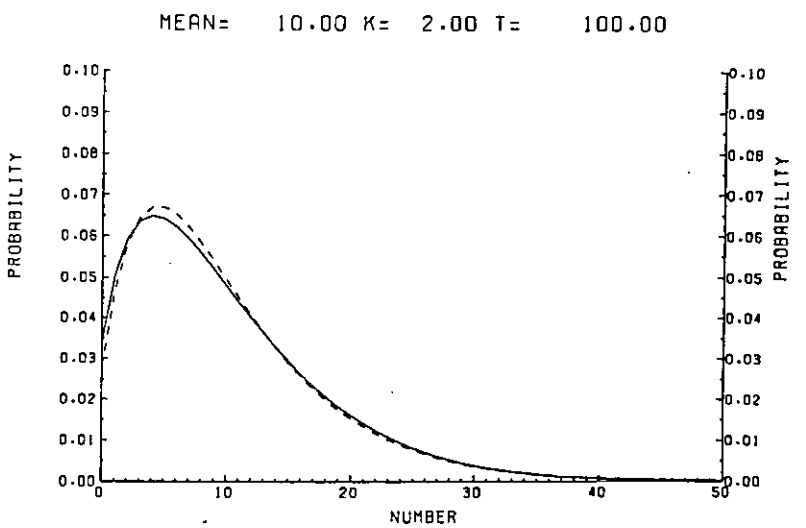
PROBABILITY
NUMBER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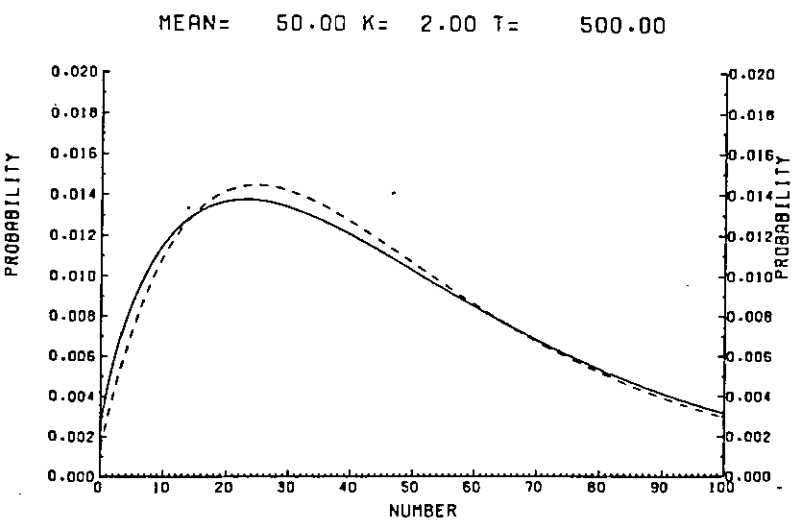
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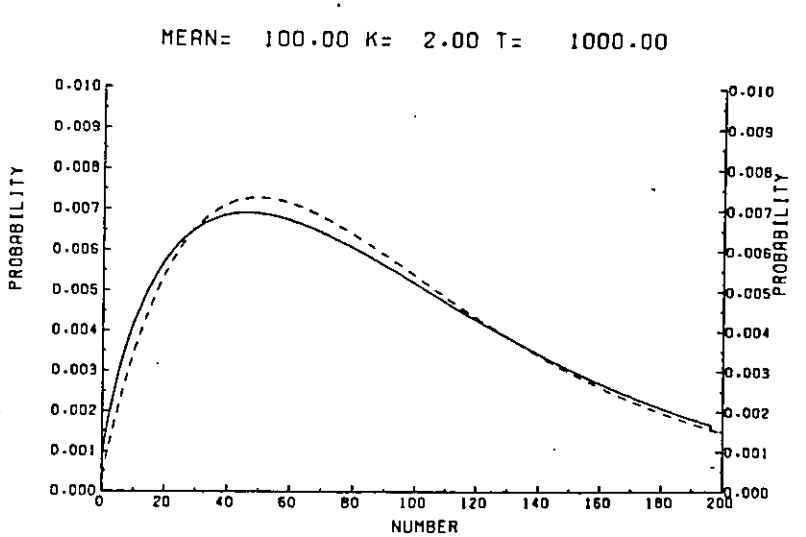
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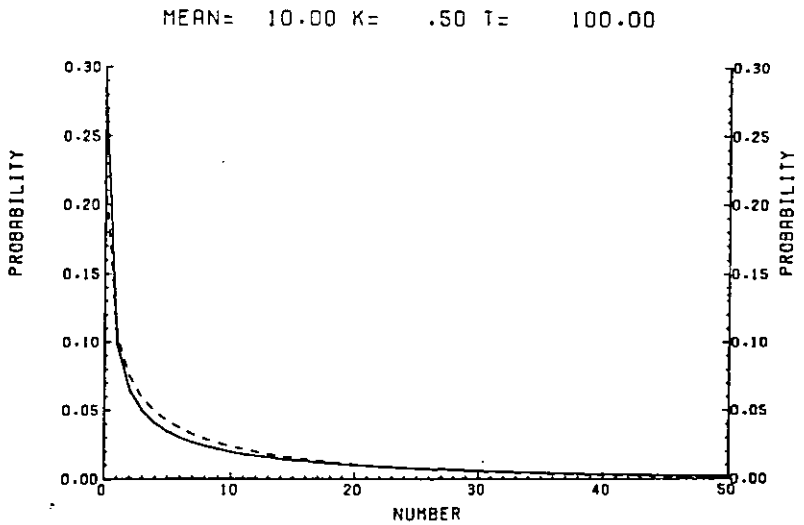
NUMBER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0.10
0.05
0.00
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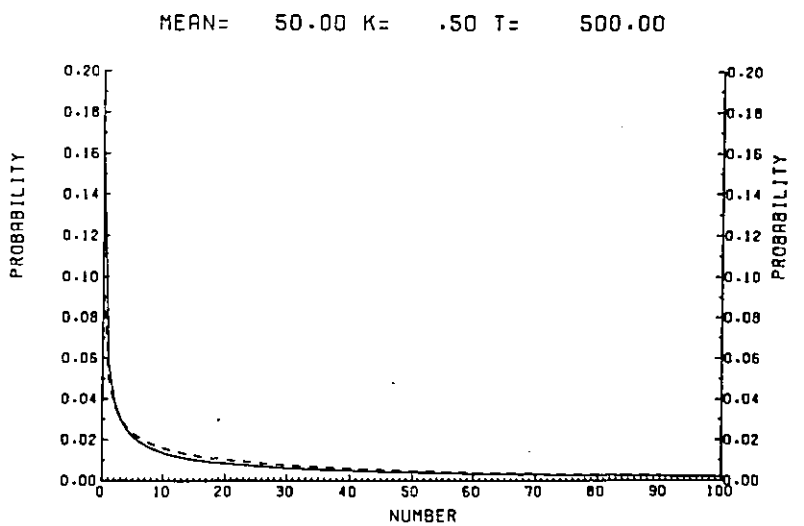
10
20
30
40
50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PROBABILITY
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0.12
0.10
0.08
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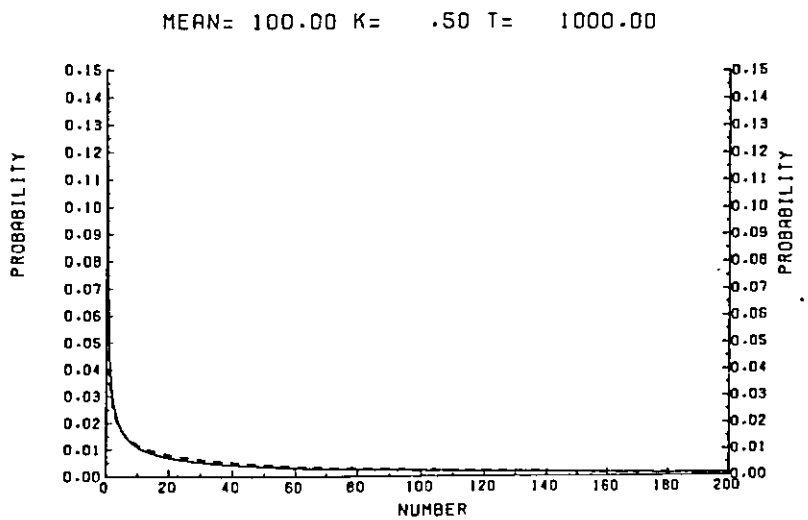
0.02
0.00
0
10
20
30
40
50
60
70
80
90
100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PROBABILITY
NUMBER
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0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
0
20
40
60
80
100
120
140
160
180
200

distorted to some extent. Non random infective stage distributions can be expected to lead to increased overdispersion (Keymer and Anderson, 1979) as will the fact that hosts recently born or added have not been exposed for as long as others to infection. Overdispersion will be reduced by the deaths of heavily infected individuals. The effect of the interplay of these factors on the distribution of parasites on hosts is examined with a simulation model in Chapter 13.

# CHAPTER 9

# EXPERIMENTAL EPIDEMICS

## 9.1 *Introduction*

The experiments described in this chapter took place over a longer term than those discussed in previous chapters. They are intended to examine experimentally the effect that the factors discussed previously have together on the dynamics of the pathogen in fish populations. As black mollies did not readily breed in the laboratory, it was necessary to introduce fresh hosts to produce a continuous epidemic. In the first series of experiments, this was done at a constant rate of one host per day, and in the second series, hosts were introduced when necessary to maintain the fish population at a constant level.

## 9.2 *Constant Host Immigration*

### 9.2.1. *Methods*

Two series of experiments are described. In each, three replicates were set up under the same initial conditions. Ten uninfected mollies were placed in 10 l tanks holding conditioned water. Tanks were aerated, but not filtered, and the bottom of each was covered with approximately 1 cm of coarse gravel. Water temperature was maintained at approximately 18$^{o}$C. Experiments were commenced by adding the same number of infective stages to each tank, approximately 3600 per tank in the first series, and 25,000 per tank in the second series. After this, uninfected hosts were added at a constant rate of one per day. Any dead fish were left in the tank for approximately 24 hours, to allow trophozoites present on them to leave and encyst. Fish were fed daily with "Aquarian"

Tropical Fish Flakes and the water level in the tanks topped up to 10 l when necessary. Every two days, initially, all fish were removed and their total parasite burden counted under anaesthesis with ethyl-m-amino benzoate (Sigma). Only trophozoites of greater diameter than 0.1 mm (representing approximately those older than four days) were counted. Frequency of counting was decreased later in the course of the experiments.

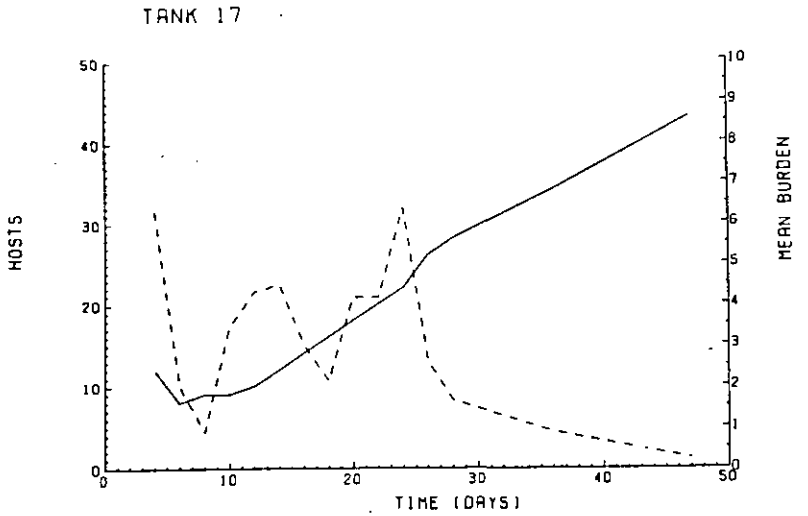
9.2.2. *Results of the first series*

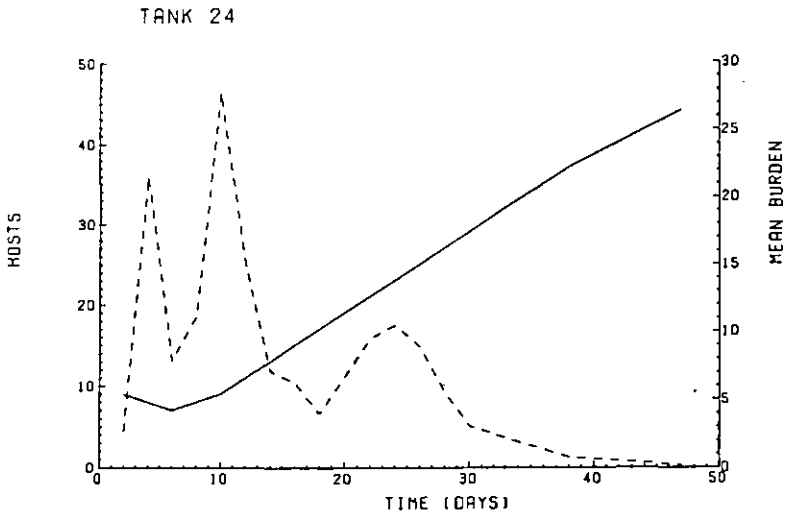
Changes with time of the number of hosts present and mean parasite burden for the first series of experiments are shown in Figure 9.1. All three replicates show broadly the same pattern. Host numbers decrease slightly initially, possibly due to secondary infections harboured by hosts prior to the beginning of the experiment, but after this, there is virtually no host mortality. Mean parasite burden initially shows a series of peaks following the generation time of the parasite, and then decreases to a low level. In no case do parasite numbers reach levels at which they might cause substantial host mortality. The extremely large potential reproductive rate of *Ichthyophthirius* (Section 4.3.5.) does not appear to be realized.

Two reasons for this failure to increase at the expected rate can be postulated. It is possible that the unfiltered water in the tanks may have become progressively more toxic to *Ichthyophthirius* during the fifty days of the experiment. Accordingly, at the completion of the experiment, water from one tank (tank 24) was kept for ten days to ensure that any *Ichthyophthirius* present had died. Ten fish were then infected in this water, ten others being infected in conditioned water. Fish infected in water from tank 24 developed burdens that were

Figure 9.1 *Results of the first series of constant immigration experiments*

The time in days since the commencement of the experiment is shown on the horizontal axis. The solid line represents the number of hosts present in the tank, and is scaled on the axis to the left of the figure. The mean trophozoite burden per host is shown as a dashed line, and scaled on the axis on the right of each graph.


TANK 17
HOSTS
MEAN BURDEN
TIME (DAYS)
0
10
20
30
40
50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HOSTS
MEAN BURDEN
TIME (DAYS)
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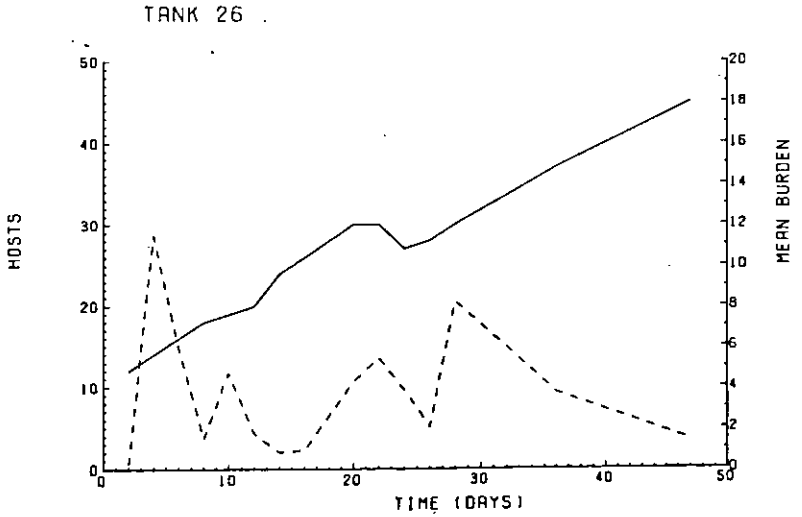
10
20
30
40
50


TANK 26
HOSTS
MEAN BURDEN
TIME (DAYS)
0
10
20
30
40
50

in fact higher than those developing in conditioned water (t = 2.28, 18 df, based on logged data) indicating that the water was not toxic to tomites. A second possibility is that the fish may have acquired resistance to infection. Ten fish were taken at random from tank 24, kept for ten days until parasite free, and then reinfected along with ten controls. The number of trophozoites establishing on the controls was significantly higher than that establishing on the hosts from the immigration-death experiment (t = 2.47, 18 df, based on logged data). Host resistance appears therefore to be the likely cause of the observed decline in parasite numbers.

The distribution of parasites on hosts was examined using the method of Bliss and Owen (1958) described in Section 8.3. These results cannot be interpreted too stringently, because successive observations of distribution of parasites are not strictly independent, but nevertheless, some important indications can be obtained. The results of the analysis are given in Figure 9.2. In all three cases, the distribution on hosts is highly overdispersed, with a value of $k$ considerably less than 1. The results are consistent with a common $k$ describing the distributions, with the exception of tank 24, where the deviation appears to be due to a single point.

### 9.2.3. *Results of the second series*

Results of the experiments commenced with a higher initial number of tomites are displayed in Figure 9.3. In each of these cases, parasite burden increases to a level where significant parasite induced mortality is caused. The *Ichthyophthirius* populations do not, however, appear to be able to persist at these high levels: every replicate shows a decline in mean parasite burden to a low level, following a peak in which there is major host mortality. Only in one tank (Tank 7)

**Figure 9.2** *Analysis of the results of the first series of constant immigration experiments, using the method of Bliss and Owen (1958).*

Two graphs are shown for each replicate:

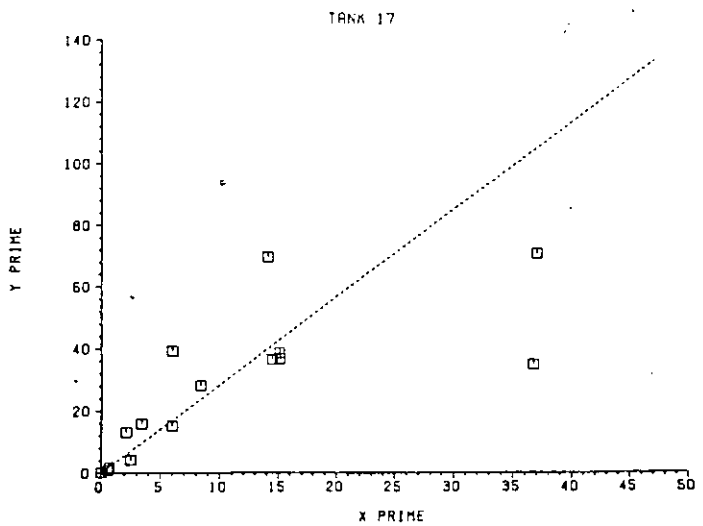
(i) $y' = s^2 - \bar{x}$ (vertical axis) is plotted against $x' = (\bar{x})^2 - s^2/N$ (horizontal axis).

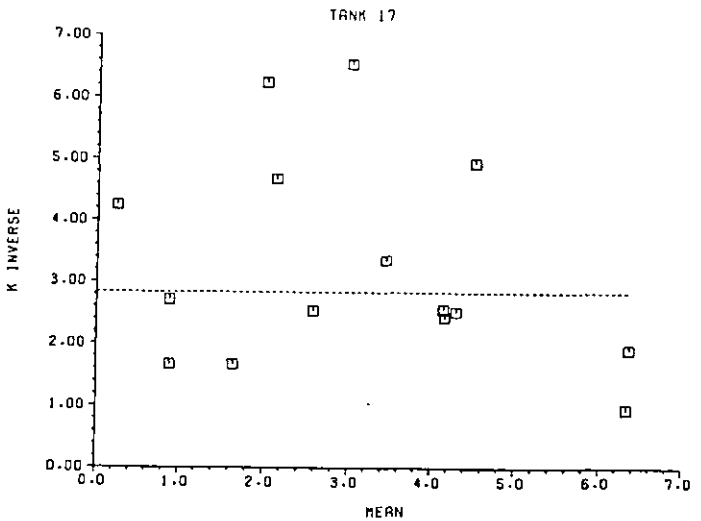
Each point is based on the distribution of parasites on hosts at one sampling occasion. The dashed line shown is the result of a weighted linear least squares regression of $y'$ on $x'$.

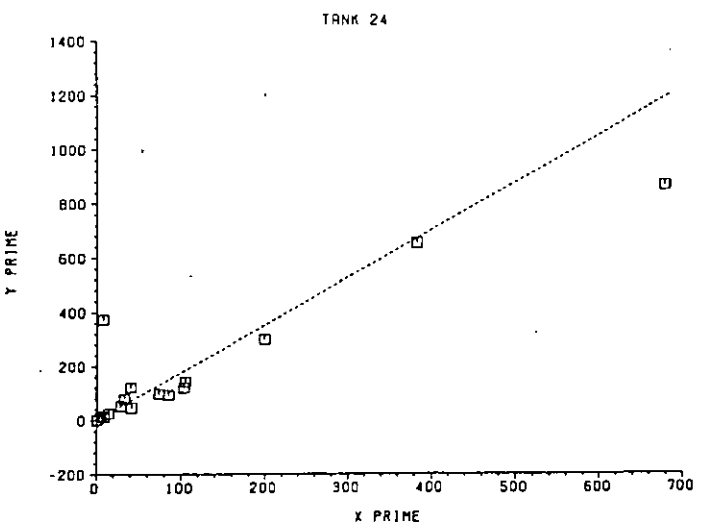
(ii) The estimated value of $1/k$ at each sampling occasion is shown, related to the sample mean. The dashed line represents the common $1/k$ estimated by Bliss and Owen's method.

Details of the analysis for each replicate follow. The $\chi^2$ is a test for homogeneity of each sample with respect to $k$ and the F, if significant, indicates a trend in $k$ with mean burden.

| Tank | Estimated $k$ | $\chi^2$ | d.f. | sig | F | d.f. | sig |
|---|---|---|---|---|---|---|---|
| 17 | 0.35 | 4.4 | 13 | NS | 0.49 | 1,12 | NS |
| 26 | 0.47 | 6.4 | 14 | NS | 0.39 | 1,13 | NS |
| 24 | 0.57 | 99 | 15 | .0001 | 0.09 | 1,14 | NS |


TANK 17
Y PRIME
X PRIME
0
20
40
60
80
100
120
140
0
5
10
15
20
25
30
35
40
45
50


TANK 17
K INVERSE
MEAN
0.00
1.00
2.00
3.00
4.00
5.00
6.00
7.00
0.0
1.0
2.0
3.0
4.0
5.0
6.0
7.0


TANK 24
Y PRIME
X PRIME
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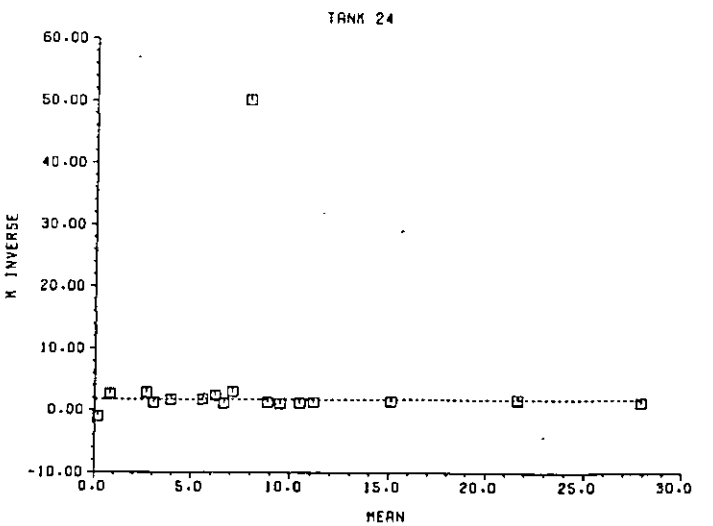
0
200
400
600
800
1000
1200
1400
0
100
200
300
400
500
600
700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K INVERSE
MEAN
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0.00
10.00
20.00
30.00
40.00
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60.00
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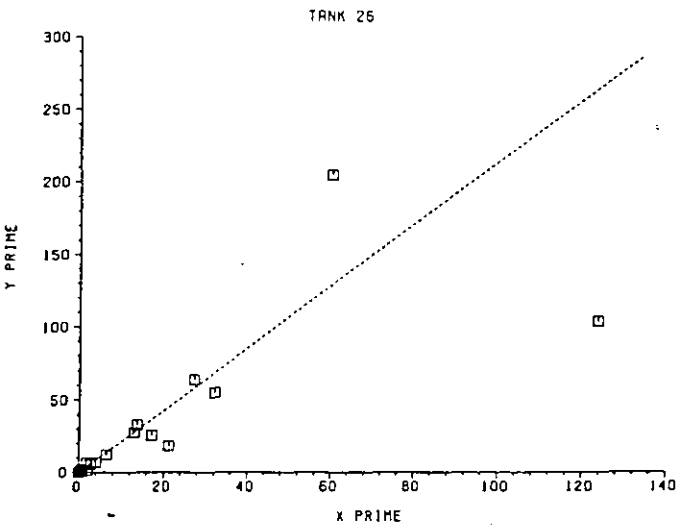
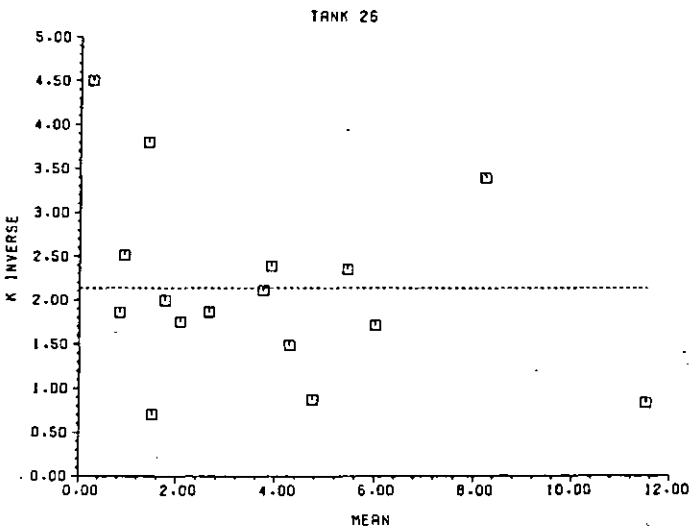
5.0
10.0
15.0
20.0
25.0
30.0


TANK 26
Y PRIME
X PRIME
0
50
100
150
200
250
300
0
20
40
60
80
100
120
140


TANK 26
K INVERSE
MEAN
0.00
0.50
1.00
1.50
2.00
2.50
3.00
3.50
4.00
4.50
5.00
0.00
2.00
4.00
6.00
8.00
10.00
12.00

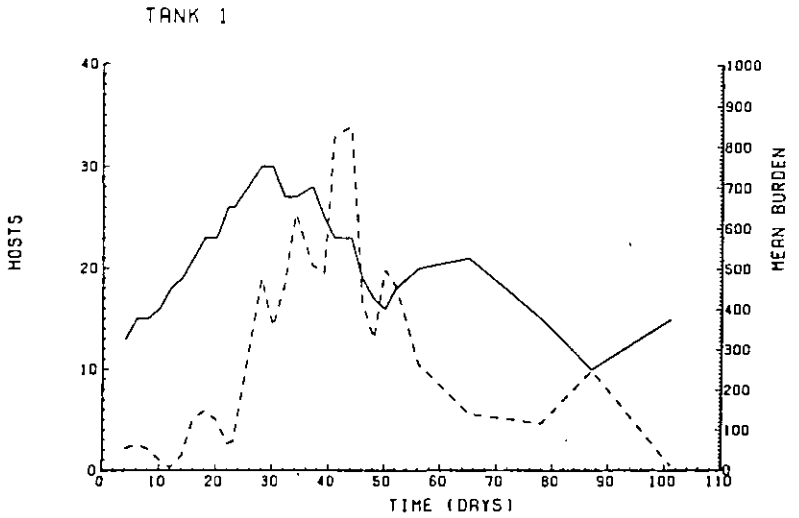
does the infection actually die out: in the other two replicates, it persists at a low level, and there are indications that further outbreaks may be possible.

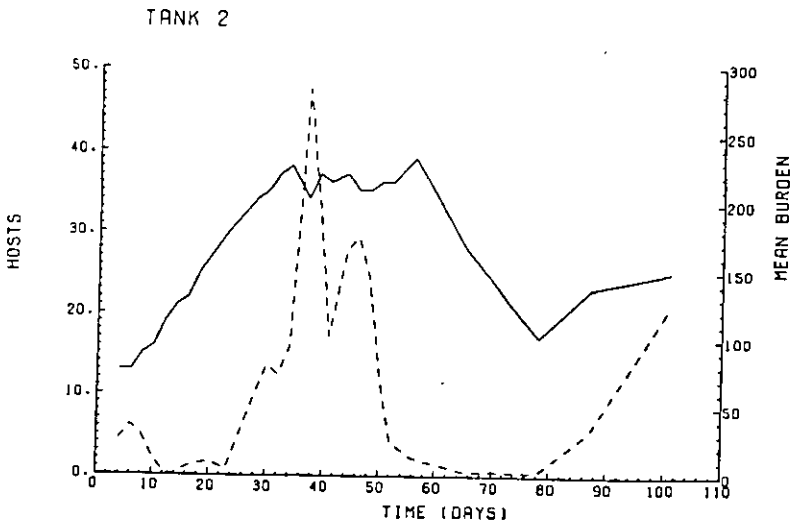
Due to the nature of the experiment, conditions did not remain constant during the course of the epidemics. The water became progressively more turbid: it is not possible to change the water without destroying all tomites and parasites undergoing encystation. Increasing host mortality with time due to crowding or progressive debilitation may also be postulated. The very low rate of mortality in tank 7 once *Ichthyophthirius* had become extinct indicates that these factors are not of overriding importance. A number of secondary infections were also inevitably present in each tank, including fungi of the genus *Saprolegnia*, the alga *Oodinium limneticum* and monogeneans of the genus *Gyrodactylus*. Whilst secondary infections added to the variability observed in results, secondary infection of lesions resulting from *Ichthyophthirius* infection is very probably a normal and important component of the parasite induced host death rate (see Chapter 7).

The distribution of parasites on hosts was again analyzed by the method of Bliss and Owen (1958). Results are shown in Figure 9.4. The highly significant values of $\chi^2$ obtained in two of the three cases indicate that, for these experiments with higher mean parasite burdens, $k$ cannot be assumed to be constant. The degree of overdispersion does not appear to be related in any simple way to mean parasite burden. In the only case in which the F test for trend is $k$ is significant, the $\chi^2$ value does not indicate a significant departure from a constant $k$ value. A more detailed discussion of these results follows in Chapter 14.

Figure 9.3 *Results of the second series of constant immigration experiments*

The time in days since commencement of the experiment is shown on the horizontal axis. The solid line represents the total number of hosts present, and is scaled on the axis to the left of the page. The mean trophozoite burden per host is shown as a dashed line, and is scaled on the axis to the right of each graph.


TANK 1
HOSTS
MEAN BURDEN
TIME (DAYS)


TANK 2
HOSTS
MEAN BURDEN
TIME (DAYS)

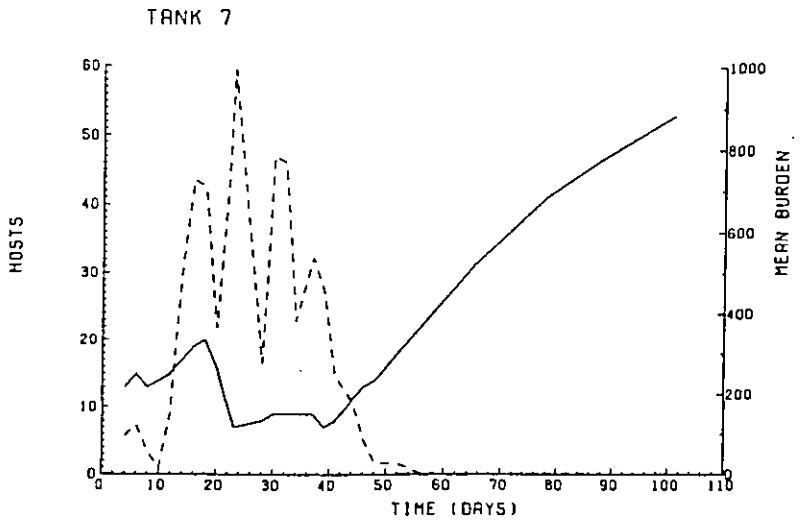
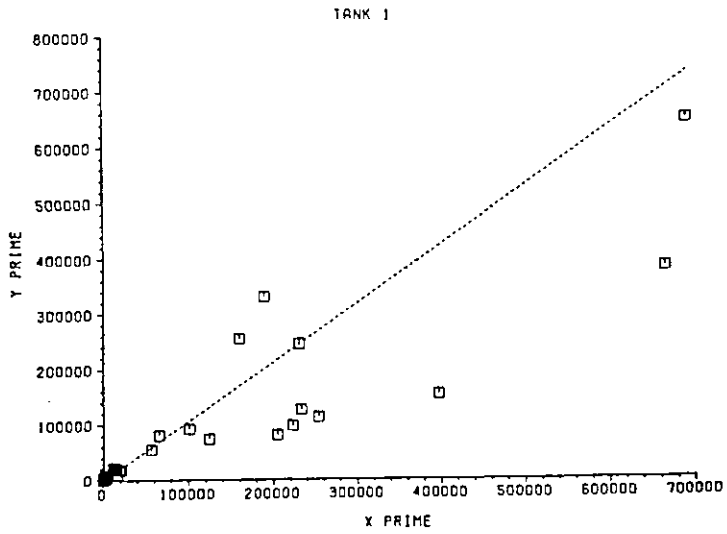

TANK 7
HOSTS
MEAN BURDEN
TIME (DAYS)

Figure 9.4 *Analysis of the results of the second series of constant immigration experiments*

Details are as described in Figure 9.2.

| Tank | Estimated $k$ | $\chi^2$ | d.f. | sig | F | d.f. | sig |
|---|---|---|---|---|---|---|---|
| 2 | 0.42 | 101.6 | 26 | .0001 | 0.094 | 1,25 | NS |
| 7 | 0.50 | 27.0 | 25 | NS | 8.34 | 1,24 | .01 |
| 1 | 0.94 | 98.5 | 26 | .0001 | 0.877 | 1,24 | NS |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Y PRIME
X PRIME
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700000
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500000
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100000
0
100000
200000
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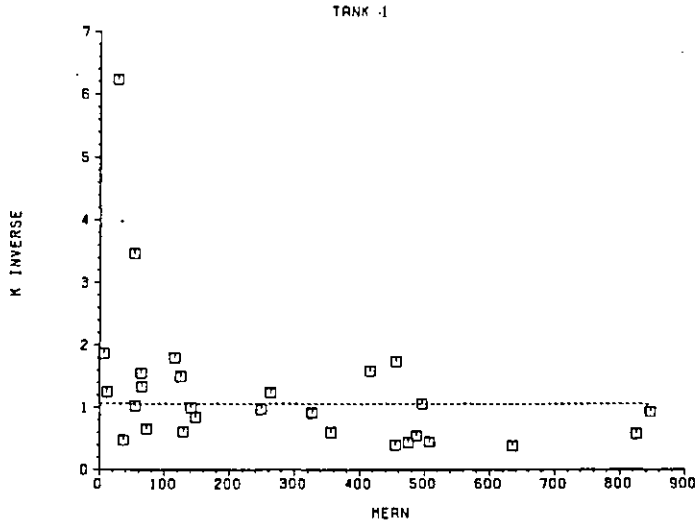
500000
600000
700000


TANK 1
K INVERSE
MEAN
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6
5
4
3
2
1
0
100
200
300
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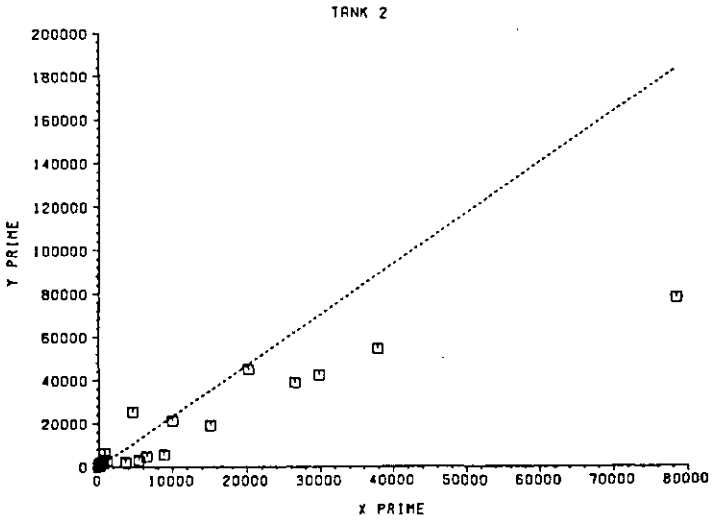
500
600
700
800
900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40000
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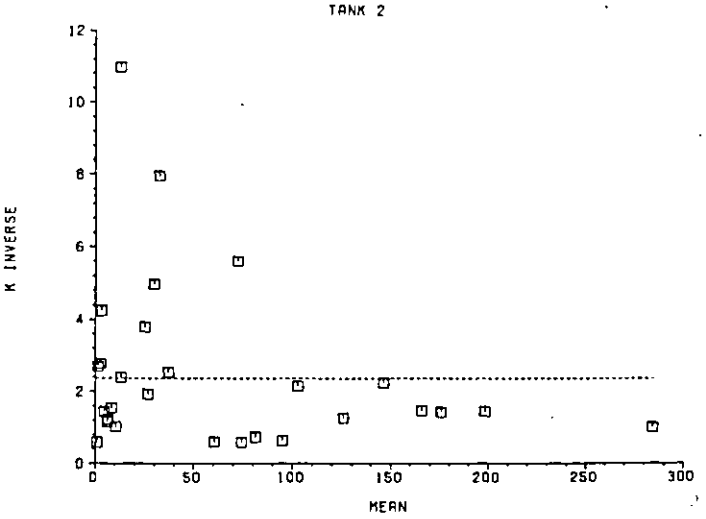
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250
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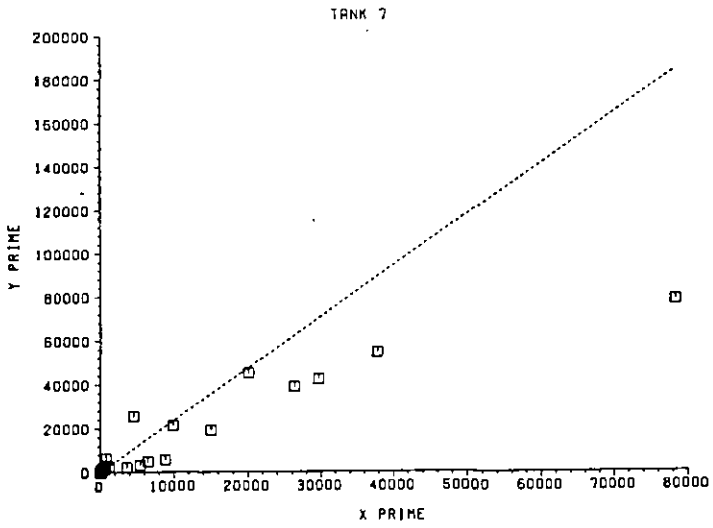
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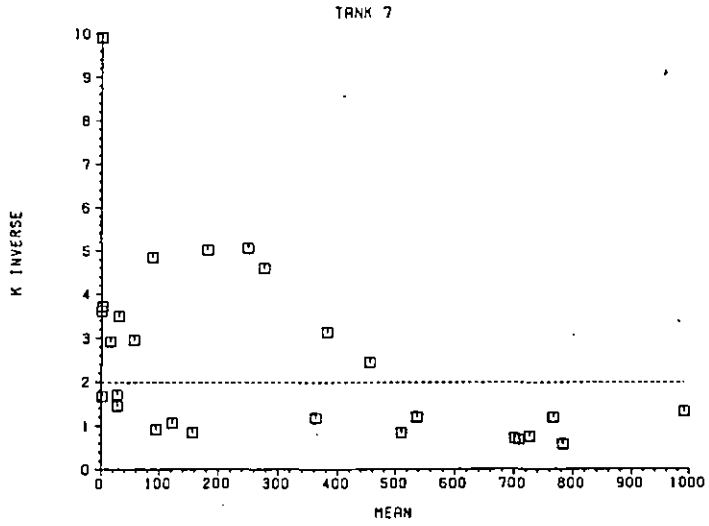
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0
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100
200
300
400
500
600
700
800
900
1000

## 9.3 *Constant Host Numbers in Tanks*

### 9.3.1. *Methods*

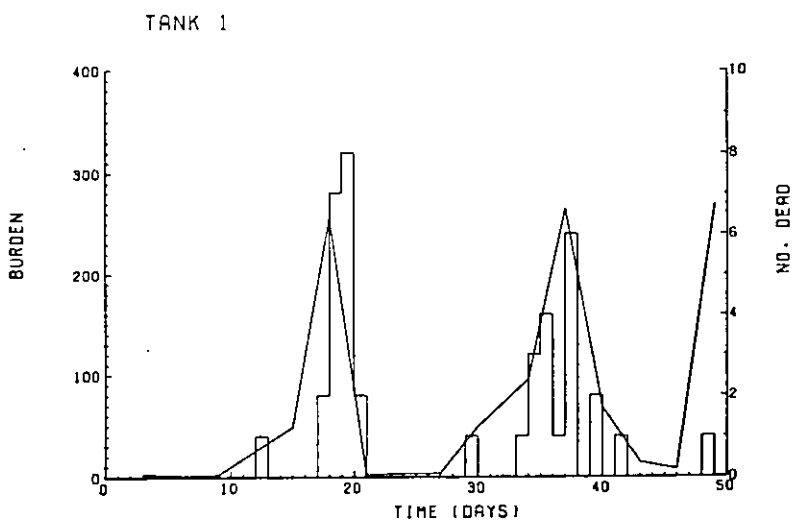
Ten fish were placed in each of eight 10 l tanks, holding conditioned water at 20°C. The bottom of each tank was covered with a layer of coarse gravel and the water aerated but not filtered. The experiment was commenced by adding a large number of tomites (36,000) to four of the tanks, and a lower number (1800) to the other four replicates. Host numbers were maintained at a constant ten live fish by daily replacing any dead hosts with uninfected mollies. Dead fish were left in the tanks for 24 hours to allow trophozoites to leave them and encyst. Fish were fed daily with "Aquarian" tropical fish flakes, and the water level topped up to 10 l with conditioned water when required. Parasite burden was assessed every three days by removing all hosts, anaesthetizing them with Ethyl-m-Aminobenzoate and counting the number of trophozoites visible within a 0.16 $cm^2$ grid, positioned on either side of the tail, just forward of the caudal peduncle, and on either side of the operculum. This method, utilizing higher magnification than that used for the previous experiment, detects most trophozoites older than one day.

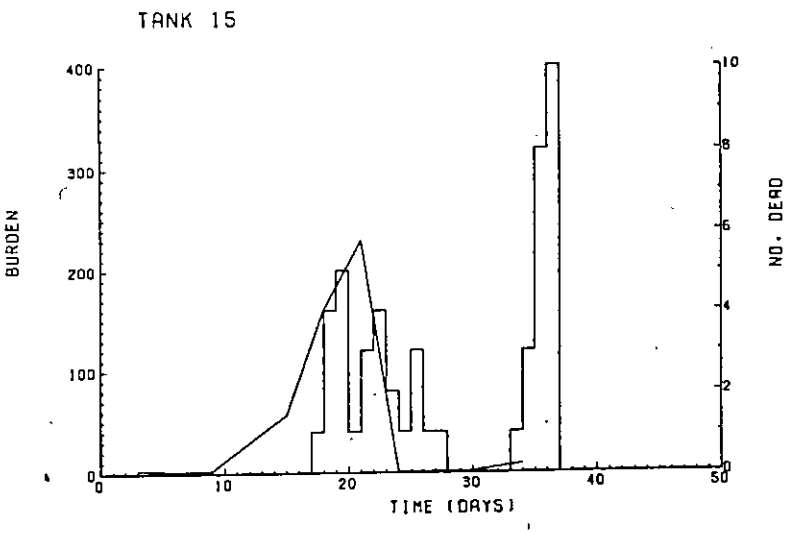
### 9.3.2. *Results*

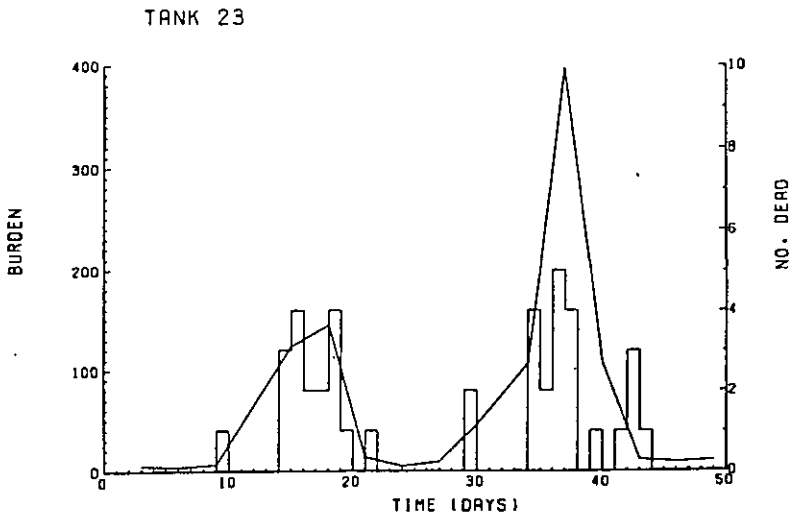
Figure 9.5 shows the changes with time in mean parasite burden and the number of hosts dying each day. One replicate (Tank 15) was discontinued after 34 days, because of a severe fungal infection that killed the entire host population. With the exception of tank 13, in which the effect is not pronounced, the remaining replicates show two or more sharp peaks in mean parasite burden associated with high levels of host mortality. Each peak is followed by a crash in mean

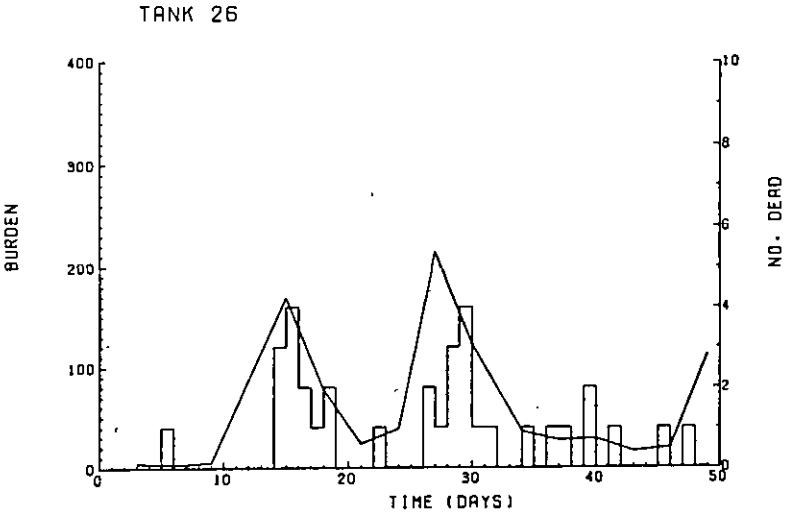
Figure 9.5 *Results of the experiment in which host numbers were maintained constant.*

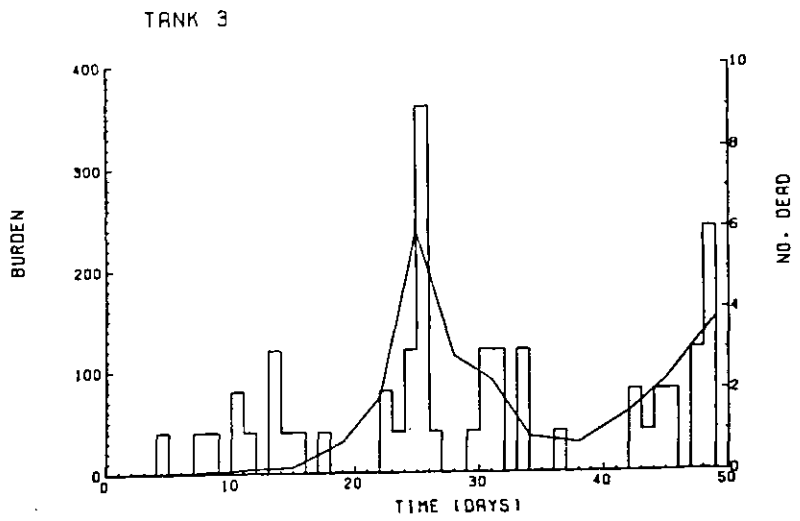
The infections in Tanks 1, 26, 15 and 23 (first page) were commenced with a large initial dose of tomites (36,000) and tanks 10, 3, 16 and 13 (second page) were subjected to a smaller initial dose (1800). In each of the eight graphs, the time in days since commencement of the experiment is shown on the horizontal axis. The number of trophozoites per 0.16 $cm^2$ of host surface on fish alive at the time of counting is scaled on the axis to the left of the figure and represented by a solid line. Number of hosts dying each day is represented by the histogram bars, and scaled on the axis to the right of the figure. The replicate in tank 15 was terminated 34 days after commencement of the experiment after a fungal infection killed all the fish in the tank.

