Endemic infection in