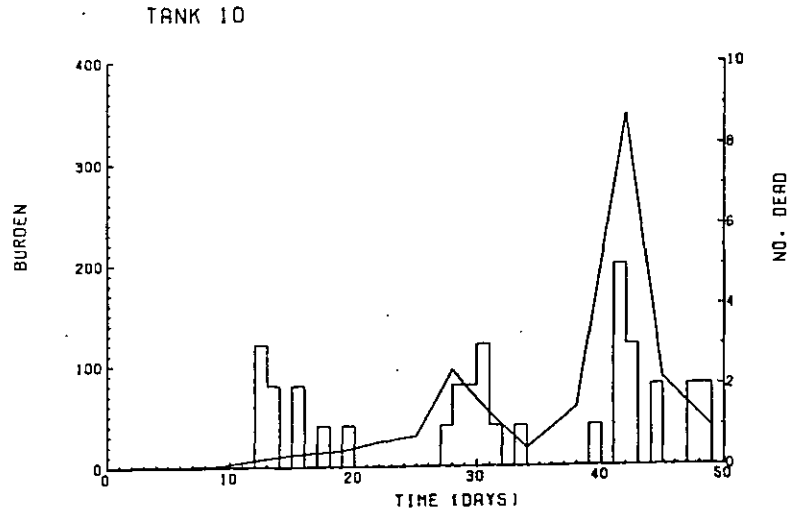

TANK 1
BURDEN
NO. DEAD
TIME (DAYS)
0
100
200
300
400
0
2
4
6
8
10
0
10
20
30
40
50

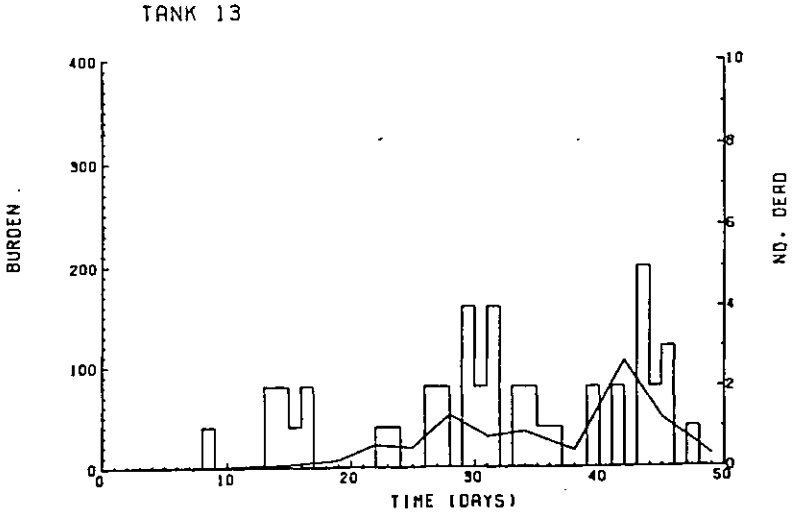

TANK 15
BURDEN
NO. DEAD
TIME (DAYS)
0
100
200
300
400
0
2
4
6
8
10
0
10
20
30
40
50

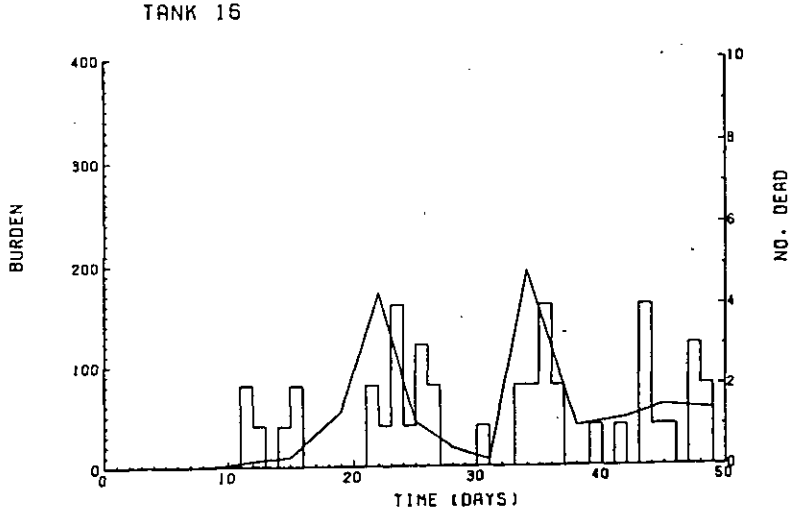

TANK 23
BURDEN
NO. DEAD
TIME (DAYS)
0
100
200
300
400
0
2
4
6
8
10
0
10
20
30
40
50


TANK 26
BURDEN
NO. DEAD
TIME (DAYS)
0
100
200
300
400
0
2
4
6
8
10
0
10
20
30
40
50


TANK 3
BURDEN
NO. DEAD
TIME (DAYS)
0
100
200
300
400
2
4
6
8
10
20
30
40
50


TANK 10
BURDEN
NO. DEAD
TIME (DAYS)
0
100
200
300
400
2
4
6
8
10
20
30
40
50


TANK 13
BURDEN
NO. DEAD
TIME (DAYS)
0
100
200
300
400
2
4
6
8
10
20
30
40
50


TANK 16
BURDEN
NO. DEAD
TIME (DAYS)
0
100
200
300
400
2
4
6
8
10
20
30
40
50

parasite burden. These peaks are not merely a reflection of the age structure of the parasite population, as were the peaks observed in the results of the previous section, since the interval between them is considerably more than the eight day generation length of the parasite. The eight replicates are not sufficiently consistent to determine whether or not these results represent convergent oscillations, diverging oscillations or stable limit cycles. There is no consistent trend toward either increase or decrease in magnitude of the peaks. The differing initial conditions do not qualitatively effect the system behaviour: the first peak in parasite burden merely takes longer to occur.

Total parasite burdens established in this experiment appear to be very much higher than those described in the previous section: the highest mean parasite burden established is in the region of 30,000 per host. Unfortunately, parasite burdens between the two series of experiments cannot be quantitatively compared, due to differences in the counting methods used. Only trophozoites older than four days were counted in the immigration-death experiments, and as can be seen in Chapter 7, hosts with infections approaching 30,000 would be most unlikely to survive long enough for the burden to be counted. Each counting method has its particular advantages. Complete census of all trophozoites older than four days is more accurate when sampling at low infection levels, and enables conclusions about distribution of parasites on hosts to be drawn. It is difficult to obtain a reliable estimate of distribution of parasites amongst fish from sample densities at four points on the host, but this method yields an estimate of the level of total parasite population, regardless of age, and is presumably more accurate than an attempted census at extremely high

parasite densities. Qualitatively, peak parasite burdens established in the constant host density experiment did appear to be considerably greater than those established in any of the constant immigration experiments.

These results are discussed more fully in Chapter 14.

# CHAPTER 10

## THE BASIC MODEL

### 10.1 *Aims of Modelling*

Ecological models can broadly be divided into two categories: "strategic" and "tactical" models (Holling, 1968). The essential feature of the strategic approach is simplicity. The intention is to include as little detail as possible, describing only the "bare bones" of an interaction. Results of the model can then be applied as generally as is possible, and insights can be gained into the effects of particular factors on the behaviour of the system under examination. The approach is exemplified by Ross (1916) who developed models with the object of identifying the factors that may lead to certain diseases displaying epidemic behaviour, and to others persisting endemically at approximately constant levels. An inescapable result of the simplicity of these models is that they are unlikely to provide accurate quantitative predictions about the behaviour of particular populations or interactions between populations (in some cases, however, predictions are remarkably accurate. See for example, Anderson and May 1982a). Tactical models, on the other hand, aim to include as much detail as possible about the system under examination, with the object of making accurate forecasts about the behaviour of the system under particular circumstances. A corollary of the inclusion of this detail is that the applicability of the model is limited, and it is often difficult to identify which factors are responsible for the behaviour observed. When digital computers first became available, there was a hope that their numerical powers might

enable the development of detailed simulation models that would revolutionize ecological thought (Holling, op cit). This dream does not seem to have been realized. The conclusion that the aims of generality and precision are, by their nature, incompatible has been reached by a number of authors (Bailey, 1967; May, 1974; Maynard Smith, 1974).

The models discussed in this and following chapters are of the strategic type, although based on the behaviour of a particular parasite. A number of the assumptions made in their construction involve what are, in the light of the previous chapters, considerable simplications of the actual biology of *Ichthyophthirius*. The models aim to examine the effect on host parasite interactions of particular features of the biology of *Ichthyophthirius* such as acquired immunity or parasite survival of host death. These are introduced one at a time so that the influence they have on the population dynamics of the system can be ascertained. Implicit in this approach is the assumption that a first approximation to the behaviour of a complex system can be gained from the behaviour of the sum of its parts. Whilst this may, in general, be an oversimplification (Holling op cit), it provides a valuable first approach (May, 1974).

The models described in this thesis are unlikely to be able to quantitively mimic the results of even controlled laboratory epidemics, but should be capable of reproducing the qualitative behaviour observed under experimental conditions. The function of this type of model is to identify important components of the population biology of the disease, and to indicate areas in which experimental investigation would prove valuable (Bradley, 1982).

## 10.2 *A brief history of the modelling of disease and parasitism*

Bernoulli (1760) was probably the first to use mathematical models to assist in the understanding of epidemics, developing a simple differential equation to describe the progress of smallpox epidemics. Although his model is remarkably similar to more recent epidemic models, further progress was restricted because of the limited understanding of the biology of infectious diseases at that time.

Modern epidemic theory is based on work in the early part of the twentieth century by Hamer (1906) and Ross. The theory of "happenings" developed by Ross (1916), in which a population is divided into affected and unaffected parts, and infection occurs at a rate proportional to the product of the number of infected and uninfected individuals, is basic to many subsequent epidemiological models. The most important result Ross obtained is that epidemics may die out due to the depletion of the susceptible host population, without necessarily involving any decrease in infectivity of the disease itself, which had been thought necessary by some previous workers (e.g. Brownlee, cited in Ross, 1916).

It was left to Kermack and McKendrick (1927) to develop one of the most important results in epidemiology. Their threshold theorem states that, for a given disease, there exists a minimum density of hosts that must be present in order for an epidemic to occur, and that in general, the epidemic ceases before all the susceptible hosts have become infected. This result is central, in a variety of guises, to the discussion in the remainder of this thesis. Kermack and McKendrick divide the host population into susceptible, infected and

immune categories and assume that infection occurs at an overall rate proportional to the product of the number of infected and susceptible hosts. In their 1927 paper, the host population is assumed to be approximately constant, but in later work (Kermack and McKendrick 1932, 1933) immigration and birth are included, and the stability of equilibrium solutions is considered.

A literature of considerable size has been built up based on these "compartmental" models. It is reviewed in detail by Bailey (1975). Many modifications of these models involve introducing stochastic effects (e.g. Bartlett, 1966) and are mathematically very sophisticated. An assumption common to most developments, however, is that the total host population can be considered to be constant.

Anderson and May (1979) have recently relaxed this assumption, allowing the host population to be a dynamic variable affected by the course of the epidemic. The construction and properties of these models are described in Appendix 4.

Such compartmental models, which divide the host population into susceptible, infected and immune classes, are most applicable when considering the population dynamics of microparasitic diseases, which are able to reproduce within their hosts. It is implicitly assumed that, after infection, the number of disease organisms within each host increases rapidly to a level determined by the host responses, (whether non specific or immunological in nature) rather than by the size of the infective dose. All hosts harbouring the disease are assumed to be equally infected. This approach is unlikely to be successful in describing the dynamics of parasites such as *Ichthyophthirius*

which are incapable of reproduction within the host. In these cases, the parasite burden is a function of the number of infective stages encountered and the burden determines both the death rate of the host and its infectivity to further hosts. It is therefore necessary to explicitly consider the dynamics of the parasite population.

Kostitzin (1934) derived a general model in which there were separate differential equations for the number of hosts harbouring different numbers of parasites, but was able to obtain analytical results only in very restricted cases. He noted "This system is not linear, and its solution presents almost insurmountable difficulties". Compared with the degree of attention given to predator prey dynamics, host parasite interactions were subsequently rather neglected by theoretical ecologists. Pielou (1969) considered the host parasite interaction to be mathematically equivalent to predator prey interactions, and that it could therefore be described by the Lotka Volterra equations:

$$dH/dt = (a_1 - b_1 P)H \qquad (10.1)$$

$$dP/dt = (-a_2 + b_2 H)P \qquad (10.2)$$

where $H$ is the number of hosts and $P$ is the number of parasites, and $a_1$, $a_2$, $b_1$ and $b_2$ are constants. It is difficult, however, to ascribe biological interpretations to the constants, as the model is not derived from parasitological principles. (It turns out that eqns. (10.1) and (10.2) are equivalent to Anderson and May's (1978) basic model, if $P$ denotes the mean parasite burden per host, and only a very small

proportion of infective stages succeed in locating hosts).

Recent examination of the host-parasite interaction has emphasized the crucial role played in the stability and regulation of parasite and host numbers by the nature of the distribution of parasites within the host population. Bradley (1972) argued that an aggregated distribution of parasites on hosts implies that a relatively small proportion of hosts harbours a large proportion of the parasite population. As the parasite population size increases, the few heavily infected hosts die, restricting the host and disease populations without massive host mortality that may lead to an equilibrium being overshot. An important advance was made by Crofton (1971b), who described a difference equation model in which it was assumed that parasites were distributed according to a negative binomial distribution amongst the host population. Imposing this distribution *a priori* provides a means of circumventing some of the insurmountable difficulties referred to by Kostitzin. Unfortunately, Crofton did not explicitly write down the equations upon which his model is based, although they have subsequently been deduced by May (1977). A feature of this model is the extremely complicated shape of the zone of attraction to its equilibrium (May, 1979). This may be the result of an unrealistic assumption made concerning the transmission of parasites which, in certain conditions, allows more parasites to become established than there are infective stages!

Anderson and May (1978) have developed a firmly biologically based model, framed as differential equations, which utilizes Crofton's assumption of a negative binomial distribution of parasites on hosts. This model forms the basis of the theoretical work in this thesis, and

a modified version is described in the following section.

## 10.3 *A Basic Model to Describe the Population Dynamics of Ichthyophthirius multifiliis*

The following model is a slightly modified version of one described by Anderson and May (1978). Its formulation and dynamical properties are described in some detail, as it forms the basis for a series of extensions, aimed at adding further biological detail, which are described in the following sections.

Hosts are assumed to reproduce, in the absence of infection, at a per capita rate, $a$, per unit of time, and to die from the effects of all factors other than parasitism at a constant per capita rate, $b$. The death rate of an infected host is taken to be augmented by an amount $\alpha i$, where $i$ is the parasite burden of the host. The total rate of loss of infected hosts due to parasitism is therefore:

$$\alpha H \sum_{i=0}^{\infty} i p(i) \qquad (10.3)$$

where $H$ is the size of the host population, and $p(i)$ the probability that a host harbours $i$ trophozoites. These assumptions lead to the following equation to describe changes in host population size.

$$dH/dt = (a-b)H - \alpha H \sum_{i=0}^{\infty} i p(i) \qquad (10.4)$$

The number of individuals of the three main developmental stages of *Ichthyophthirius* at time $t$ will be denoted by the variables $P(t)$ representing trophozoites on the host, $C(t)$ representing the encysting stage, and $T(t)$ representing the free living infective tomites. The

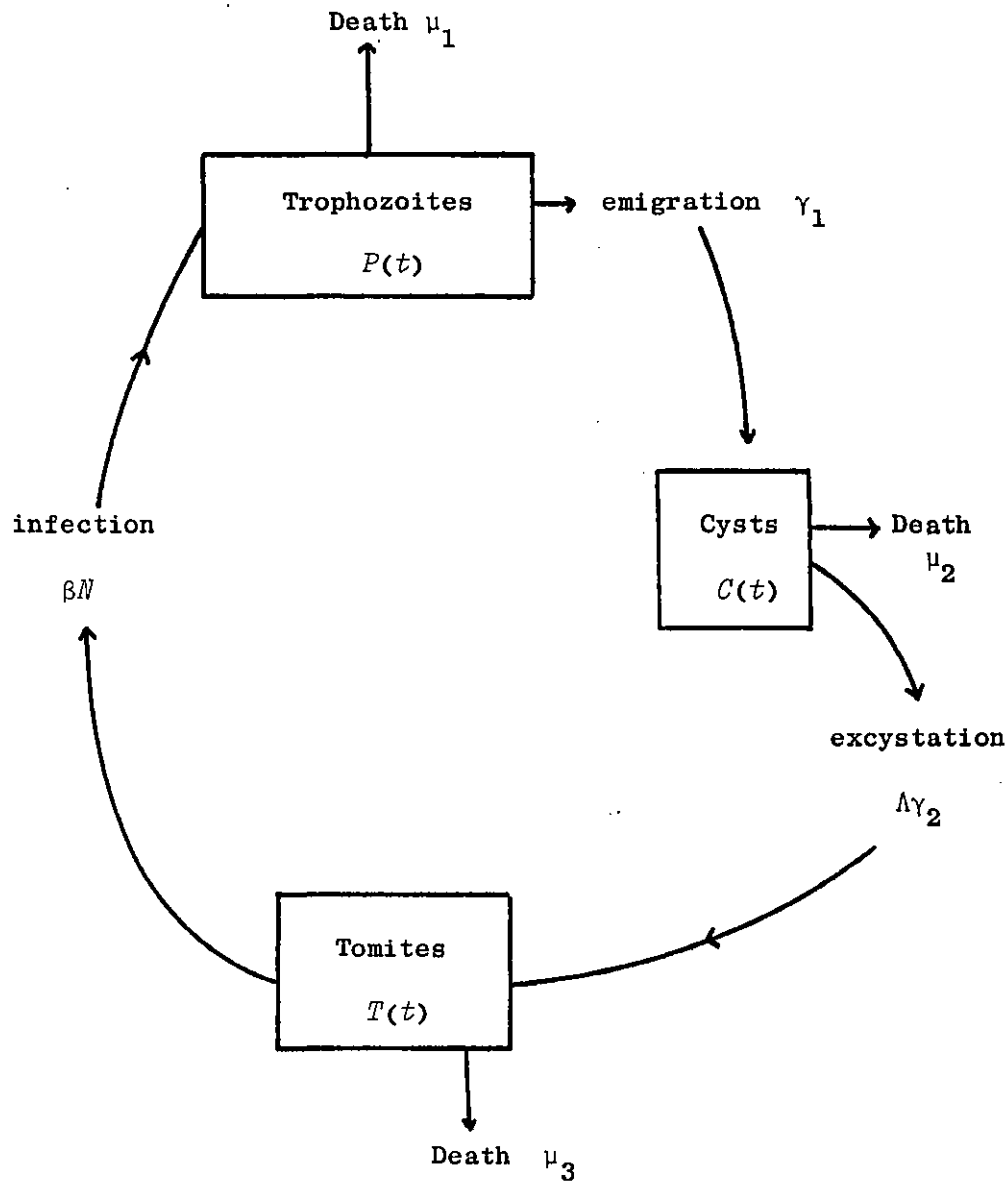
life cycle is shown schematically in Fig. 10.1. Age structure of these life history stages is not considered in this basic model. The number of trophozoites leaving the host in a small time interval, $\Delta t$ is assumed to be simply $\gamma_1 P(t)\Delta t$ where $\gamma_1$ is constant and is the reciprocal of the mean time spent on the host.. Similar assumptions are made concerning the rate at which cysts rupture to release tomites, which is expressed as $\gamma_2 C(t)$. Exactly $\Lambda$ tomites are assumed to be produced per rupturing cyst. Cysts and tomites are assumed to have constant death rates of $\mu_2$ and $\mu_3$ respectively. Trophozoite mortality has two components, death of trophozoites on hosts at a per capita rate $\mu_1$ and mortality due to the death of the host. Initially, it is assumed that no parasite survives the death of its host. Under the above assumptions, the net loss of trophozoites due to host mortality is:

$$H \sum_{i=0}^{\infty} \left[ i(b+\alpha i)\ p(i) \right] = HbE(i) + \alpha H E(i^2) \qquad (10.5)$$

where $E(i)$ and $E(i^2)$ are the expected values of $i$ and $i^2$. $E(i)$ is simply the mean parasite burden, $P/H$, but evaluation of $E(i^2)$ requires some knowledge of the nature of the distribution of the parasites within the host population. Following Anderson and May (1978) it is assumed that this distribution is negative binomial in form, and that the parameter, $k$, of this distribution, which varies inversely with the degree of parasite overdispersion, is constant. In this case,

$$E(i^2) = P/H + \left[(k+1)/k\right](P/H)^2 \qquad (10.6)$$

**Figure 10.1** *Schematic life cycle of Ichthyophthirius*


Death $\mu_1$
Trophozoites
$P(t)$
emigration $\gamma_1$
Cysts
$C(t)$
Death
$\mu_2$
excystation
$\Lambda\gamma_2$
Tomites
$T(t)$
Death $\mu_3$
infection
$\beta N$

Infection is taken to occur as a result of the result of binary collision between infective stage and host at a rate β per tomite, per host, per unit of time.

These assumptions lead to the following four coupled differential equations to describe the dynamic behaviour of $H$, $P$, $C$ and $T$ with respect to time:

$$dH/dt = (a-b)H - \alpha P \qquad (10.7)$$

$$dP/dt = \beta HT - (\gamma_1+\mu_1+\alpha+b)P - \alpha[(k+1)/k][P^2/H] \qquad (10.8)$$

$$dC/dt = \gamma_1 P - (\gamma_2+\mu_2)C \qquad (10.9)$$

$$dT/dt = \Lambda\gamma_2 C - \beta NT - \mu_3 T \qquad (10.10)$$

Table 10.1 provides a summary of the identification of the various parameter symbols.

In the system represented by the above set of equations, the intrinsic rate of increase of the host population $(a-b)$ is small compared with the rate of parasite population change (at 20°C, the total generation time of *Ichthyophthirius* is around one week). If the parasite population size is small, so that the rate of parasite induced host death is low, parasite population dynamics can be considered assuming that the host population level is essentially constant. The cyst and tomite stages are short lived compared with the trophozoite stage (Table 2.1) and so it can be assumed, as a first

Table 10.1 *Parameters of the Model Defined by eqns. (10.7) - (10.8)*

| Parameter | Biological interpretation | units |
|---|---|---|
| $a$ | birth rate of hosts | per host, per unit of time |
| $b$ | disease independent death rate of hosts | " " |
| $\alpha$ | increment in the rate of parasite induced mortality per trophozoite | per trophozoite, per host, per unit of time |
| $\beta$ | rate of tomite infection of hosts | per tomite, per host per unit of time |
| $\gamma_1$ | rate of trophozoite emigration from hosts | per trophozoite, per unit of time |
| $\mu_1$ | rate of trophozoite death on hosts | per trophozoite, per unit of time |
| $\gamma_2$ | rate of excystation | per cyst, per unit of time |
| $\mu_2$ | death rate of cysts | " " |
| $\Lambda$ | number of tomites produced per excystation | per cyst |
| $\mu_3$ | death rate of tomites | per tomite, per unit of time |
| $k$ | parameter of negative binomial distribution inversely describing the degree of aggregation of trophozoites on hosts | |

approximation, that the number of cysts and tomites are at levels in equilibrium with the current trophozoite population size. Setting eqns. (10.9) and (10.10) to zero and substituting into eqn. (10.8), the following condition can therefore be found for $P$ to be able to increase (at low parasite burdens, the second order term, $\alpha[(k+1)/k][P^2/H]$, can be neglected)

$$\Lambda(\gamma_1\gamma_2)H/[(\gamma_2+\mu_2)(\mu_3/\beta + H)d] > 1 \qquad (10.11)$$

Here, $d$ is defined for notational convenience as

$$d = \alpha + b + \gamma_1 + \mu_1 \qquad (10.12)$$

The dimensionless quantity

$$R_o = \Lambda(\gamma_1\gamma_2)H/[(\gamma_2 + \mu_2)\ (\mu_3/\beta + H)d] \qquad (10.13)$$

is the basic reproductive rate of the parasite (MacDonald, 1965; Anderson and May 1981) and can be thought of in biological terms as the mean number of trophozoites in the next generation established from each trophozoite in the current generation. It can readily be seen that in order for $R_o > 1$, and for the parasite to be able to persist in the host population, the host density must exceed a critical level, $H_T$, where

$$H_T = (\mu_3/\beta) \ / \ \left[\Lambda\gamma_1\gamma_2/[(\gamma_2+\mu_2)d] \ \ -1\right] \qquad (10.14)$$

This is the threshold host population necessary for disease persistence

(Kermack and McKendrick, 1927). If $\Lambda\gamma_1\gamma_2/[(\gamma_2+\mu_2)d] < 1$, no host density exists great enough to enable disease persistence. For notational convenience it is useful to define another dimensionless quantity

$$\hat{R} = \Lambda\ \gamma_1\gamma_2/[(\gamma_2+\mu_2)\ d] \qquad (10.15)$$

$\hat{R}$ is simply the level to which $R_o$ asymtotes at very high host densities and, in biological terms, is the mean number of infective stages produced per trophozoite establishing on a host.

Returning to the case where $H$ is a dynamic variable, an equilibrium may be found by setting eqns. (10.7) to (10.10) to zero. There is a trivial equilibrium with $H$ and $P = 0$, which is unstable, and there may be an equilibrium with both parasites and hosts present with a host population, $H^*$, where:

$$H^* = (\mu_3/\beta)/\left[\hat{R}/[1+(k+1)(a-b)/(kd)]-1\right] \qquad (10.16)$$

and an equilibrium parasite population, $P^*$, where:

$$P^* = H^*(a-b)/\alpha \qquad (10.17)$$

Clearly

$$\hat{R} > 1 + (k+1)(a-b)/(kd) \qquad (10.18)$$

for a non trivial equilibrium to exist. If $\hat{R} < 1$, the parasite is unable to persist and will decline in numbers to extinction whilst host numbers increase exponentially at a rate $r = a-b$. In the region

$1 < \hat{R} < 1 + (k+1)(a-b)/(kd)$, the parasite is able to persist in the host population, but is unable to regulate its growth: both parasite and host populations increase exponentially. In the case of *Ichthyophthirius* and probably most host parasite systems, the time scale on which parasite populations change is very much shorter than that on which host populations change (i.e. $d >> a-b$).

This region is therefore likely to be small unless the parasites are very highly aggregated in their distribution within the host population ($k \to 0$). Figure 10.2 shows the region of the $\Lambda$ - $k$ parameter space within which this type of behaviour occurs.

Consider now the case in which eqn. (10.18) is satisfied, and an equilibrium with both hosts and parasites present does exist. The local stability of the equilibrium may be determined by considering the eigen values of the community matrix (May, 1974), but this approach is algebraically cumbersome and biological interpretation of the results is difficult when the model consists of four equations. Some progress can be made by assuming that the tomite stage operates on a time scale that is short in comparison with the other life history stages (i.e. $\mu_3 >> \mu_1$; $\mu_3 >> \mu_2$). The tomite population is essentially always in equilibrium with the current numbers of cysts and hosts at a level

$$T^* = (\Lambda\gamma_2 C)/(\mu_3+\beta H) \qquad (10.19)$$

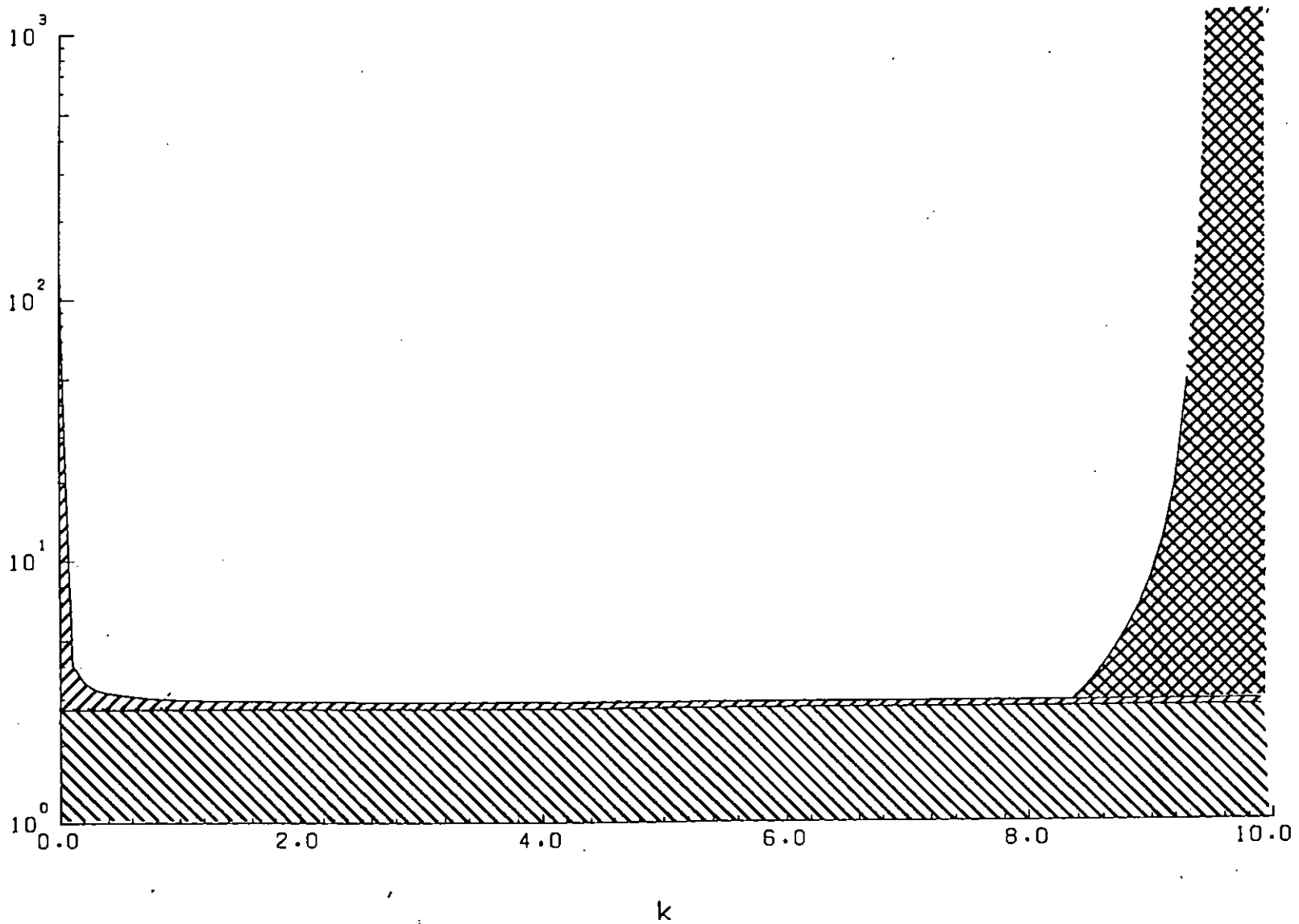
obtained by setting eqn. (10.10) to zero. Equation (10.8) then becomes

$$dP/dt = \lambda\gamma_2 HC/(H + \mu_3/\beta) - dP - \alpha P^2(k+1)/(kH) \qquad (10.20)$$

**Figure 10.2.** *The dynamical behaviour of eqns. (10.7), (10.9) and (10.20)*

The figure shows a portion of the $\Lambda$ - $k$ parameter space of the model defined by eqns. (10.7), (10.9) and (10.20). In the unshaded region, the system possesses a stable equilibrium. In the cross hatched ( XXX ) region to the right of the graph, equilibrium solutions exist, but they are unstable, and the system follows diverging oscillations. The parasite is able to persist in the host population, but is unable to regulate it in the narrow region with right sloping hatching ( /// ). Below this region, the reproductive potential of the parasite is insufficient to allow it to persist in the host population. This part of the $\Lambda$-$k$ parameter space is shown by leftward sloping hatching ( \\\ ). Parameter values used are:

$a = 0.02$, $b = 0.01$, $\alpha = 0.001$, $\gamma_1 = 0.167$, $\mu_1 = 0.05$, $\mu_2 = 1$, $\gamma_2 = 1$


$10^3$
$10^2$
$10^1$
$10^0$
Λ
0.0
2.0
4.0
6.0
8.0
10.0
k

The quantity $\mu_3/\beta$, the death rate of the tomites divided by their infectivity, is an inverse measure of the transmission efficiency of the disease, and it is convenient to define:

$$H_o = \mu_3/\beta \qquad (10.21)$$

At a given host density, $H$, a proportion $H/(H + H_o)$ of tomites are able to locate a host before death. The quantity $H_o$ is discussed in some detail in Section 5.4. In particular, it should be recalled that $\beta$ and hence $H_o$ depend not only on the biology of the host and parasite, but also on the size of the water body containing the populations.

The non trivial equilibrium of the system described by equations (10.7), (10.20) and (10.9) is shown in Appendix 5 to be stable, provided:

$$[\gamma_2 + \mu_2 + (a-b)/k + df]\,[\hat{R}/(kf) - df/(\mu_2+\gamma_2)] > df[\hat{R}/f-1] \qquad (10.22)$$

where $$f = 1 + (k+1)(a-b)/(kd) \qquad (10.23)$$

The right hand side of eqn. (10.22) is positive if the equilibrium exists (see eqn. 10.18). The left hand side will be negative, and the equilibrium therefore unstable, if the distribution of parasites within the host population approaches a random (Poisson) pattern ($k$ large) or if the cyst stage is sufficiently long lived ($\gamma_2+\mu_2$ small). The cyst stage of *Ichthyophthirius* is relatively short lived compared with the time spent on the host by the trophozoite or the lifespan of the host, and the first term on the left hand side of eqn. (10.22) will therefore approximate $\gamma_2+\mu_2$, except for extreme degrees of overdispersion ($k$ small). Hence, eqn. (10.22) is approximately:

$$d/[(\gamma_2+\mu_2)f] \ll 1/k \qquad (10.24)$$

The regions of the $\Lambda$ - $k$ parameter space in which the equilibrium is stable or unstable are shown in Fig. 10.2.

May and Anderson (1978) considered the effect on the stability of a host parasite system of long lived infective stages, in contrast to the system described above, in which there is an incubation period before short lived infective stages are produced. The precise condition for stability is different, but if it is assumed that the life span of the infective stages is short compared with that of the parasites on the host, and that $\hat{R}$ is considerably greater than 1, a very similar condition to eqn. (10.24):

$$d/\mu_3 < 1/k \qquad (10.25)$$

is obtained. If a time delay of exactly $\tau$ days occurs in transmission (i.e., the cyst stage requires exactly $\tau$ days) the condition for stability becomes

$$d\,\tau \lesssim 1/k \qquad (10.26)$$

(May and Anderson, 1978).

The conclusion that the system is unstable if the ratio of time delay in transmission to time spent on the host exceeds $1/k$ appears to be robust and not dependent on the precise details of the model formulation. It should be noted, however, that if the condition defined in eqn. (10.25) is violated in a system with long lived infective stages, stable limit cycles ensue, whereas violation of eqn. (10.22)

results in the system described above oscillating in a divergent manner (Fig. 10.3).

Given that the time spent in the cyst stage and the lifespan of the tomites of *Ichthyophthirius* are both short compared with the time spent on hosts by trophozoites (Table 2.1), and that most parasites are overdispersed on hosts, typically with $k < 1$ (Anderson and May, 1978), time delays are unlikely to cause instability in the *Ichthyophthirius* system.

As a basic model of *Ichthyophthirius* dynamics, one may therefore use a two equation system obtained by "collapsing" eqns. (10.9) and (10.10) into eqn. (10.8)

$$dH/dt = (a-b)H - \alpha P \qquad (10.27)$$

$$dP/dt = \lambda\gamma HP/(H_o+H) - (\gamma+\mu+\alpha+b)P - \alpha(k+1)P^2/(kH) \qquad (10.28)$$

with parameters as defined in Table 10.2.

By analogy with the four equation model (equations 10.7 - 1010) one may define:

$$d = \gamma+\mu+\alpha+b \; ; \qquad (10.29)$$

the basic reproductive rate

$$R_o = \lambda\gamma H/\left[(H_o+H)d\right]; \qquad (10.30)$$

Table 10.2 *Parameters of the Model Defined by Eqns (10.27) - (10.28)*

| Parameter | Biological interpretation | units |
|---|---|---|
| $a$ | birth rate of hosts | per host, per unit of time |
| $b$ | disease independent death rate of hosts | " " |
| $\alpha$ | increment in the rate of parasite induced mortality per trophozoite | per trophozoite, per host, per unit of time |
| $\lambda$ | number of tomites produced per trophozoite leaving the host | per trophozoite |
| $\gamma$ | rate of trophozoite emigration from hosts | per trophozoite, per unit of time. |
| $\mu$ | death rate of trophozoites on hosts | per trophozoite per unit of time |
| $H_o$ | saturation term in transmission | dimensionless |
| $k$ | parameter of negative binomial distribution | dimensionless |

and $\hat{R} = \lambda\gamma/d$ (10.31)

The threshold host population for disease maintenance is simply

$$H_T = H_o/[\hat{R}-1] . \quad (10.32)$$

Once again, there is a trivial equilibrium with $H = P = 0$, and an equilibrium with hosts and parasites present, where:

$$H^* = H_o/ \left[\hat{R}/[1+(k+1)(a-b)/(kd)] -1 \right] \quad (10.33)$$

and $P^* = H^*(a-b)/\alpha$ ; (10.34)

provided

$$\hat{R}[1 + (k+1)(a-b)/(kd)] > 1 \quad (10.35)$$

which will be stable if $k$ is finite and positive. The approach to this equilibrium is oscillatory (Fig. 10.4).

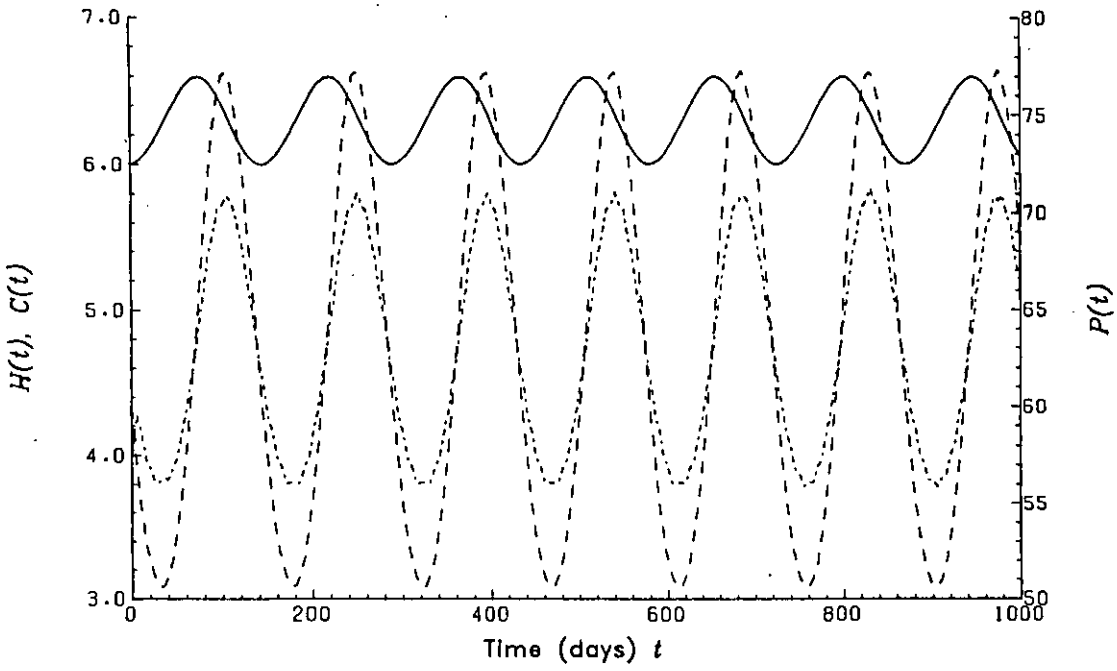
A counter intuitive result of this basic model is that the conditions for the existence and stability of the equilibria do not involve the transmission efficiency of the parasite ($H_o$). This is because of the unrealistic assumption made of unlimited host population growth in the absence of parasitism. Provided the relation (10.35) holds, whatever the transmission efficiency (the value of $H_o$) host density will increase until transmission is sufficient for parasite maintenance (as $H \to \infty$, $H/(H_o+H) \to 1$). If host density is in any way restricted, independent of parasitism, $H_o$ assumes a crucial

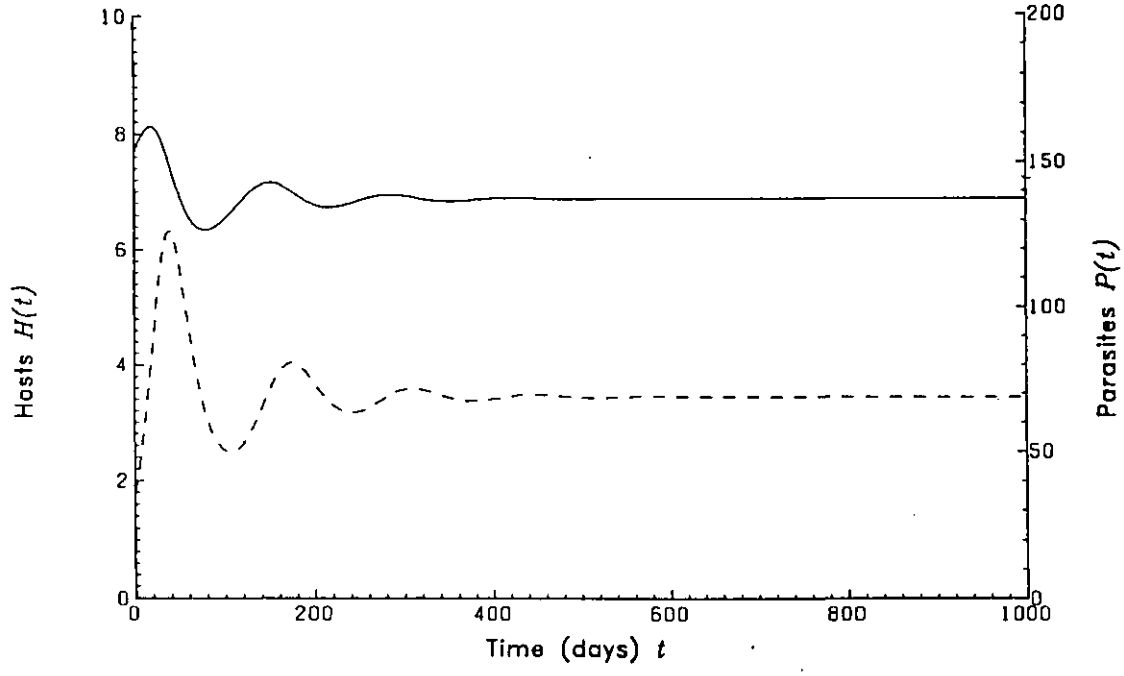
Figure 10.3 *An unstable solution of the basic model*

A solution is shown of the model defined by eqns. (10.7), (10.9) and (10.20), with condition (10.22) not satisfied. The solid line represents the number of hosts $H(t)$ and is scaled according to the axis to the left of the figure. The trophozoite population $P(t)$, is shown as an evenly dashed line, and scaled according to the axis to the right of the figure. The cyst population, $C(t)$, is shown as an unevenly dashed line, and scaled on the axis to the left of the figure. Parameter values are those used in Fig. 10.2, with $\Lambda$ =50, $k$ = 10 and $H_o$ = 100. Note that the divergence of the oscillations is extremely slow if the cyst stage is short ($\mu_2$ and $\gamma_2$ large). This figure represents nearly neutral oscillations and does not show a stable limit cycle.

Figure 10.4 *A numerical solution of eqns. (10.27) and (10.28)*

A time dependent solution is shown of the model defined by eqns. (10.27) and (10.28). The number of hosts, $H(t)$, is shown as a solid line, and is scaled according to the axis to the left of the figure. The number of parasites $P(t)$, is shown as a dashed line, and is scaled on the axis to the right of the figure. Parameter values used are: $a$ = 0.02, $b$ = 0.01, $\alpha$ = 0.001, $\gamma$ = 0.15, $\mu$ = 0.05, $\lambda$ = 25.0, $H_o$ = 100. , $k$ = 0.5.


7.0
6.0
5.0
4.0
3.0
80
75
70
65
60
55
50
0
200
400
600
800
1000
H(t), C(t)
P(t)
Time (days) t


10
8
6
4
2
0
200
150
100
50
0
0
200
400
600
800
1000
Hosts H(t)
Parasites P(t)
Time (days) t

role in determining the conditions for parasite persistence. This will be discussed more fully in the following section. Another surprizing result is the limited importance of the magnitude of the per capita pathogenicity of the parasite, $\alpha$, to the dynamics of the system, which should be contrasted with the central role the magnitude of $\alpha$ plays in the disease model (Appendix 4. ). Provided the increment in host mortality caused by a single parasite is small compared with the natural loss rate of the parasites ($\mu+\gamma >> \alpha$), the conditions for an equilibrium to exist (eqn. 10.35) and the equilibrium burden ($H^*$) are affected very little by changes in $\alpha$. Per capita parasite pathogenicity acts only as a scaling factor that determines the equilibrium mean parasite burden, $M^*$ (eqn. 10.34). The biological explanation of this is straightforward. The only density dependent constraint on mean parasite burden occurs through the pathogenicity itself. Mean parasite burden, $M$, is thus able to increase until host mortality sufficient to regulate the system occurs. It is the effect of the product $\alpha M$ on the host population which is of importance, rather than $\alpha$, the effect of each individual parasite.

## 10.4 *Constant host immigration*

The model described by eqns. (10.27) and (10.28) can be modified to allow for modes of host population growth other than exponential increase. If hosts are introduced at a constant rate $A$ per unit time, and suffer a constant disease independent per capita mortality of $b$, eqn. (10.27) becomes:

$$dH/dt = A - bH - \alpha P \qquad (10.36)$$

Equation (10.28) remains unchanged. In the absence of parasitism, there

is an equilibrium at

$$H^* = A/b \qquad (10.37)$$

at which point the constant death rate, $b$, balances the immigration rate $A$. The threshold host population level, $H_T$, below which parasites cannot persist remains unchanged from the basic model (eqn. 10.32) and hence the disease is only able to invade a host population if

$$A/b > H_o/[\hat{R} - 1] \qquad (10.38)$$

Note the crucial role played in this case by the transmission efficiency, $H_o$. If eqn. (10.38) is satisfied, a stable equilibrium with host numbers, $H^*$, less than $A/b$ and parasites present exists. This point is shown graphically in the phase plane (Maynard Smith, 1974) in Fig. 10.5 (See appendix 6 for equations for $H^*$ and $P^*$ and stability analysis).

## 10.5 *Logistic Host Population Growth*

A closer approximation to the behaviour of real populations may be obtained by assuming that the host population increases in a logistic fashion in the absence of parasitism. Host population growth is assumed to be subject to density dependent constraints other than parasitism, so that the per capita host death rate is $(b + \kappa H)$ rather than $b$ as in eqns (10.27) and (10.28). The constant $\kappa$ determines the degree of density dependence in host population growth. The following equations are obtained to describe the dynamics of the system:

$$dH/dt = (a-b)H - \kappa H^2 - \alpha P \qquad (10.39)$$

$$dP/dt = \lambda\gamma HP/[H_o+H] - (\alpha+b+\mu+\gamma)P - \kappa HP - \alpha(k+1)P^2/(kH) \quad (10.40)$$

In the absence of infection, there is a stable host equilibrium of

$$H^* = (a-b)/\kappa \quad (10.41)$$

Determination of the conditions under which an equilibrium with both hosts and parasites present may exist is facilitated by expressing eqns.(10.39)and(10.40)in terms of the mean parasite burden $M = P/H$. Thus:

$$dH/dt = H[a-b - \kappa H - \alpha M] \quad (10.42)$$

$$dM/dt = M[\lambda\gamma H/(H_o+H) - (\alpha+\gamma+\mu+a) - \alpha M/k] \quad (10.43)$$

Equation (10.43) is positive in value for small $M$ provided

$$H > H_o/[\lambda\gamma/(\alpha+\gamma+\mu+a) - 1] \quad (10.44)$$

It is important to note that this threshold condition is not the same as that for $dP/dt$ to be positive: there may be a small range of $H$ values within which total parasite numbers, $P$, can increase, but at a slower rate than host numbers, $H$, so that $dM/dt$ is negative. The condition for an equilibrium to exist with parasites present ($M^* > 0$) is obtained from eqns. (10.41) and (10.44) and is:

$$(a-b)/\kappa > H_o/[\lambda\gamma/(\alpha+a+\gamma+\mu) - 1] \quad (10.45)$$

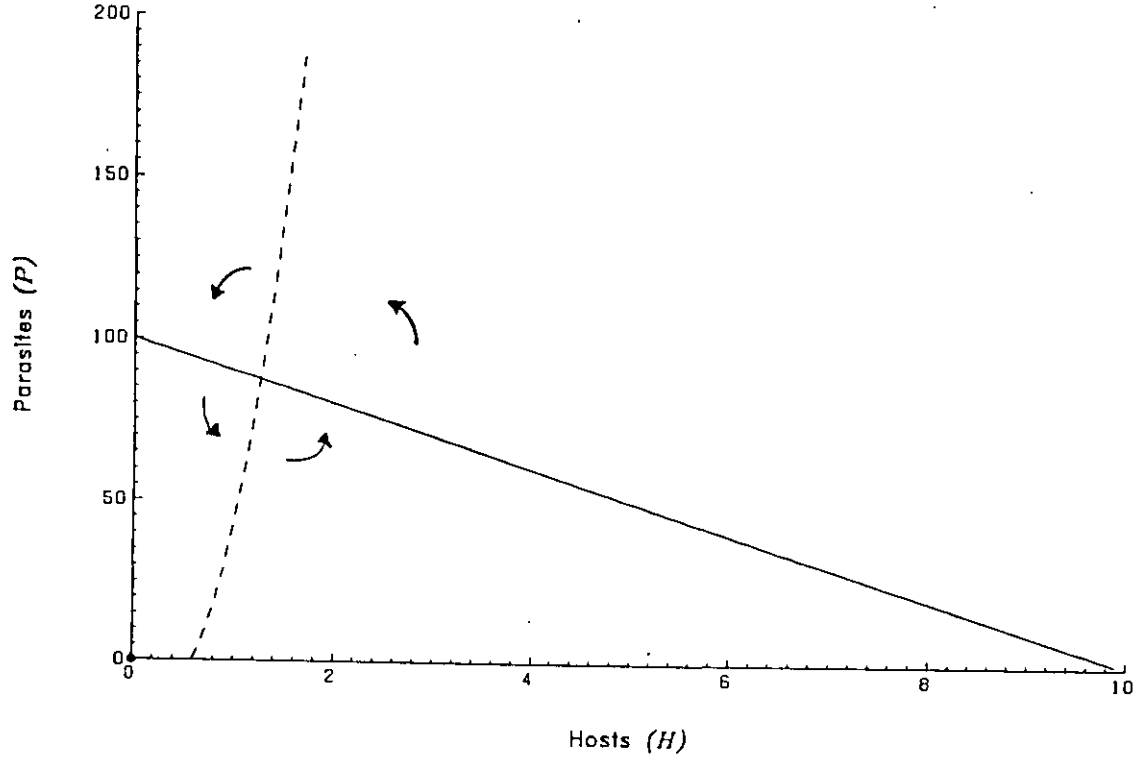
If this equilbrium exists, it can be shown to be stable (Anderson, 1979a). The system is illustrated with the aid of a phase plane in Figure 10.6.

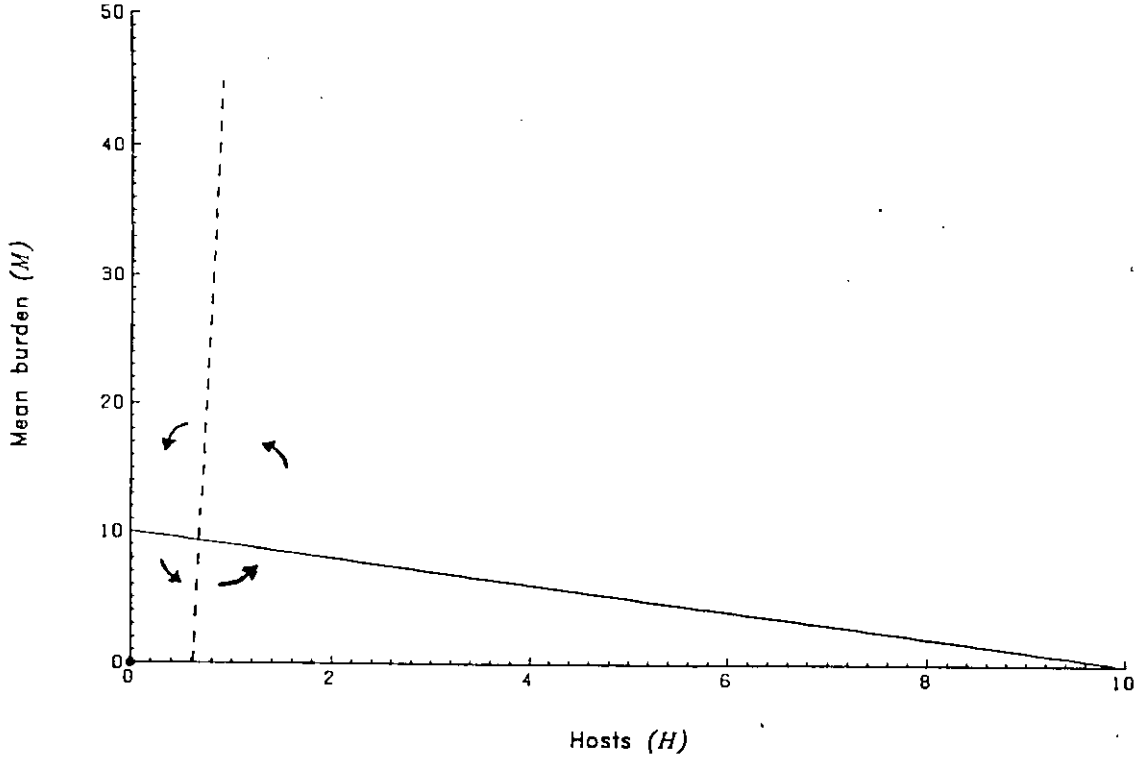
Figure 10.5 *Phase diagram analysis of eqns (10.36) and (10.28)*

Along the solid line shown, $dH/dt = 0$ (ie eqn. 10.36 = 0), and along the dashed line, $dP/dt = 0$ (ie eqn. 10.28 = 0). The equilibrium of the system is given by the intersection of the two lines. The arrows drawn show the trajectory of the solution of the system in each of the four quadrants. Parameter values used are: $A = 0.10$, $b = 0.01$, $\alpha = 0.001$, $\gamma = 0.15$, $\mu = 0.05$ $\lambda = 25.$, $k = 0.5$, $H_o = 10.$

Figure 10.6 *Phase diagram analysis of eqns. (10.39) and (10.40)*

Along the solid line shown, $dH/dt = 0$ (eqn. 10.39 = 0) and along the dashed line, $dM/dt = 0$ (eqn. 10.40 = 0). As in the figure above, the equilibrium is given by the intersection of the two lines, and the arrows show the trajectory of the solution of the system. Parameter values used are: $a = 0.02$, $b = 0.01$, $\kappa = 0.001$, $\alpha = 0.001$, $\gamma = 0.15$, $\mu = 0.05$, $\lambda = 25.$, $k = 0.5$, $H_o = 10.$


200
150
100
50
0
Parasites (P)
0
2
4
6
8
10
Hosts (H)


50
40
30
20
10
0
Mean burden (M)
0
2
4
6
8
10
Hosts (H)

# CHAPTER 11

## PARASITE SURVIVAL OF HOST DEATH

### 11.1 *Introduction*

The host-parasite models developed by Anderson and May (1978) assume that the death of a host results in the death of any parasites that it might be harbouring. They argue that this component of the death rate of parasites contributes to the regulation and stability of both host and parasite populations. As has been discussed in the previous chapters, *Ichthyophthirius* trophozoites are not necessarily killed by the death of their host: most are able to leave the fish, successfully encyst and produce infective stages, although more tomites are produced per parasite if the trophozoites are able to grow to full maturity on the host.

Although many parasites, such as cestodes, are killed by the death of their host, *Ichthyophthirius* is by no means unique in being capable of surviving the death of its host. Many microparasites, particularly those infecting insects, do not die with their host. Examples are fungi of the genus *Entomophora* (Bell, 1974) or the nuclear polyhedrosis viruses (Smith, 1976). These pathogens typically have very high reproductive potentials within their host, and are best modelled by modifications to the susceptible-infected-immune framework commonly employed in studies of viral and bacterial diseases (e.g. Bailey, 1975; Anderson and May 1980). Amongst metazoans, arthropod ectoparasites are often capable of surviving the death of their host (Marshall, 1981). Many do not reproduce on their host: or as do most ticks; larvae, nymphs and adults may utilize different host species (Savory, 1977). The deleterious effects that ectoparasites

may have on their hosts' survival and fecundity should not be underestimated (Marshall, op.cit.). Models that do not consider the size of the parasite burden of ectoparasites are unlikely to be successful in describing their dynamics. Some parasites of fish, other than *Ichthyophthirius*, are also capable of surviving the death of their host. An example is the monogean *Gyrodactylus*.

## 11.2 *A simple model of survival of host death*

The following model, whilst developed specifically to describe the dynamics of *Ichthyophthirius* populations, may also serve, with suitable modifications, to describe the dynamics of some of the parasites discussed above.

In the basic model of *Ichthyophthirius* dynamics (eqns 10.27 and 10.28), it was assumed that each trophozoite leaving the host gave rise to $\lambda$ tomites. Suppose that each trophozoite on a host which dies produces $x\lambda$ tomites, where $x$ varies between 0 and 1. This proportional survival may arise from a combination of two factors: some trophozoites dying with the host, and fewer tomites being produced because some trophozoites, forced to leave the host after its death, will be immature. In mathematical terms, assuming each trophozoite on a dying host produces $x\lambda$ tomites is equivalent to assuming that a proportion $x$ of trophozoites survive, each of which produces $\lambda$ tomites. In the model described by eqns. (10.27) and (10.28) the rate of loss of parasites due to their hosts dying is:

$$(\alpha + b)\, P + \left[\alpha(k+1)/k\right]\ \left[P^2/H\right] \qquad (11.1)$$

The rate of production of tomites from these trophozoites will therefore be:

$$\lambda x \quad [\ (\alpha+b)P \ + \ \alpha(k+1)\ P^2/\ (k\ H)] \qquad (11.2)$$

Following the lines of model construction outlined in Chapter 10, the following three differential equations may be obtained to describe the interaction between host and parasite.

$$dH/dt = (a-b)H - \alpha P \qquad (11.3)$$

$$dP/dt = \beta HT - (\alpha+b+\gamma+\mu)P - \alpha(k+1)\ P^2/(kH) \qquad (11.4)$$

$$dT/dt = \lambda[\ \gamma P + x(\alpha+b)P \ + \ (k+1)\ P^2/(kH)] - \mu_2 T - -\beta HT \qquad (11.5)$$

The parameters and variables of this model are defined in Table 11.1.

Following the arguments of Chapter 10, if the life expectancy of the tomites is short, their population level may be assumed to be in equilibrium with the current host and parasite populations at:

$$T^* = \lambda P[\gamma + x(\alpha+b) + x\alpha(k+1)P/kH] \ / \ [\mu_2+\beta H] \qquad (11.6)$$

Hence,

$$\frac{dP}{dt} = \frac{\lambda PH[\gamma+x(\alpha+b) \ + \ x\alpha(k+1)P/(kH)]}{(H_o + H)} - (\alpha+b+\alpha+\mu)P - \frac{\alpha(k+1)P^2}{kH} \qquad (11.7)$$

where $H_o = \mu_2/\beta$

The analysis of the system is simplified if eqns (11.6) and (11.7) are expressed in terms of the new variables, $N = H/H_o$ and $M = P/H$ .

$$\frac{dN}{dt} = N\ (a-b - \alpha M) \qquad (11.8)$$

Table 11.1 *Variables and Parameters used in Models in Chapter 11*

| *Variable* | *Biological Interpretation* |
|---|---|
| $H$ | Number of Hosts |
| $P$ | Number of trophozoites |
| $T$ | Number of tomites |
| $M$ | Mean parasite burden ($P/H$) |
| $N$ | Rescaled host population ($H/H_o$) |
| $t$ | time |

| *Parameter* | *Biological interpretation* | *units* |
|---|---|---|
| $a$ | birth rate of hosts | per host, per unit of time |
| $b$ | disease independent death rate of hosts | per host, per unit of time |
| $\alpha$ | increment in rate of parasite induced mortality per trophozoite | per trophozoite, per host, per unit of time |
| $\lambda$ | number of tomites produced per trophozoite leaving the host | per trophozoite |
| $\gamma$ | rate of trophozoite emigration from hosts | per trophozoite per unit of time |
| $\mu$ | death rate of trophozoites on hosts | per trophozoite, per unit of time |
| $\mu_2$ | death rate of tomites | per tomite, per unit of time |
| $\beta$ | rate of tomite infection hosts | per tomite, per host per unit of time |
| $H_o$ | saturation term in transmission ($\mu_2/\beta$) | |
| $k$ | parameter of negative binomial distribution | |
| $A$ | rate of introduction of hosts | per unit of time |
| $\kappa$ | parameter determining the degree of density dependent constraint on host population growth | (per host)$^2$, per unit of time |
| $x$ | proportion of trophozoites surviving host death | |
| $\phi$, $\omega$ | parameters of eqn. (11.21) | |

$$\frac{dM}{dt} = M\left[\frac{\lambda N[\gamma+x(\alpha+b+\alpha M(k+1)/k)]}{(N+1)} - (\gamma+a+\alpha+\mu) - \frac{\alpha M}{k}\right] \qquad (11.9)$$

The basic reproductive rate of the parasite (see Anderson, 1981) $R_o$, is obtained from eqn. (11.9), where:

$$R_o = \lambda N[\gamma+x(\alpha+b)] \;/\; [(N+1)(\gamma+\alpha+a+\mu)] \qquad (11.10)$$

As is expected, parasite survival of host death increases $R_o$ by a factor $x(\alpha+b)$ in the numerator of this expression. (In the absence of survival of host death, the basic reproductive rate, $R_o$, is simply: $R_o = \lambda N\gamma/[(N+1)(\gamma+\alpha+a+\mu)]$ ). Equations (11.8) and (11.9) will have an equilibrium with the parasite present if:

$$\lambda[\gamma+x(\alpha+b+(k+1)(a-b)/k)] > \gamma+a+\alpha+\mu+(a-b)/k \qquad (11.11)$$

The host population at equilibrium, $N^*$, is given by

$$N^* = \left[\frac{1}{\frac{\lambda[\gamma+x(\alpha+b+(k+1)(a-b)/k)]}{[\gamma+a+\alpha+\mu+(a-b)/k]} - 1}\right] \qquad (11.12)$$

and the equilibrium mean parasite burden, $M^*$, is

$$M^* = (a-b)/\alpha \qquad (11.13)$$

It is shown in Appendix 7 that this equilibrium is stable if:

$$x < \gamma/[(k+1)(\gamma+a+\alpha+\mu) - (\alpha+b)] \qquad (11.14)$$

As the life expectancy of trophozoites is short, $\gamma$ is likely to be an order of magnitude greater than the other parameters in eqn. (11.14),

and eqn. (11.14) can therefore be approximated by

$$x < 1/(k+1) \qquad (11.15)$$

It is clear from eqn. (11.14) that the equilibrium cannot be stable if the tomite production of trophozoites on dead hosts is equal to that of trophozoites leaving the host at maturity. Trophozoites must leave the host in any case to reproduce, and if host death has the effect of forcing them to leave more rapidly, without decreasing their tomite production, death of the host will actually increase the reproductive rate of the parasite. As with all the models discussed throughout this thesis, stability of the host parasite interaction is enhanced if the parasites are highly aggregated within the host population. Equation (11.15) shows that the interaction will be unstable as the distribution of parasites on hosts approaches a Poisson form. Without parasite survival of host death, the equilibrium is neutrally stable if parasites are distributed randomly on hosts (Anderson and May, 1978) and any survival whatsoever is sufficient to induce instability. At the other extreme, even as overdispersion becomes very great indeed ($k \to 0$), an equilibrium with parasites present will not be stable if $x > \gamma/(\gamma+a-b+\mu)$. Numerical studies indicate that if eqn. (11.14) is not satisfied, the system will exhibit diverging oscillations (Fig. 11.1).

Equations (11.8) and (11.9) can be easily modified so that the host population grows in a logistic manner in the absence of infection. The per capita host death rate in a population of size $N$ is assumed to be $b+\kappa N$ where $b$ is defined as before, and $\kappa$ is a parameter determining the extent of the disease independent constraints on host population

growth. In the absence of infection, the equilibrium density of hosts, $K$, is simply $K = (a-b)/\kappa$. The system is described by the following equations:

$$\left.\begin{aligned} dN/dt &= N\left[(a-b) - \kappa N - \alpha M\right] \\ \frac{dM}{dt} &= M\left[\frac{\lambda N}{(N+1)}\left[\gamma+x(b+\alpha+\kappa N) + x\alpha(k+1)M/k\right] - (\gamma+a+\alpha+\mu) - \frac{\alpha M}{K}\right] \end{aligned}\right\} \quad (11.16)$$

Full algebraic analysis of eqns. (11.16) is cumbersome. A sufficient condition for the existence of an equilibrium with parasites present can be obtained without difficulty where:

$$\lambda(\gamma+x(b+\alpha))/(\gamma+a+\alpha+\mu) > \kappa/(a-b) + 1 \quad (11.17)$$

If eqn. (11.17) is satisfied, numerical studies show that the equilibrium established with parasites present is locally unstable if $x$ is sufficiently large. In this case, in contrast to the system described by eqns. (11.8) and (11.9), the system described by eqns. (11.16) will follow stable limit cycles[1] (Fig. 11.2).

Similar behaviour is also observed if it is assumed that hosts are introduced into the system at a constant rate $A$, and that they die at a per capita rate, $b$. In this case,

$$\frac{dN}{dt} = A - bH - \alpha MN \quad (11.18)$$

---

[1] An analytical technique for detecting closed orbits in two dimensional systems exists (the Poincare - Bendixon criteria; Clark, 1976), but these and other limit cycles discussed in this thesis were detected numerically. Time dependent solutions of the equations were found to follow increasing oscillations until the limit cycle solution was reached, if commenced close to the (unstable) equilibrium values, but were found to converge towards the limit cycle if started with initial conditions sufficiently far from the equilibrium.

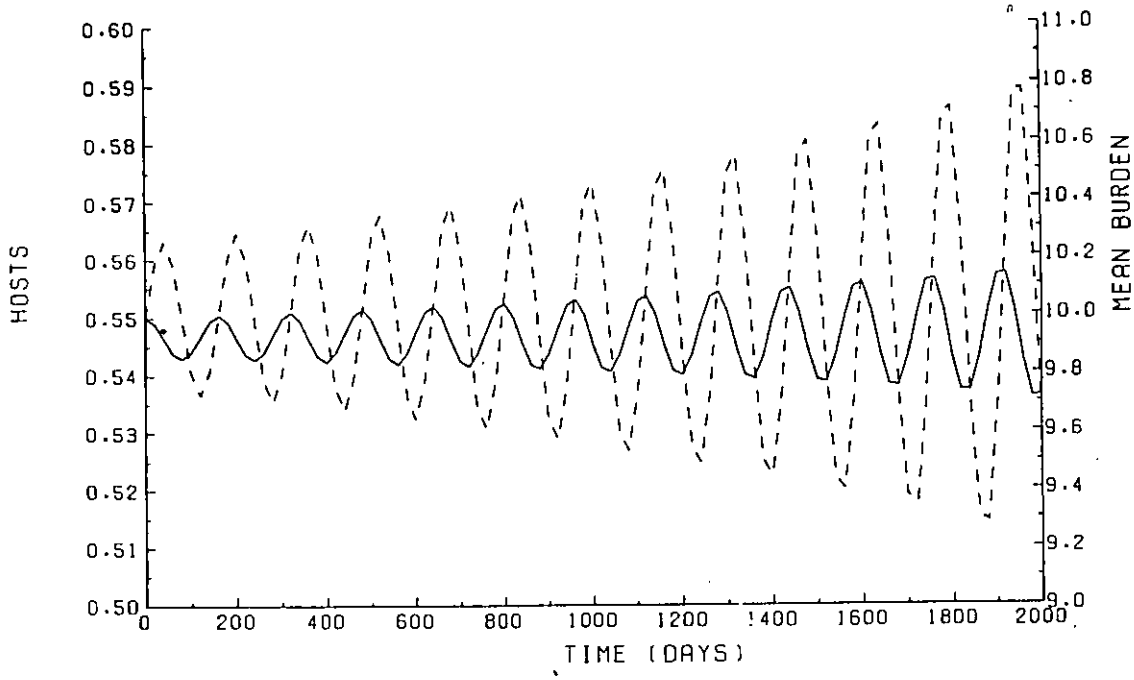
Figure 11.1 *Increasing Oscillations Generated from eqns (11.8) and (11.9)*

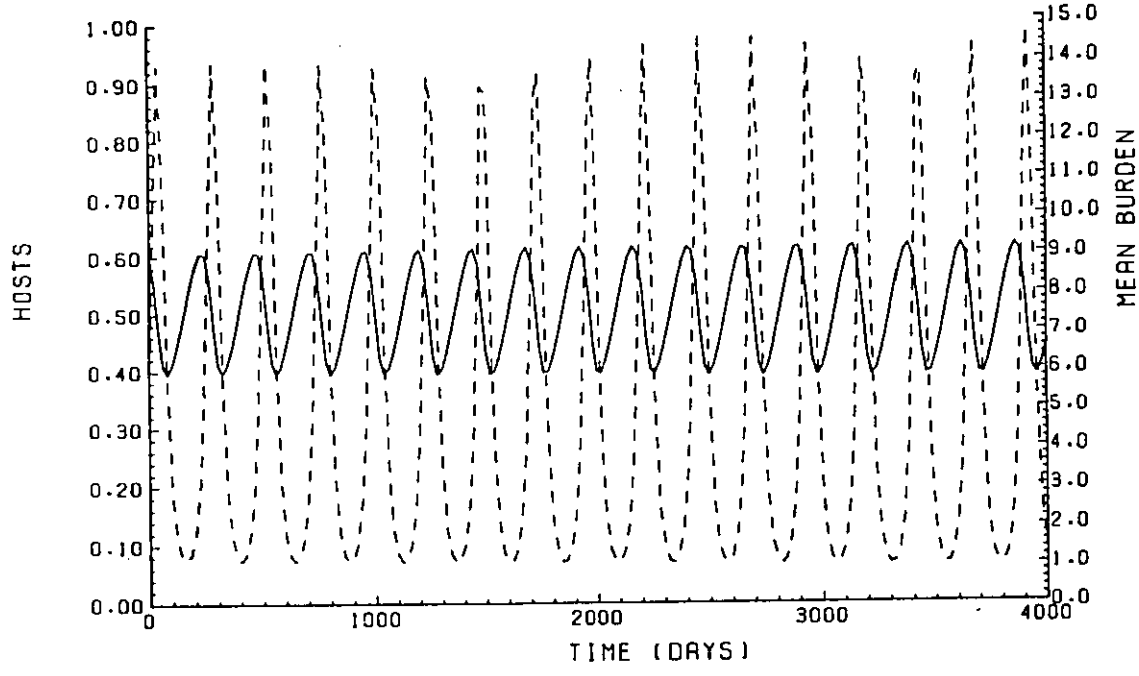
The increasing oscillations shown are generated from eqns. (11.8) and (11.9) if eqn. (11.14) is not satisfied. The solid line represents the size of the host population, $N(t)$, and is scaled on the axis to the left of the figure. The dashed line represents the mean parasite burden, $M(t)$, and is scaled on the axis to the right of the figure. Parameter values used are: $a = 0.02$, $b = 0.01$, $\alpha = 0.001$, $\lambda = 4.0$, $\gamma = 0.15$, $\mu = 0.05$, $k = 0.5$, $x = 0.5$. Other numerical solutions with these same parameter values and differing starting conditions failed to show any evidence of the existence of a stable limit cycle.

Figure 11.2 *Stable limit cycle generated from eqns (11.16)*

The stable limit cycle shown is generated from eqns. (11.16) when parasite survival of host death is high. The variables are represented in the same way as in Fig. 11.1. Parameter values used for this solution are: $a = 0.02$, $b = 0.01$, $\kappa = 0.01$, $\alpha = 0.001$, $\lambda = 4.0$, $\gamma = 0.15$, $\mu = 0.05$, $k = 0.5$, $x = 0.76$. In the absence of parasitism, the equilibrium level of the host population is 1.0.

There appears to be a secondary oscillation in peak mean parasite burden, but this is an artifact. Although the integration procedure used maintained accuracy in both variables to approximately 0.001, only results at every tenth day are plotted. The peak plotted value of $M(t)$ therefore does not necessarily correspond to its actual peak value in that cycle.


HOSTS
MEAN BURDEN
TIME (DAYS)
0.60
0.59
0.58
0.57
0.56
0.55
0.54
0.53
0.52
0.51
0.50
11.0
10.8
10.6
10.4
10.2
10.0
9.8
9.6
9.4
9.2
9.0
0
200
400
600
800
1000
1200
1400
1600
1800
2000


HOSTS
MEAN BURDEN
TIME (DAYS)
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
15.0
14.0
13.0
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
0
1000
2000
3000
4000

and

$$\frac{dM}{dt} = M\left[ \frac{\lambda N}{(N+1)} \left[\gamma + x(\alpha+b) + x\alpha(k+1)M/k\right] - (\gamma+\mu+\alpha) - \frac{\alpha M}{k} - \frac{A}{N} \right] \qquad (11.19)$$

In the absence of infection, there is a host equilibrium population of $K = A/b$. An equilibrium with parasites present will exist if

$$\lambda\left[\gamma+x(\alpha+b)\right]/(\gamma+\mu+\alpha+b) > b/A + 1 \qquad (11.20)$$

Numerical studies again show that this equilibrium may be locally unstable, and the system may follow stable limit cycles if $x$ is sufficiently large (Fig. 11.3).

11.3 *Parasite survival decreasing with mean parasite burden*

The results of Section 4.3.3 show clearly that the age of a trophozoite determines the number of infective stages it produces. This means that the number of tomites produced per trophozoite on a host that dies will be determined by the age structure of the trophozoite population on the particular fish in question. The higher the parasite burden on a host, the more rapidly it will die, and thus the lower the average age of its trophozoites. One should therefore expect that the proportion of parasites surviving host death should be related in an inverse manner to the parasite burden of the host. Ideally, this situation should be examined by an age dependent model in partial differential equation form (Oster, 1977). An approximate model may be obtained by assuming that the proportion of trophozoites surviving host death (or equivalently, the number of tomites produced relative to a mature trophozoite) is a function, $s(i)$, which decreases with increasing parasite burden, $i$. The following function has this property:

$$s(i) = \phi \quad \exp(-\omega i) \tag{11.21}$$

where $\omega > 0$ and $0 < \phi < 1$. The expected rate of survival of host death, $S(M)$, is hence

$$S(M) = E(i\phi(b+\alpha i) \exp(-\omega i)) \tag{11.22}$$

If the distribution of parasites amongst the host population can be described by a negative binomial distribution with parameter $k$, eqn. (11.22) can be evaluated via the generating function of the probability distribution (see Appendix 8) and is found to be

$$S(M) = \phi M z \left[1+M(1-z)/k\right]^{-(k+2)} \left[ b+\alpha+\left[M/k\right]\left[\alpha(kz+1)+b(1-z)\right]\right] \tag{11.23}$$

where $z = \exp(-\omega i)$

If the host population grows in an exponential fashion in the absence of parasitism, the following equations will describe temporal changes in the variables $N(t)$ and $M(t)$:

$$dN/dt = N ( (a-b) - \alpha M) \tag{11.24}$$

$$dM/dt = M\left[\left[\lambda N/(N+1)\right]\left[\gamma+S(M)/M\right] - (\gamma+\mu+\alpha+a) - \alpha M/k\right] \tag{11.25}$$

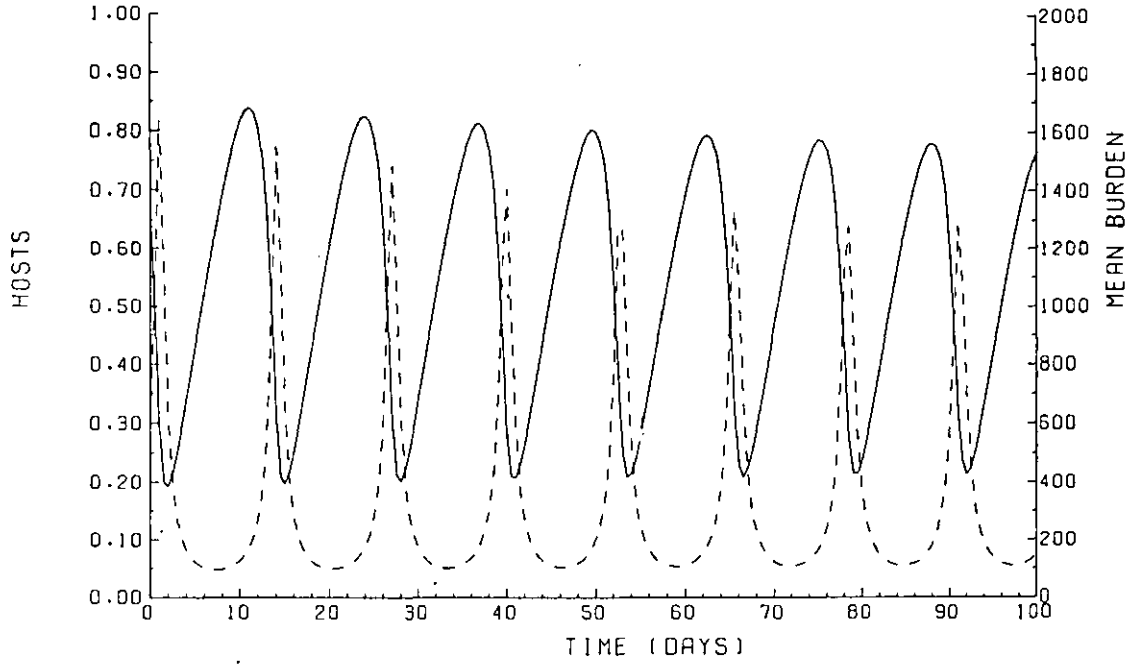
If equations (11.24) and (11.25) have a non trivial equilibrium, it may be unstable if $\phi$ is large and $\omega$ is small. Figure 11.4 shows the region within the $\phi$-$\omega$ parameter space in which the equilibrium is locally unstable. In contrast to the model described by eqns. (11.8) and (11.9),

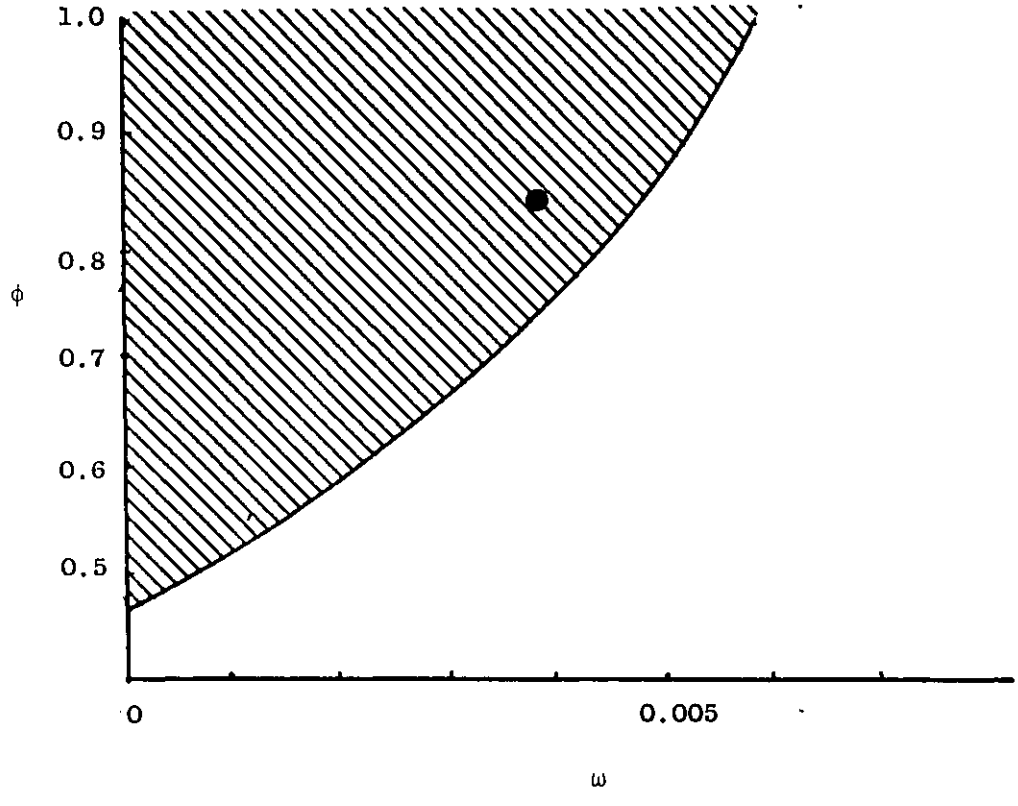
Figure 11.3 *Stable limit cycle generated from eqns. (11.18) and (11.19)*

An example is shown of the stable limit cycles generated from eqns. (11.18) and (11.19) when parasite survival of host death is high. The variables $N(t)$ and $M(t)$ are represented in the same way as in Fig. 11.1. Parameter values used are: $A$ = 0.15, $b$ = 0.01, $\alpha$ = 0.001, $\lambda$ = 4.0, $\gamma$ = 0.15, $\mu$ = 0.05, $k$ = 0.5, $x$ = 0.85.

Figure 11.4 *Stability regions of the model defined by eqns. (11.24) and (11.25)*

Stability regions in the $\phi$ - $\omega$ plane for the model defined by eqns. (11.24) and (11.25) are shown. In the hatched region to the left of the figure, the system will exhibit stable limit cycles. The equilibrium with parasites present is locally stable to the right of the figure. Parameter values used are: $a$ = 0.02, $b$ = 0.01, $\alpha$ = 0.001, $\lambda$ = 4.0, $\gamma$ = 0.15, $k$ = 0.5, $\mu$ = 0.05. The parameters used in Fig. 11.5 are shown by a solid circle.


HOSTS
MEAN BURDEN
TIME (DAYS)
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
2000
1800
1600
1400
1200
1000
800
600
400
200
0
0
10
20
30
40
50
60
70
80
90
100


1.0
0.9
0.8
0.7
0.6
0.5
ϕ
0
0.005
ω

parameter combinations that result in locally unstable equilibria lead to the system following stable limit cycles rather than diverging oscillations (Fig. 11.5). Figure 11.5 should be contrasted with Fig. 11.6, which shows a time dependent solution of eqns. (11.24) and (11.25) with $\omega$ chosen so that parasite survival of host death falls off more rapidly with increasing parasite burden.

## 11.4 *Conclusions*

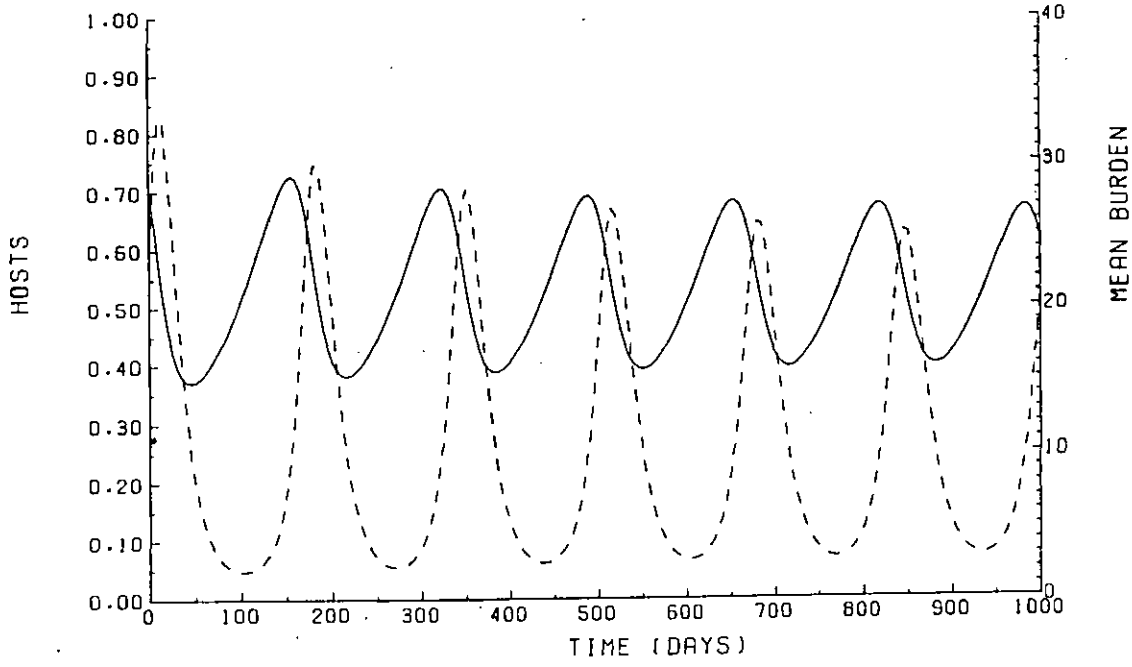
Parasite survival of host death is a strongly destabilizing factor. If sufficient tomites are produced from trophozoites on hosts that die, stable limit cycles may be induced in populations which have stable equilibria in the absence of parasitism.

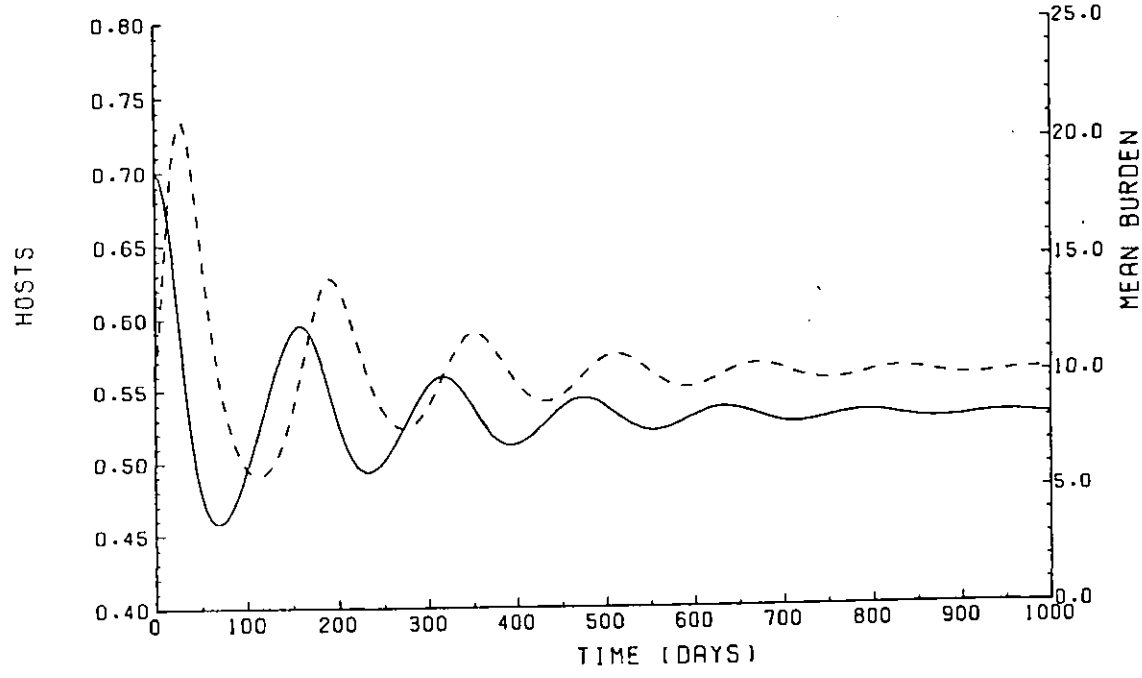
Figure 11.5 *Stable limit cycle generated from eqns. (11.24) and (11.25)*

A time dependent solution is shown of the system described by eqns. (11.24) and (11.25), displaying stable limit cycles. The solid line represents the number of hosts, and is scaled on the axis to the left of the figure. The mean parasite burden per host is represented by a dashed line, and is scaled according to the axis to the right of the figure. Parameter values used are: $a = 0.02$, $b = 0.01$, $\alpha = 0.001$, $\lambda = 4.0$, $\gamma = 0.15$, $k = 0.5$, $\mu = 0.05$, $\phi = 0.83$, $\omega = 0.0039$.

Figure 11.6 *A stable solution of eqns. (11.24) and (11.25).*

A time dependent solution is shown of the system described by eqns. (11.24) and (11.25), converging to a stable equilibrium. The variables $N(t)$ and $M(t)$ are represented in the same manner as in Fig. 11.5. Parameter values used are also the same, except $\phi = 0.8838$ and $\omega = 0.0100$. These parameters produce the same equilibrium values of $N$ and $M$ as do those used in the previous figure.


HOSTS
MEAN BURDEN
TIME (DAYS)
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
40
30
20
10
0
0
100
200
300
400
500
600
700
800
900
1000


HOSTS
MEAN BURDEN
TIME (DAYS)
0.80
0.75
0.70
0.65
0.60
0.55
0.50
0.45
0.40
25.0
20.0
15.0
10.0
5.0
0.0
0
100
200
300
400
500
600
700
800
900
1000

# CHAPTER 12

# MODELLING OF THE IMMUNE RESPONSE

## 12.1 *Introduction*

Most vertebrates are capable of mounting some type of immunological defence against infective agents (Roitt, 1980). The immune response is of particular importance in the dynamics of microparasitic disease agents (in the sense of Anderson and May, 1979) such as bacteria and viruses, which have extremely large potential rates of increase within the host. In such cases, the period during which a host is refractory to further infection is often much longer than the course of a single infection (Bailey, 1975). Many models aiming to describe the dynamics of microparasitic diseases in vertebrates include an immune class, into which hosts pass when they recover from infection (Ross and Hudson, 1917 , Bailey, 1975; Anderson and May 1979, also see Appendix 4).

*Ichthyophthirius*, although a protozoan, behaves more like a macroparasite in that it is incapable of reproduction on its host. For the reasons detailed in Chapter 10, models dividing the host population into infected, susceptible and immune categories are therefore inappropriate to describe the dynamics of the disease. A further complication arises when considering the modelling of immunity to macroparasitic infections. Both the rate of gaining immunity and pathogenicity are functions of the parasite burden, and thus the proportion of hosts becoming immune before death is liable to depend on the level of infection (Bradley, 1972). Anderson and May (1978), in their discussion of the population dynamics of host parasite interactions, do not specifically

include an immune class. Immunity to many metazoan parasites is of short duration, relative to the life expectancy of an individual parasite, and often incomplete (Weinmann, 1970). It is therefore reasonable to consider such an immune response as being included within the density dependent constraints on parasite burden within individual hosts. Immunity to some cestode infections (for example to *Hymenolepis nana* and *Taenia saginata*) has been reported as lasting for a considerable time after the removal of the parasite from the host (Weinmann, 1970). Immunity to *Ichthyophthirius*, whilst incomplete, lasts considerably longer than the life expectancy of an individual trophozoite (Hines and Spira, 1974c, Chapter 6, this study) and is maintained in the absence of parasites. An immune class of hosts with parasite burdens very much less than those of susceptible hosts may therefore develop within fish populations. In this chapter, the consequences of adding an immune class to the basic model of Chapter 10 are considered.

## 12.2 *A Simple Model of Immunity*

The model described below introduces immunity into the host parasite model in the simplest conceivable manner. Many of the assumptions made are gross oversimplifications of the biology of the immune response to *Ichthyophthirius* as described by Hines and Spira (1974c), Goven et al (1980) or as indicated by the results of the experiments described in Chapter 6. The intention is to provide a simple baseline from which to discuss more realistic modifications, and to elucidate some of the general features of the effects of an immune response on the dynamics of a host parasite interaction.

It is initially assumed that parasites are distributed in a

random (Poisson) pattern within the susceptible host population. If such a distribution occurs in a host parasite system without immunity, any non trivial equilibrium is neutrally stable (Anderson and May, 1978). Making this assumption will thus highlight any effect that the immune response may have on the stability of the host-parasite interaction. The overall distribution of parasites within the total host population will take the form of a Poisson distribution with added zeroes, and hence is overdispersed in form (Pielou, 1969).

The basic model described by eqns. (10.27) and (10.28) is modified by the inclusion of an immune class, $I$, of hosts, which tomites may infect, but upon which they cannot survive to become trophozoites. Susceptible hosts are assumed to enter this class at a rate determined by their parasite burden. In this simplest case, the rate is assumed to be $\eta i$; a function directly proportional to the host's current parasite burden, $i$. Immune hosts are assumed to lose resistance at a constant per capita rate of $\nu$ per unit time. Immunity is assumed to be total, and all trophozoites on a host when it enters the immune class are assumed to die.

Denoting the number of susceptible hosts by $S$, the number of immune hosts by $I$, the number of trophozoites on hosts by $P$ and the number of tomites by $T$, the following four differential equations are obtained to describe temporal changes in $S$, $I$, $P$ and $T$:

$$dS/dt = a(S+I) - bS - (\alpha+\eta)P + \nu I \qquad (12.1)$$

$$dI/dt = \eta P - bI - \nu I \qquad (12.2)$$

$$dP/dt = \beta ST - (b+\gamma)P - P^2(\alpha+\eta)/S \qquad (12.3)$$

$$dT/dt = \lambda P - \mu T - \beta T(S+I) \qquad (12.4)$$

Construction of this model closely follows the lines described in Section 10.3. Both classes of host are assumed to reproduce at the same rate, $a$ per unit time (all offspring are susceptible). Tomites may be removed from the system by dying or by infecting either a susceptible or immune host. Parameter definitions vary slightly from those used in Chapter 10. Table 12.1 outlines the new definitions.

As with eqns. (10.7) - (10.10), the system may be reduced to three equations by assuming that the life expectancy of the free swimming tomites is much shorter than that of the trophozoites or hosts, so that the tomite population is essentially in equilibrium with the current host and trophozoite populations. It is also convenient to sum eqns. (12.1) and (12.2), to express the system in terms of the total host population, $H$, the number of immune hosts, $I$, and the number of parasites, $P$. Hence:

$$dH/dt = (a-b)H - \alpha P \qquad (12.5)$$

$$dI/dt = \eta P - (b+\nu)I \qquad (12.6)$$

$$dP/dt = \lambda P(H-I)/(\mu/\beta + H) - (b+\gamma+\alpha)P - (\alpha+\eta)P^2/(H-I) \qquad (12.7)$$

Equations (12.5 - 12.7) can be analyzed in a similar fashion to eqns. (10.27) and (10.28). The threshold host population necessary for the introduction of the disease is found to be:

Table 12.1 *Parameters used in Chapter 12*

| Parameter | Biological meaning | Units |
|---|---|---|
| $a$ | birth rate of hosts | per host, per unit of time |
| $b$ | disease independent death rate of hosts | per host, per unit of time |
| $\alpha$ | increment in rate of parasite induced mortality per trophozoite | per trophozoite, per host, per unit of time |
| $\eta$ | increment in rate of entering the immune class per trophozoite | per trophozoite, per host, per unit of time |
| $\beta$ | rate of tomite infection of hosts | per tomite, per host, per unit of time |
| $\lambda$ | rate of tomite production by trophozoites | per trophozoite, per unit of time |
| $\gamma$ | rate of loss of trophozoites from hosts | per trophozoite per unit of time |
| $\mu$ | death rate of tomites | per tomite, per unit of time |
| $\nu$ | rate of loss of immunity | per host, per unit of time |
| $\kappa$ | parameter determining the degree of density dependent constraint on host population growth | $(\text{per host})^2$, per unit of time |
| $H_o$ | saturation term in transmission ($\mu/\beta$) | |
| $c$ | Asymtotic rate of gaining immunity | per host, per unit of time |
| $\zeta$ | parameter determining per parasite increment in the rate of gaining immunity | per trophozoite |
| $k$ | parameter of negative binomial distribution | |
| $s$ | survival of tomites infecting resistant hosts relative to survival on susceptibles | |

$$H_T = \mu/\beta \; / \; \left[\lambda/(b+\gamma+\eta+\alpha) - 1\right] \qquad (12.8)$$

Two conditions must be satisfied if an equilibrium is to exist:

$$\rho > 0 \qquad (12.9)$$

where $\rho = 1 - \eta(a-b)/[\alpha(b+\nu)]$ (12.10)

and $\lambda > (b+\gamma+\alpha+\eta)/\rho + (\alpha+\eta)(a-b)/(\alpha\rho^2)$ (12.11)

If this equilibrium exists, it can be shown to be always stable (Appendix 9, see also Fig. 12.1). Increasing the rate of gaining immunity, $\eta$, relative to the rate of host induced mortality, $\alpha$, decreases the ability of the parasite to regulate the host population to a low level (Fig. 12.2). If the rate of gaining immunity is large enough, eqns. (12.9) and (12.11) are no longer satisfied, and the parasite alone cannot regulate host population growth. Parasites against which hosts rapidly develop immunity can regulate host populations only if the rate of loss of immunity is rapid (Fig. 12.3). Equations (12.9) and (12.11) should be compared with the condition for the existence of an equilibrium in the related disease model (Appendix 4);

$$\alpha > (a-b)\left[1 + \gamma/(b+\nu)\right] \qquad (12.12).$$

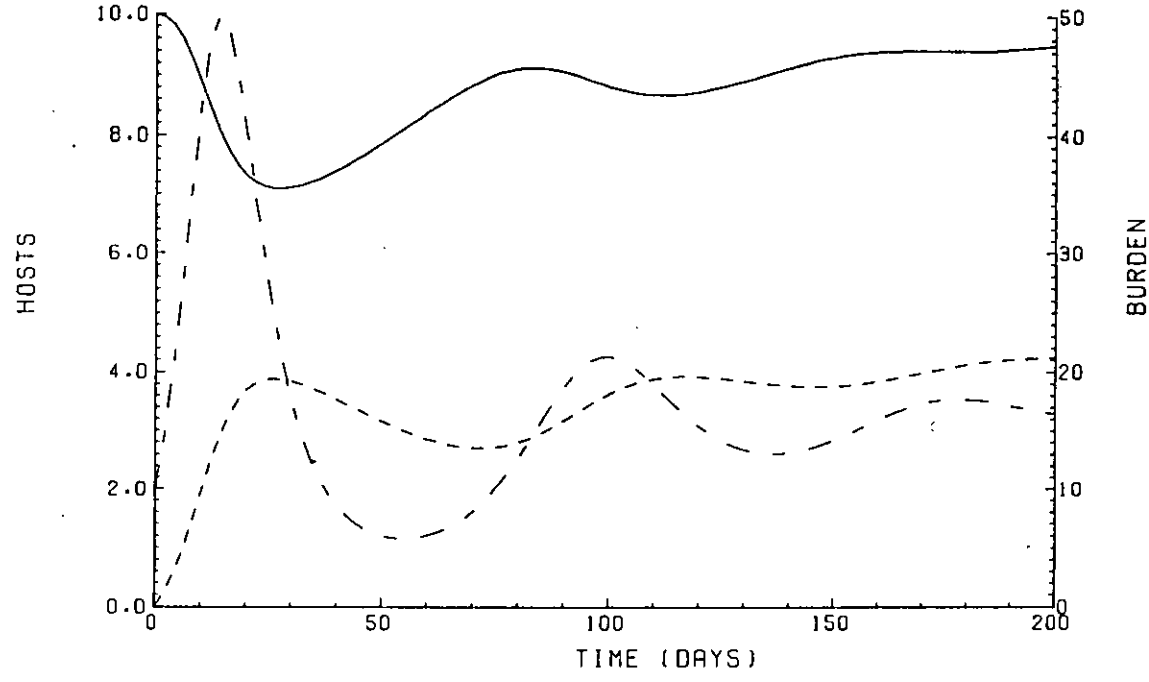
Here $\gamma$, the rate of recovery from infection is analogous to $\eta$, the per capita rate of entering the immune class. Conditions (12.9) and (12.12) are very similar. In the disease model, the rate of parasite

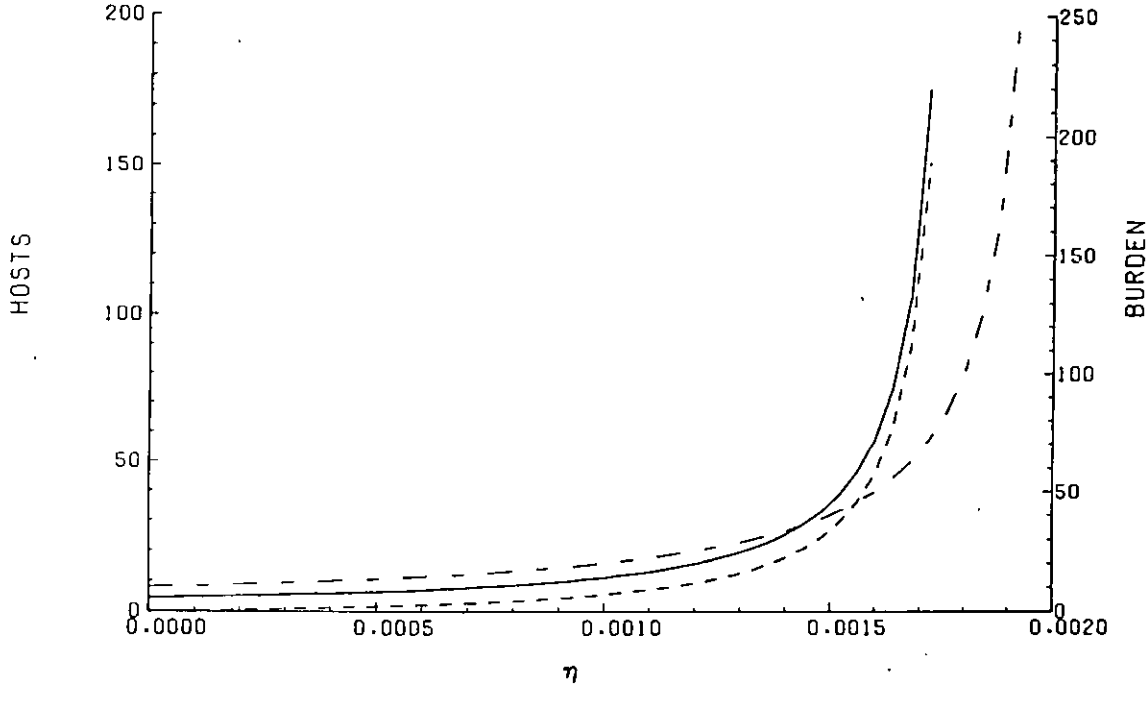
Figure 12.1 *A solution of eqns. (12.5) - (12.7) with* $\eta = 0.001$

The time dependent solution of eqns. (12.5) - (12.7) is shown, with parameter values chosen so that an equilibrium with the parasite present exists (eqns. (12.9) - (12.11) satisfied). The solid line represents the total number of hosts, $H(t)$, and the evenly dashed line, the number of immune hosts, $I(t)$. Both these variables are scaled on the axis to the left of the figure. The unevenly dashed line represents the mean parasite burden per susceptible host, and is scaled on the axis to the right of the figure. Parameter values used are: $a = 0.02$, $b = 0.01$, $\alpha = 0.001$, $\lambda = 4.0$, $H_o = 100.$, $\nu = 0.01$, $\eta = 0.001$, $\gamma = 0.15$. The equilibrium is at $H^* = 11.23$, $I^* = 5.12$, $M^* = 20.0$.

Figure 12.2 *The effect of increasing* $\eta$ *on equilibrium values of eqns. (12.5) - (12.7)*

The effect is shown of increasing the rate of gaining immunity on the equilibrium of the system described by eqns. (12.5)-(12.7). The solid line represents the equilibrium total host population, $H^*$, and the evenly dashed line, the number of immune hosts at equilibrium, $I^*$. These are scaled on the axis to the left of the figure. The unevenly dashed line shows the equilibrium value of the mean parasite burden per susceptible host, $M^*$, and is scaled on the axis to the right of the figure. The values of parameters other than $\eta$ are the same as those used in Fig. 12.1.


HOSTS
BURDEN
TIME (DAYS)
10.0
8.0
6.0
4.0
2.0
0.0
50
40
30
20
10
0
0
50
100
150
200


HOSTS
BURDEN
η
200
150
100
50
0
250
200
150
100
50
0
0.0000
0.0005
0.0010
0.0015
0.0020

induced death must exceed $a-b$, the natural rate of host population growth, in order to control the host population. In addition, $\alpha$ must be sufficiently large in comparison with the rate of gaining immunity, $\gamma$, that the number of immune hosts does not increase without limit.

The absolute size of $\alpha$, the increment in parasite induced mortality per parasite, is not of importance in the host-parasite model (for reasons discussed in Chapter 10), but $\alpha$ must also be sufficiently large relative to $\eta$, the per capita rate of gaining immunity. If the parasite induced death rate is sufficiently great, the disease will be able to regulate the host population provided the parasite growth rate outstrips that of the host (eqn. 12.11). Should either eqn. (12.9) or (12.11) not be satisfied, but

$$\lambda > b+\gamma+\alpha+\eta \qquad (12.13)$$

hosts become immune at a rate rapid enough to escape regulation by the disease and hosts and parasites both increase in an unbounded manner. (Figs. 12.4 and 12.5). Whether or not both eqns. (12.9) and (12.11) are violated does not appear to qualitatively affect the behaviour of the system. If eqn. (12.13) is not satisfied, no host population level exists which is sufficient to enable parasite increase, and parasite numbers decline to extinction while host numbers increase without limit (Fig. 12.6).

## 12.3 *Host Population Growth Restricted*

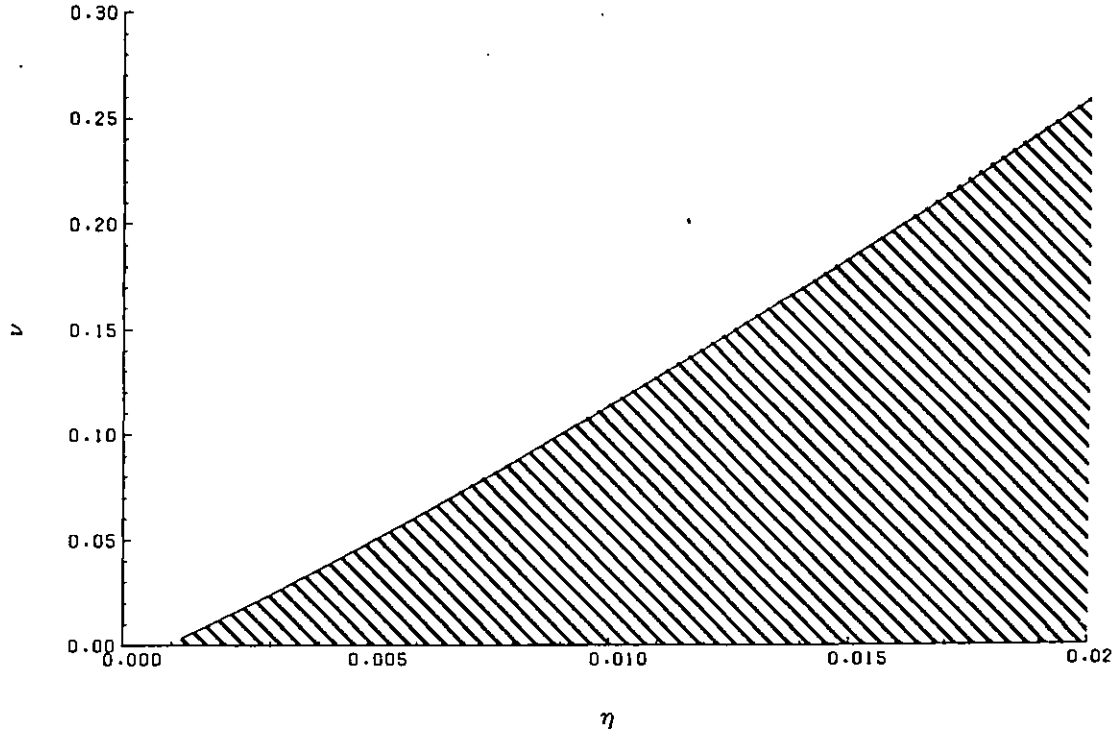
Natural populations of hosts do not increase without limit if not regulated by parasitism. A better approximation to the behaviour

Figure 12.3 *The region of the η - ν parameter space within which eqns. (12.5) - (12.7) have an equilibrium solution*

In the hatched region of the η - ν parameter space below the line shown the parasite is unable to regulate the host population. Above the line, immune hosts are removed from the system by recovery at a rate high enough that the parasite can stably regulate host population growth. The figure is obtained from eqn. (12.11), with $a$ = 0.02, $b$ = 0.01, α = 0.001, λ = 4.0 and γ = 0.15.

Figure 12.4 *A solution of eqns. (12.5) - (12.7) with η = 0.0019*

The time dependent solution of eqns. (12.5) - (12.7) is shown, with parameter values chosen so that eqns. (12.9) and (12.13) are satisfied, but eqn. (12.11) is not satisfied. The solid line represents the total number of hosts, $H(t)$ and the evenly dashed line, the number of immune hosts $I(t)$. Both these variables are scaled on the axis to the left of the figure. The mean parasite burden on susceptible hosts, $M(t)$, is represented by an unevenly dashed line, and is scaled on the axis to the right of the figure. Parameter values used are: $a$ = 0.02, $b$ = 0.01, α = 0.001, λ = 4.0, γ = 0.15, $H_o$ = 100., ν = 0.01, η = 0.0019. Initial conditions are $H(o)$ = 10., $I(o)$ = 0., $M(o)$ = 10.


0.30
0.25
0.20
0.15
0.10
0.05
0.00
ν
0.000
0.005
0.010
0.015
0.02
η

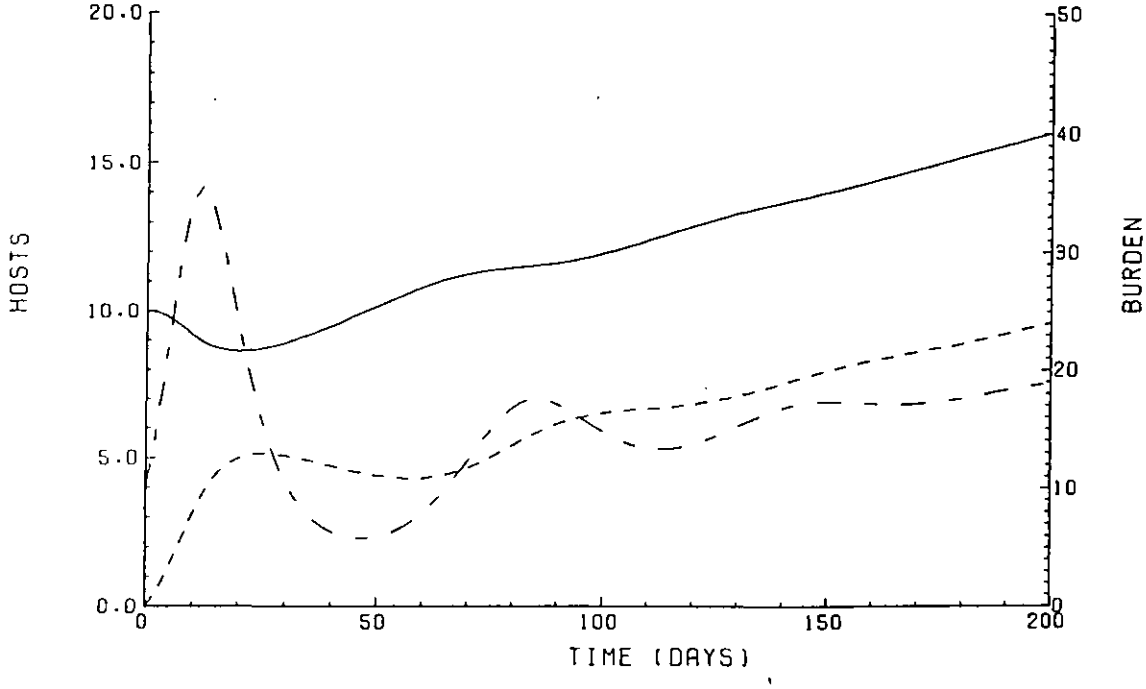
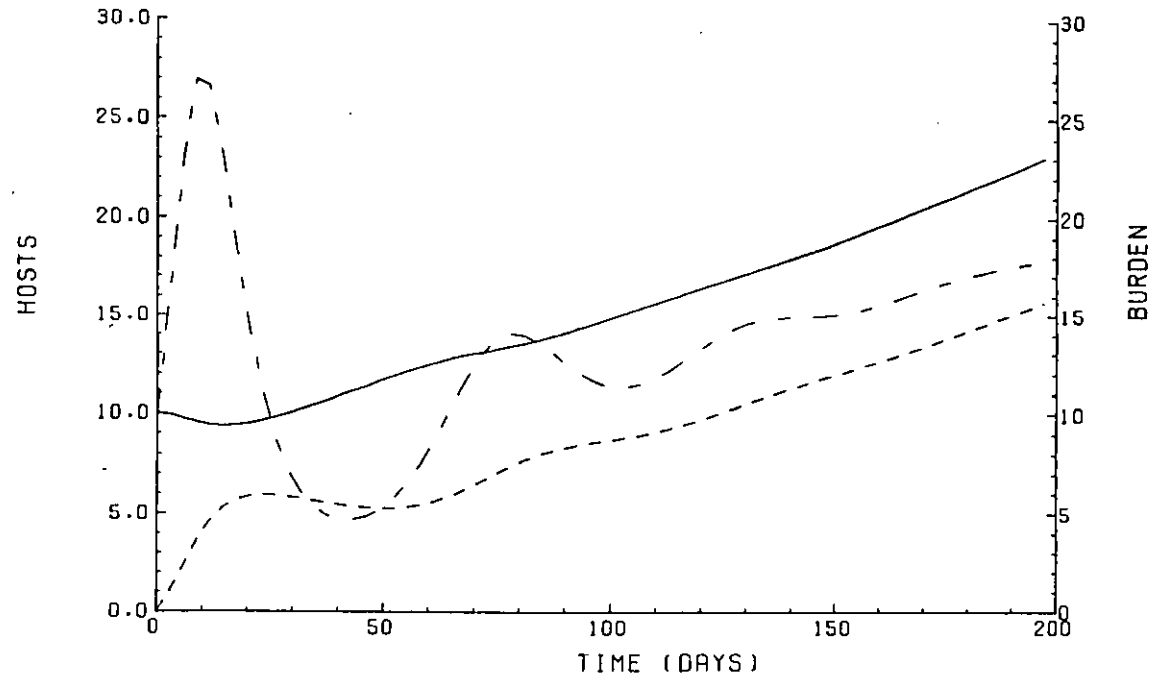

20.0
15.0
10.0
5.0
0.0
HOSTS
50
40
30
20
10
0
BURDEN
0
50
100
150
200
TIME (DAYS)

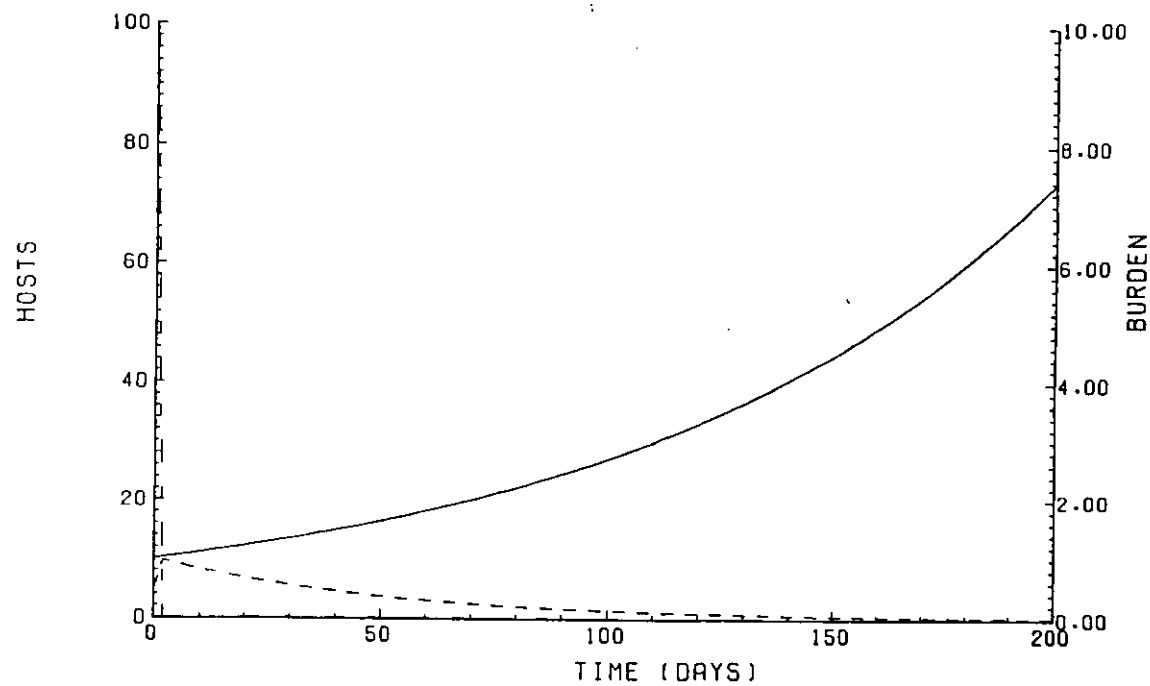
Figure 12.5 *A solution of eqns. (12.5) - (12.7) with* $\eta = 0.003$

The time dependent solution of eqns. (12.5) - (12.7) is shown, with parameters chosen so that eqn. (12.13) is satisfied, but eqns. (12.9) and (12.11) are not satisfied. Details are as for Fig. 12.4, except that $\eta = 0.003$.

Figure 12.6 *A solution of eqns. (12.5) - (12.7) with* $\eta = 4.0$

The time dependent solution of eqns. (12.5) - (12.7) is shown, with parameter values chosen so that eqn.(12.13) is not satisfied. Details are as for Fig. 12.4, except that $\eta = 4.0$.


HOSTS
BURDEN
TIME (DAYS)
30.0
25.0
20.0
15.0
10.0
5.0
0.0
0
50
100
150
200
30
25
20
15
10
5
0


HOSTS
BURDEN
TIME (DAYS)
100
80
60
40
20
0
0
50
100
150
200
10.00
8.00
6.00
4.00
2.00
0.00

of real host-parasite interactions may be obtained by assuming that host populations grow in a logistic fashion in the absence of parasitism, in such a way that the parasite independent death rate of hosts is $b + \kappa H$, where $H$ is the size of the host population, and $\kappa$ is a parameter determining the degree of density dependence in host population growth. The temporal changes in total host population, $H$, the number of immune hosts, $I$, and the number of parasites, $P$, may be described by the following equations:

$$dH/dt = (a-b)H - \kappa H^2 - \alpha P \qquad (12.15)$$

$$dI/dt = \eta P - (b+\nu)I - \kappa HI \qquad (12.16)$$

$$dP/dt = \lambda(H-I)P/(H+H_o) - (\alpha+\eta+\gamma+b)P - \kappa PH - (\alpha+\eta)P^2/(H-I) \qquad (12.17)$$

where $H_o$ is defined as $\mu/\beta$.

Analysis of the system is facilitated if it is defined in terms of new variables $M(t)$, the mean parasite burden on susceptible hosts, and $Q(t)$, the proportion of hosts that are immune.

Hence:

$$dH/dt = H\left[a-b - \kappa H - \alpha M(1-Q)\right] \qquad (12.18)$$

$$dQ/dt = M(1-Q)(\eta+\alpha Q) - (a+\nu)Q \qquad (12.19)$$

$$dM/dt = M\left[\lambda H(1-Q)/(H+H_o) - (\gamma+\alpha+\eta-\nu) - (a+\nu)/(1-Q)\right] \qquad (12.20)$$

It is also possible to rescale the total host population in terms

of $H_o$. If $N = H/H_o$ the first term in eqn. (12.20) becomes $N(1-Q)/(N+1)$ and eqn. (12.18) becomes $dN/dt = N[a-b - \kappa H_o N - \alpha M(1-Q)]$ It is therefore the size of the saturation term, $H_o$, relative to $\kappa$, the degree of density dependence, which is of importance in this model.

Two equilibrium states exist for this model. There is a disease free equilibrium at which

$$H^* = (a-b)/\kappa \qquad (12.21)$$

and $Q$ and $M$ are both zero. If

$$H > 1/\left[\lambda/(\gamma+\alpha+\eta+a) - 1\right] \qquad (12.22)$$

eqn. (12.20) will be positive if $M$ and $Q$ are small. An equilibrium with parasites present will therefore exist if the value of $H^*$ given by eqn. (12.21) exceeds the threshold value given by eqn. (12.22): i.e.

$$\lambda/(\gamma+\alpha+\eta+a) > \kappa/(a-b) + 1 \qquad (12.23)$$

Figure 12.7a shows the effect on the equilibrium values of the variables $H$, $I$ and $M$ of increasing $\eta$, the per parasite increment in the rate of gaining immunity. The equilibrium mean parasite burden on susceptible hosts, $M^*$, increases initially with $\eta$: at this stage, $H^*$ is low, the density dependent constraints introduced by $\kappa$ are unimportant and the system behaves essentially like that illustrated in Fig. 12.2. Once the equilibrium host density reaches levels at which density dependent constraints begin to act, $M^*$ declines with increasing $\eta$. The overall mean parasite burden on all hosts decreases monotonically

with increasing $\eta$ (Fig. 12.7b).

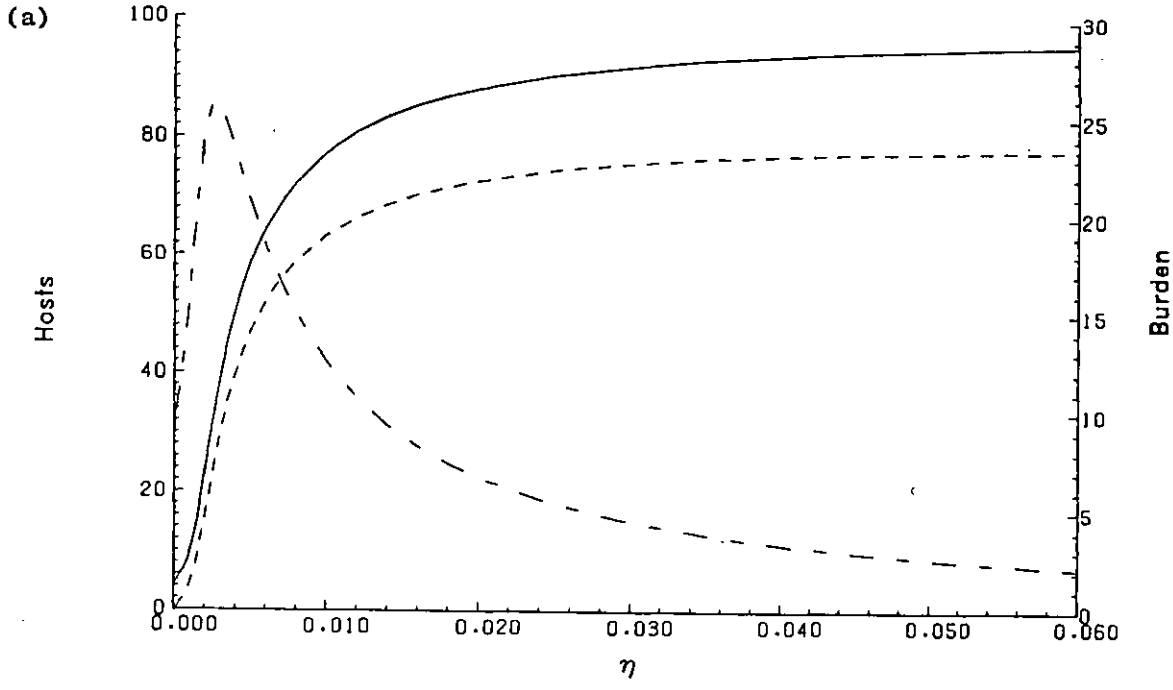
The most interesting prediction of this model is that, for a wide range of values of $\eta$, the parasites persist at low levels, with most hosts immune and the host population only slightly depressed below its disease free carrying capacity, $(a-b)/\kappa$. This type of behaviour occurs if $\eta$ is large enough so that eqn. (12.9) is not satisfied, provided $\eta$ is small compared with $\gamma$ (eqn. 12.23 satisfied). Such behaviour might well account for the low levels of *Ichthyophthirius* observed in most natural situations. These patterns are difficult to generate from the simple disease models (Appendix 4; see also Fig. 12.8). To be a satisfactory model for *Ichthyophthirius* dynamics, however, the model must also be capable of generating outbreak epidemic behaviour.

Figure 12.9 shows the effect on the equilibrium values, $H^*$, $I^*$ and $M^*$, of changing $\kappa$, the degree of density dependence in host population growth. This particular parameter is of interest as it might be expected to vary greatly in natural populations. In the presence of parasitism, changes in $\kappa$ do not necessarily produce changes in the equilibrium host population size of a magnitude that would be expected in disease free populations. Under the conditions defined in Fig. 12.9, for example, halving $\kappa$ from 0.002 to 0.001 increases the total host population very little. Mean parasite burden, however, shows a considerable increase as the system shifts from being regulated mostly by disease independent factors to being controlled to a greater extent by the parasite. Sharp decreases in $\kappa$ do not, however, appear to generate outbreaks of infection. Figure 12.10 shows the time dependent solution of equations (12.18 - 12.20)

Figure 12.7. *The effect of increasing η on the equilibrium of eqns. (12.18) - (12.20)*

The effect is shown of increasing the per parasite increment in the rate of gaining immunity, η, on the equilibrium described by eqns. (12.18) - (12.20). The total equilibrium host population, $H^*$, is shown as a solid line, and scaled according to the axis to the left of the figure. For convenience, the equilibrium number of immune hosts, $I^*$, is shown, rather than the equilibrium proportion of immune hosts, $Q^*$. $I^*$ is represented by the evenly dashed line, and is scaled on the axis to the left of the figure. In Fig. 12.7a, the equilibrium mean parasite burden per susceptible host, $M^*$, is shown, and is represented by an unevenly dashed line and scaled according to the axis to the right of the figure. The overall equilibrium mean parasite burden per host is shown, represented in the same manner, in Figure 12.7b. In both cases, the parameter values used are: $a$ = 0.02, $b$ = 0.01, $\alpha$ = 0.001, $\kappa$ = 0.0001, $\lambda$ = 4.0, $\gamma$ = 0.15, $H_o$ = 100., $\nu$ = 0.01. With these parameter values, the disease free equilibrium host population level is 100.

(a)


Hosts
Burden
η
0
20
40
60
80
100
0
5
10
15
20
25
30
0.000
0.010
0.020
0.030
0.040
0.050
0.060

(b)

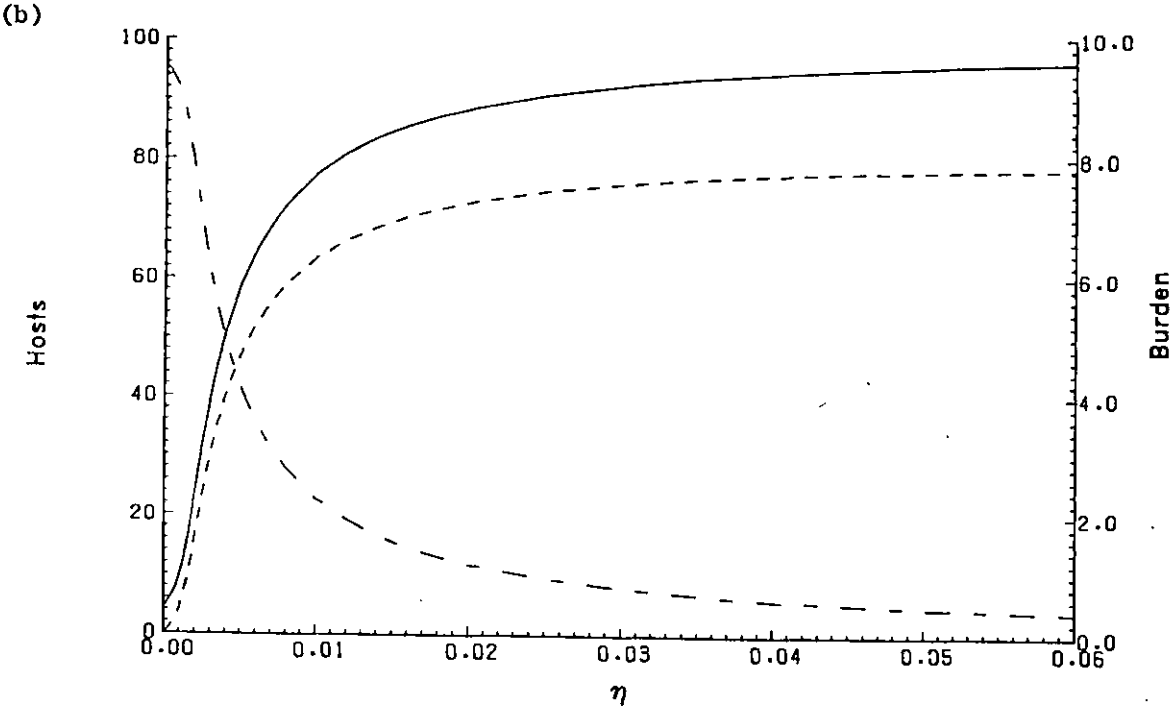

Hosts
Burden
η
0
20
40
60
80
100
0.0
2.0
4.0
6.0
8.0
10.0
0.00
0.01
0.02
0.03
0.04
0.05
0.06

Figure 12.8 *The effect of increasing the rate of joining the immune class on equilibria of a compartmental model*

The figure shows the effect of increasing the rate of entering the immune class, $\gamma$, on the equilibrium of a microparasitic disease model. The model is defined by the following equations.

$$dN/dt = (a-b)N - \kappa N^2 - \alpha Y$$

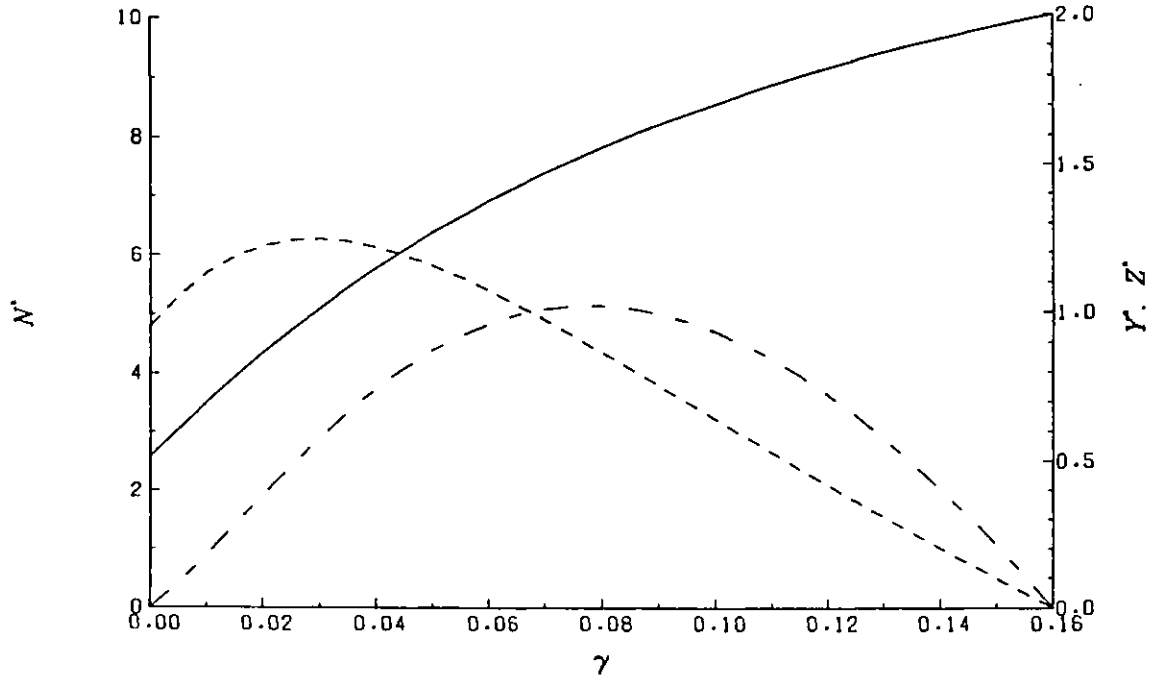
$$dY/dt = \beta Y(N-Y-Z) - (b+\kappa N)Y - (\alpha+\gamma)Y$$

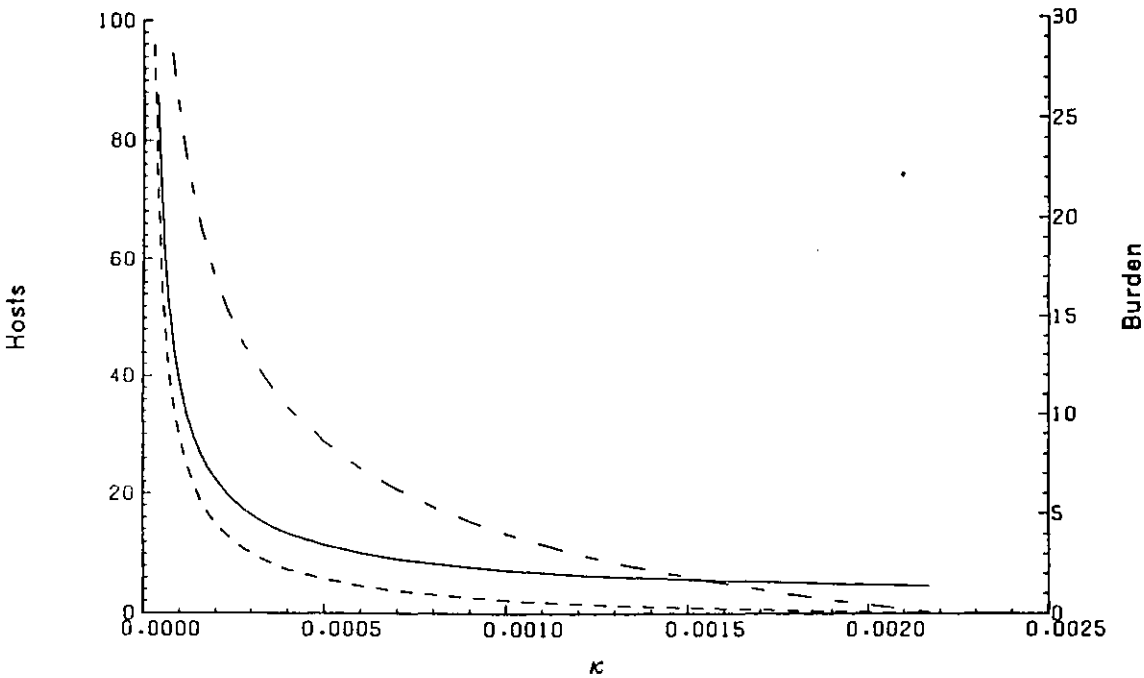
$$dZ/dt = \gamma Y - (b+\kappa N)Z - \nu Z$$

(see Appendix 4). The equilibrium total host population, $N^*$, is shown as a solid line and is scaled on the axis to the left of the figure; the equilibrium number of infected hosts, $Y^*$, is shown as an evenly dashed line and scaled on the axis to the right of the figure, and the equilibrium number of immune hosts, $Z^*$, is shown as an unevenly dashed line, and scaled on the axis to the right of the figure. Parameter values used are: $a = 0.02$, $b = 0.01$, $\kappa = 0.001$, $\beta = 0.02$, $\alpha = 0.02$, $\nu = 0.05$.

Figure 12.9 *The effect of changing $\kappa$ on the equilibrium solutions of eqns. (12.18) - (12.20)*

The effect is shown of changing $\kappa$ (the degree of non parasite induced density dependent constraint on host population growth) on the equilibrium of the system described by eqns. (12.18) - (12.20). As in Figs. 12.7, $H^*$ is shown by a solid line and $I^*$ by an evenly dashed line. Both these variables are scaled on the axis to the left of the figure. $M^*$ is shown by an unevenly dashed line, and is scaled on the axis to the right of the figure. Parameter values used are: $a = 0.02$, $b = 0.01$, $\alpha = 0.001$, $\lambda = 4.0$, $\gamma = 0.15$, $H_0 = 100.$, $\eta = 0.003$, $\nu = 0.01$.


N*
Y*, Z*
γ
0
2
4
6
8
10
0.0
0.5
1.0
1.5
2.0
0.00
0.02
0.04
0.06
0.08
0.10
0.12
0.14
0.16


Hosts
Burden
κ
0
20
40
60
80
100
0
5
10
15
20
25
30
0.0000
0.0005
0.0010
0.0015
0.0020
0.0025

when $\kappa$ is reduced from 0.001 to 0.0002. All the variables increase almost monotonically toward the new equilibrium. This should be contrasted with the increase in mean parasite burden caused by doubling of the number of susceptible hosts present, the effect of which is shown in Fig. 12.11.

## 12.4 *The Effect of the Rate of Development of Immunity Reaching a Maximum Level*

An unsatisfactory feature of the model just described is that both the rate of parasite induced death and the rate at which infected hosts become immune are linearly dependent on the parasite burden per host. This means that the probability of a host becoming immune before it dies is independent of its parasite burden. The experiments described in Chapter 6 indicate that black mollies require of the order of 10 days to develop an immune response to *Ichthyophthirius*. Whilst hosts with heavy parasite burdens may become immune somewhat faster than lightly infected hosts, the nearly tenfold difference in mean parasite burden between the two infected groups in Fig. 6.2 is not reflected in a tenfold difference in the time taken for an immune response to become established. The experiments of Chapter 7 indicate, however, that parasite induced mortality is approximately linearly related to the parasite burden. Hosts with light parasite burdens are therefore likely to become immune to further infection, whereas heavily infected hosts are likely to die before immunity can become established.

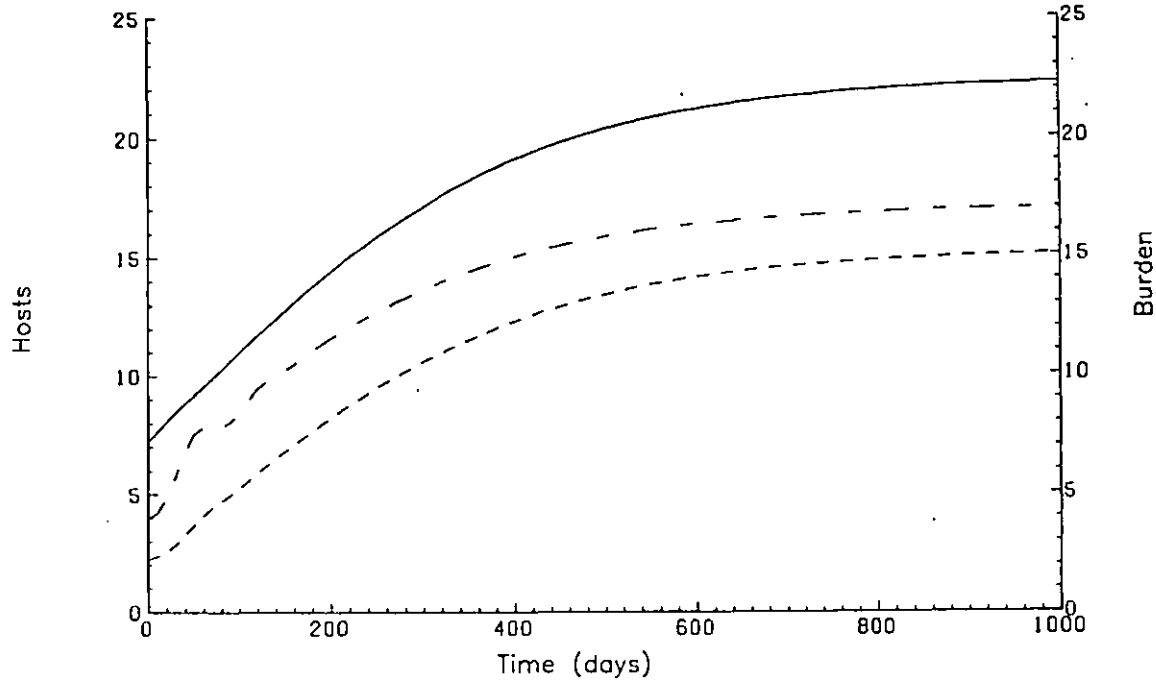
In this section, the model of immunity is modified so that the rate at which hosts enter the immune class initially increases approximately linearly with parasite burden, but as mean parasite burden

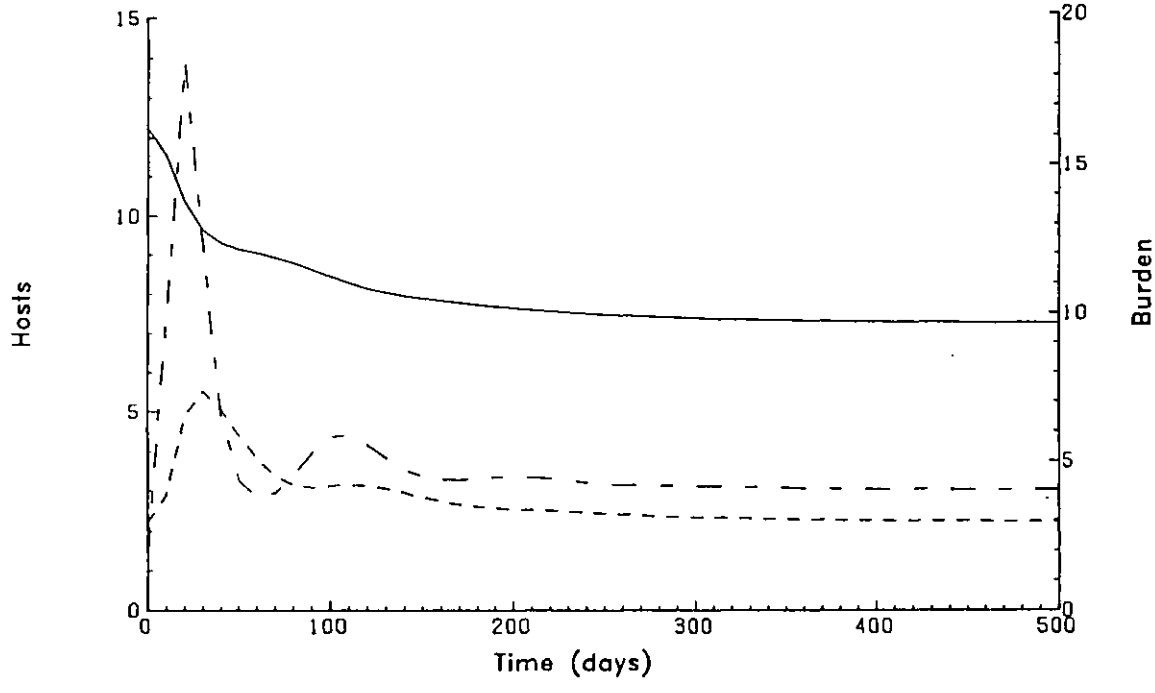
Figure 12.10 *The effect of changing κ on the solution of eqns. (12.18) - (12.20)*

A time dependent solution of the system described by eqns. (12.18) - (12.20) is shown. The numerical integration was performed with $\kappa = 0.0002$, but commenced at the equilibrium reached if $\kappa = 0.001$. Total number of hosts, $H(t)$, is shown as a solid line, and scaled on the axis to the left of the figure. The number of immune hosts, $I(t)$, is shown as an evenly dashed line and scaled on the same axis. The mean parasite burden per susceptible host, $M(t)$, is shown as an unevenly dashed line, and scaled according to the axis to the right of the figure. Parameter values other than κ are: $a = 0.02$, $b = 0.01$, $\alpha = 0.001$, $\lambda = 4.0$, $\kappa = 0.0002$, $H_o = 100.$, $\gamma = 0.15$, $\eta = 0.003$, $\nu = 0.01$.

Figure 12.11 *The effect of doubling the number of susceptibles on the solution of eqns. (12.18) - (12.20).*

The effect of doubling the number of susceptible hosts on the time dependent behaviour of the system described by eqns. (12.18) - (12.20) is shown. The total numbers of parasites and immune hosts at the beginning of the integration were set equal to their equilibrium values, but the number of susceptible hosts was doubled. Representation of the three variables $H(t)$, $I(t)$ and $M(t)$ follows that of Fig. 12.10. Parameter values used are: $a = 0.02$, $b = 0.01$, $\alpha = 0.001$, $\lambda = 4.0$, $\kappa = 0.001$, $H_o = 100.$, $\gamma = 0.15$, $\eta = 0.003$, $\nu = 0.01$.


Hosts
Burden
Time (days)
0
5
10
15
20
25
0
200
400
600
800
1000


Hosts
Burden
Time (days)
0
5
10
15
20
0
100
200
300
400
500

increases, the rate asymtotes to a constant level. To mirror this assumption, the rate per unit time with which a host with $i$ parasites enters the immune class is assumed to be:

$$r(i) = c\left[1 - \exp(-\zeta i)\right] \tag{12.25}$$

At low parasite burdens the rate, $r(i)$, is approximately $c\zeta i$, but at high parasite densities, it asymtotes to the rate $c$ per unit time. Hosts will enter the immune class at an overall rate

$$S \sum_{i=0}^{\infty} c\ (1-\exp(-\zeta i))p(i) \tag{12.26}$$

where $S$ is the size of the susceptible host population, and $p(i)$ is the probability of a susceptible host harbouring $i$ trophozoites. If it is assumed that all trophozoites on hosts becoming immune die, the rate of loss of trophozoites due to immunity is

$$S \sum_{i=0}^{\infty} ci\ (1-\exp(-\zeta i))p(i) \tag{12.27}$$

Expressions (12.26) and (12.27) clearly depend on the nature of the distribution of parasites on susceptible hosts. If the probability generating function of this distribution can be specified, they may be explicitly determined as outlined in Appendix 8. In the case of a Poisson distribution for parasites within the susceptible host population, expression (12.26) becomes

$$Sc\ \{\ 1 - \exp\left[-M(1- \exp(-\zeta))\right]\ \} \tag{12.28}$$

and expression (12.27) is

$$ScM \{ 1- \exp [-\zeta - M(1- \exp(-\zeta))] \} \quad (12.29)$$

Assuming that the loss of immunity, $\nu$, occurs at a constant rate per immune host, and that all other assumptions made in deriving eqns. (12.5 - 12.7) hold, the following equations may be obtained to describe the system:

$$dN/dt = N [(a-b) - \alpha M(1-Q)] \quad (12.30)$$

$$dQ/dt = c(1-Q)(1- \exp(-Mf)) - (a+\nu)Q + \alpha MQ(1-Q) \quad (12.31)$$

$$dM/dt = M\{\lambda N(1-Q)/(N+1)-(\gamma+\alpha-\nu)-(a+\nu)/(1-Q)-fc \exp(-Mf)\} \quad (12.32)$$

where $f$ is defined for notational convenience as

$$f = 1- \exp(- \zeta) \quad (12.33)$$

The system represented by eqns. (12.30 - 12.33) is difficult to handle analytically. It is possible to obtain the reproductive rate, $R_o$, at a given population size:

$$R_o = \lambda N/ \{(N+1)(\gamma+\alpha+a+fc)\} \quad (12.34)$$

but the equations for an equilibrium with parasites present are transcendental in form and must be solved by numerical means. This was accomplished using a program that minimized the sum of the squared

values of eqns. (12.30) - (12.32). The numerical approach will not necessarily locate multiple equilibria, a problem which is considered with respect to a slightly more complex model in the following section.

Qualitatively, the equilibrium behaviour of this model is very similar to the simpler model described by eqns. (12.5 - 12.7). Figure 12.12 shows the effect on $N^*$ (the equilibrium value of the total number of hosts), $I^*$ (the number of immune hosts) and $M^*$, (the mean burden on susceptible hosts) of increasing $c$, the asymtotic rate of gaining immunity. The general form of Fig. 12.12 closely resembles that of Fig. 12.2, the analagous figure for the simple model. High values of $c$ may mean that the parasite is unable to control the host population growth. All equilibria located numerically were found to be locally stable. As with the simple model, the model described by eqns. (12.30 - 12.32) can be made more biologically realistic by assuming that, in the absence of infection, the host population grows in a logistic fashion. Equation (12.30) then becomes

$$dN/dt = N\left[a-b-\kappa N - \alpha M(1-Q)\right] . \qquad (12.35)$$

Equations (12.31) and (12.32) remain unchanged. A condition for parasite persistence may be obtained from eqns. (12.21) and (12.34).

$$\lambda/\{\gamma+\alpha+a+ c\left[1- \exp(-\zeta)\right]\} > 1 + \kappa/(a-b) \qquad (12.36)$$

The immune response may have an important influence on whether a parasite can persist in the host population if relatively few parasites elicit a strong immune response ($\zeta$ large) and if the time required to develop immunity is of shorter or of similar duration to

the life expectancy of the parasite on the host ($c \gtrsim \gamma$).

Figure 12.13 shows the effect of increasing $c$ on the equilibrium values of the population variables. There is a striking resemblance between Fig. 12.6 and 12.13. The equilibrium behaviour of the simple, analytically tractable model is very similar qualitatively to the more complex, but more biologically realistic model.

The behaviour of the two models in their approach to equilibrium, however, is very different. It is possible that in natural conditions, *Ichthyopthirius* may persist at low levels within host populations, being regulated by a strong immune response. The natural situation may therefore correspond to a point around the value of $\eta = 0.03$ in Fig. 12.6, with the host population close to its carrying capacity, most hosts immune, and a low mean parasite burden on the susceptible hosts. The approach to this equilibrium, as predicted by the simple model (eqns. 12.18 - 12.20), if the parasite is introduced into a host population at its disease free carrying capacity,is shown in Fig. 12.14.

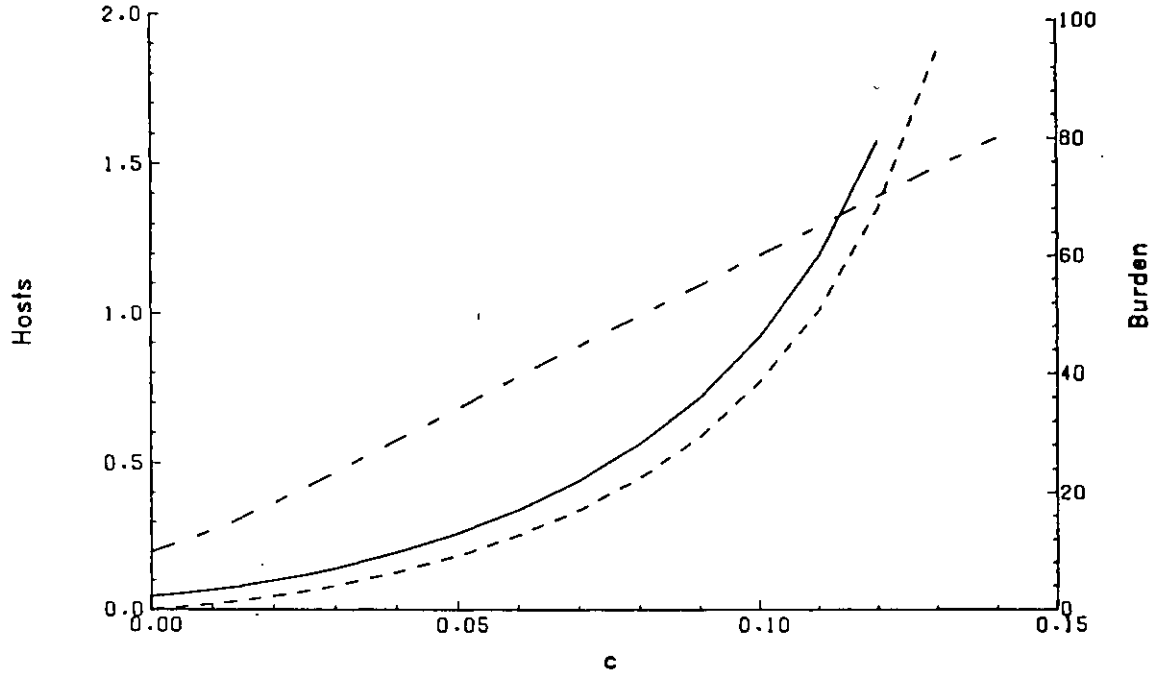
The peak mean parasite burden reached is approximately 40 per host, and the host population does not decrease to a level below its equilibrium with the pathogen present. This should be contrasted with Fig. 12.15, which shows the approach to equilibrium of the model described by eqns. (12.35), (12.31) and (12.32), commencing with the same initial starting conditions. In this case, the peak mean parasite burden reached is very much higher, at nearly 500 per host, and the host population level is depressed to a level considerably below its equilibrium. This is closer to the outbreak behaviour apparently displayed by *Ichthyophthirius* in natural conditions. (Hopkins, 1959;

Figure 12.12 *The effect of increasing* c *on the equilibrium of eqns. (12.30) - (12.32).*

The equilibrium total host population, $N^*$, is shown as a solid line and is scaled according to the axis to the left of the figure. The evenly dashed line represents the number of immune hosts present at equilibrium, $I^*$, and is also scaled according to the axis to the left of the figure. The equilibrium mean parasite burden on susceptible hosts, $M^*$, is shown as an unevenly dashed line, and is scaled according to the axis to the right of the figure. Parameter values used are: $a = 0.02$, $b = 0.01$, $\alpha = 0.001$, $\lambda = 4.0$, $\gamma = 0.15$, $\zeta = 0.1$, $\nu = 0.01$. This figure should be compared with Fig. 12.2.

Figure 12.13 *The effect of increasing* c *on the equilibrium of eqns. (12.31), (12.32) and (12.35)*

Equilibrium values of the variables $N^*$, $I^*$ and $M^*$ are represented in the same way as in Fig. 12.12. Parameter values used are, $a = 0.02$, $b = 0.01$, $\alpha = 0.001$, $\lambda = 4.0$, $\gamma = 0.15$, $\kappa = 0.01$, $\zeta = 0.1$, $\nu = 0.01$. This figure should be compared with Fig. 12.7, noting that, in Fig. 12.7, $H^*$ is shown with $H_o = 100$, and hence $\kappa = 0.01$ in Fig. 12.13 is equivalent to $\kappa = 0.0001$ in Fig. 12.7.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1.5
1.0
0.5
0.0
Hosts
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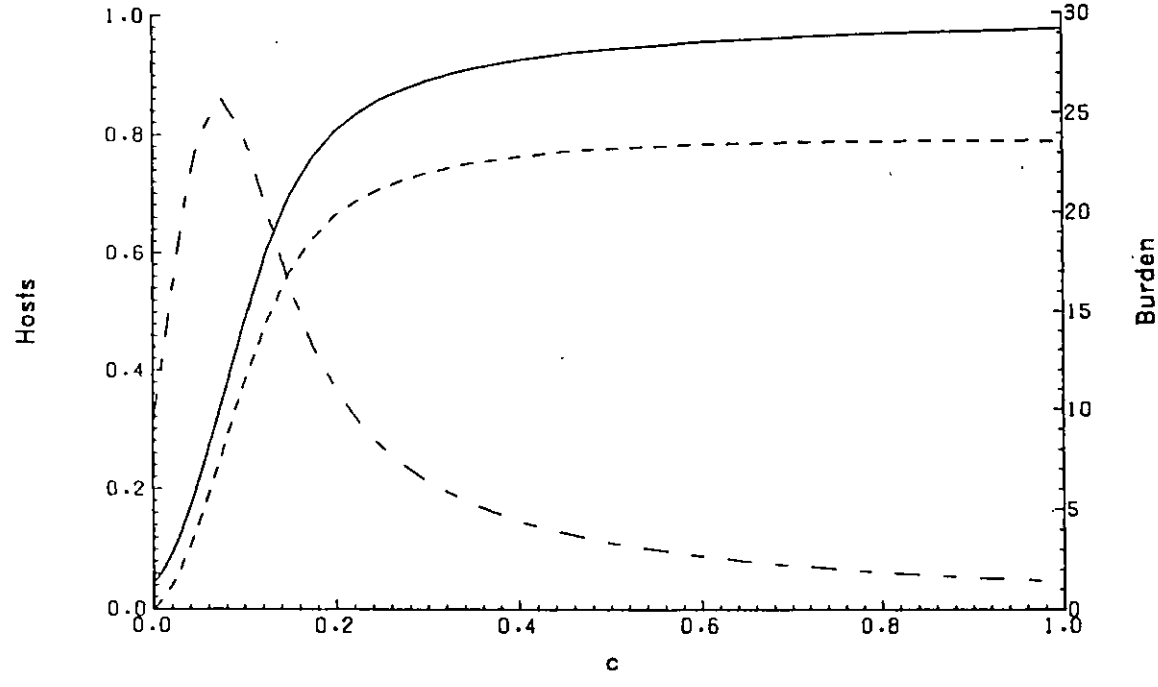
60
40
20
0
Burden
0.00
0.05
0.10
0.15
c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0.6
0.4
0.2
0.0
Hosts
30
25
20
15
10
5
0
Burden
0.0
0.2
0.4
0.6
0.8
1.0
c

Elser, 1955). The reason that this behaviour occurs can be seen in Fig. 12.16, which shows the rate of development of immunity as a function of parasite burden for the two different models. In order to make the results shown in Figs. 12.14 and 12.15 as comparable as possible, the rate of development of immunity is the same for both models at burdens equal to the equilibrium mean burden of the simple model with the parameter values used in Fig. 12.14. When parasite burdens are considerably above this level, the linear model predicts very rapid growth in the number of immune hosts, thus depressing the parasite population and stabilizing the system. The more complex model, however, does not predict this increase in the per capita rate of gaining immunity, and there is not this extremely rapid rate of increase in the number of immune hosts. The parasite population instead is depressed by parasite induced host mortality.

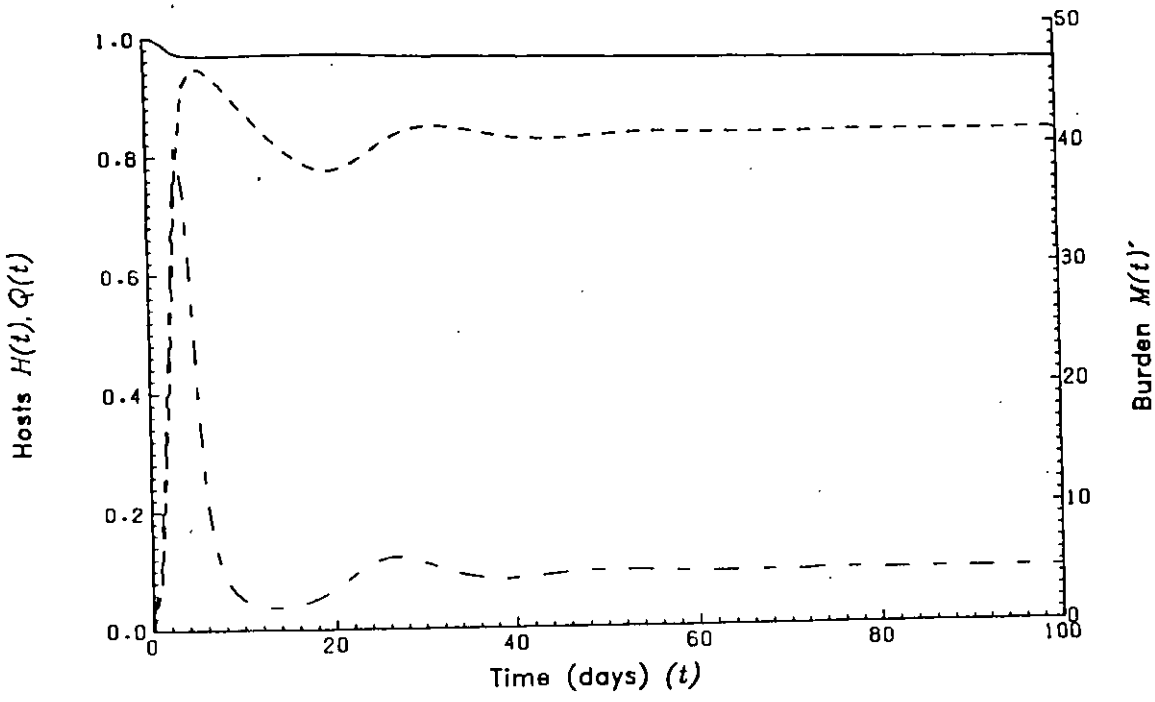
The numerical approach used to find the equilibria of this model will not necessarily locate multiple equilibrium points. It was thought conceivable that an immune response of this type might result in two stable equilibrium points, one close to the carrying capacity of the host population, with parasites regulated at a low level by the immune response, and a second, lower equilibrium where host numbers were controlled by parasite induced death, and mean parasite burdens were high enough so that the rate of parasite induced death dominated the rate of gaining immunity. Such behaviour might correspond to the behaviour of the "natural enemy ravine" model postulated by Southwood and Comins (1976). (Certain forms of non linearity in the parasite induced death rate are known to result in multiple equilibrium states (Anderson, 1979b)). In order to see if this might occur, a slightly modified version of the model described by eqns. (12.35), (12.31) and

Figure 12.14 *The approach to equilibrium of the simple model of immunity*

A time dependent solution of eqns. (12.18) - (12.20) is shown. The integration is commenced with a mean parasite burden of 1 per host, and with the host population, consisting entirely of susceptible hosts, at the disease free carrying capacity. The solid line represents $H(t)$, the total number of hosts present, which is scaled on the axis to the left of the figure. The evenly dashed line represents $Q(t)$, the proportion of hosts that are immune, and is also scaled on the axis to the left of the figure. The mean parasite burden per susceptible host, $M(t)$, is shown as an unevenly dashed line, and is scaled on the axis to the right of the figure. Parameter values used are: $a = 0.02$, $b = 0.01$, $\kappa = 0.01$, $\alpha = 0.001$, $\lambda = 4.0$, $\gamma = 0.15$, $\nu = 0.01$, $H_o = 1.0$, $\eta = 0.03$. The equilibrium with hosts present is at $H^* = 0.92$, $Q^* = 0.82$ and $M^* = 45$. This figure should be compared with Fig. 12.15.

Figure 12.15 *The approach to equilibrium of the more complex model of immunity*

A time dependent solution of eqns. (12.35), (12.31) and (12.32) is shown. As with Fig. 12.14, the integration was commenced with $M(0) = 1$, $Q(0) = 0$ and $N(0) = 1$ (Note that in this case, the rescaled host population, $N(t) = H(t)/H_o$, is used). The host population, proportion of hosts immune and mean burden on susceptible hosts are represented in the same manner as in Fig. 12.14. Parameter values used are: $a = 0.02$, $b = 0.01$, $\kappa = 0.01$, $\alpha = 0.001$ $\lambda = 4.0$, $\gamma = 0.15$, $\nu = 0.01$, $c = 0.2$, $\zeta = 0.25$. The equilibrium with parasites present is at $N^* = 0.91$, $Q^* = 0.83$ and $M^* = 5.4$. This figure should be compared with Fig. 12.14.


1.0
0.8
0.6
0.4
0.2
0.0
0
20
40
60
80
100
50
40
30
20
10
0
Hosts H(t), Q(t)
Burden M(t)
Time (days) (t)

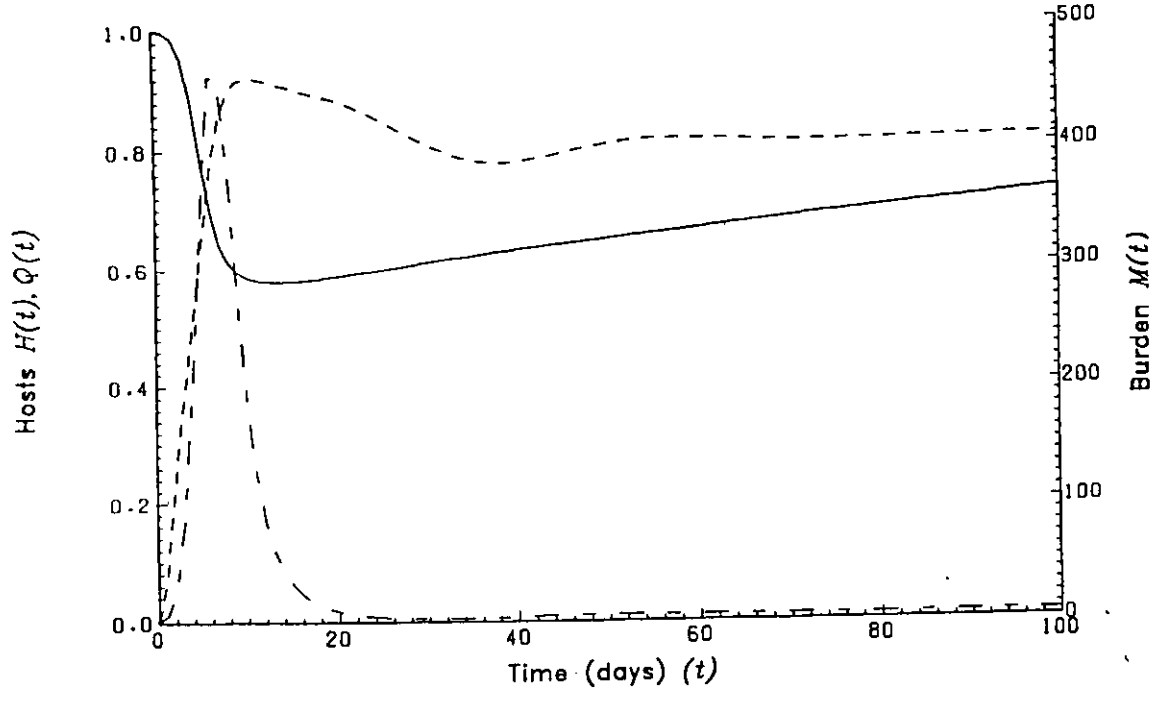
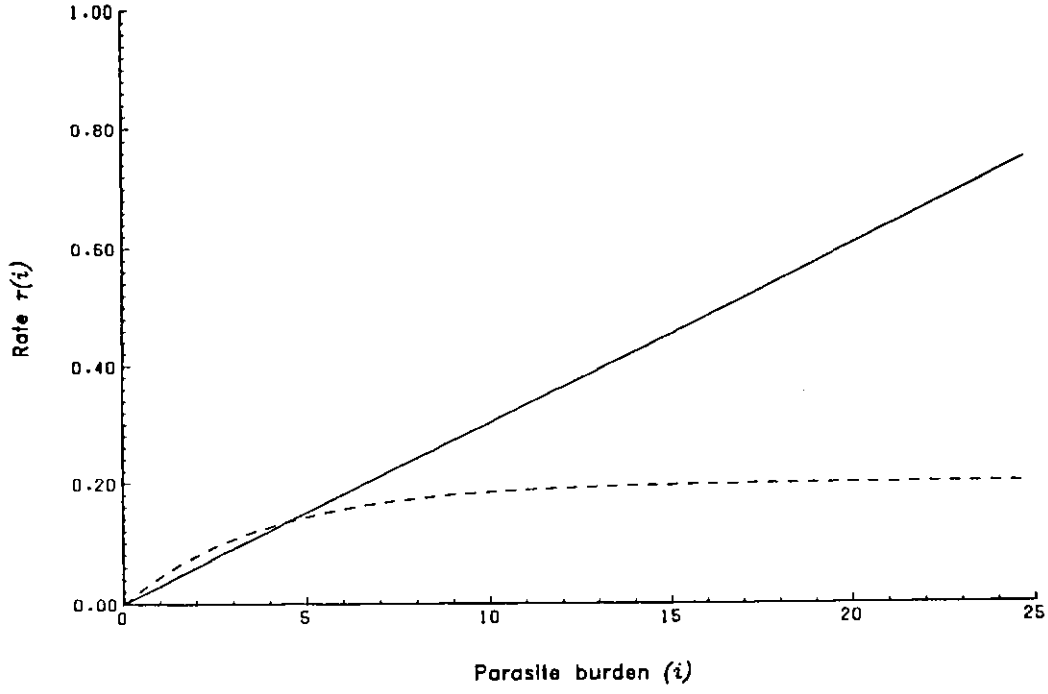

1.0
0.8
0.6
0.4
0.2
0.0
0
20
40
60
80
100
500
400
300
200
100
0
Hosts H(t), Q(t)
Burden M(t)
Time (days) (t)

Figure 12.16 *Rates of development of immunity used in Figs. 12.14 and 12.15.*

The relationship is shown between the rate of development of immunity, $r(i)$, and the parasite burden per host, $i$, in the models used to generate Figs. (12.14) and (12.15). The solid line shows the linear relationship assumed in eqns. (12.18) - (12.20) (Fig. 12.14) and the dashed line shows the relationship assumed in eqns. (12.35), (12.31) and (12.32) (Fig. 12.15).


1.00
0.80
0.60
0.40
0.20
0.00
Rate r(i)
0
5
10
15
20
25
Parasite burden (i)

(12.32) was used. The distribution of parasites on susceptible hosts was assumed to follow a negative binomial distribution. (If the distribution of parasites on hosts follows a Poisson distribution, an equilibrium in which immunity and density dependent constraints on host population are unimportant will be only neutrally stable (see Chapter 10).

Assuming that the distribution of parasites on susceptible hosts may be described by a negative binomial distribution with parameter $k$, the following equations may be obtained to describe the system:

$$dN/dt = N\{(a-b) - \kappa N - \alpha M(1-Q)\} \qquad (12.37)$$

$$dQ/dt = c\ (1-Q)\ (1 - f(M)^{-k}) - (\nu+a)\ Q + \alpha MQ(1-Q) \qquad (12.38)$$

$$dM/dt = M\left[\lambda N(1-Q)/(N+1) - (\gamma+\alpha-\nu) - cf(M)^{-(k+1)}\left[f(M)-\exp(-\zeta)\right]\right] \qquad (12.39)$$

$$\left[-(a+\nu)/(1-Q) - \alpha M/k\right]$$

Here $f(M) = 1 + (M/k)\ (1 - \exp(-\zeta))$.

All parameters and variables are as defined for eqns. (12.35), (12.31) and (12.32). At equilibrium, $N$ can be expressed in terms of $M$ and $Q$ using eqn. (12.37)

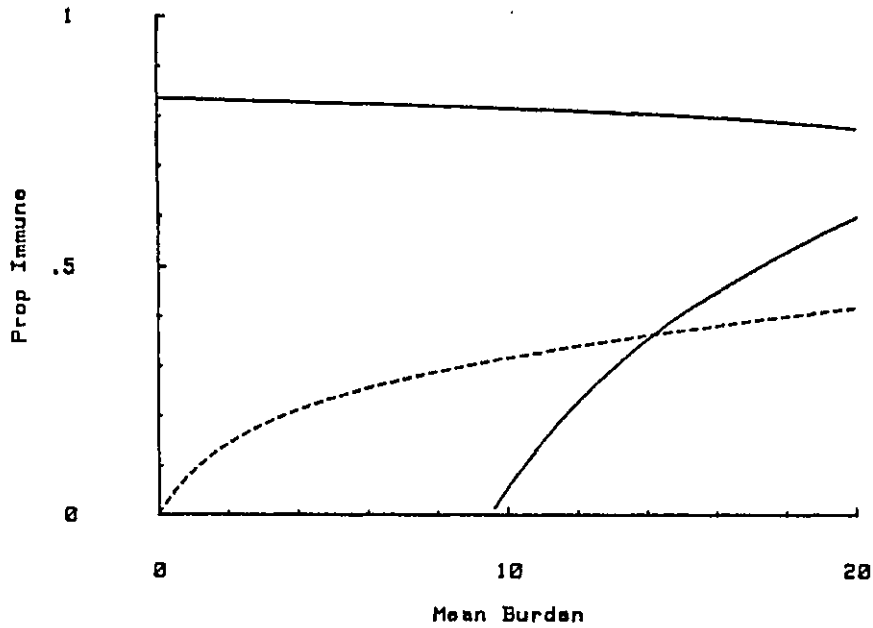
$$N = \left[a-b - \alpha M(1-Q)\right]/\kappa \qquad (12.40)$$

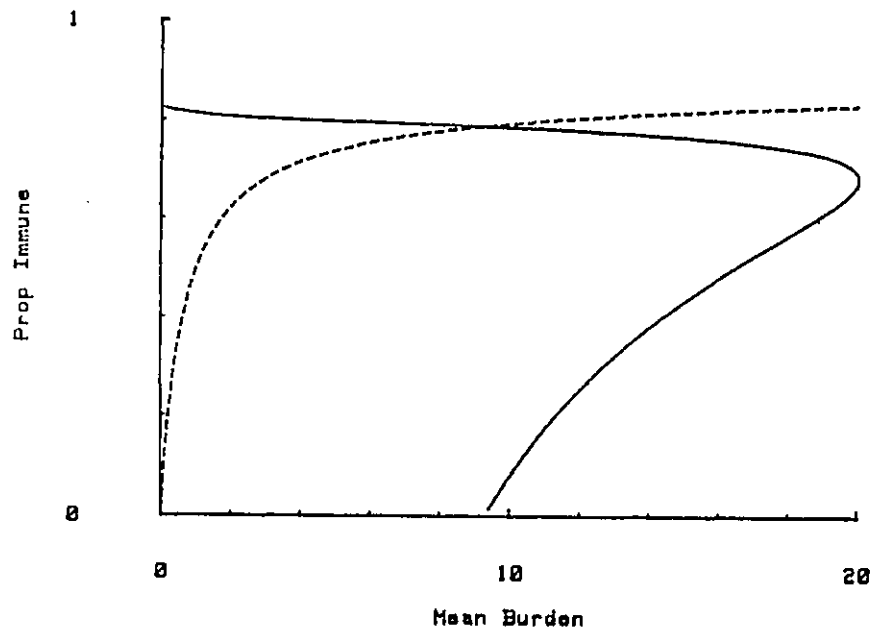
Figure 12.17 shows the curve in the $Q$ - $M$ plane along which $dQ/dt = 0$, and the curve along which $dM/dt = 0$, if $N$ is given by eqn. (12.40). Any equilibrium in the plausible region of solutions ($M^* > 0$, $0 \leq Q^* \leq 1$)

Figure 12.17 *The number of solutions to eqns. (12.37) - (12.39)*

Curves in the $M$ - $Q$ plane along which $dM/dt = 0$ (Solid line) and $dQ/dt = 0$ (dashed line) are shown. The mean burden, $M$, is shown on the horizontal axis, and the proportion of immune hosts, $Q$ is scaled on the vertical axis. The figure is based on eqns. (12.37) to (12.39). The derivation of the curves, $dM/dt = 0$ and $dQ/dt = 0$ is given in Appendix 10. At any point on the solid curve, $N$ is given by eqn. (12.4). At any intersection of the two lines shown, the eqns. (12.37) to (12.39) have an equilibrium (eqn. (12.39) has singular values when $M = 0$ which are not plotted).

Parameter values used for (a) are: $a = 0.02, b = 0.01$, $\alpha = 0.001$ $\lambda = 4.0$, $\gamma = 0.15$, $k = 0.5$, $\kappa = 0.01$, $\nu = 0.01, \zeta = 0.2$, $c = 0.02$. Parameter values used for (b) are the same, except $c = 0.2$.


1
.5
0
Prop Immune
0
10
20
Mean Burden


1
0
Prop Immune
0
10
20
Mean Burden

will be shown as an intersection of the two curves. It should be emphasized that Fig. 12.17 is not a phase plane in the sense of Maynard Smith (1974). The figure represents the surface within three dimensional phase space on which $N$ is at equilibrium with respect to $M$ and $Q$. Trajectories of the system with time cannot therefore be drawn on the figure, as they will not necessarily remain within the plane. A wide range of parameter combinations were examined, but in no case was more than one non trivial solution ($M \neq 0$) found. It therefore does not appear that the system described by eqns. (12.37) - (12.39) has multiple stable states.

## 12.5 *Partial Immunity*

The experiments of Chapter 6 show that the immunity to *Ichthyophthirius* developed by black mollies is not complete. Partial immunity only of hosts to parasitic infection appears to be the rule rather than the exception (Weinmann, 1970; Cohen, 1974).

In this section, the model described by eqns. (12.5 - 12.7) is modified to allow for only partial resistance of immune hosts to further infection.

It is assumed that parasites are distributed in a random fashion within the populations of both susceptible and resistant hosts, and that the rate of transition from the susceptible to the resistant class is linearly dependent on parasite burden. Trophozoites currently on a host are assumed to survive this transition, and resistant hosts are assumed to rejoin the susceptible class at a constant per capita rate, $\nu$, independent of their parasite burden. The proportion of tomites surviving to form trophozoites on resistant hosts is assumed to be lower, by a factor $s$, than the proportion surviving on susceptible

hosts. A minimum of four variables is required to describe the system. Two classes of host must be specified; $H_1(t)$, fully susceptible hosts, and $H_2(t)$, resistant hosts. The parasite population must likewise be divided into two categories; $P_1(t)$, parasites on susceptible hosts, and $P_2(t)$, parasites on the resistant fish. Using the parameters defined in Table 12.1, the following four equations may be derived to describe temporal changes in the above variables:

$$\frac{dH_1}{dt} = a\ (H_1 + H_2) - bH_2 - (\alpha+\eta)P_1 + \nu H_2 \qquad (12.41)$$

$$\frac{dH_2}{dt} = \eta P_1 - (\nu+b)H_2 - \alpha P_2 \qquad (12.42)$$

$$\frac{dP_1}{dt} = \frac{\lambda(P_1 + P_2)H_1}{(H_1 + H_2 + H_o)} - (\gamma+\eta+\alpha+b)P_1+\nu P_2 - \frac{(\alpha+\eta)P_1^2}{H_1} \qquad (12.43)$$

$$\frac{dP_2}{dt} = \frac{\lambda s(P_1+P_2)H_2}{(H_1 + H_2 + H_c)} - (\gamma+\alpha+\nu+b)P_2 - \frac{\alpha P_2^2}{H_2} + \eta P_1 (1+ \frac{P_1}{H_1}) \qquad (12.44)$$

The parameters are as defined in Table 12.1. Unfortunately, it is difficult to make analytical progress with these four equations. The threshold host population necessary for parasite persistence is:

$$H_T = H_o / \{\lambda/(\alpha+b+\gamma) - 1 \qquad (12.45)$$

If immunity acts only to restrict further establishment of parasites, and does not affect parasites already on the host, the rate of acquisition of immunity does not affect the conditions for parasite persistence. Figures 12.18 - 12.21 show numerical integrations of eqns. (12.41 - 12.44) with a number of differing values of $s$ and $\eta$. Unlike the simple model (eqns. 12.5 - 12.7), a rapid rate of gaining immunity

does not appear to enable hosts to entirely escape control by the parasite, because resistant hosts continue to harbour some infection (Fig. 12.19). Apart from this, the behaviour of the model is not qualitatively different from the behaviour of eqns. (12.5 - 12.7). Some insight into the behaviour of this system may be gained by considering Fig. 10.2, which shows the behaviour of the basic model of *Ichthyopthirius* (eqns. 10.7, 10.9 and 10.20) in terms of the number of tomites establishing per trophozoite leaving the host, $\Lambda$, and the degree of overdispersion, $k$. An increase in the value of $s$, the relative susceptibility of the resistant hosts, has the effect of decreasing the degree of heterogeneity of the host population, and thus makes the system less stable (compare Figs 12.18 and 12.21). Low values of $s$, and high rates of entering the resistant class ($\eta$ large), however, have the effect of decreasing the reproductive potential of the parasite, and increasing the degree of heterogeneity, thus diminishing the net impact of the parasite on host survival (the majority of parasites are harboured by a few hosts) (Fig. 12.19).

## 12.6 *Conclusions and Discussion*

The previous analysis suggests that host immunity acts to stabilize host-parasite interactions. Too high a rate of development of immunity (relative to the rate at which the parasite increases host mortality) may lead to a parasite being unable to control the population of its host. The conditions determining whether or not a parasite can control a host population are similar to those produced by the microparasitic model of immunity discussed by Anderson and May (1979).

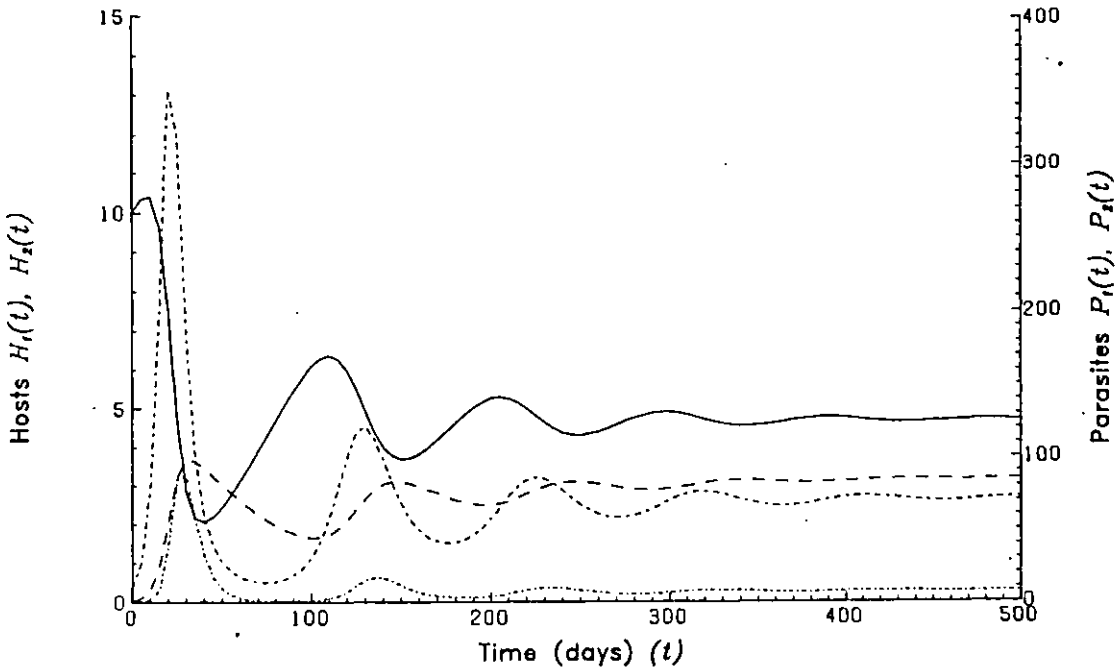
If host populations are regulated by density dependent constraints other than parasitism, both the simple and more complex models make

Figure 12.18 *Partial immunity ($s = 0.01$, $\eta = 0.001$)*

A time dependent solution of the system represented by eqns. (12.41) - (12.44) is shown. The solid line represents the number of fully susceptible hosts ($H_1(t)$), and the evenly dashed line the number of resistant hosts ($H_2(t)$). These two variables are scaled on the axis to the left of the figure. The two unevenly dashed lines represent the number of parasites on susceptible hosts ($P_1(t)$) and the number of parasites on resistant hosts ($P_2(t)$), which is represented by the more finely subdivided line. Both these variables are scaled on the axis to the right of the figure. Parameter values used are: $a = 0.02$, $b = 0.01$, $\alpha = 0.001$, $\gamma = 0.15$, $\lambda = 4.0$, $H_o = 100.$, $\nu = 0.01$, $s = 0.01$ and $\eta = 0.001$.

Figure 12.19 *Partial immunity ($s = 0.01$, $\eta = 0.01$)*

A time dependent solution of the system represented by eqns. (12.41) to (12.44) is shown. The variables are represented in the same way as in Fig. 12.18. The parameters used are also the same, except $s = 0.01$ and $\eta = 0.01$. If the integration is continued, an equilibrium is eventually reached at approximately $H_1^* = 9.8$, $H_2^* = 143.$, $P_1^* = 400.$, $P_2^* = 1130.$


15
10
5
0
0
100
200
300
400
500
400
300
200
100
0
Hosts $H_1(t)$, $H_2(t)$
Parasites $P_1(t)$, $P_2(t)$
Time (days) $(t)$

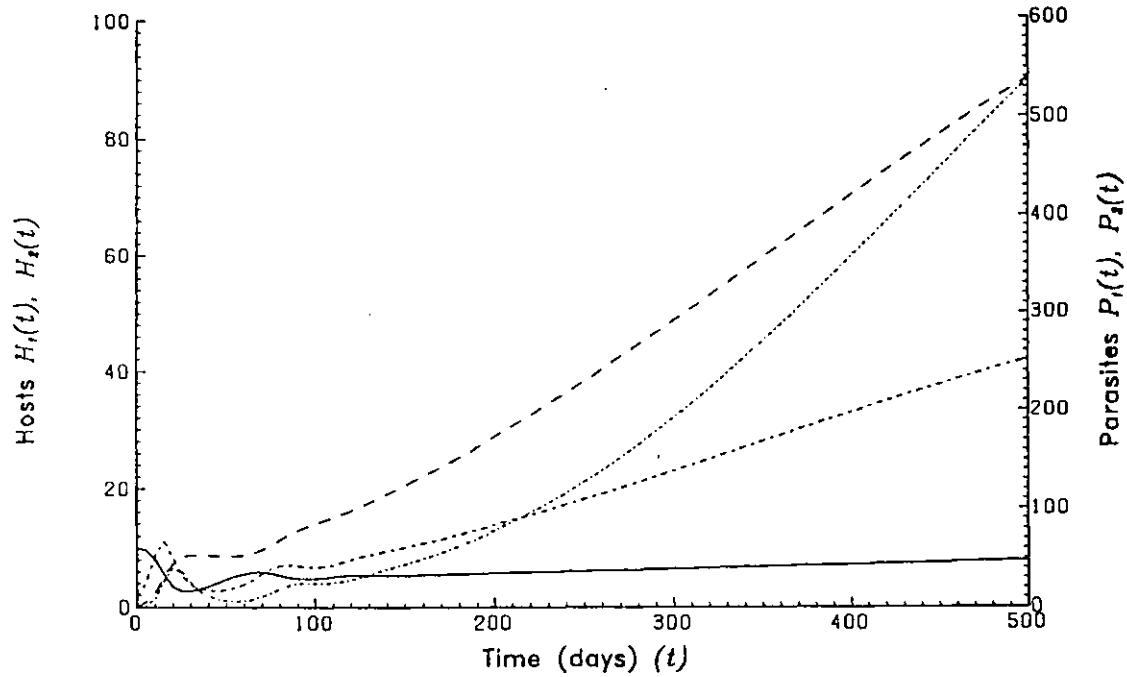
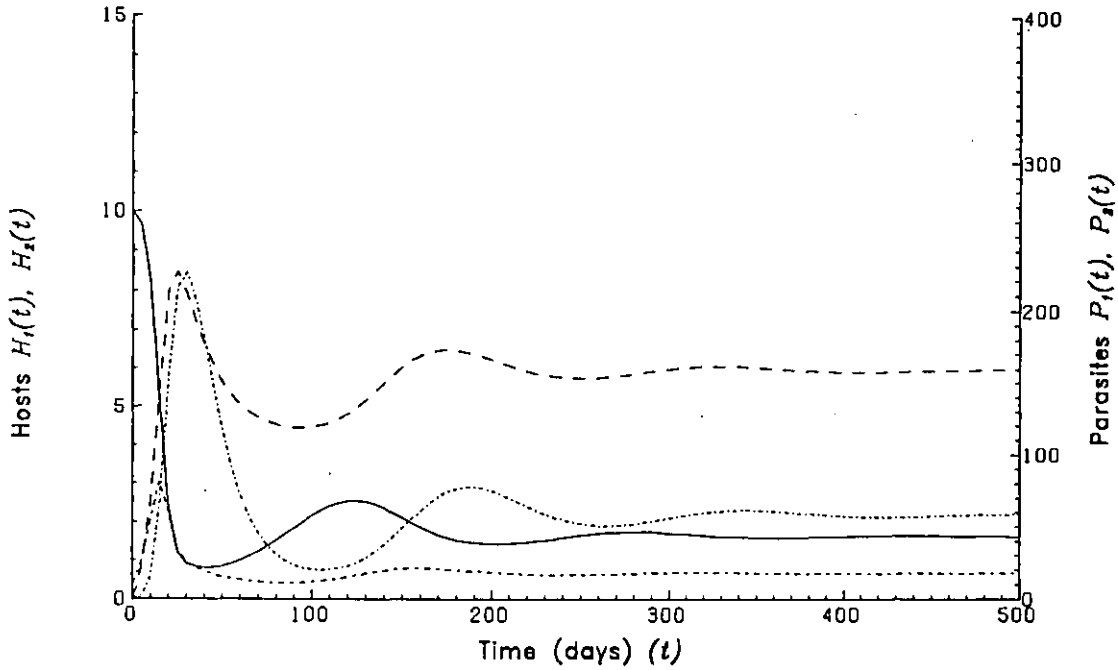

100
80
60
40
20
0
0
100
200
300
400
500
600
500
400
300
200
100
0
Hosts $H_1(t)$, $H_2(t)$
Parasites $P_1(t)$, $P_2(t)$
Time (days) $(t)$

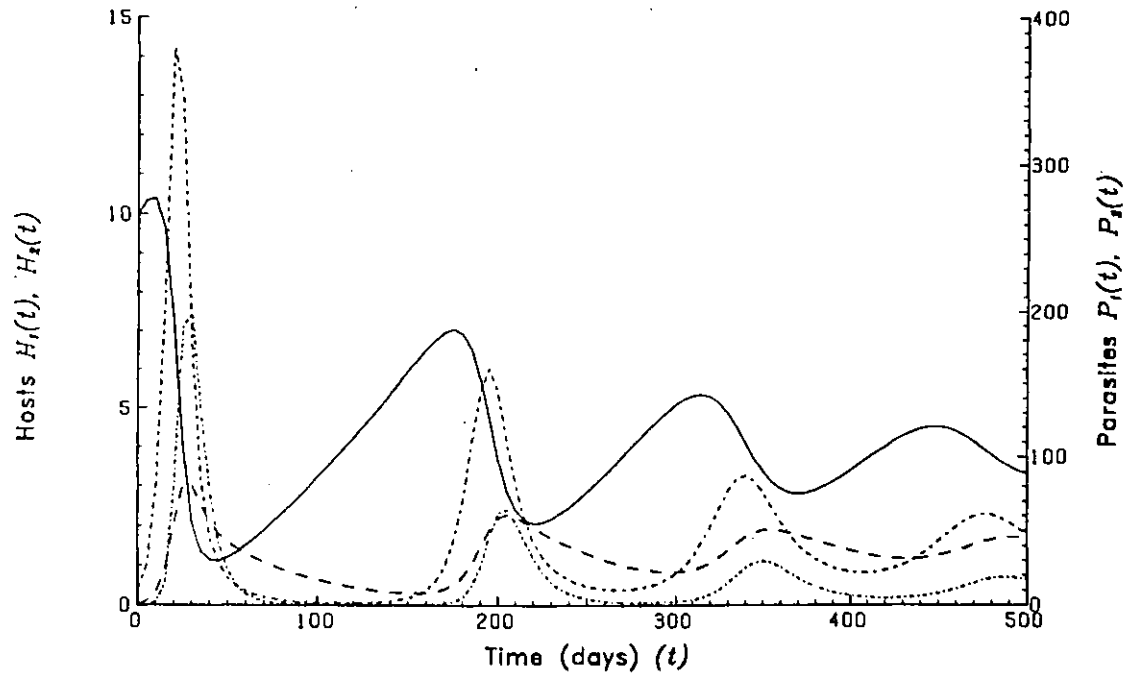
Figure 12.20. *Partial immunity (s = 0.5, η = 0.01)*

A time dependent solution of the system represented by eqns. (12.41) - (12.44) is shown. The variables are represented in the same way as in Figs. 12.18 and 12.19, and the same initial conditions as Fig. 12.18 are used. Parameter values are also the same, except $s = 0.5$ and $\eta = 0.01$.

Figure 12.21. *Partial immunity (s = 0.5, η = 0.001)*

A time dependent solution of the system represented by eqns. (12.41) - (12.44) is shown. The variables are represented in the same way as Fig. 12.18, and the same initial conditions are used. Parameter values are also the same, except $s = 0.5$ and $\eta = 0.001$.


15
10
5
0
0
100
200
300
400
500
400
300
200
100
0
Hosts $H_1(t)$, $H_2(t)$
Parasites $P_1(t)$, $P_2(t)$
Time (days) $(t)$


15
10
5
0
0
100
200
300
400
500
400
300
200
100
0
Hosts $H_1(t)$, $H_2(t)$
Parasites $P_1(t)$, $P_2(t)$
Time (days) $(t)$

similar predictions about the equilibrium established. If the rate of gaining immunity is rapid, the parasite will be maintained at a low level in a host population close to its carrying capacity, with most hosts immune. The results of the experiments discussed in Chapters 6 and 7 indicate that the rate of development of immunity to *Ichthyophthirius* is rapid compared to the parasite induced death rate at low parasite burdens. Weinmann (1958) reports that immunity to *Hymenolepis nana* develops rapidly in mice after exposure to low numbers of eggs of the cestode. High rates of development of immunity may therefore be responsible for the low levels of parasite infection observed for some parasites in natural conditions.

The more complex model discussed, in which the rate of development of immunity reaches a maximum value as parasite burden rises, predicts outbreaks of parasites under certain conditions. Stochastic factors may lead to parasites present at low levels being eliminated from host populations. In such cases, the number of immune hosts will decline and the host population will increase to its disease free carrying capacity. Parasites reintroduced to such populations will increase rapidly, before the number of immune hosts builds up, and produce high levels of host mortality. The simpler model, in which immunity is a linear function of parasite burden, is unable to generate such behaviour.

The most obvious shortcoming of the models discussed in detail is that the immunity to infection is assumed to be solid, whereas experimental evidence suggests that hosts remain partially susceptible to *Ichthyophthirius*. Limited analysis of a model in which immunity

gives only partial resistance to future infection reveals few qualitative differences in behaviour. One difference results from hosts that become immune not killing the parasites they currently harbour. In this case, the immune response does not diminish the ability of a parasite to invade a host population. This will further increase the region of parameter values within which the parasite is maintained endemically within the host population.

The model of partial immunity examined assumes a constant rate of loss of partial immunity. It is probable, however, that a degree of resistance to infection may be maintained by continual re-exposure to infection. Aron and May (1982) have developed a model for the dynamics of malaria which includes this feature. Hosts are assumed to lose their partial resistance to infection if not reinfected for a period $\tau$. The model is difficult to generalize to cases in which the host population is a dynamic variable as, in the Aron and May model, it is assumed that the per capita rate of infection is constant.

# CHAPTER 13

# THE EFFECT OF DIFFERENCES IN HOST SUSCEPTIBILITY ON POPULATION DYNAMICS

## 13.1 *Introduction*

In the basic models of host parasite interactions developed by Anderson and May (1978) and discussed in Chapter 10 of this thesis, it is assumed that the distribution of parasites within the host population may be described by a negative binomial distribution with the parameter $k$, which determines the degree of overdispersion, independent of the mean parasite burden. It is shown in Section 8.3 that reasonable assumptions about host susceptibility lead to parasite burdens established from single infections following distributions that are approximately negative binomial in form, with $k$ independent of the mean parasite burden. In the case discussed, infective stages were assumed to be randomly distributed between hosts with differing susceptibilities to infection.

In natural populations of hosts and parasites, many factors may affect the distribution of parasites within the host population. Reproduction within the host will obviously result in considerable aggregation of parasites, but even restricting the discussion to parasites incapable of reproduction without leaving the host, a great number of possible causes of overdispersion remain. Clumped infective stage distributions will be reflected in overdispersion in the parasite distribution amongst hosts (Keymer and Anderson, 1979) or equivalently host lifestyle or behaviour may result in non random encounters with infective stages. Hetereogeneity in parasite burdens can also be generated by the age structure of a host population: younger hosts

have been exposed to infection for a shorter time than older hosts, and if parasites are long lived, mean parasite burden will increase with age (Anderson, 1982). Conversely, prevalence of infection may decrease with host age if older hosts acquire resistance to infection (Aron and May, 1982). Immunity to infection has been discussed as a source of overdispersion in the previous chapter.

Other factors may act to decrease heterogeneity in parasite burdens per host. These are the density dependent constraints, of various forms, that act on parasites within individual hosts. Under laboratory conditions, if the rate of infection is high, and the number of infective stages low, distributions that are underdispersed may become established (eg Anderson, Whitfield and Mills,1977). Death of heavily infected animals lops off the upper tail of the distribution of parasites on hosts, and might be expected to decrease the degree of overdispersion.

With this range of possible influences on parasite distributions, the relationship between the degree of parasite overdispersion and mean parasite burden is likely to be extremely complex, and dependent to a large extent on the biology of the particular system in question. This chapter restricts its attention to one factor only - the effect of differing host susceptibility to infection on overdispersion when host population size is a dynamic variable.

## 13.2 *A Deterministic Model*

As a beginning to examining this problem, it will be assumed that there are two types of host: those that are susceptible to infections and those that are fully resistant. If these two classes cannot be

distinguished, apart from the size of their parasite burden, the overall distribution of parasites within the host population will appear to be aggregated. Random infective stage distribution will result in the distribution of parasites within the host population having the form of a Poisson distribution with added zeroes. The mean and variance of this distribution are given by Peilou (1969).

Suppose that a proportion, $x$, of all hosts born are equally susceptible to infection, the remainder being totally resistant. This proportion is assumed to be fixed: the probability of being born susceptible does not depend on the susceptibility of the parents. Representing the susceptible hosts by $H_1(t)$ and the resistant hosts by $H_2(t)$, the following equations may be obtained to describe temporal changes in the variables $H_1(t)$, $H_2(t)$ and the number of parasites, $P(t)$.

$$dH_1/dt = ax(H_1 + H_2) - bH_1 - \alpha P \qquad (13.1)$$

$$dH_2/dt = a(1-x)\ (H_1 + H_2) - bH_2 \qquad (13.2)$$

$$dP/dt = \lambda PH_1/(H_1 + H_2 + H_o) - (\gamma+\alpha+b)P - \alpha P^2/H_1 \qquad (13.3)$$

Construction of the model and parameter definitions follow those outlined in Chapter 10. It is immediately clear that, for an equilibrium to exist, there is a lower limit on $x$, the proportion of susceptible hosts. If

$$a(1 - x) > b \qquad (13.4)$$

the number of resistant hosts will increase without limit, and the parasite will not be able to persist in the host population. The system is best analyzed by introducing new variables: $H = H_1 + H_2$, the total host population; $R = H_2/H$, the proportion of resistant hosts; and $M = P/H_1$, the mean parasite burden on susceptible hosts. Equations (13.1) - (13.3) then become

$$dH/dt = H\left[(a-b) - \alpha M(1 - R)\right] \qquad (13.5)$$

$$dR/dt = a(1-x) - aR + \alpha MR(1 - R) \qquad (13.6)$$

$$dM/dt = M\left[\lambda H(1-R)/(H+H_o) - (\gamma+\alpha) - ax/(1-R)\right] \qquad (13.7)$$

A close resemblance between eqns. (13.5 - 7) and eqns. (12.18 - 20) is evident.

An equilibrium, which can be shown to be stable (Appendix 11), will exist if condition (13.4) does not hold, and if

$$\lambda(1 - a(1-x)/b) > \alpha+\gamma+ax + a^2(1-x)/\left[b-a(1-x)\right] \qquad (13.8)$$

At this equilibrium, the proportion of resistant hosts, $R^*$, is

$$R^* = a(1-x)/b \qquad (13.9)$$

and the mean burden on susceptibles, $M^*$, is

$$M^* = b(a-b)/\left[\alpha(b-a(1-x))\right] \qquad (13.10)$$

An interesting feature of the model is that the overall mean parasite burden (obtained from eqns. (13.9) and (13.10)) is $(a-b)/\alpha$, the same as in the basic model (eqns. (10.27) and (10.28)) and is unaffected by the proportion of hosts that are resistant to infection. At equilibrium, the variance in parasite numbers per host, var $(M)$ is

$$\text{var}(M) = \left(\frac{a-b}{\alpha}\right)\left[1 + \frac{a(a-b)(1-x)}{\alpha[b-a(1-x)]}\right] \qquad (13.11)$$

using formulae given by Pielou (1969)..

A quantity which can be used as a useful measure of the degree of overdispersion is

$$k' = (\bar{x})^2 / (s^2 - \bar{x}) \qquad (13.12)$$

where $\bar{x}$ and $s^2$ are the mean and variance of the burden. If the distribution is negative binomial, this is the parameter $k$ which inversely describes the degree of overdispersion (in the case under discussion here, $k'$ does not have this direct meaning, as the distribution is not strictly negative binomial).

At equilibrium, $k'$ is found to be

$$k' = x/(1-x) - (a-b)/[a(1-x)] \qquad (13.13)$$

This should be compared with the $k'$ which would result from a single infection of a population of hosts when a proportion, $x$, are susceptible to infection:

$$k' = x/(1-x) \qquad (13.14)$$

(obtained from eqn. 8.3).

For any combination of parameters which permits the existence of a stable equilibrium, the model predicts that a greater degree of overdispersion exists at the equilibrium of a free running population than is produced by a single infection of a population with the same initial proportion of susceptible hosts. The equilibrium mean susceptibility to infection, $1-R^*$, is also always less than the susceptibility of the same host population before the introduction of infection. In this very simple model, both the mean susceptibility to infection and the degree of overdispersion of parasites within the host population are dynamic variables (Fig. 13.1). The expression giving $k'$ at any particular time, $k' = (1-R)R$, does not explicitly involve the mean parasite burden, $M$. The next section considers a more complex simulation model, in which the distribution of susceptibility of hosts to infection is a continuous function.

## 13.3 *A Simulation Model*

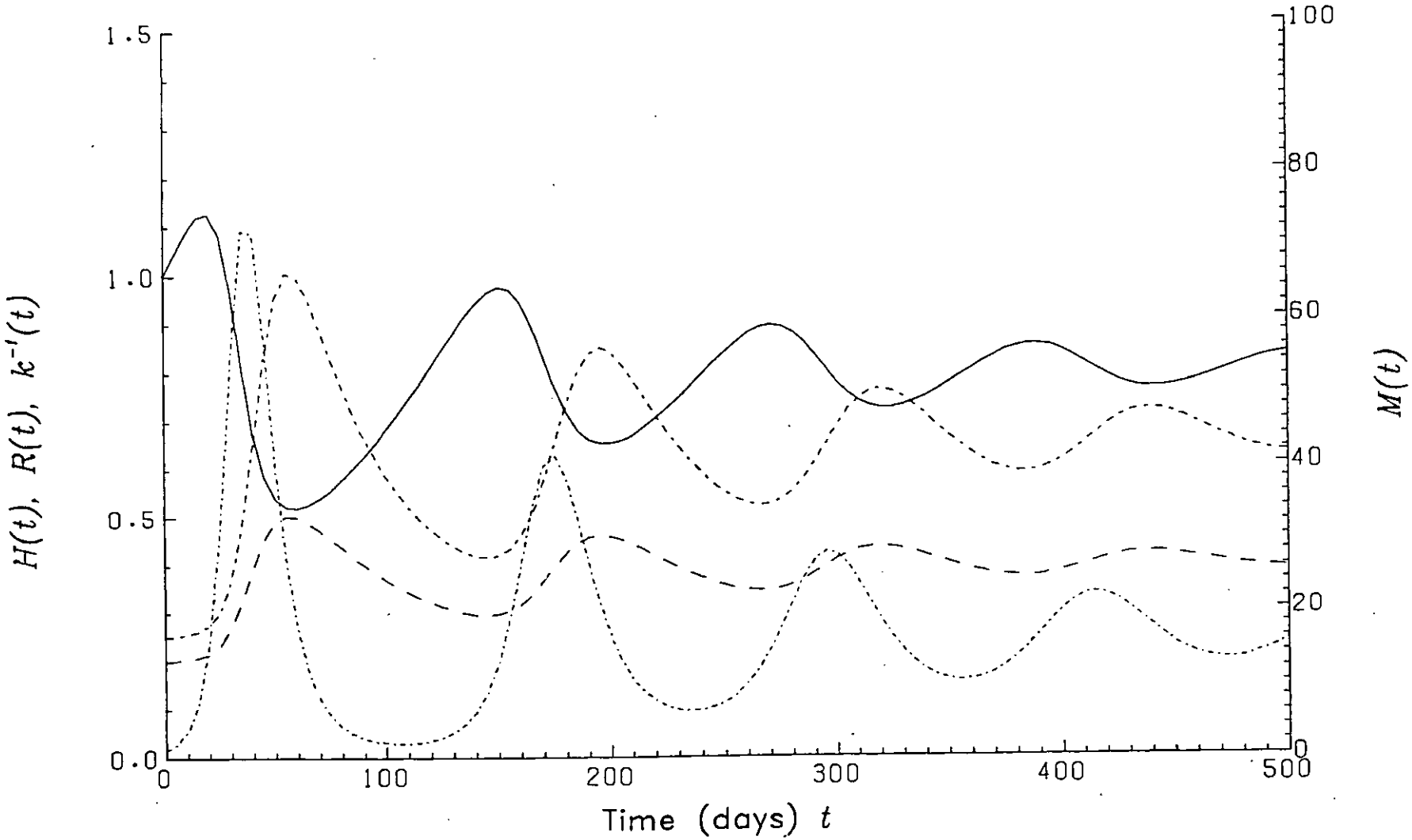
The model developed in the previous section allows a limited degree of difference in susceptibility between hosts, but imposes, *a priori*, a Poisson distribution of parasites on the susceptible hosts. Parasite induced mortality will have a tendency to decrease the heterogeneity of parasite burdens on susceptible hosts by removing hosts with heavy parasite burdens. This is a difficult factor to handle within the constraints of these purely deterministic models. This simulation model attempts to examine the results of this dynamic interplay between parasite induced death reducing overdispersion and differing susceptibilities of hosts increasing it. This interplay is an important feature of natural host parasite systems (Anderson and

Figure 13.1. *Results of the deterministic model in which a proportion of hosts are resistant to infection*

A numerical solution is shown of the system described by eqns. (13.5) - (13.7) (in which a fixed proportion of all hosts both are resistant to infection). Three variables are scaled on the axis to the left of the figure, the total host population, $H(t)$ (shown as a solid line); the proportion of resistant hosts, $R(t)$ (shown as an evenly dashed line); and the inverse of the parameter $k'$ (determined from the equation $k^{-1} = R(t)/(1 - R(t))$, and shown as the unevenly dashed line with the longer length dashes). The mean parasite burden on susceptible hosts, $M(t)$, is scaled according to the axis to the right of the figure, and is shown as the unevenly dashed line with dashes of a shorter length than $k^{-1}(t)$.

Parameter values used are: $a = 0.02$, $b = 0.01$, $\alpha = 0.001$, $\lambda = 4.0$, $\gamma = 0.15$, $H_o = 10.0$, $x = 0.8$; and initial conditions are $H(o) = 1.$, $R(o) = 0.2$, $M(o) = 1.0$.

Note that whilst both $R(t)$ and $k^{-1}(t)$ change in an oscillatory fashion, both the proportion of resistant hosts and the degree of overdispersion are always greater than at the introduction of the parasite.


1.5
1.0
0.5
0.0
0
100
200
300
400
500
Time (days) t
H(t), R(t), k⁻¹(t)
M(t)
100
80
60
40
20
0

Gordon, 1982). The model is not intended to be any more realistic or detailed than the simple models discussed in Chapter 10. The object is to construct a model identical, as far as is possible, to eqns. (10.27) and (10.28), but instead of imposing a negative binomial distribution on the parasite population, the distribution is generated by the dynamics of the interaction between host and parasite concomitant with differing host susceptibilities to infection. As far as is possible, the model is deterministic. Stochastic elements are introduced only when necessary to consider the distribution of parasites on hosts. The model is thus hybrid in structure, containing deterministic and stochastic elements.

13.3.1. *Model structure*

The simulation is commenced at time 0 with $H(0)$ hosts. Each of these is assumed to have a susceptibility, $s$, to infection between 0 and 1. The susceptibility, $s_j$ of the $j$th host present initially, and of all hosts added during the course of the infection is determined by sampling from a beta distribution, which has a probability density function:

$$B(s) = \frac{\Gamma(l+m)}{\Gamma(m)\Gamma(l)} \; s^{l-1} \; (1-s)^{m-1} \qquad (13.15)$$

The parameters $l$ and $m$ together specify the mean and variance of the distribution. For the simulation, values of $l = 0.5$ and $m = 4$ were used. If an infection is commenced with a mean of $T$ tomites encountering each host, these parameter values will result in a distribution approximately negative binomial in form being established, with mean of $0.11T$ and $k = 0.5$.

Initially, $T(0)$ tomites are assumed to be present to commence the simulation, and the number of tomites establishing on the $j$th host is determined by sampling from a Poisson distribution with mean

$$\bar{x}_j = s_j T(0) \,/\, H(0) \tag{13.16}$$

After initialization, the system is run with a finite step length of $\tau$ days. Iterations proceed as follows:

(i) Each host has a probability $q$ of surviving from the previous step, where

$$q = \exp(-[b+\alpha i]\tau) \tag{13.17}$$

Here $b$ is the instantaneous disease independent rate of host death, and $\tau$ the per parasite increment in parasite induced mortality.

(ii) Hosts are assumed to have a deterministic instantaneous per capita rate of increase, $a$. The number of new fish present, $H_a$, is thus

$$H_a = H(t)\ [\exp(a\tau) - 1] \tag{13.18}$$

where $H(t)$ is the number of hosts present after mortality in (i). If the result of eqn. (13.18) is not an integer, it is rounded down and the fractional part added to an accumulator which is added to $H_a$ for the next iteration. New hosts are assigned susceptibilities from the beta distribution specified in eqn. (13.15).

(iii) The parasite burden of each host, $i_j(t+\tau)$, is determined by the following equation:

$$i_j(t+\tau) = i_j(t) \exp\left[-(\gamma+\mu)\tau\right] + I_j \qquad (13.19)$$

where $\mu$ is the instantaneous per capita death rate of trophozoites on the host, and $\gamma$ is their per capita emigration rate. $I_j$ is generated from a Poisson distribution with mean

$$T(t)\, s_j / \left[H(t) + H_o\right] \qquad (13.20)$$

where $T(t)$ is the number of tomites present at time $t$, $s_j$ is the susceptibility of the $j$th host to infection, $H(t)$ is the size of the host population at time $t$ and $H_o$ is a saturation term.

(iv) $T(t+\tau)$, the number of tomites present at the next time interval, is

$$T(t+\tau) = \frac{\lambda\gamma}{(\gamma+\mu)} \sum_{j=1}^{H} \left[1 - e^{-(\gamma+\mu)\tau}\right] i_j(t) \qquad (13.21)$$

where $\lambda$ is the number of infective stages produced per trophozoite.

To an accuracy of terms in $\tau^2$, this system is an analogue of

$$dH/dt = H(a-b-\alpha M) \qquad (13.22)$$

$$dM/dt = \lambda \bar{s}\, HM\gamma/(H+H_o) - M(\gamma+\mu+b) - \alpha E(i^2) \qquad (13.23)$$

in which $\bar{s}$ and $E(i^2)$ are dynamic variables.

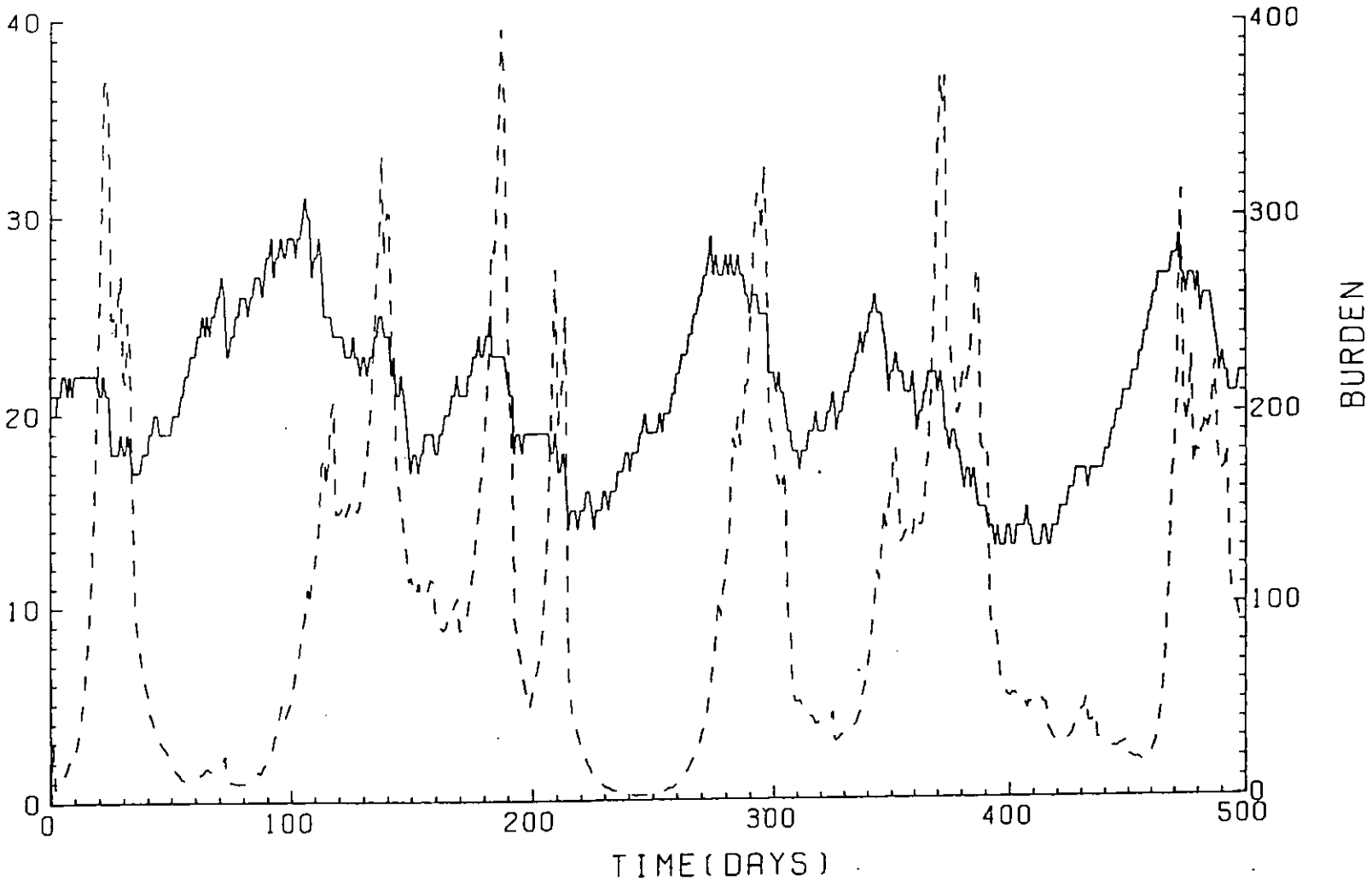
13.3.2 *Simulation results*

Figure 13.2 shows the changes in number of hosts and mean parasite burden during the course of one simulation run. A strong tendency toward oscillatory behaviour is evident, increases in mean parasite burden leading to crashes in host population numbers (as in all the host-parasite models discussed in this thesis, the actual peak in mean parasite burden lags behind the peak in host population). Other simulation runs produced qualitatively similar results. In no case did the oscillation appear to damp out to a stable equilibrium. White noise (uncorrelated stochastic variation) in the parameters governing the behaviour of a two species predator prey system may lead to maintenance of oscillations in a case where the equilibrium would otherwise be stable (May, 1974). The stochastic variation in this model has a similar effect, although random variations introduced in host susceptibility may be maintained for more than one time period, until the removal of the host from the system. There is therefore serial correlation in the varying terms. Similar results have also been obtained by Bartlett (1957) for models of recurrent epidemic behaviour, demographic stochasticity acting to pump otherwise damped oscillations.

The changes in host susceptibility with time during the course of the simulation run of Fig. 13.2 are shown in Fig. 13.3. Recalling that the expected susceptibility of hosts at birth is 0.11, it is clear that the mean susceptibility of the host population is at a level very much lower than this. A beta distribution with the values of $l$ and $m$ used in this simulation is strongly positively skewed, and the few

Figure 13.2 *Results of the simulation model*

The figure shows the results of the simulation described in Section 13.3. The number of hosts present is shown as a solid line, and is scaled on the axis to the left of the figure. The dashed line represents the mean parasite burden per host, and is scaled on the axis to the right of the figure. Parameter values used are: $a$ = 0.02, $b$ = 0.01, $\alpha$ = 0.001, $\lambda$ = 300, $\gamma$ = 0.15, $\mu$ = 0.05, $H_o$ = 100, $\tau$ = 1.0. The simulation was commenced with $T(0) = 10^4$ and $H(0)$ = 20. (If the step length, $\tau$, is reduced to 0.5, the interval between parasite outbreaks remains at around 100 days).


40
30
20
10
0
0
100
200
300
400
500
TIME(DAYS)
400
300
200
100
0
BURDEN

highly susceptible outliers are rapidly eliminated from the population due to parasite induced death. This lowered susceptibility is not the result of natural selection in the normal sense of the term: the distribution of susceptibility of offspring to infection remains constant. In a real population, the offspring of hosts resistant to infection would themselves tend to be resistant, further reducing the overall susceptibility of the host population. There is also some evidence that susceptibility is a dynamic variable, being subject to other than purely random perturbations. When parasite burden is low, there is less selective pressure against highly susceptible hosts, and mean susceptibility tends to rise. This behaviour resembles that of the simple model discussed in Section 13.2.

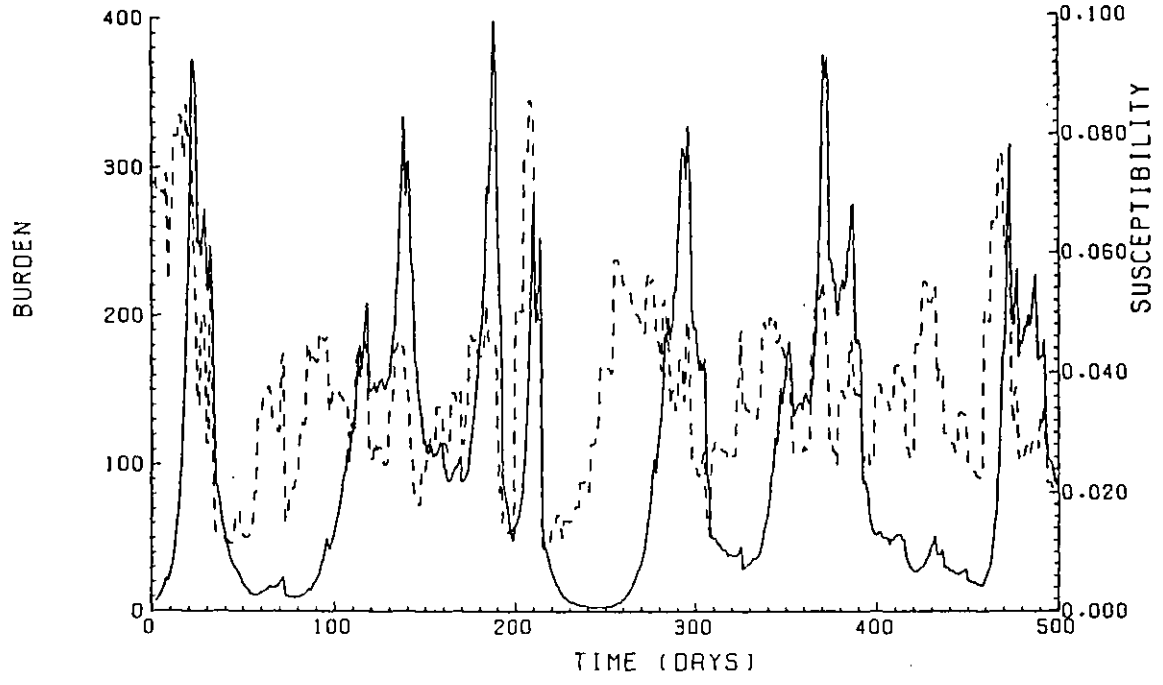
The changes with time in the degree of overdispersion of the parasite distribution are shown in Fig. 13.4. Overdispersion is measured in this case by the inverse of the moment estimate of $k$, determined according to eqn. (8.4). Estimates of $k$ for fairly small populations show a high degree of variability (Taylor et al 1979). The degree of variability of the plotted values of $1/k$ does not necessarily reflect the same degree of variability in the underlying degree of overdispersion. There is no clear evidence of a relationship between the degree of aggregation, as measured by $1/k$, and the mean parasite burden per host. At one point only, the distribution approaches randomness, but for most of the course of the simulation, the distribution of parasites on hosts is highly aggregated, to a similar or greater degree than the distribution with which the simulation was commenced.

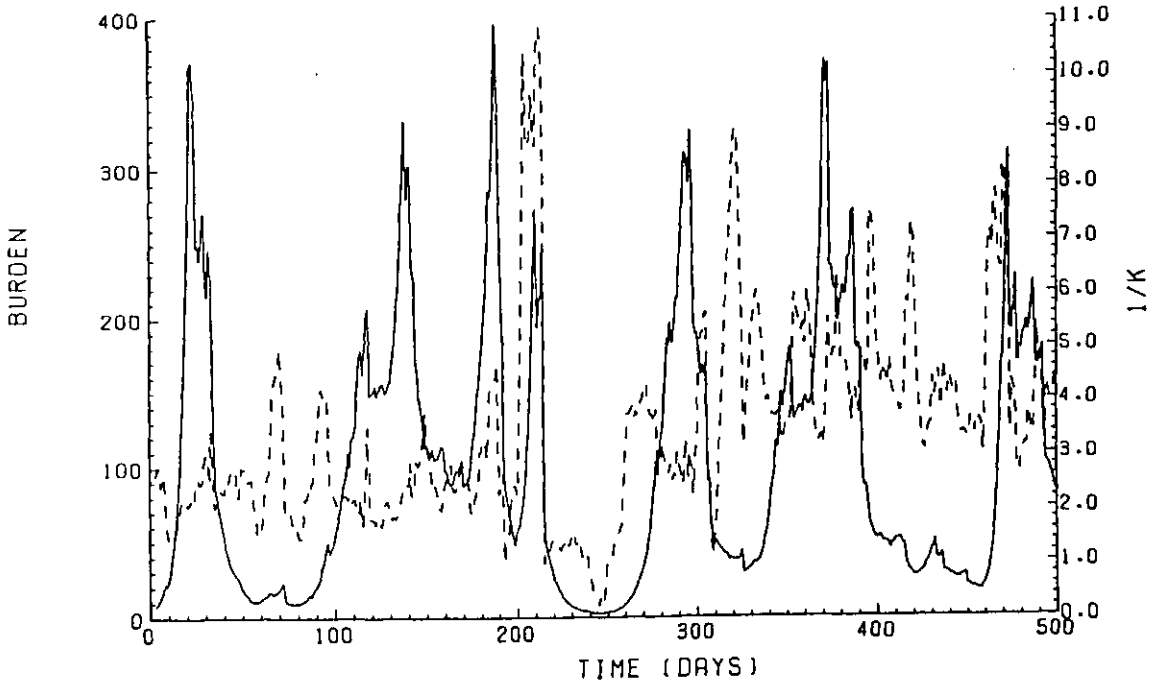
Figure 13.3 *Changes in average host susceptibility during a simulation run.*

The changes in host susceptibility during the course of the simulation run of Fig. 13.2 are shown. The solid line, scaled on the axis to the left of the figure, represents the mean parasite burden per host, and the dashed line, scaled on the axis to the right of the figure, represents the mean susceptibility to infection of the host population. The expected susceptibility of hosts at "birth" is 0.11.

Figure 13.4 *The behaviour of $k^{-1}$ during a simulation run*

The figure shows the changes with time of $k^{-1}$ during the course of the simulation run of Fig. 13.2. The solid line represents the mean parasite burden per host, and is scaled on the axis to the left of the figure. The inverse of $k$, calculated using eqn. (8.4), is shown as a dashed line, and is scaled on the axis to the right of the figure. The expected value of $k^{-1}$ at the commencement of the simulation is 2.0.


BURDEN
SUSCEPTIBILITY
TIME (DAYS)
0
100
200
300
400
500
0.000
0.020
0.040
0.060
0.080
0.100


BURDEN
1/K
TIME (DAYS)
0
100
200
300
400
500
0.0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
9.0
10.0
11.0

# CHAPTER 14

## MODELLING THE EXPERIMENTAL EPIDEMICS

### 14.1 *Constant Host Immigration*

A number of difficulties arise in attempting to compare the experimental results of Section 9.2 with the predictions of the models discussed in the previous chapters. The simple models do not take into account the age structure of parasite or host populations, but most of the results discussed in Chapters 4 - 7 are a consequence of age dependent processes acting on the trophozoites.

The basic model (eqns 10.7 - 10.10) assumes that each trophozoite leaving the host gives rise to $\Lambda$ tomites. The mean number of tomites produced per trophozoite leaving the host was found to be 334 in Section 4.3.4, and this provides a convenient estimate of $\Lambda$. If host density is high, and density dependent constraints are unimportant, the basic model predicts that the change in the number of trophozoites can be described by the following equation:

$$dP(t)/dt = (\Lambda\gamma s - \gamma)P(t) \qquad (14.1)$$

where $P(t)$ is the number of trophozoites at time $t$, $s$ is the proportion of tomites that develop to form trophozoites and $\gamma$ is the rate of emigration of trophozoites from the host. In tanks of the size used in the long term experiments, the experimental results of Chapter 5 indicate that changing host density has little effect on the overall reproductive success of the parasite. The maximum rate of reproduction per unit of time, $r_o$, is hence:

$$r_o = \gamma(\Lambda s - 1) \qquad (14.2)$$

If $s$ is estimated as 0.063 (Section 5.4) and $\gamma$ is estimated as 0.13 per day (Section 4.3.1), $r_o$ is found to be 2.61 per day. This value is an order of magnitude greater than the $r_o$ of 0.391 per day calculated from the Euler equation, using the observed form of the trophozoite survival function, $l(x)$ (See Section 4.3.5). This major discrepancy occurs because of the assumption of a constant emigration rate of trophozoites made in the basic model. The actual form of the trophozoite survival function, $l(x)$, is compared with the form assumed by the model in Fig. 14.1. The model predicts a proportion of trophozoites leaving the host shortly after infection that is considerably higher than the true figure. If all these trophozoites are assumed to produce the same number of tomites as mature trophozoites are observed to produce, the model will considerably overestimate the reproductive rate of the parasite.

The experimentally observed form of $l(x)$, is bracketed between a step function, in which all trophozoites leave the host at age $1/\gamma$ and the exponential decay function assumed by the model (Fig. 14.1). The step function leads to an Euler relation:

$$1 = R \exp(-r_o/\gamma) \qquad (14.3)$$

where $R$ is the number of second generation trophozoites produced per trophozoite in the previous generation (the basic reproductive rate). The maximum rate of reproduction per unit of time, $r_o$, is therefore given by

$$r_o = \gamma \ln(R) \qquad (14.4)$$

Figure 14.1 *Comparison of forms of the survival function, $l(x)$*

The experimentally observed form of the trophozoite survival function $l(x)$ is compared with the exponential decay assumed by the simple models and with a step function. The evenly dashed line represents the equation:

$$l(x) = \exp\left[a/b\ \left[1-\exp(bx)\right]\right]$$

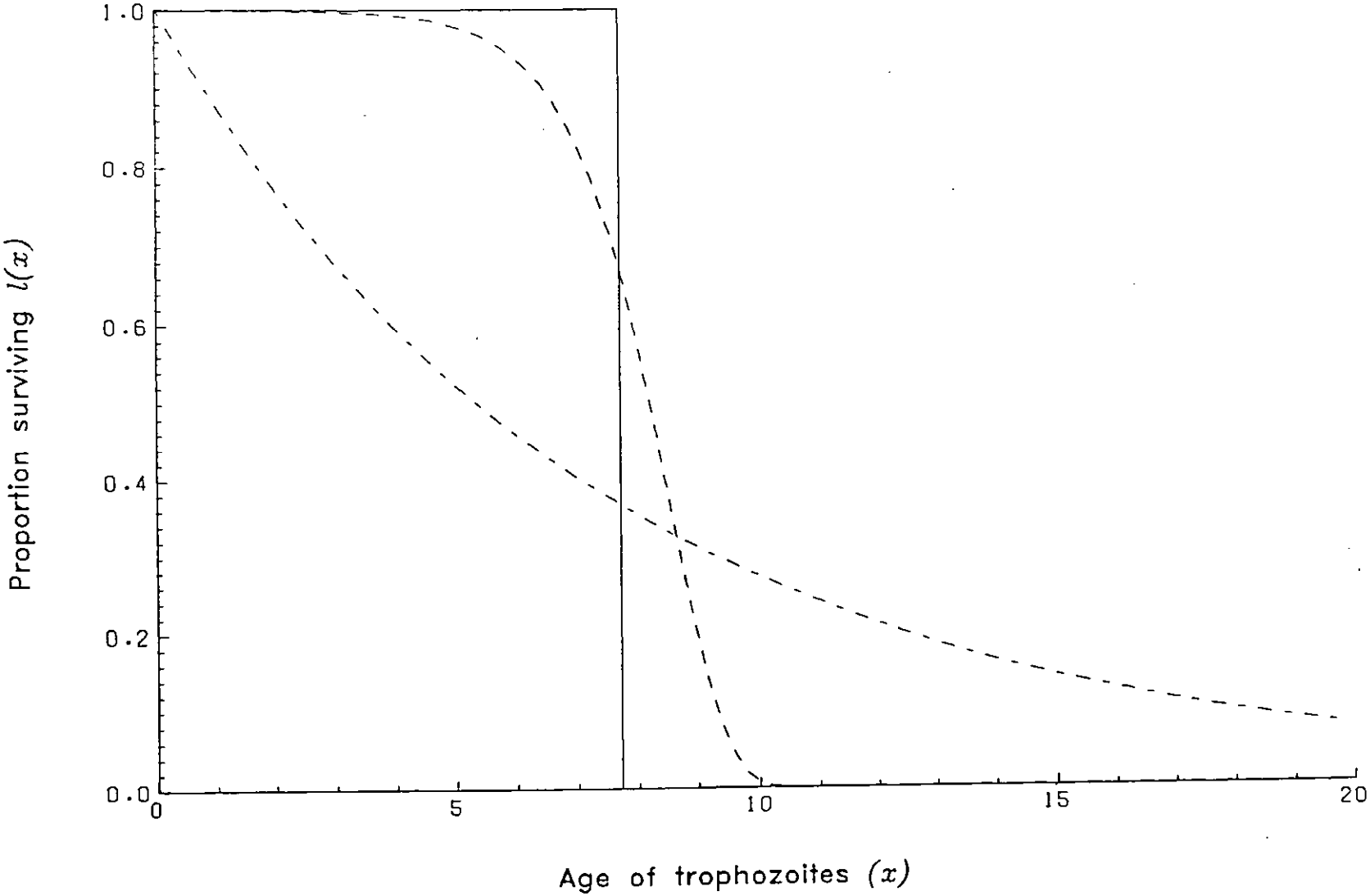
where $a = 1.04 \times 10^{-4}$ and $b = 1.084$ (The parameters found to closely approximate the experimental results in Section 4.3.1). The unevenly dashed line represents the function

$$l(x) = \exp\left[-\gamma x\right]$$

where $\gamma = 0.130$ (the inverse of the life expectancy of a trophozoite) This is the survivorship function assumed by the basic host-parasite models. The solid line represents the step function

$$l(x) = \begin{cases} 1 & x < 7.7 \\ 0 & x > 7.7 \end{cases}$$

where 7.7 is the life expectancy of a trophozoite.


1.0
0.8
0.6
0.4
0.2
0.0
0
5
10
15
20
Proportion surviving $l(x)$
Age of trophozoites $(x)$

In Section 4.3.5, this was found to provide a good approximation to the value of $r_o$ generated from the experimentally observed $l(x)$. Comparison of eqn. (14.4) with eqn. (14.2) shows that the exponential survival function provides a reasonable estimate of $r_o$ if $\ln(R)$ can be approximated by $(R-1)$. In the experimental systems under examination here, the host density is high and $R$ is therefore considerably greater than 1, leading to the simple model greatly overestimating the reproductive rate of the parasite.

There is no entirely satisfactory way around this problem, but a better numerical agreement between the models and experimental data can be obtained by estimating $\Lambda$ so that the maximum reproductive rate, $r_o$, is at the level found in Section 4.3.5. If this is done, $\Lambda$ is estimated as 63.6 tomites per trophozoite.

A second major problem is estimation of the rate of parasite induced mortality, $\alpha$. Figure 7.1 shows that this rate is very strongly dependent on the age of the trophozoites. An approximate, averaged estimate of the increment in the rate of parasite induced mortality per trophozoite per day of $1 \times 10^{-5}$ was obtained, but as discussed in Section 7.6, there are a variety of reasons why this is liable to be a considerable underestimate. The relationship between mean death rate and parasite burden was found to be approximately linear, but appeared to break down at low parasite burdens per host. Unfortunately, the results of the immigration death experiments are concerned with parasite burdens in this low range. A further complication is that only trophozoites older than three days were counted in the immigration death experiments, whereas the results of Chapter 7 are concerned with estimates of the total number of trophozoites per host. Precise

quantification of the effect that all these factors might have on the value of $\alpha$ is difficult. For the purposes of the numerical results that follow, a value of $\alpha$ of $1 \times 10^{-4}$ (per host, per trophozoite, per day) was used.

The results of the immigration experiments shown in Figs. 9.1 and 9.3 follow a similar pattern. There is an initial increase in mean parasite burden, which leads to host mortality if the initial infective dose is high, but subsequently the mean parasite burden declines and the host population increases to a level where it does not appear to be depressed to any great extent by the parasite.

The predictions of the simple model without immunity (eqns. 10.28 and 10.36) are shown in Fig. 14.2. The equilibrium host numbers are low, and the mean parasite burden per host is high. This does not closely correspond to the observed behaviour of the experimental systems. The simple model of immunity, (eqns. 12.5 - 12.7) in which the rate of gaining resistance to infection in a linear function of parasite burden, is useful in describing some of the general features of the effect of immunity in a host parasite interaction, but is not suitable for use in modelling this particular system. The results of the experiments in Chapter 6 show that the rate of gaining immunity is not a linear function of parasite burden. It is therefore difficult to estimate a plausible value for $\eta$, the per parasite increment in the rate of gaining resistance to infection. The time required for hosts to acquire resistance appears to be approximately ten days, and is only slightly longer with burdens of 15 parasites per host than with burdens of 150. The rate of gaining resistance may therefore be approximately of the form used in eqns. (12.30 - 12.32):

Figure 14.2 *The predicted behaviour of the constant immigration experiments, if the immune response is not allowed for.*

A numerical solution is shown of a simple immigration-death model, with no acquired immunity. The model is defined by the following equations, closely based on eqns. (10.36) and (10.28).

$$dH/dt = A - bH - \alpha MH$$

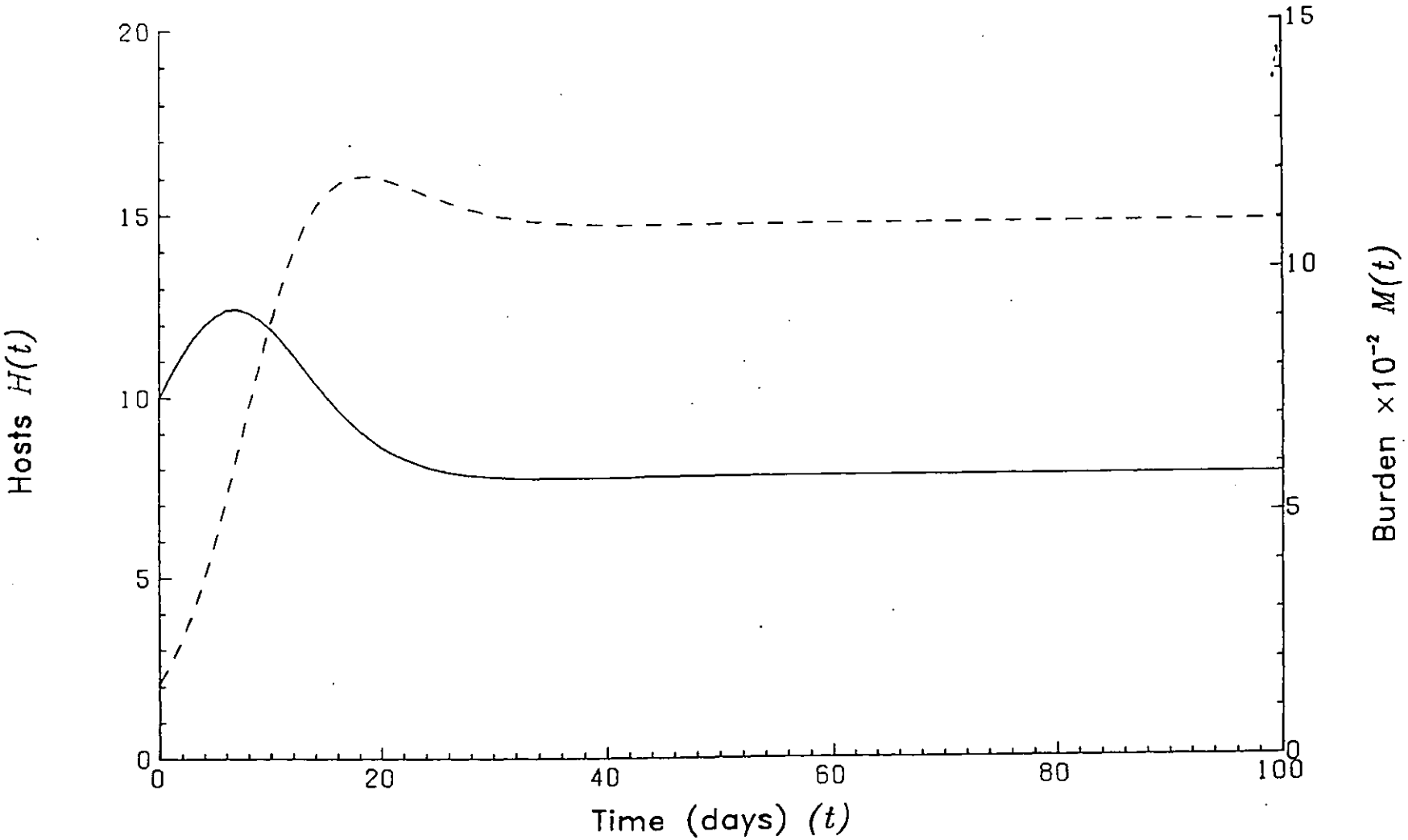
$$dM/dt = M \{\lambda H/(H_o+H) - (\gamma+\alpha+ A/H) - \alpha M/k \}$$

The total number of hosts, $H(t)$, is shown as a solid line, and is scaled according to the axis to the left of the figure. The mean parasite burden per host, $M(t)$, is shown as a dashed line, and is scaled on the axis to the right of the figure.

Parameters used

| Parameter | Biological interpretation | Value |
|---|---|---|
| $A$ | rate of host introduction | 1 day$^{-1}$ |
| $b$ | disease independent death rate | 0.02 per host, day$^{-1}$ |
| $\alpha$ | Parasite induced death rate | 0.0001 per parasite, per host, day$^{-1}$ |
| $\lambda$ | Rate of trophozoite reproduction | 0.52 per trophozoite, day$^{-1}$ |
| $H_o$ | Saturation term in infectivity | 0.32 |
| $\gamma$ | Emigration rate of trophozoites | 0.13 per trophozoite |
| $k$ | Parameter of negative binomial distribution describing degree of overdispersion of parasites on hosts. | 0.5 |

The solution was commenced with initial conditions of $H(0) = 10$, $M(0) = 151.0$. (The initial conditions at the commencement of the second series of immigration/


20
15
10
5
0
0
20
40
60
80
100
Time (days) (t)
Hosts H(t)
15
10
5
0
Burden ×10⁻² M(t)

$$r(i) = c(1 - \exp(-\zeta i)) \qquad (14.5)$$

where $r(i)$ is the rate of gaining immunity with a parasite burden of $i$, with $c$ approximately equal to 0.1 per day, and $\zeta$ estimated by 0.1 per trophozoite.

The predictions of eqns. (12.30 - 12.32), modified to account for constant host introduction, are shown in Fig. 14.3. The model captures certain qualitative features of the observations (it predicts an equilibrium host population close to the disease free carrying capacity, and a low equilibrium mean parasite burden) but with these parameter values, the model will not generate the initial high levels of mean parasite burden and parasite induced death shown in Fig. 9.3. A number of important features of the biology of *Ichthyophthirius* are not included in this model. Parasite survival of host death and the partial nature of resistance will tend to increase the reproductive rate of the parasite, while the selective removal of more susceptible hosts by parasite induced death will tend to decrease the reproductive potential of the parasite. A close quantitative fit of the model to the experimental data should not therefore be expected.

In these relatively small experimental systems, stochastic elements can also be expected to have a considerable influence on the course of the epidemics. Figure 14.4 shows the results of four runs of the simulation model described in Chapter 13, modified to allow for constant introduction of hosts, and with parameter values similar to those used in Fig. 14.2. This model does not include an immmune response, but the mean susceptibility of the host population decreases with time, as the more susceptible hosts are more rapidly removed by

**Figure 14.3** *The predicted behaviour of the constant immigration experiments, when an immune response is included.*

Numerical solutions are shown of an immigration death model with an immune response of the form of eqn. (14.5). The model is defined by the following equations:

$$\frac{dH}{dt} = A - bH - \alpha M(H-I)$$

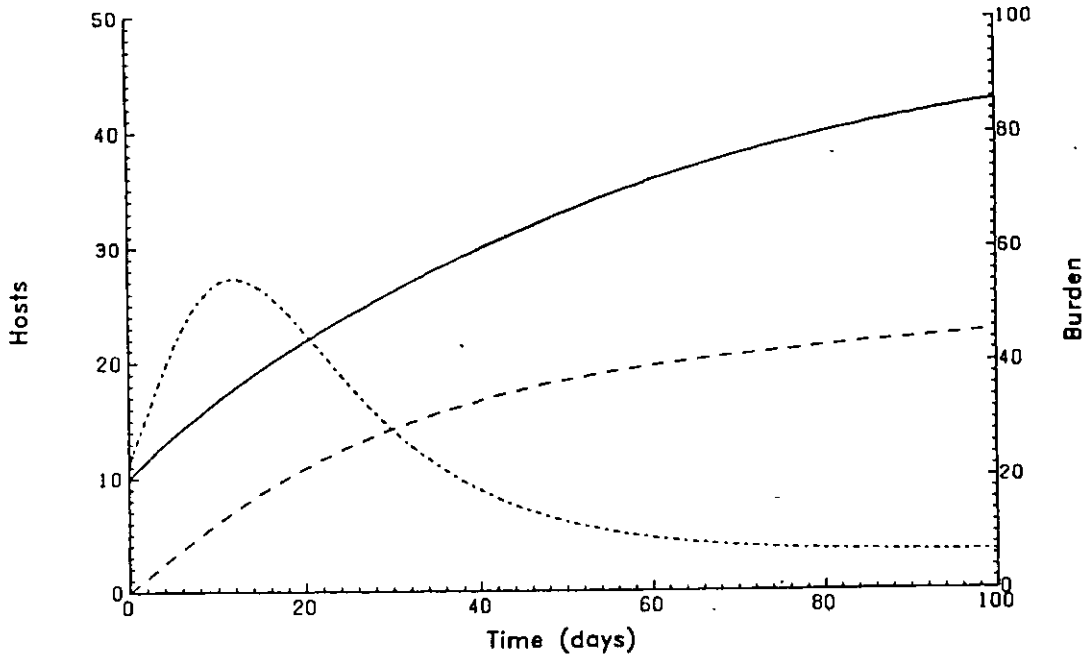
$$\frac{dI}{dt} = c(H-I)(1-f(M)^{-k}) - (\nu+b)I$$

$$\frac{dM}{dt} = M\left[\frac{\lambda(H-I)}{(H+H_o)} - (\gamma+\alpha) - cf(M)^{-(k+1)}\left[f(M)-e^{-\zeta}\right] - \frac{\nu I}{(H-I)} - \frac{\alpha M}{k}\frac{-A}{(H-I)}\right]$$

where $f(M) = 1 + \left[M/k\left[1 - \exp(-\zeta)\right]\right]$

The parameter values used are the same as those of Fig. 14.2, with the following additional parameters to describe the immune response: $c$ (the rate of gaining immunity at high parasite burdens) = 0.1 per day; $\zeta$ (the parameter describing the increment in gaining immunity per parasite) = 0.1 per day; $\nu$ (the rate of loss of immunity) = 0.02 per day. The total host population, $H(t)$, is represented by a solid line, and scaled according to the axis to the left of the figure, as is $I(t)$, the number of immune hosts (represented by an evenly dashed line. The mean parasite burden, $M'(t)$ is shown as an unevenly dashed line and is scaled according to the axis to the right of the figure. In order to facilitate comparison with figs. 9.1 and 9.3, the overall mean parasite burden is shown, $M'(t) = M(t)\ (H(t) - I(t))/H(t)$. Figure 14.3a shows a solution with initial conditions: $H(0) = 10.$, $I(0) = 0.$, $M(0) = 22.7$ (approximating the starting conditions in Fig. 9.1) and Fig. 14.3b shows a solution with starting conditions: $H(0) = 10.$, $I(0) = 0.$, $M(0) = 151$ (approximating the initial conditions in Fig. 9.3).

(a)


Hosts
Burden
Time (days)
0
10
20
30
40
50
0
20
40
60
80
100
0
20
40
60
80
100

(b)

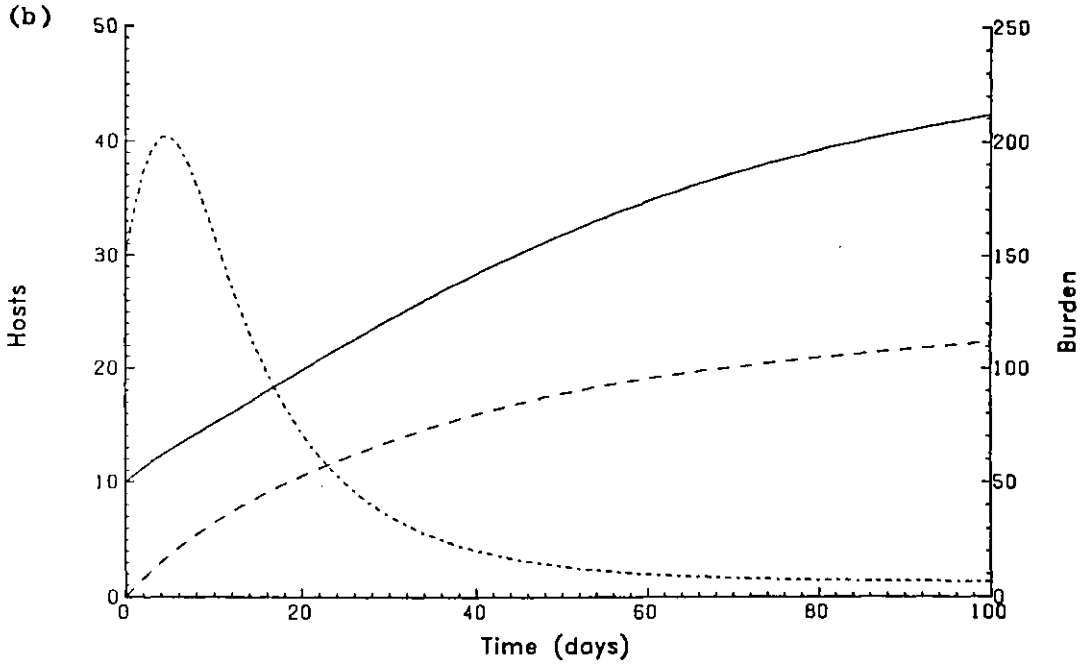
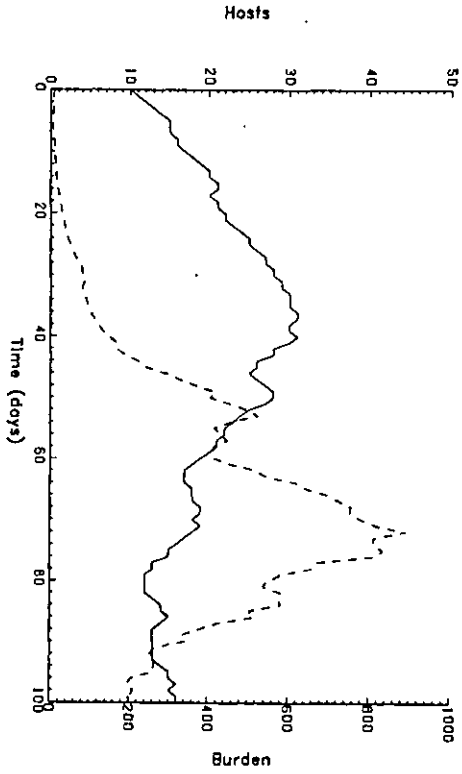

Hosts
Burden
Time (days)
0
10
20
30
40
50
0
20
40
60
80
100
0
50
100
150
200
250

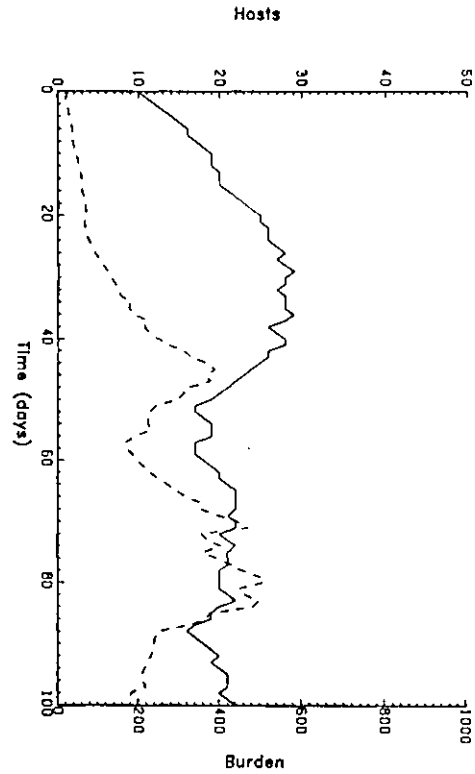
Figure 14.4 *Solutions of the simulation model with constant immigration*

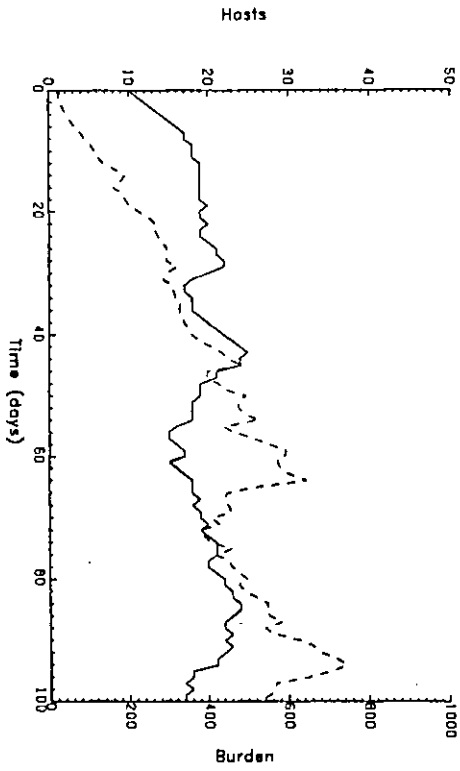
Four solutions are shown of the simulation model described in Chapter 13, modified to allow for host introductions at a constant rate. The total host population is shown as a solid line, and scaled according to the axis to the left of the figure. The mean trophozoite burden per host is shown as a dashed line, and scaled according to the axis to the right of the figure.

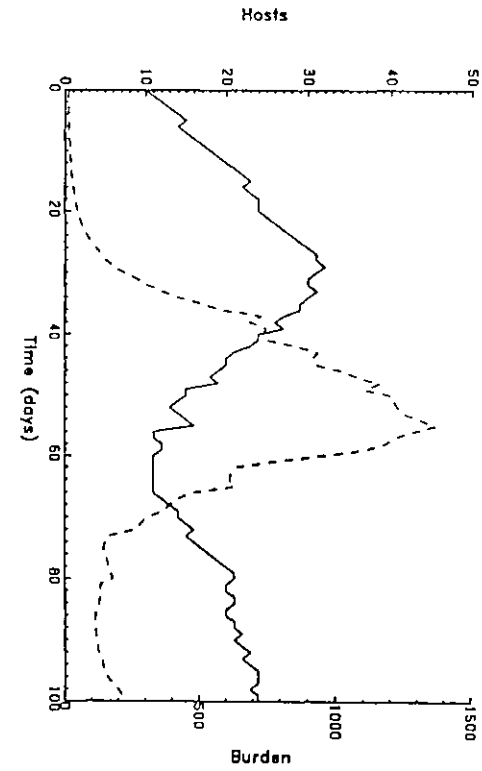
The parameters used correspond as closely as possible to the conditions of the immigration/death experiments described in Chapter 9. The immigration rate of susceptible hosts is 1 per day, and their susceptibility to infection is determined from a beta distribution with $l$ = 1 and $m$ = 16, parameters which result in a mean susceptibility to infection of 0.06 and would generate a $k$ = 1.13 after a single infection. Other parameters used (defined in Chapter 13) are: $b$ = 0.02 per day, $\alpha$ = 0.0001 per trophozoite per day, $\gamma$ = 0.13 per day, $\mu$ = 0.05 per day, $H_o$ = 0.32, $\lambda$ = 69. The initial number of hosts present in each case in 10, and the initial number of tomites is 3600., corresponding to the conditions at the beginning of the first series of constant immigration experiments.

Note the range of behaviour generated from identical starting conditions.


Hosts
Time (days)
Burden


Hosts
Time (days)
Burden


Hosts
Time (days)
Burden


Hosts
Time (days)
Burden

parasite induced death. A considerable degree of variation is evident between the simulation runs (which were started with identical initial conditions) showing the importance of stochastic effects.

## 14.2 *Constant Host Numbers*

The results of the second series of experiments, in which the total host numbers were held constant, show oscillatory behaviour in mean parasite burdens (Fig. 9.5). There does not appear to be any tendency for parasite numbers to settle to a stable equilibrium value. Although this particular experimental arrangement was set up with the intention of investigating the immune response (it was hoped that, by holding total host numbers constant, the effect of changes in the proportion of hosts that are immune could be investigated), the immune response is almost certainly not of importance in this case. The rate of turnover of hosts is extremely rapid, because of the very high rates of host death in these experiments (Fig. 9.5). Few fish survive the ten days required for an immune response to become significant (Chapter 6). The oscillations may result from the way in which the age of a trophozoite at the time of the death of its host affects its reproductive potential (Section 7.5).

The models described in Chapter 11 can be modified to deal with hosts being replaced as soon as they die. If a constant proportion of trophozoites survive host death, irrespective of the parasite burden, the following two equations (eqns (11.4) and (11.5), with $H$ a constant, rather than a dynamic variable) will describe the changes with time in the number of parasites, $P(t)$, and tomites, $T(t)$:

$$dP/dt = \beta HT - (\alpha+b+\mu+\alpha)P - \alpha(k+1)P^2/(kH) \qquad (14.6)$$

$$dT/dt = \lambda[\gamma P + x(\alpha+b)P + (k+1)P^2/(kH)] - \mu_2 T - \beta TH \qquad (14.7)$$

where the parameters are as defined as in Chapter 11. A non trivial equilibrium ($P \neq 0$, $T \neq 0$) can exist provided $H$ is greater than a threshold level

$$H > \mu_2/\beta/[\gamma\lambda/(\alpha+\mu+\gamma+b)] \qquad (14.8)$$

An equilibrium parasite burden exists only if

$$\lambda Hx/(\mu_2+H) < 1 \qquad (14.9)$$

otherwise parasite numbers increase rapidly without limit. Any equilibrium that does exist is locally stable (Appendix 12) and numerical studies indicate that the approach to equilibrium is rapid. The model is not capable of describing the behaviour of the experimental system. Similarly, the more complex model, eqns.(11.24 and 11.25), in which the level of parasite survival decreases with increasing parasite burden, can be modified to apply in cases where host numbers are constant such that:

$$dP/dt = \beta HT - (\gamma+\mu+\alpha+b)P - \alpha(k+1)P^2/(kH) \qquad (14.10)$$

$$dT/dt = \lambda\gamma P - \lambda HS(M) - \beta HT - \mu_2 T \qquad (14.11)$$

where $S(M)$ is defined in eqn. (11.23).

This system, unlike eqns. (14.6) and (14.7), always has a non trivial equilibrium value, provided $H$ is above the threshold level

(eqn. 14.8) and this equilibrium is always locally stable (Appendix 12). Once again, numerical studies indicate that the approach to equilibrium is rapid. This model is also incapable of generating the behaviour observed in the experimental system. It appears that in this case, the age structure of the parasite population cannot be disregarded.

14.2.1. *A difference equation model*

A simple way to include age structure is to frame the problem as a difference equation model, in which all generations are entirely distinct (as opposed to a differential equation model, in which generations are assumed to totally overlap). Figure 7.6 shows the number of tomites produced per unit of host surface area related to the trophozoite density. If one host is maintained in a small container, and immediately replaced should it die, the number of second generation trophozoites per unit of host surface area will be equal to the tomite production shown on Fig. 7.6, multiplied by the susceptibility to infection of the hosts. Figure 7.6 is thus a Ricker curve (Varley, Gradwell and Hassell, 1973) for parasite numbers on a single host. The figure cannot, however, be simply used in this way for ten hosts. Within a population of ten fish, differences in host susceptibility to infection will result in a range of parasite burdens if each host is exposed to the same number of infective stages. The results discussed in Chapter 8 indicate that, for a single infection the number of parasites per host follows a negative binomial distribution with parameter $k$ (inversely describing the degree of overdispersion) of approximately 2. This variability will result in the relationship between the mean parasite density on hosts and the expected number of tomites produced being rather different from Fig. 7.6.

When the trophozoite density on a single fish is very high, the expected number of tomites produced is low. If the mean density of trophozoites on the surface of fish in a population is at this same level, a few hosts will have lower parasite densities, and consequently produce more tomites, thereby increasing the average. The result is that the curve is flattened out in the manner shown in Fig. 14.5.

The curve shown in Fig. 14.5 was obtained in the following manner. The number of tomites produced per unit of surface area of a single host is

$$T(P) = O(P).P \tag{14.12}$$

where $O(P)$ is the tomite production of each trophozoite, if the trophozoite density is $P$ per unit of host surface area. The average number of trophozoites produced per unit of host surface area in a population of fish with mean trophozoite density $\bar{d}$ will therefore be:

$$\bar{T}(\bar{d}) = \int_0^\infty T(P).\ f(P|\bar{d})\ dP \tag{14.13}$$

where $f(P|\bar{d})$ is the probability distribution of $P$, given that the mean trophozoite density is $\bar{d}$. The probability distribution of the total number of trophozoites per host can reasonably be assumed to follow a negative binomial distribution, but this will not be the distribution of trophozoite density per unit of area of the surface of a fish, which may take non integral values. The probability distribution $f(P|\bar{d})$ was approximated by a log normal distribution with

mean $\bar{d}$ and variance $(\bar{d})^2/2$. (If the distribution of parasites amongst the host population follows a negative binomial distribution with $k = 2$, and the absolute parasite numbers per host are substantial, the variance in parasite density is approximately $(\bar{d})^2/2$. A log normal distribution is used as it can only adopt positive values). A proportion, $\bar{s}$, of the tomites produced is expected to form trophozoites in the next generation, where $\bar{s}$ is mean proportion of tomites infecting hosts that are able to form trophozoites (in tanks of the size used, virtually all tomites will locate a host before death [Chapter 5]). The number of second generation parasites, $N_{t+1}$, per unit area of host surface is therefore given by:

$$N_{t+1} = \bar{s}\bar{T}(N_t) \qquad (14.14)$$

where $N_t$ is the mean number of trophozoites per unit area of host surface in the previous generation, and $\bar{T}(N_t)$ is obtained by numerical integration of eqn. (14.13).

Although the derivation of the curve defined by eqn. (14.14) is complex, the curve may be closely approximated by a simple model of density dependence in single species used by Maynard Smith and Slatkin (1973).

$$N_{t+1} = \lambda N_t / \left[1 + (\upsilon N_t)^{w}\right] \qquad (14.15)$$

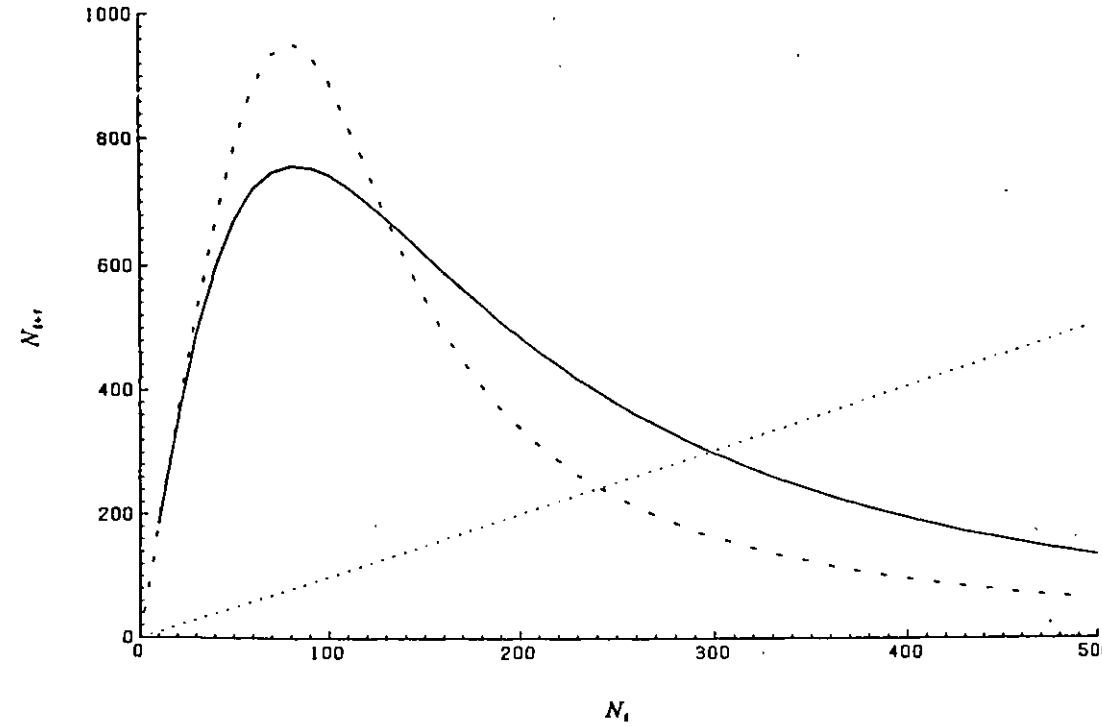
Values of the three parameters $\lambda$, $\upsilon$ and $w$ were estimated from 50 equally spaced points on the solution curve of eqn. (14.14) using a non linear least squares procedure (Appendix 1). The resulting curve is compared with the actual solution of eqn. (14.14) in Fig. 14.6.

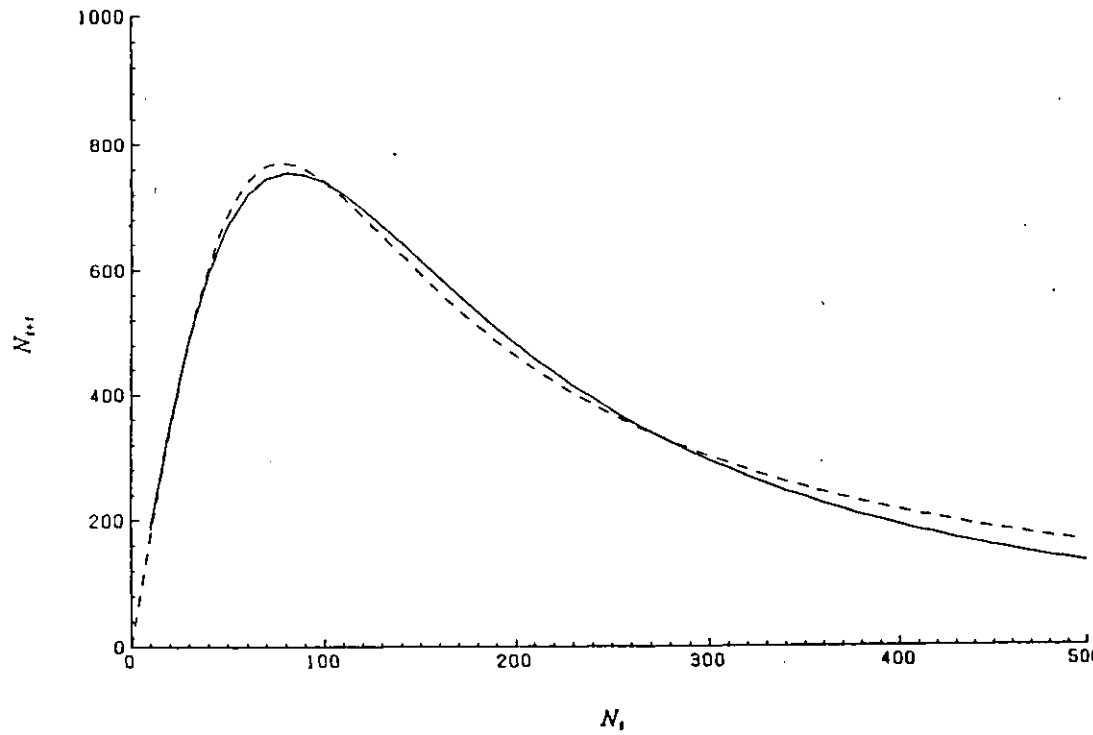
Figure 14.5 *The Ricker curve for trophozoite numbers*

The relationship shown is that expected between the number of second generation parasites, $N_{t+1}$, and the number of first generation parasites, $N_t$, if hosts are replaced at death. $N_t$ and $N_{t+1}$ are expressed per 0.16 $cm^2$ of host surface area. The dashed line represents the relationship expected if only one host is present. The solid line is the relationship expected between the mean parasite densities in successive generations in a population of fish, and is the solution of eqn. (14.15). Along the dotted line, $N_{t+1} = N_t$.

Figure 14.6 *The fit of the Maynard Smith - Slatkin model to the Ricker curve*

The solution of eqn. (14.14), shown as a solid line, is compared with the solution of eqn. (14.15) (the Maynard Smith - Slatkin model), shown as a dashed line. Estimates of the parameters of eqn. (14.15) are as follows: $\lambda = 17.8$, $v = 0.012$, $w = 2.25$.


1000
800
600
400
200
0
0
100
200
300
400
500
$N_{t+1}$
$N_t$


1000
800
600
400
200
0
0
100
200
300
400
500
$N_{t+1}$
$N_t$

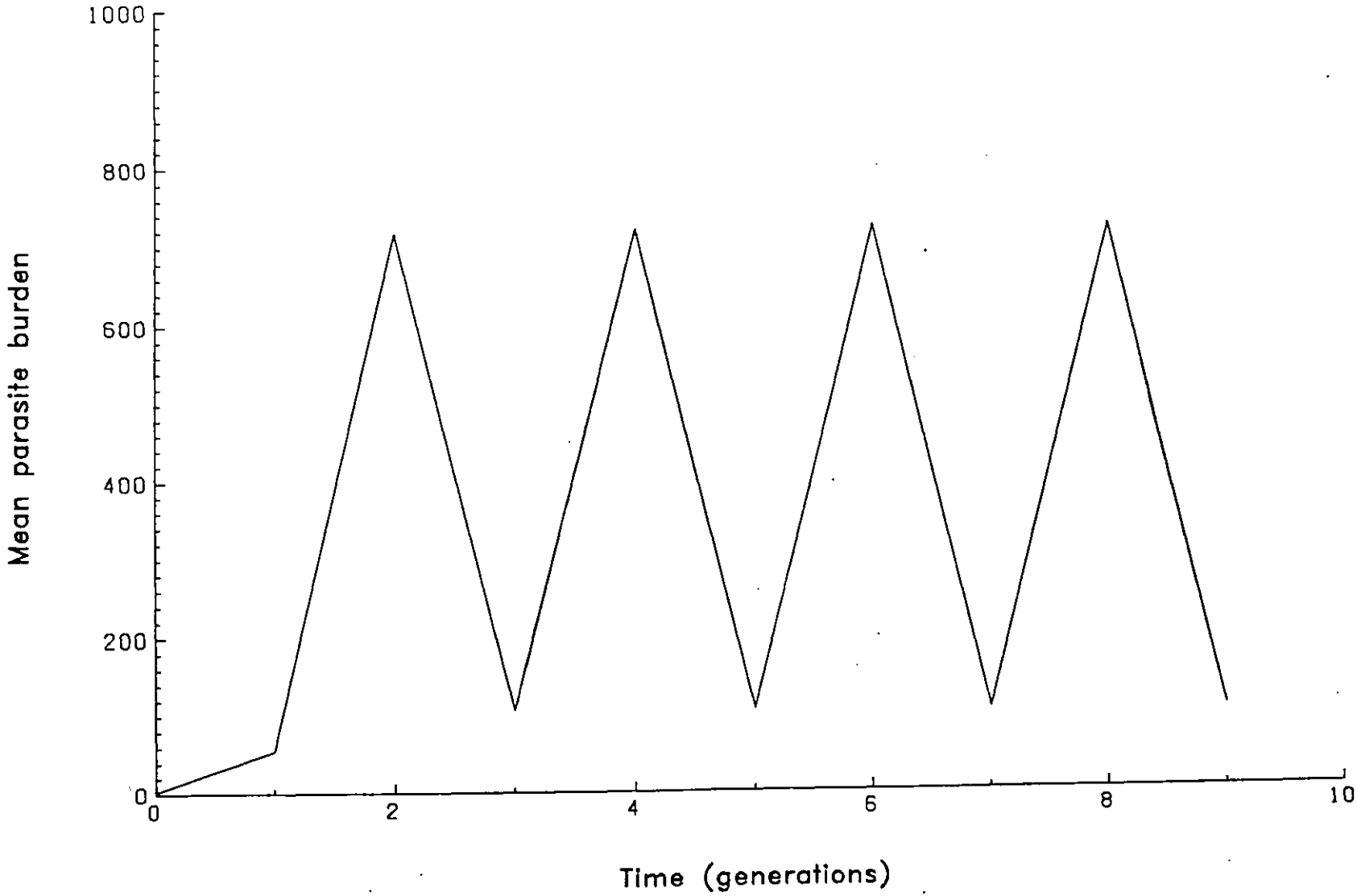
The simple difference equation model defined by eqn. (14.15) generates a two point stable limit cycle given the estimated values of $\lambda$, $v$ and $w$ (Fig. 14.7). The qualitative predictions of the model are in accord with the experimental results shown in Fig. 9.5. The quantitative results of the model, however, do not agree closely with the experimental results, predicting parasite densities considerably in excess of those observed, at both high and low points of the cycle. A possible reason for this lack of agreement is that the production of the Ricker curve involves extrapolating the relationship between parasite burden and death rate that was observed in Chapter 7 (eqn. 7.2) beyond the range of parasite burdens that could be examined. Small differences in the tail of the curve have large effects on the actual range of the oscillations. Even assuming that the relationship of eqn. (7.2) can reasonably be extrapolated, eqn. (14.15) overestimates the value of $N_{t+1}$ at high values of $N_t$.

An unusual feature of this experimental system is that the generation time of the parasite is a function of its population size. When parasite burdens are low, host mortality is slight, and the generation time is approximately the 7.7 days that the parasite remains on the host(determined in Section 4.3.1),but at high parasite densities, it is approximately the life expectancy of the infected hosts. Although the host mortality observed in the experiments does occur in clear bursts (Fig. 9.5), there is not total synchrony in host deaths, and the parasite generations will also overlap to a degree.

Within these limitations, the qualitative fit of this very simple model is encouraging.

Figure 14.7 *The numerical solution of eqn. 14.15.*

The two point limit cycle generated by eqn (14.15) is shown. The horizontal axis shows the time in generations, and the vertical axis shows the mean number of trophozoites per 0.16 $cm^2$ of host body surface. The solution was started with an initial value of 3.11, equivalent to the upper initial starting value of the experiments described in Section 9.3.


Mean parasite burden
1000
800
600
400
200
0
0
2
4
6
8
10
Time (generations)

# CHAPTER 15

## GENERAL DISCUSSION

### 15.1 *Seasonality and Age Structure*

As a parasite of poikilothermic hosts, *Ichthyophthirius* is particularly susceptible to seasonal changes in temperature. All of the parameters governing the life history of the disease are highly temperature dependent (see Table 2.1). Without the inclusion of seasonal variation, the models discussed in Chapters 10 - 13 are therefore unlikely to accurately describe the course of infection in natural conditions. A related problem, implicit in the use of simple differential equation models, is that the age structure of both parasite and host populations is ignored.

There are several levels at which seasonal factors can be incorporated into models of this type. The simplest approach, employed by Fretwell (1972) is to consider two sets of parameters to describe the system, a "winter" set and a "summer" set. A separate equilibrium will exist for each combination of parameters. If the response time of the system is rapid (relative to the frequency of seasonal change) both "summer" and "winter" equilibria may be reached in turn. The approach to equilibrium of the models discussed in this thesis, however, is generally via damped oscillations, and it is unlikely that either equilibrium would be reached, the system heading toward one equilibrium and then toward the other, actually attaining neither equilibrium state. On a slightly higher level of complexity, the parameters in the models may be made to oscillate periodically with time by the use of trigonometric functions. This approach has been

used by Dietz (1976) to consider the dynamics of infectious disease in seasonal environments. If the response time of the system is fast, it may merely track the cyclic changes in the parameters, but if the response is slow, Dietz showed that recurrent outbreaks of disease may occur at lower frequencies than the frequency of the cyclical variations. In other words, annual cycles may cause biennial or triennial outbreaks of disease.

The problem of age structure is in some ways related to that of seasonality. In both cases, the coefficients involved in the differential equations become functions of time. The results of the experiment in which host numbers were kept constant, discussed in Chapter 14, show how simple models assuming constant coefficients may be unable to generate the qualitative behaviour observed in real systems. In discrete time models, age structure can be handled by the matrix techniques discussed by Beddington (1974). In continuous time, with overlapping generations, the situation is more difficult. Age structure can crudely be taken into account by framing the model as differential-delay equations (Oster (1977) shows how a fully age structured model of Nicholson's blowflies can be approximated by a single differential delay equation). In general terms, the inclusion of time delays is destabilizing, and may result in cycles of a longer period than the delay itself (for example, see Gurney, Blythe and Nisbett, 1980).

Seasonality and age structure are considered together in a complex model by Oster and Takahashi (1974), using balance equations involving partial differentials. The most important conclusions reached are in accord with rather simpler models that include age structure and seasonality separately. Both factors may individually

induce cycles longer in length than either the time delay or the period of the seasonality, and these cycles may resonate, resulting in peaks separated by a longer time period again.

The development of such equations for the dynamics of *Ichthyophthirius* is beyond the mathematical scope of this thesis. Age dependent models couched in partial differential equation form are highly complex, both to formulate and analyze. They tend to have all the disadvantages of simulation models, such as the necessity to fully estimate birth and death rate functionals, and the difficulty of obtaining results in an algebraic form that enables general conclusions to be drawn. A particular problem is introduced by the importance of the distribution of parasites on hosts in host parasite interactions. In the simple differential equation models, the variance of parasite numbers per host was required, and this was obtained by assuming a negative binomial distribution for parasites on hosts. In addition to this, the age dependent models require the postulation of some functional form for the covariance between parasites of different ages on hosts.

The general effect of age structure and seasonality, together with stochastic perturbations (Bartlett, 1957) is likely to be that factors leading to damped oscillations in the simple models will tend to produce cyclic behaviour in real situations.

## 15.2 *Susceptibility to Infection*

Two fundamental factors that determine the transmission efficiency of a parasite are the host density and the susceptibility to infection of the host population. A major contribution made by Anderson and May

(1978, 1979) toward the understanding of host parasite interactions was that they allowed the total host population to be a dynamic variable, rather than fixing it at a constant level, as had most previous workers (Bailey, 1975). The experimental results of Chapter 5 indicate, however, that the host density does not have a major effect on the overall reproductive success of *Ichthyophthirius* in the particular experimental conditions used in this study. This thesis has therefore concentrated, both experimentally and theoretically, on the susceptibility of the host population to infection and on the factors that may cause it to be a dynamic variable.

In the parasitological literature, there are a number of different uses of the terms "resistant" and "susceptible" (Wakelin, 1978). The level of resistance of an organism to a parasite or disease is in some cases measured by the host's ability to survive infection, rather than its ability to prevent establishment of the parasite. These two factors may well be correlated, and are difficult to separate experimentally for certain classes of infection. No such problem exists with *Ichthyophthirius*, as the number of trophozoites a host is harbouring can be easily counted. Susceptibility throughout this discussion is defined according to the usage of Chapter 8: the susceptibility to infection of a host is simply the proportion of infective stages encountering the host that are able to establish on it.

The experiments described in Chapters 5 and 8 show that, even under ideal conditions, a majority of tomites are overcome by the defences of the host, and that there is considerable variation in susceptibility between hosts in a single infection. Differences in

susceptibility to infection may come from several sources. The fish used in these experiments were obtained from a commercial supplier and some may have been exposed to infection before purchase (*Ichthyophthirius* infection was not, however, detected on any black mollies at the time of purchase). As the results of Chapter 6 show, black mollies can acquire a degree of immunity to *Ichthyophthirius* from slight infections, and this resistance may last for a considerable length of time after exposure. It is therefore possible that the fish used in these experiments may have been resistant to infection because of previous exposure. The physiological status of a fish may also affect its susceptibility to infection. Pickering and Christie (1980) reported that sexually mature male brown trout *Salmo trutta* had higher levels of *Ichthyophthirius* infection than immature fish of either sex or mature females. (No evidence of black mollies showing sexual dimorphism in susceptibility to infection was found in the present study). It is likely that at least some of the variability in susceptibility observed is genetic in origin.

When considering the genetic control of susceptibility to infection, it is important to distinguish between innate resistance, the degree of resistance to an initial infection, and acquired immunity, developed subsequent to a previous exposure. The degree of innate resistance of a host and its ability to acquire immunity are both to an extent genetically determined (Wakelin, 1978) but they may be controlled from quite unrelated loci (Blackwell, Freeman and Bradley,1980). The observation made in Section 6.3 that the parasite burden developed on individual black mollies after an initial infection is not correlated with the burden established after a challenge infection suggests that in this case too, the factors determining

innate and acquired resistance may be distinct.

Little information is available at present concerning the genetic basis of innate resistance to infection (Wakelin, 1978). One of the few established cases is the protection afforded against malaria by the sickle cell allele in man. *Ichthyophthirius* tomites may fail to successfully infect a naive host for a number of reasons. The mucus layer may prevent them from reaching the epithelium of the fish, infective stages may be unable to penetrate the epidermis, or they may be overcome by non specific host defences once in the dermis. One might therefore expect the degree of resistance to infection to be mediated by a number of different genes.

Rather more information is available on the genetic aspects of acquired resistance to infection. In some cases certain hosts appear to be totally unable to mount an immune response (Wassom, DeWitt and Grundmann, 1974, Wakelin 1975) and the variability in acquired resistance appears to be mediated via a single gene. Inbred laboratory lines of host may clearly have more limited sources of variability than natural populations. The observations of Wassom et al, in which a single autosomal dominant gene was found to be responsible for the ability of deer mice *Peromyscus maniculatus* to develop resistance to the cestode *Hymenolepis citelli*, are of particular interest, being based on experiments with wild strains of the mice. In other cases, a number of genes are implicated in variability observed in the ability to mount an immune response (Wakelin, 1978). The high variance in parasite burdens per host established in the second infection compared with the control infection in Section 6.3 suggests that the black mollies used in these experiments

differed in their ability to acquire resistance to reinfection, but the degree of replication is insufficient to determine whether the distribution of trophozoites on reinfected hosts is bimodal (as it would be if a single dominant gene controlled resistance).

No direct evidence was obtained in this study concerning the heritability or genetic basis of either innate or acquired resistance because of the difficulty of getting *Poecilia latipinna* to breed under laboratory conditions. The related guppy *Poecilia reticulata* breeds prolifically in the laboratory, and although parasite burdens might prove harder to count on a fish that is not black, it would prove a good subject for studies on the heritability of resistance. The *Ichthyophthirius*-fish system is ideal for this type of work: levels of infection can be determined without the destruction of the host, and innate and acquired resistance can be readily distinguished.

The second part of this thesis considers some of the mechanisms that may lead to dynamic changes in the overall susceptibility to infection of a host population during the course of an epidemic. The immigration-death experiments of Chapter 9 show that, in controlled conditions, the parasite was unable to persist at high densities because of increasing host resistance to infection. As was seen in Chapter 14, this increase in resistance may have occurred through a combination of two factors: increasing acquired immunity to infection, discussed in Chapter 12, and rapid elimination of the more susceptible elements of the host population (Chapter 13).

The main conclusion of the models of acquired immunity in Chapter 12 is that a rapid rate of gaining immunity will lead to parasites being maintained endemically at low levels within host populations.

This conclusion is in accord with the observations of Wassom and co-workers (Wassom, Guss and Grundmann, 1973; Wassom, DeWitt and Grundmann, 1974) who obtained experimental evidence that the existence of acquired immunity of the deer mouse *Peromyscus maniculatus* to *Hymenolepis citelli* was responsible for the extremely low rate of prevalence (1.4%) of the cestode in populations of the mouse. Whilst *Ichthyophthirius* appears to be endemically present in very low numbers in natural fish populations, no evidence is available to suggest that it is immunity which is responsible. This could prove a suitable area for future research.

The models discussed in Chapter 13 show that a parasite may depress the mean susceptibility to infection of a host population without the involvement of an immune response. Provided hosts differ in their innate resistance to infection, the removal of highly susceptible members of a population due to parasite induced death will rapidly reduce the overall susceptibility of the hosts to infection. When parasite burdens are low, the rate of removal of highly susceptible hosts will be reduced, and the overall susceptibility to infection will rise. This may result in cyclical changes in the overall resistance of the host population to infection (Comparison of the deterministic and stochastic models in Chapter 13 suggests that stochastic variability may be necessary to maintain the cycles). The model does not involve any explicit genetic structure, although it is assumed implicitly that the variability in susceptibility to infection is genetic in origin.

In real populations, the probability distribution of innate susceptibility to infection amongst offspring will not be independent

of the susceptibility of their parents. Information on the rate at which resistance to infection might be expected to evolve is rather limited at present. Given that a polymorphism exists, the rate of evolution will clearly be a function of the number of genes involved in determining resistance, and the degree of linkage between them. Two experiments discussed by Wakelin (1978) suggest that even under continuous stringent artificial selection, significant increases in resistance may only occur over a time span of several generations. In real conditions, selection pressure will only be great at times of parasite outbreak, and there may be costs associated with resistance. The rate of evolution is therefore liable to be considerably slower than in artificial conditions. Changes in the susceptibility of the host population to infection caused by the mechanism discussed in Chapter 13 are likely to occur on a timescale very much faster than that of evolutionary change.

## 15.3 *Coevolution*

The preceeding discussion shows that the innate susceptibility of a host population cannot be assumed to remain constant. Given genetic variability in susceptibility to infection, evolution can be expected to occur. Genetic variability affecting virulence of the parasite is also likely to exist. The resulting problems of coevolution are of great importance in the understanding of host parasite systems. Hamilton (1980) has gone as far as to suggest that coevolution of pathogens and hosts may have been responsible for the evolution of sex itself.

In many basic parasitological texts, it is assumed that parasite and host should coevolve toward an amensal relationship, in which the

parasite has little influence on the host. The best documented example of coevolution actually occurring is the classic study of rabbits and myxamatosis in Australia (Fenner and Radcliffe, 1965). In this case, rabbits have become more resistant to the disease, but the most common form of the virus is of intermediate virulence, and is not the least virulent form present. Pathogenicity and transmissability of a parasite or pathogen may usually be correlated (Anderson and May, 1982b)which will limit the progress of evolution toward decreased virulence. Taking *Ichthyophthirius* as an example, the trophozoite affects the host primarily by the physical damage it causes to the epithelium. It is difficult to conceive of a way in which the amount of damage caused per parasite could be reduced without decreasing its reproductive potential. It is easy to show (see Appendix 13) that a form of the parasite with an increased reproductive rate will always be able to invade a population of the parasite which is less fecund. (If the two forms are both aggregated in the host population, independent of each other, however, a stable polymorphism may become established.

A number of simple models of coevolution of parasites and hosts have been proposed (Gillespie, 1975, Pimentel and Levin, 1981; Lewis, 1981a, 1981b). The *Ichthyophthirius* fish interaction would prove extremely suitable to examine both the predictions of these models, and the assumptions on which they are based.

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## APPENDIX 1 *Non Linear Curve Fitting*

The results of a number of the experiments described in this thesis are analyzed with the aid of a non linear curve fitting procedure available on a Hewlett Packard 9845 desktop computer.

The procedure is based on the assumption that each experimental data point may be described by a specified function of the independent variables, together with an additive error term, i.e.

$$Y_i = f(\beta_1, \beta_2, \cdots \beta_m; X_{1i}, X_{2i} \cdots X_{ki}) + \varepsilon \qquad \text{(A1.1)}$$

where $Y_i$ is the $i$th observation of the dependent variable, and $X_{1i}$ $X_{ki}$ are the $i$th values of the $k$ independent variables. The specified function $f$ involves $m$ parameters, $\beta_1 \cdots \beta_m$. The error term $\varepsilon$ ideally has a mean of zero and is normally distributed.

The purpose of the procedure is to obtain values of the parameters $\beta_1 \cdots \beta_m$ so that the quantity, $Q$ is minimized, where

$$Q = \sum_{i=1}^{N} \left[ Y_i - f(\beta_1 \cdots \beta_m; \; X_{1i} \cdots X_{ki}) \right]^2 \qquad \text{(A1.2)}$$

($N$ is the number of observations of $Y$)

In principle, the intention is identical to that of conventional linear regression : to find parameters so that the sum of the squares of the deviations of the observed values from the model is minimized. This may be done analytically for a linear model, but in general, only an approximate answer can be obtained iteratively for a non linear model. A further difference is that, whereas the least squares estimators in

a linear model are also the maximum likelihood estimators (provided $\varepsilon$ is normal, Hogg and Craig, 1970), this is not necessarily true in the non linear case.

The iterative procedure used is described by Marquadt (1963). The Hewlett Packard program continues iteration until the change in each parameter between successive iterations, $\delta_j$, satisfies the following relation:

$$|\delta_j| \; / \; [0.001 + |\beta_j|] \; < \; \Delta \qquad \text{(A1.3)}$$

The tolerance, $\Delta$, was set at 0.005 in all cases. Condition (A1.3) requires that the error in each parameter is less than $5 \times 10^{-6}$ if $\beta_j$ is small, or if $\beta_j$ is large, less than 0.5% of the absolute value of $\beta_j$.

The program produces confidence limits for parameters, but these are liable to be misleading. They are based on the assumption that within the range of the error, the function may be satisfactorily represented by the linear terms of a Taylor series expansion. Given the highly non linear nature of most of the functions fitted, and the degree of error associated with the data, these linearized confidence limits are liable to be very inaccurate.

APPENDIX 2 *Raw data from parasite induced mortality experiments*

The mean number of trophozoites per 0.16 $cm^2$ of host surface area is given in the column to the left of the page, and the number of days since infection the host survived is given in the column to the right.

| Trophozoite density | Days survived | Trophozoite density | Days survived |
|---|---|---|---|
| 5.5 | >10 | 28.5 | 4 |
| 6 | >10 | 30 | 9 |
| 6.5 | >10 | 34 | 9 |
| 6.5 | >10 | 37 | 8 |
| 10 | 7 | 41 | >10 |
| 11 | >10 | 43 | 9 |
| 12.5 | >10 | 43 | 9 |
| 12.5 | >10 | 44 | 8 |
| 17 | >10 | 44.5 | 8 |
| 18 | >10 | 47 | 9 |
| 18.5 | >10 | 48.5 | 7 |
| 20 | >10 | 50.5 | >10 |
| 22 | 9 | 52 | >10 |
| 22.5 | 8 | 52.5 | 4 |
| 23.5 | >10 | 53 | 6 |
| 24.5 | 9 | 53.5 | 8 |
| 27 | >10 | 53.5 | 4 |
| 54.5 | 9 | 144 | 6 |
| 54.5 | 6 | 155.5 | 6 |
| 54.5 | 6 | 158.5 | 5 |
| 57.5 | 5 | 168.5 | 3 |
| 58.5 | 9 | 170 | 6 |
| 62.5 | >10 | 175 | 5 |
| 66 | 5 | 176 | 9 |
| 67.5 | 9 | 176 | 4 |
| 67.5 | 6 | 182 | 4 |

APPENDIX 2 (Cont)

| Trophozoite density | Days survived | Trophozoite density | Days survived |
|---|---|---|---|
| 68.5 | >10 | 183 | 3 |
| 69 | 9 | 186.5 | 5 |
| 70 | 9 | 188.5 | 4 |
| 72 | 7 | 190.0 | 4 |
| 73.5 | 6 | 199.5 | 6 |
| 73.5 | 8 | 212.5 | 3 |
| 74 | 5 | 212.5 | 4 |
| 76.5 | >10 | 214 | 4 |
| 77 | 9 | 228.5 | 4 |
| 78.5 | 10 | 228.5 | 3 |
| 80 | >10 | 233.3 | 3 |
| 80.5 | 8 | 235.5 | 9 |
| 85 | 6 | 236 | 3 |
| 90 | 6 | 242 | 4 |
| 90.5 | 7 | 254 | 7 |
| 92 | 5 | 256.5 | 5 |
| 93.5 | >10 | 285 | 5 |
| 104.5 | 4 | 294 | 7 |
| 108 | 7 | 322.5 | 4 |
| 112.5 | 9 | 322 | 5 |
| 112.5 | 5 | 346 | 5 |
| 114 | 5 | 346 | 8 |
| 124.5 | 7 | 348.5 | 3 |
| 131 | 5 | 351.5 | 6 |
| 140 | 5 | 388 | 3 |
| 553 | 4 | 395.5 | 4 |
| 706 | 5 | 409 | 3 |

## APPENDIX 3 *The effect of heterogeneity in host susceptibility on parasite distribution*

Suppose $m$ infective stages are placed in an arena with $N$ hosts, and that all will eventually encounter a host. If all hosts are uniform in their attractiveness to infective stages, the distribution of infective stages amongst hosts is positive binomial, with mean $m/N$ and variance $m/N(1-1/N)$.

Provided $N$ is reasonably large, this may be approximated by a poisson distribution with parameter $m/N$. The poisson distribution may also arise with many other sets of assumptions: for example, hosts exposed to a sample from a suspension with a known mean concentration of infective stages.

Suppose only a proportion of the infective stages encountering a host survive to form detectable parasites: let each infective stage have a probability, $s$, of surviving. It can be easily shown that the probability of a host with 'susceptibility', $s$, having exactly $p$ parasites is also poisson (Feller, 1968).

$$P\{P = p\} = \left[\exp\ (-Ts)(Ts)^{p}\right]/p! \qquad \text{(A3.1)}$$

where $T$ is the mean number of infective stages which the host encounters.

Consider now the total population of hosts. Assume each has a 'susceptibility', $s$, between 0 and 1 to infection, and that a probability density function, $f(s)$ describes the probability that a particular host has susceptibility $s$.

Equation (A3.1) above is in fact $P\{P = p|S = s\}$. The joint p.d.f. of

$S$ and $P$ is thus:

$$g(p,s) = \exp(-Ts)(Ts)^p . f(s)/p! \quad \begin{bmatrix} p = 0,1,2 \\ 0 < s < 1 \end{bmatrix}, \qquad \text{(A3.2)}$$

$$= 0 \qquad \text{elsewhere}$$

and the marginal distribution of $P$, the number of parasites on a host is:

$$P\{P = p\} = \int_0^1 [\exp(-Ts)(Ts)^p/p!]\, .\, f(s)\, ds, \qquad \text{(A3.3)}$$

which has a probability generating function, $(E(z^p))$, $P(z)$:

$$P(z) = \int_0^1 f(s) \exp(Ts(z-1))\, ds, \qquad \text{(A3.4)}$$

from which one may obtain:

$$\left.\frac{dP(z)}{dz}\right|_{z=1} \equiv E(P) = TE(s), \qquad \text{(A3.5)}$$

and

$$\left.\frac{d^2P(z)}{dz^2}\right|_{z=1} \equiv E(P^2) - E(P) = T^2E(s^2). \qquad \text{(A3.6)}$$

Hence:

$$\text{var}\ (P) = T^2\ \text{var}\ (s) + TE(s). \qquad \text{(A3.7)}$$

## APPENDIX 4 *Microparasitic disease models*

This appendix describes the behaviour of some simple models reviewed by Anderson and May (1979, 1981) which divide the host population into susceptible, infected and immune categories. The biological basis of the models is described in detail in Anderson and May (1981) and is not repeated here.

### *Basic Assumptions*

Suppose that there are $X$ susceptible hosts and $Y$ infected hosts present in a population. To derive equations to describe the progress of an epidemic, the following assumptions are made:

(i) Infection occurs as a result of binary collision between infected and susceptible hosts at a rate $\beta XY$, where $\beta$ is a transmission constant (the inverse of $\beta$ is proportional to the average time interval between susceptible and infectious host contacts

(ii) Uninfected hosts die at a constant per capita rate of $b$ per unit of time.

(iii) The per capita death rate of infected hosts is $b$, augmented by an amount $\alpha$, representing the deleterious effect of infection.

(iv) Infected hosts may recover and rejoin the susceptible class at a rate $\gamma$ per host, per unit of time (where $1/\gamma$ is the average duration of infectiousness).

### *Constant Host Numbers*

Initially, suppose that the host population remains approximately constant at a level $H$. (ie $H = X + Y$).

The following equation will then suffice to describe temporal changes in $Y$:

$$dY/dt = Y\,[(\beta H - \alpha - b - \gamma) - \beta Y\,] \qquad \text{(A4.1)}$$

Equation (A4.1) has a time dependent solution which is logistic in form.

In order for $Y$ to be able to increase, $dY/dt$ must be positive when $Y$ is small, and hence:

$$\beta H/(\alpha+b+\gamma) \;>\; 1 \qquad \text{(A4.2)}$$

The basic reproductive rate of the disease, which is the average number of secondary infections produced per newly infected host is therefore:

$$R_o \;=\; \beta H/[\alpha+b+\gamma] \qquad \text{(A4.3)}$$

It can be seen that condition (A4.2) is equivalent to requiring that $R_o > 1$. Condition (A4.2) may also be used to obtain the threshold host density, $H_T$, below which the pathogen cannot persist in the host population (Kermack and McKendrick, 1927) where,

$$H_T \;=\; (\alpha+b+\gamma)/\beta \qquad \text{(A4.4)}$$

*Exponential host population growth*

Suppose that both classes of host have a per capita growth rate $a$, so that the total host population grows at a rate $r = a-b$ in the absence

of infection. The following equations then describe temporal changes in $X$ and $Y$.

$$dX/dt = a(X+Y) - bX - \beta XY + \gamma Y \qquad \text{(A4.5)}$$

$$dY/dt = \beta XY - (\alpha+b+\gamma)Y \qquad \text{(A4.6)}$$

or, summing eqns. (A4.5) and (A4.6) to yield an equation for changes in the total host population, $H(t)$:

$$dH/dt = (a-b) - \alpha Y \qquad \text{(A4.7)}$$

$$dY/dt = \beta(H-Y)Y - (\alpha+b+\gamma)Y \qquad \text{(A4.8)}$$

$R_o$ and $H_T$ are still given by eqns. (A4.3) and (A4.4), but in this case, if $H < H_T$, the population increases in size until $H_T$ is exceeded. The disease is able to stably regulate the host population, provided:

$$\alpha > (a-b) \qquad \text{(A4.9)}$$

If eqn. (A4.9) is satisfied, the equilibrium host population is $H^*$ where:

$$H^* = \alpha(\alpha+b+\gamma)/[\beta(\alpha-(a-b))] \qquad \text{(A4.10)}$$

At this equilibrium, the number of infected hosts is $Y^*$, where:

$$Y^* = (a-b)H^*/\alpha \qquad \text{(A4.11)}$$

If eqn. (A4.9) is not satisfied, the host population grows at an exponential rate of less than $a$-$b$ and the proportion of infected hosts increases toward unity.

*Constant Host Immigration*

If host numbers increase by a constant number, $A$, of hosts per unit of time, eqn. (A4.7) becomes:

$$dH/dt = A - bH - \alpha Y \qquad \text{(A4.12)}$$

Equation (A4.8) remaining unchanged.

In the absence of infection, eqn. (A4.12) has an equilibrium at:

$$H^* = A/b \qquad \text{(A4.13)}$$

The disease will therefore be able to invade the host population if $A/b > H_T$, where $H_T$ is defined in eqn. (A4.4).

$$\text{ie} \quad A/b > (\alpha+b+\gamma)/\beta \qquad \text{(A4.14)}$$

A highly pathogenic disease ($\alpha$ large) must therefore be highly infectious ($\beta$ large) if it is not to be eliminated from the system because infected hosts die before they can infect another host. If condition (A4.14) is satisfied, the host population is regulated to a level less than $A/b$, and is smallest when $\alpha$ is of intermediate size.

*Logistic Host Population Growth*

A more realistic assumption is that the host population increases in a logistic fashion in the absence of parasitism. It may be assumed

that the death rate per capita of hosts, is $b + \kappa H$, where $\kappa$ is a parameter describing the effect of density dependent influences other than parasitism on host population growth.

Hence:

$$dH/dt = H(a-b) - \kappa H^2 - \alpha Y \qquad \text{(A4.15)}$$

$$dY/dt = Y\left[(\beta-\kappa)H - (b+\gamma+\alpha) - \beta Y\right] \qquad \text{(A4.16)}$$

There is a disease free host population equilibrium at

$$K = (a-b)/\kappa \qquad \text{(A4.17)}$$

and in order for eqn. (A4.16) to be positive, and for the disease to increase within the host population,

$$H > (b+\gamma+\alpha)/(\beta-\kappa) \qquad \text{(A4.18)}$$

and $\kappa < \beta$

There will therefore be a (stable) equilibrium with the disease persisting, provided

$$(a-b)/\kappa > (b+\gamma+\alpha)/(\beta-\kappa) \qquad \text{(A4.19)}$$

(Actual equilibrium values of $H^*$ and $Y^*$ are given by Anderson and May, 1981).

*Immunity and Exponential Growth.*

Vertebrate hosts are usually able to develop some type of immunological

defence against microparasitic infections. It is therefore necessary to include an immune class, $Z$, in these models of disease. Hosts are assumed to enter this class at the net rate $\gamma Y$ at which they recover from infection, and to leave the class either by dying, at a per capita rate $b$, or by loss of immunity at a rate $\nu$, after which they rejoin the susceptible class. The system may be described by the following equations:

$$dH/dt = (a-b)H - \alpha Y \qquad \text{(A4.20)}$$

$$dY/dt = \beta(H-Y-Z)Y - (\alpha+b+\gamma)Y \qquad \text{(A4.21)}$$

$$dZ/dt = \gamma Y - (b+\nu)Z \qquad \text{(A4.22)}$$

The threshold host population necessary for disease persistence, $H_T$, is still given by eqn. (A4.4) and $R_o$ is still given by eqn. (A4.3).

The disease is able to regulate the host population if

$$\alpha > (a-b)\left[1 + \gamma/(b+\nu)\right] \qquad \text{(A4.23)}$$

in which case, the total host population, $H^*$, reaches a stable equilibrium where:

$$H^* = \frac{\alpha(\alpha+b+\gamma)}{\beta\left[\alpha - \frac{(a-b)}{\left[1 + \gamma/(b+\nu)\right]}\right]} \qquad \text{(A4.24)}$$

Equations (A4.23) and (A4.24) should be contrasted with eqns. (A4.9)

and (A4.10), the corresponding equations when there is no immune class. The existence of immunity makes population regulation more difficult: the pathogenicity must exceed the intrinsic birth rate of the hosts weighted by an addition factor $\gamma/(b+\nu)$. The parasite cannot control the host population if the rate of recovery from infection, $\gamma$, is sufficiently large compared with the rate of loss of immune hosts $(b+\nu)$. The equilibrium host population is greater than the equilibrium established with equivalent parameters but no immunity (Compare eqns. (A4.10) and (A4.24)).

*Immunity and Constant Host Immigration*

If hosts are introduced at a constant rate, $A$, eqn. (A4.20) becomes:

$$dH/dt = A - bH - \alpha Y \qquad \text{(A4.25)}$$

eqns. (A3.21) and (A4.22) remaining unchanged. The conditions for the existence of an equilibrium with the disease present to exist are identical to the model described by eqns (A4.12) and (A4.8), but the equilibrium host population size is greater (In eqns. (A4.25), (A4.21) and (A4.22), $\gamma$ represents the rate at which hosts recover and become immune, whereas in eqns. (A4.12) and (A4.8) $\gamma$ represents the rate at which hosts recover and rejoin the susceptible class).

*Immunity and logistic host population increase*

The following differential equations will describe the system:

$$dH/dt = (a-b)H - \kappa H^2 - \alpha Y \qquad \text{(A4.26)}$$

$$dY/dt = \beta(H-Y-Z) - (b+\kappa H)Y - (\alpha+\gamma)Y \qquad \text{(A4.27)}$$

$$dZ/dt = \gamma Y - (b+\kappa H)Z - \nu Z \qquad \text{(A4.28)}$$

where $\kappa$ is defined in the same way as in eqns. (A4.15) and (A4.16) The conditions for an equilibrium with the disease present to exist are again identical to those for the model without immunity defined by eqns. (A4.15) and (A4.16). In contrast to the model with exponential population growth defined by eqns. (A4.20) to (A4.23), the rate of loss of immunity has no bearing on whether or not an equilibrium with the disease present exists.

APPENDIX 5 *Stability Analysis of the Basic Model*

Assuming that the tomites are short lived compared with the other life history stages, the model can be defined by the following three equations.

$$\frac{dH}{dt} = (a-b)H - \alpha P \qquad \text{(A5.1)}$$

$$\frac{dP}{dt} = \frac{\Lambda\gamma_2 HC}{H_o + H} - dP - \frac{\alpha(k+1)P^2}{kH} \qquad \text{(A5.2)}$$

$$\frac{dC}{dt} = \gamma_1 P - (\gamma_2+\mu_2)C \qquad \text{(A5.3)}$$

Variables and parameters are as defined in Table 10.1

The non trivial equilibrium of the system occurs at

$$H^* = H_o/(S-1); \quad P^* = H^*(a-b)/\alpha \; ; \quad C^* = \gamma_1 P^*/(\gamma_2+\mu_2) \qquad \text{(A5.4)}$$

$$\text{where } S = \Lambda\gamma_2\,\gamma_1 /\{ [\gamma_2+\mu_2][d + (k+1)(a-b)/k] \} \qquad \text{(A5.5)}$$

and exists provided $S > 1$.

The local stability of this equilibrium can be determined following standard lines (May, 1974). The elements of the "community matrix" (May, 1974) are as follows (defining $f = dH/dt$, $g = dP/dt$, $h = dC/dt$).

$$\left.\begin{aligned} a_{11} &= \partial f/\partial H = (a-b); \; a_{12} = \partial f/\partial P = -\alpha \; ; \; a_{13} = \partial f/\partial C = 0 \\ a_{21} &= \partial g/\partial H = \Lambda\gamma_2 CH_o/(H+H_o)^2 + \alpha(k+1)P^2/(kH^2) \end{aligned}\right| \qquad \text{(A5.6)}$$

$$\left.\begin{aligned} a_{22} &= \partial g/\partial P = -\left[d + 2\alpha(k+1)P/(kH)\right] \\ a_{23} &= \partial g/\partial C = \Lambda\gamma_2 H/(H_o+H) \\ a_{31} &= \partial h/\partial H = 0;\quad a_{32} = \partial h/\partial P = \gamma_1 \;;\; a_{33} = \partial h/\partial C = -(\gamma_2+\mu_2) \end{aligned}\right\} \quad \text{(A5.6)}$$

At equilibrium, $a_{21}$, $a_{22}$ and $a_{23}$ may be simplified to

$$a_{21} = \left[(a-b)/\alpha\right]\left[\, d + 2\,(k+1)(a-b)/k - \left[d + (k+1)(a-b)/k\right]/\, S \,\right] \quad \text{(A5.7)}$$

$$a_{22} = -\left[d + 2\,(k+1)(a-b)/k\right] \quad \text{(A5.8)}$$

$$a_{23} = \gamma_2/S \quad \text{(A5.9)}$$

The eigen values, $\lambda$, of the matrix satisfy the following equation:

$$\lambda^3 - \lambda^2(a_{11}+a_{22}+a_{33}) + \lambda(a_{11}a_{22} + a_{11}a_{33} + a_{22}a_{33} - a_{32}a_{23} - a_{21}a_{12}) \quad \text{(A5.10)}$$

$$+ a_{21}a_{12}a_{33} + a_{11}a_{23}a_{32} - a_{11}a_{22}a_{33} = 0$$

For stability, the Routh Hurwitz criteria require that:

$$\text{(i)}\quad a_{11} + a_{22} + a_{33} < 0 \quad \text{(A5.11)}$$

$$\text{(ii)}\quad a_{12}a_{21}a_{33} + a_{11}a_{23}a_{32} - a_{11}a_{22}a_{33} > 0 \quad \text{(A5.12)}$$

$$\text{(iii)}\quad -(a_{11}+a_{22}+a_{33})(a_{11}a_{22} + a_{11}a_{33} + a_{22}a_{33} - a_{32}a_{23} - a_{21}a_{12}) > \quad \text{(A5.13)}$$

$$a_{12}a_{21}a_{33} + a_{11}a_{23}a_{32} - a_{11}a_{22}a_{33}$$

Condition (A5.11) requires that

$$(a-b) < d + 2(k+1)(a-b)/k + \gamma_2+\mu_2 \qquad \text{(A5.14)}$$

which is true if $a > b$

Condition (A5.12) requires that

$$\Lambda\gamma_2\gamma_1 \Big/ \left[ (\gamma_2+\mu_2)(d + (k+1)(a-b)/k) \right] > 1$$ which must be true

for the equilibrium to exist (see eqn. A5.5).

Condition (A5.13) is therefore crucial. The right hand side of condition (A5.13) is:

$$a_{12}a_{21}a_{33} + a_{11}a_{23}a_{32} - a_{11}a_{22}a_{33} = S(a-b)\left[\Lambda\gamma_1\gamma_2-[\gamma_2+\mu_2][d+(k+1)(a-b)/k]\right]$$

and the left hand side of condition (A5.13) is:

$$a_{11}a_{22} + a_{11}a_{33} + a_{22}a_{33} - a_{32}a_{23} - a_{21}a_{12}=(a-b)\left[ (\gamma_2+\mu_2)/k -[d+(k+1)(a-b)/k]/S\right]$$

Condition (A5.13) thus requires:

$$[\gamma_2+\mu_2 + df + (a-b)/k][\Lambda\gamma_1\gamma_2/(kdf)-df] > [\Lambda\gamma_1\gamma_2 - (\gamma_2+\mu_2)df] \qquad \text{(A.5.15)}$$

where $f = 1 + (k+1)(a-b)/(kd)$.

## APPENDIX 6 *Stability Analysis of the Host Parasite Model with Constant Host Introduction*

The model is defined by the equations:

$$dH/dt = A - bH - \alpha P \qquad \text{(A6.1)}$$

$$dP/dt = \lambda\gamma HP/(H_o+H) - dP - \alpha(k+1)P^2/(kH) \qquad \text{(A6.2)}$$

where $d = \gamma+\mu+\alpha+b$

As discussed in the main body of the text, an equilibrium with the parasite present exists if:

$$A/b > H_o/\left[(\lambda\gamma/d) - 1\right] \qquad \text{(A6.3)}$$

If this equilibrium exists, the equilibrium number of parasites, $P^*$, may be found in terms of the equilibrium host population, $H^*$, (using eqn. (A6.1)) where

$$P^* = \left[A - bH^*\right]/\alpha \qquad \text{(A6.4)}$$

On substitution of eqn. (A6.4) into eqn. (A6.1), it is found that $H^*$ satisfies the following quadratic equation:

$$(H^*)^2\left[k\lambda\gamma/(k+1) - dk/(k+1) - b\right] - H^*\left[H_o dk/(k+1) + A - bH_o\right] - H_o A = 0 \qquad \text{(A6.5)}$$

Defining $f = dH/dt$ and $g = dP/dt$, the partial derivatives of $f$ and $g$

with respect to $H$ and $P$ are as follows at equilibrium:

$$\left.\begin{array}{l} \partial f/\partial H = -\,b \qquad \partial f/\partial P = -\,\alpha \\ \\ \partial f/\partial H = \lambda H_o/(H_o+H^*)^2 + \alpha(k+1)(P^*)^2/[k(H^*)^2] \\ \\ \partial g/\partial P = -\,P^* \quad \alpha(k+1)/(kH^*) \end{array}\right\} \qquad \text{(A6.6)}$$

For two equations, the Routh Hurwitz criteria (May, 1974) require that:

$$\text{(i)} \qquad \partial f/\partial H + \partial g/\partial P < 0 \qquad \text{(A6.7)}$$

$$\text{(ii)} \quad (\partial f/\partial H)\,(\partial g/\partial P) > (\partial f/\partial P)\,(\partial g/\partial H) \qquad \text{(A6.8)}$$

Condition (A6.7) is clearly satisfied, as both $\partial f/\partial H$ and $\partial g/\partial P$ are negative at equilibrium. Condition (A6.8) requires that the product of two terms that are negative at equilibrium is greater than the product of one positive and one negative term. It is therefore also always satisfied at any equilibrium with parasites present that exists.

## APPENDIX 7 *Local Stability Analysis of the Model with Constant Parasite Survival of Host Death and Exponential Host Population Growth*

**The model is defined by the equations:**

$$dN/dt = N( (a-b) - \alpha M) \tag{A7.1}$$

$$dM/dt = M\left[\lambda N \left[\gamma+x(\alpha+b+\alpha M(k+1)/k)\right]/(N+1)-(\gamma+a+\alpha+\mu)-\alpha M/k\right] \tag{A7.2}$$

**and has a non trivial equilibrium where**

$$N^* - 1/\left[\lambda\left[\gamma+x(\alpha+b) + x(k+1)(a-b)/k\right]/\left[\gamma+a+\alpha+\mu+ (a-b)/k\right] \ -1 \ \right] \tag{A7.3}$$

**and**

$$M^* = (a-b)/\alpha \tag{A7.4}$$

Defining $f = dN/dt$ **and** $g = dM/dt$, **the partial derivatives of** eqn. (A7.1) **and eqn. (A7.2) with respect to** $N$ **and** $M$ **at equilibrium are given below.**

$$\partial f/\partial N = 0 \tag{A7.5}$$

$$\partial f/\partial M = -\alpha N^* \tag{A7.6}$$

$$\partial g/\partial N = \lambda(a-b)/\left[\alpha(1+N^*)^2\right] \left[\gamma+x(\alpha+b) + x(a-b)/(k+1)/k\right] \tag{A7.7}$$

$$\partial g/\partial M = \left[(a-b)/k\right] \left[ \lambda N^{*} x\ (k+1)/(1+N^{*}) - 1 \right] \qquad \text{(A7.8)}$$

The Routh Hurwitz criteria (May, 1974) require that, for stability:

$$\partial f/\partial N + \partial g/\partial M < 0 \qquad \text{(A7.9)}$$

and $(\partial f/\partial N)(\partial g/\partial M) - (\partial f/\partial M)(\partial g/\partial N) > 0$ (A7.10)

As $\partial f/\partial N = 0$ (eqn. A7.5), these conditions are simply:

$$\partial g/\partial M < 0 \qquad \text{(A7.11)}$$

and $(\partial f/\partial M)(\partial g/\partial N) < 0$ (A7.12)

Condition (A7.12) is true if any equilibrium exists, as eqn. (A7.6) is negative and eqn (A7.7) positive.

Condition (A7.11) requires that

$$\lambda N^{*}\ x(k+1)/(1+N^{*}) < 1 \qquad \text{(A7.13)}$$

Now

$$\lambda N^{*}/(1+N^{*}) = \left[(\gamma+a+\alpha+\mu) + (a-b)/k\right]/\left[\gamma+x(\alpha+b) + x(a-b)(k+1)/k\right] \qquad \text{(A7.14)}$$

Hence condition (A7.13) requires

$$x\left[(k+1)(\gamma+a+\alpha+\mu) - (\alpha+b)\right] < \gamma \qquad \text{(A7.15)}$$

## APPENDIX 8 The expected values of $e^{-\zeta i}$, $ie^{-\zeta i}$ and $i^2e^{-\zeta i}$

The probability generating function of a probability distribution, $P(z)$ is defined as

$$P(z) = E(z^i) \quad , \quad 0 < z < 1, \quad i = \{0,1,2.-\} \tag{A8.1}$$

It is implicit in this definition that $E(e^{-\zeta i})$ is simply

$P(z)$ evaluated at $z = e^{-\zeta i}$

$$\text{Now, } \frac{dP(z)}{dz} = E(iz^{i-1}) \tag{A8.2}$$

$$\text{and hence } E(ie^{-\zeta i}) = z\frac{dP(z)}{dz} \quad , \tag{A8.3}$$

evaluated at $z = e^{-\zeta i}$ ,

$$\text{and } \frac{d^2P(z)}{dz} = E(i(i-1)z^{i-2})$$

$$= E(i^2z^{i-2}) - E(iz^{i-2}). \tag{A8.4}$$

$$\text{Hence, } E(i^2e^{-\eta i}) = \frac{z^2d^2P(z)}{dz} + \frac{zP(z)}{dz} \tag{A8.5}$$

again evaluated at $z = e^{-\zeta i}$

In particular, if $i$ follows a poisson distribution,

$$P(z) = \exp(-m[1-z]) \tag{A8.6}$$

where $m$ is the mean of the distribution,

and $E(iz^i) = zm \exp(-m[1-z])$ (A8.7)

If $i$ follows a negative binomial distribution with mean $m$ and parameter $k$ inversely describing the degree of overdispersion:

$$P(z) = [1 + m(1-z)/k]^{-k} \quad \text{(A8.8)}$$

$$E(iz^i) = zm\,[1 + m(1-z)/k]^{-(k+1)}; \quad \text{(A8.9)}$$

and $E(i^2z^i) = mz(1 + m/k + mz)(1 + m(1-z)/k)^{-(k+2)}$ (A8.10)

## APPENDIX 9 *Local stability analysis of the simple model of immunity*

**The model is defined by the following equations:**

$$f \equiv dH/dt = (a-b)H - \alpha P \qquad \text{(A9.1)}$$

$$g \equiv dP/dt = \lambda P(H-I)/(H_o+H) - (b+\gamma+\alpha+\eta)P - (\alpha+\eta)P^2/(H-I) \qquad \text{(A9.2)}$$

$$h \equiv dI/dt = \eta P - (b+\nu)I \qquad \text{(A9.3)}$$

**with parameters as defined in Table 12.1.**

A non trivial equilibrium exists if eqns. (12.9) and (12.11) are satisfied. The equilibrium host population, $H^*$, is given by:

$$H^* = \frac{H_o}{\left[\dfrac{\lambda\rho}{[d + (\alpha+\eta)(a-b)/(\alpha\rho)]}\right]^{-1}} \qquad \text{(A9.4)}$$

where $\rho = 1 - \eta(a-b)/[\alpha(b + \nu)]$

and $d = b + \gamma + \alpha + \eta$

The equilibrium number of immune hosts, $I^*$ is

$$I^* = H^* \eta (a-b)/[\alpha(b+\nu)] \qquad \text{(A9.5)}$$

and the parasite population at equilibrium is:

$$P^* = H^*(a-b)/\alpha$$

At equilibrium, the partial derivatives of eqns. (A9.1) - (A9.3) with respect to each of the three variables, $H$, $P$ and $I$, are as follows:

$$\left.\begin{aligned}
&\partial f/\partial H = (a-b) \quad ; \quad \partial f/\partial P = -\alpha \; ; \quad \partial f/\partial I = 0 \\
&\partial g/\partial H = \lambda P(H_o+I)/(H+H_o)^2 + (\alpha+\eta)P^2(H-I)^2 \\
&\partial g/\partial P = \lambda(H-I)/(H_o+H) - (b+\gamma+\alpha+\eta) - 2(\alpha+\eta)P/(H-I) \\
&\qquad = -P(\alpha+\eta)/(H-I) \\
&\partial g/\partial I = -\lambda P/(H_o+H) - (\alpha+\eta)P^2/(H-I)^2 \\
&\partial h/\partial H = 0 \; ; \; \partial h/\partial P = \eta \quad ; \quad \partial h/\partial I = -(\nu+b)
\end{aligned}\right\} \qquad \text{(A9.7)}$$

The eigen values, $\Lambda$, of the community matrix (May, 1974) are given by:

$$\begin{vmatrix} \partial f/\partial H - \Lambda & \partial f/\partial P & 0 \\ \partial g/\partial H & \partial g/\partial P - \Lambda & \partial g/\partial I \\ 0 & \partial h/\partial P & \partial h/\partial I - \Lambda \end{vmatrix} = 0 \qquad \text{(A9.8)}$$

$$\Rightarrow \quad (\frac{\partial h}{\partial I}-\Lambda)|A| - \frac{\partial h}{\partial P}(\frac{\partial f}{\partial H}-\Lambda)\frac{\partial g}{\partial I} = 0 \qquad \text{(A9.9)}$$

$$\text{where } |A| = \Lambda^2 - \Lambda(\frac{\partial f}{\partial H} + \frac{\partial g}{\partial P}) + \frac{\partial f}{\partial H}\frac{\partial g}{\partial P} - \frac{\partial f}{\partial P}\frac{\partial g}{\partial H} \qquad \text{(A9.10)}$$

Let $b_1 = -\frac{\partial f}{\partial H} - \frac{\partial g}{\partial P}$ (A9.11)

now, $b_1 = -(a-b) + (a-b)(\alpha+\eta)/\alpha\rho$

using eqns. (A9.4) - (A9.7). As $\rho < 1$, $b_1$ is always positive.

Let $b_2 = \frac{\partial f}{\partial H}\frac{\partial g}{\partial P} - \frac{\partial f}{\partial P}\frac{\partial g}{\partial H}$ (A9.12)

now, $b_2 = \frac{-(a-b)^2(\alpha+\eta)}{\alpha\rho} + \alpha\left[\frac{\lambda P(H_o+I)}{(H+H_o)^2} + \frac{(\alpha+\eta)(a-b)^2}{(\alpha\rho)^2}\right]$

from eqns. (A9.4) - (A9.7)

Hence, $b_2 = \frac{(a-b)^2(\alpha+\eta)(1-\rho)}{\alpha\rho^2} + \frac{\alpha\lambda P^*(H_o+I)}{(H+H_o)^2}$ (A9.13)

which is always positive.

Equation (A9.9) can therefore be written as:

$$\Lambda^3 + \Lambda^2\left(b_1 - \frac{\partial h}{\partial I}\right) + \Lambda\left[b_2 - b_1\frac{\partial h}{\partial I} - \frac{\partial h}{\partial P}\frac{\partial g}{\partial I}\right] + \frac{\partial f}{\partial H}\frac{\partial h}{\partial P}\frac{\partial g}{\partial I} - b_2\frac{\partial h}{\partial I} = 0 \quad \text{(A9.14)}$$

In order for the equilibrium to be stable, the Routh Hurwitz criteria (May, 1974) require that three conditions be satisfied:

(i) $b_1 - \frac{\partial h}{\partial I} > 0$ (A9.15)

as $b_1 > 0$ and $\partial h/\partial I < 0$, eqn. (A9.15) is always satisfied if an equilibrium exists.

$$\text{(ii)} \quad \frac{\partial f}{\partial H}\,\frac{\partial h}{\partial P}\,\frac{\partial g}{\partial I} - b_2\,\frac{\partial h}{\partial I} > 0 \qquad \text{(A9.16)}$$

Using eqns. (A9.13) and (A9.4) to (A9.7), the left hand side of eqn. (A9.16) may be written:

$$\frac{(\nu+b)(\alpha-b)^2(\alpha+\eta)(1-\rho)}{\alpha\rho^2} + \frac{\alpha(\nu+b)\lambda P^*(H_o+I)}{(H+H_o)^2} - \frac{\eta(\alpha-b)\lambda P^*}{(H+H_o)} - \frac{\eta(\alpha-b)(\alpha+\eta)(P^*)^2}{(H-I)^2}$$

$$= \frac{\lambda P^* H_o \alpha(\nu+b)}{(H_o+H)^2} \qquad \text{(A9.17)}$$

Hence condition (A9.16) is always satisfied if an equilibrium exists.

$$\text{(iii)} \quad \left(b_1 - \frac{\partial h}{\partial I}\right)\left(b_2 - b_1\frac{\partial h}{\partial I} - \frac{\partial h}{\partial P}\frac{\partial g}{\partial I}\right) > \frac{\partial f}{\partial H}\frac{\partial h}{\partial P}\frac{\partial g}{\partial I} - b_2\frac{\partial h}{\partial I} \qquad \text{(A9.18)}$$

multiplying inequality (A9.18) out, and placing (+) below positive components, and (-) below negative components, one may obtain:

$$b_1 b_2 - b_1^2\frac{\partial h}{\partial I} - b_1\frac{\partial h}{\partial P}\frac{\partial g}{\partial I} + b_1\left[\frac{\partial h}{\partial I}\right]^2 + \frac{\partial h}{\partial P}\frac{\partial g}{\partial I}\frac{\partial h}{\partial I} > \frac{\partial f}{\partial H}\frac{\partial h}{\partial P}\frac{\partial g}{\partial I}$$

$$(+)(+) - [(+)(-)] - [(+)\ (+)\ (-)] + [(+)\ (+)] + [(+)\ (-)\ (-)] > (+)\ (+)\ (-)$$

$$\text{ie} \qquad (+) \quad > \quad (-)$$

Hence condition (A9.18) is always satisfied if an equilibrium exists and thus the equilibrium of eqns (A9.1) - (A9.3) is always stable, if it exists.

APPENDIX 10 *Derivation of the relations dM/dt = 0 and dQ/dt = 0*

The model under consideration is described by the following equations:

$$\frac{dN}{dt} = N\left[(a-b) - \kappa N - \alpha M(1-Q)\right] \qquad \text{(A10.1)}$$

$$\frac{dQ}{dt} = c(1-Q)(1-f(M)^{-k}) - (\nu+a)Q + \alpha MQ(1-Q) \qquad \text{(A10.2)}$$

$$\frac{dM}{dt} = M\left[\frac{\lambda N(1-Q)}{N+1} - (\gamma+\alpha+a) - cf(M)^{-(k+1)}\left[f(M)-e^{-\zeta}\right] - \frac{(a+\nu)Q}{(1-Q)} - \frac{\alpha M}{k}\right] \qquad \text{(A10.3)}$$

where $f(M) = 1 + \frac{M}{k}(1-e^{-\zeta})$

At equilibrium, eqns. (A10.1) - (10.3) are equal to 0. From eqn. (A10.1), one may obtain the equilibrium total host population, $N^*$, in terms of the equilibrium mean proportion of immune hosts $Q^*$, and the equilibrium mean parasite burden on susceptible hosts, $M^*$:

$$N^* = (a-b) - \alpha M^*)/\kappa + \alpha M^* Q^*/\kappa \qquad \text{(A10.4)}$$

Substitution of eqn. (A10.4) into eqn. (A10.3) produces the following equation, involving up to cubic terms in $Q$:

$$(Q^*)^3 \quad \frac{\lambda\alpha M^*}{\kappa}$$

$$+ (Q^*)^2\left\{\frac{\lambda}{\kappa}\left[(a-b) - 3\alpha M^*\right] - \frac{\alpha M^*}{\kappa}\left[\nu-\gamma-\alpha-cf(M^*)^{-(k+1)}\left[f(M^*)-e^{-\zeta}\right] - \frac{\alpha M^*}{k}\right]\right.$$

$$+ Q^* \left\{ \begin{array}{l} - \frac{\alpha M^*}{k} \left[ \gamma+\alpha+a+cf(M^*)^{-(k+1)} \left[ f(M^*)-e^{-\zeta} \right] + \frac{\alpha M^*}{k} \right] \\ + \frac{\lambda}{\kappa} \left[ 3\alpha M^* - 2(a-b) \right] \\ - \frac{\alpha M^*}{\kappa} \left[ \nu-\gamma-\alpha-cf(M^*)^{-(k+1)} \left[ f(M^*)-e^{-\zeta} \right] - \frac{\alpha M^*}{k} \right] \end{array} \right\}$$

$$+ \frac{\lambda}{\kappa} \left[ (a-b) - \alpha M^* \right]$$

$$- \kappa \left\{ \gamma+\alpha+a+cf(M^*)^{-(k+1)} \left[ f(M^*)-e^{-\zeta} \right] + \frac{\alpha M^*}{k} \right\} \left[ (a-b) - \alpha M^* + \kappa \right]$$

$$= 0 \qquad \text{(A10.5)}$$

Hence, for any value of $M^*$, there are three values of $Q^*$ at which $dM/dt = 0$.

Similarly, setting eqn. (A10.2) to zero produces the following expression, containing up to quadratic terms in $Q$:

$$(Q^*)^2 \alpha M^* + Q^* \left[ \nu+a-\alpha M^* + c(1-f(M)^{-k}) \right] - c(1-f(M)^{-k}) = 0 \qquad \text{(A10.6)}$$

## APPENDIX 11 *Stability Analysis of the model with a fixed proportion of hosts resistant at birth*

The model is defined by the following three equations:

$$\frac{dH}{dt} = H\left[(a-b) - \alpha M(1-R)\right] \qquad \text{(A11.1)}$$

$$\frac{dR}{dt} = a(1-x) - aR + \alpha MR(1-R) \qquad \text{(A11.2)}$$

$$\frac{dM}{dt} = M\left[\frac{\lambda H(1-R)}{(H+H_o)} - (\gamma+\alpha+a+x) - \frac{axR}{(1-R)}\right] \qquad \text{(A11.3)}$$

Equations (A11.1) - (A11.3) have a non trivial equilibrium if

$$b > a(1-x) \qquad \text{(A11.4)}$$

and $$\lambda(b-a(1-x))/b > \alpha+\gamma+ax+\left[a^2x(1-x)\right] / \left[b-a(1-x)\right] \qquad \text{(A11.5)}$$

The equilibrium values of $H$, $R$ and $M$ are:

$$H^* = \frac{H_o}{\left[b\left[\frac{\lambda(b-a(1-x))}{\gamma+\alpha+ax+\frac{a^2x(1-x)}{(b-a(1-x))}}\right]^{-1}\right]} \qquad \text{(A11.6)}$$

$$R^* = a(1-x)/b \qquad \text{(A11.7)}$$

$$M^* = b(a-b)/\left[\alpha(b-a(1-x))\right] \qquad \text{(A11.8)}$$

Letting $dH/dt = f$, $dR/dt = g$ and $dM/dt = h$, the partial derivatives of

$f$, $g$ and $h$ with respect to $H$, $R$ and $M$ have the following values at the equilibrium:

$$\frac{\partial f}{\partial H} = 0 \; ; \quad \frac{\partial f}{\partial R} = \alpha M^* H^* \; ; \quad \frac{\partial f}{\partial M} = -\alpha H^* (1-R^*)$$

$$\frac{\partial g}{\partial H} = 0 \; ; \quad \frac{\partial g}{\partial R} = -a+\alpha M^* (1-2R^*) \; ; \quad \frac{\partial g}{\partial M} = \alpha R^* (1-R^*) \qquad \text{(A11.9)}$$

$$\frac{\partial h}{\partial H} = \frac{\lambda M^* (1-R^*) N_o}{(H^*+H_o)^2} \; ; \quad \frac{\partial h}{\partial R} = -\frac{\lambda H^*}{(H_o+H^*)} - \frac{ax}{(1-R^*)^2} ; \quad \frac{\partial h}{\partial M} = 0$$

The eigen values of the community matrix are therefore given by the following equation (evaluated at the equilibrium):

$$\begin{vmatrix} -\Lambda & \partial f/\partial R & \partial f/\partial M \\ 0 & \partial g/\partial R-\Lambda & \partial g/\partial M \\ \partial h/\partial H & \partial h/\partial R & -\Lambda \end{vmatrix} = 0 \qquad \text{(A11.10)}$$

ie

$$\Lambda^3 - \Lambda^2 \frac{\partial g}{\partial R} - \Lambda\left[\frac{\partial h}{\partial R}\frac{\partial g}{\partial M} + \frac{\partial h}{\partial H}\frac{\partial f}{\partial M}\right] + \frac{\partial h}{\partial H}\left[\frac{\partial f}{\partial M}\frac{\partial g}{\partial R} - \frac{\partial f}{\partial R}\frac{\partial g}{\partial M}\right] = 0 \qquad \text{(A11.11)}$$

The Routh-Hurwitz criteria require that:

$$\text{(i)} \quad \partial g/\partial R < 0 \qquad \text{(A11.12)}$$

Now, at equilibrium, $\partial g/\partial R = a- +\alpha M^* (1-2R^*)$

$$= -b-\alpha M^* R^* \qquad \text{(A11.13)}$$

which is always < 0

$$\text{(ii)}\quad \frac{\partial h}{\partial H}\left[\frac{\partial f}{\partial M}\frac{\partial g}{\partial R} - \frac{\partial f}{\partial R}\frac{\partial g}{\partial M}\right] > 0 \qquad \text{(A11.14)}$$

As $\frac{\partial h}{\partial H} > 0$, eqn. (A11.14) requires that

$$\frac{\partial f}{\partial M}\frac{\partial g}{\partial R} > \frac{\partial f}{\partial R}\frac{\partial g}{\partial M} \qquad \text{(A11.15)}$$

Which, using eqns. (A11.9) and eqn. (A11.13), requires that, at equilibrium,

$$b + \alpha M^{*} R^{*} > \alpha M^{*} R^{*} \qquad \text{(A11.16)}$$

which is always true if the equilibrium exists.

$$\text{(iii)}\quad \frac{\partial g}{\partial R}\left[\frac{\partial g}{\partial M}\frac{\partial h}{\partial R} + \frac{\partial h}{\partial H}\frac{\partial f}{\partial M}\right] > \frac{\partial h}{\partial H}\left[\frac{\partial f}{\partial M}\frac{\partial g}{\partial R} - \frac{\partial f}{\partial R}\frac{\partial g}{\partial M}\right] \qquad \text{(A11.17)}$$

$$\frac{\partial g}{\partial R}\frac{\partial h}{\partial R} > -\frac{\partial h}{\partial H}\frac{\partial f}{\partial R}$$

$$(-)\ (-) > -\ (+)\ (+)$$

which is always true.

Hence any non trivial equilibrium of eqns. (A11.1) - (A11.3) is stable if it exists.

## APPENDIX 12 *Stability analysis of models in which the parasite survives host death, and host numbers are maintained constant*

### (a) *Proportion of trophozoites surviving host death is constant*

The model is defined by the following equations.

$$dP/dt = \beta HT - (\alpha+b+\gamma+\mu)P - \alpha(k+1)P^2/(kH) \qquad \text{(A12.1)}$$

$$dT/dt = \lambda\left[\gamma P - x(\alpha+b)P + x(k+1)P^2/(kH)\right] - \mu_2 T - \beta HT \qquad \text{(A12.2)}$$

If a non trivial steady state exists, the equilibrium number of tomites, $T^*$, is given by:

$$T^* = \lambda P^* \left[\gamma+x(\alpha+b) + x\alpha(k+1)(P^*)^2/(kH)\right] / (\mu_2+\beta H) \qquad \text{(A12.3)}$$

substituting eqn. (A12.3) into eqn. (A12.1) and setting it to 0:

$$\frac{P^*\alpha(k+1)}{kH}\left[1 - \frac{\lambda H x}{H_o+H}\right] = \frac{H\lambda}{H_o+H}\left[\gamma+x(\alpha+b)\right] - (\alpha+b+\gamma+\mu) \qquad \text{(A12.4)}$$

where $H_o = \mu_2/\beta$

It can be seen that an equilibrium will exist if

$$x < (H_o+H)/(\lambda H) \qquad \text{(A12.5)}$$

and $$H > \frac{H_o}{\{\lambda\left[\gamma+x(\alpha+b)\right]/(\alpha+b+\gamma+\mu) - 1\}} \qquad \text{(A12.6)}$$

Defining $f = dP/dt$ and $g = dT/dt$, the partial derivatives of $f$ and $g$ with respect to $P$ and $T$ are:

$$\left.\begin{aligned} \partial f/\partial P &= -(\alpha+b+\gamma+\mu) - 2P\alpha(k+1)/(kH); \quad \partial f/\partial T = \beta H \\ \partial g/\partial P &= \lambda\left[\gamma+x\left[\alpha+b+2\alpha(k+1)P/(kH)\right]\right] ; \quad \partial g/\partial T = -(\mu_2+\beta H) \end{aligned}\right\} \qquad \text{(A12.7)}$$

With two equations, the Routh Hurwitz criteria are (May, 1974);

$$\partial f/\partial P + \partial g/\partial T < 0 \qquad \text{(A12.8)}$$

and $(\partial f/\partial P)(\partial g/\partial T) > (\partial f/\partial T)(\partial g/\partial P)$ (A12.9)

Condition (A12.8) is clearly always true

Condition (A12.9) requires that:

$$(\mu_2+\beta H)(\alpha+b+\gamma+\mu+2P\alpha(k+1)/(kH)) > \lambda\beta H\left[\gamma+x\left[\alpha+b+2\alpha(k+1)P/(kH)\right]\right] \qquad \text{(A12.10)}$$

$$\Rightarrow \quad \frac{2\alpha(k+1)P}{kH}\left[1 - \frac{\lambda Hx}{H+H_o}\right] > \frac{\lambda H}{H_o+H}\left[\gamma+x(\alpha+b)\right] - (\alpha+b+\gamma+\mu) \qquad \text{(A12.11)}$$

Comparison of eqn. (A12.4) with condition (A12.11) shows that at equilibrium, condition (A12.11) requires that

$$\frac{\alpha(k+1)\ P}{kH} > 0 \qquad \text{(A12.12)}$$

which is always true. Hence any equilibrium is stable.

(b) *Proportion of trophozoites surviving host death declines with increasing burden*

The model is defined by the following equations:

$$dP/dt = \beta HT - (\gamma+\alpha+\mu+b)P - \alpha(k+1)P^2/(kH) \qquad \text{(A12.13)}$$

$$dT/dt = \lambda\gamma P + \lambda HS(M) - \beta HT - \mu_2 T \qquad \text{(A12.14)}$$

where $S(M)$ is as defined by eqn. (11.23)

If an equilibrium exists, the number of tomites is given by:

$$T^* = \lambda\left[\gamma P + HS(M)\right] / (\mu_2+\beta H) \qquad \text{(A12.15)}$$

substituting eqn. (A12.15) into eqn. (A12.13) gives the following equation for the equilibrium value of $P$

$$\frac{\lambda H}{(H_o+H)}\left[\gamma + S(M^*)/M^*\right] = (\alpha+\mu+\gamma+b) + \frac{\alpha(k+1)P^*}{Hk} \qquad \text{(A12.16)}$$

where $H_o = \mu_2/\beta$ and $M^* = P^*/H$

Provided

$$\frac{\lambda\gamma H}{(H_o+H)} > (\alpha+\mu+\gamma+b) \qquad \text{(A12.17)}$$

ie. $H > H_T$, eqn. (A12.16) will always have a solution, because $S(M) \to 0$ as $M \to \infty$.

Defining $f = dP/dt$ and $g = dT/dt$, the partial derivatives of $f$ and $g$ with respect to $P$ and $T$ are:

$$\left.\begin{aligned} &\partial f/\partial P = -(\gamma+\alpha+\mu+b) - 2\alpha(k+1)P/(kH) \;;\; \partial f/\partial T = \beta H \\ &\partial f/\partial P = \lambda\gamma + \lambda\, \partial S(M)/\partial M \;;\quad \partial g/\partial T = -(\beta H+\mu_2) \end{aligned}\right\} \qquad \text{(A12.18)}$$

The Routh Hurwitz criteria are as defined in conditions (A12.8) and (A12.9).

As $\partial f/\partial P < 0$ and $\partial g/\partial T < 0$, condition (A12.8) is obviously satisfied. Condition (A12.9) requires that:

$$(\beta H+\mu_2)(\gamma+\mu+\alpha+b+2\alpha(k+1)M/k) > \beta H(\lambda\gamma+\lambda dS(M)/dM) \qquad \text{(A12.19)}$$

$$\Rightarrow \left[\lambda H/(H_o+H)\right]\left[\gamma+dS(M)/dM\right] < \gamma+\mu+\alpha+b+2\alpha(k+1)M/k \qquad \text{(A12.20)}$$

Comparison of condition (A12.20) with eqn. (A12.16) shows that condition (A12.20) will always hold at equilibrium if

$$dS(M)/dM < S(M)/M \qquad \text{(A12.21)}$$

$S(M)$ may be written as $MV(M)$, where $V(M)$ is a function of $M$ which is strictly monotonically decreasing with increasing $M$.

Now.

$$dS(M)/dM = V(M) + MdV(M)/dM \qquad \text{(A12.22)}$$

As $V(M)$ is monotonically decreasing, $dV(M)/dM < 0$, and hence $dS(M)/dM < S(M)/M$, and the equilibrium is stable.

APPENDIX 13 *Two forms of a parasite, one with a higher reproductive rate than the other.*

Suppose there are two types of parasite, $P_1$ and $P_2$, and that the rate of increase of $P_1$ ($\lambda_1$) is greater than that of $P_2$ ($\lambda_2$), but the per capita pathogenicity of each is the same. Using the same parameter definitions as in Chapter 12 (Table 12.1), and denoting the number of $P_1$ parasites on a host by $i$, and the number of $P_2$ parasites by $j$, the following equations will describe the behaviour of the system:

$$\frac{dH}{dt} = (a-b)H - \alpha(P_1 + P_2) \qquad \text{(A13.1)}$$

$$\frac{dP}{dt} = \frac{\lambda_1 P_1 H}{(H+H_o)} - (b+\gamma)P_1 - \alpha H E(i^2) - \alpha H E(ij) \qquad \text{(A13.2)}$$

$$\frac{dP}{dt} = \frac{\lambda_2 P_2 H}{(H+H_o)} - (b+\gamma)P_2 - \alpha H E(j^2) - \alpha H E(ij) \qquad \text{(A13.3)}$$

If both $P_1$ and $P_2$ parasites are independently randomly distributed,

$$\frac{dP_1}{dt} = \frac{\lambda_1 P_1 H}{(H_o+H)} - (b+\gamma+\alpha)P_1 - \frac{\alpha P_1^2}{H} - \frac{\alpha P_1 P_2}{H} \qquad \text{(A13.4)}$$

$$\frac{dP_2}{dt} = \frac{\lambda_2 P_2 H}{(H_o+H)} - (b+\gamma+\alpha)P_2 - \frac{\alpha P_2^2}{H} - \frac{\alpha P_1 P_2}{H} \qquad \text{(A13.5)}$$

at equilibrium, $dP_1/dt = 0$ and $dP_2/dt = 0$. If neither $P_1$ or $P_2 = 0$ then:

$$\lambda_1 H/(H+H_o) = (b+\gamma+\alpha) + \alpha P_1/H + \alpha P_2/H \qquad \text{(A13.6)}$$

and

$$\lambda_2 H(H+H_o) = (b+\gamma+\alpha) + \alpha P_2/H + \alpha P_1/H \qquad \text{(A13.7)}$$

If $\lambda_1 \neq \lambda_2$, eqns. (A13.6) and (A13.7) cannot be simultaneously satisfied. One of $P_1$ or $P_2$ must equal 0. This is merely a manifestation of the competitive exclusion principle.

Suppose, however, that the distributions of $i$ and $j$ are still independent, but that the distributions of $i$ and $j$ are individually aggregated. This is unlikely to be the case if overdispersion is generated primarily by differences in host susceptibility to infection, but may be an adequate first approximation, if overdispersion is generated by hosts passing through clouds of infective stages.

Assuming that the degree of overdispersion of each parasite may be described by a negative binomial distribution, and (for simplicity) that a common $k$ will describe both distributions, eqns. (A13.2) and (A13.3) become:

$$dP_1/dt = \lambda_1 P_1 H/(H_o+H) - (b+\gamma+\alpha)P_1 - \alpha(k+1)P_1^2/(kH) - \alpha P_1 P_1/H \qquad \text{(A13.8)}$$

$$dP_2/dt = \lambda_2 P_2 H/(H_o+H) - (b+\gamma+\alpha)P_2 - \alpha(k+1)P_2^2/(kH) - \alpha P_1 P_2/H \qquad \text{(A13.9)}$$

Assume that $\lambda_1 > \lambda_2$

At an equilibrium with only $P_1$ present,

$$P_1^* = H\left[\frac{\lambda_1 H}{(H_o+H)} - (b+\alpha+\gamma)\right]\frac{k}{\alpha(k+1)} \qquad \text{(A13.10)}$$

At this point,

$$\frac{dP_2}{dt} = P_2 \left[ \frac{\lambda_2 H}{H+H_o} - (b+\gamma+\alpha) - \frac{\alpha P_1^*}{H} \right] \qquad \text{(A13.11)}$$

ie $$\frac{dP_2}{dt} = P_2 \left[ \frac{\lambda_2 H}{H+H_o} - (b+\gamma+\alpha) - \frac{k}{(k+1)} \left[ \frac{\lambda_1 H}{H+H_o} - (b+\gamma+\alpha) \right] \right] \qquad \text{(A13.12)}$$

Even though $\lambda_1 > \lambda_2$, eqn. (A13.12) may be positive, provided $k$ is sufficiently small. The form of the parasite with the lower reproductive rate may therefore be able to invade a population of the other form of the parasite. If eqn. (A13.12) is positive when $P_2$ is small, eqn. (A13.8) will also be positive when $P_1$ is small, and an equilibrium with both forms of the parasite present must exist.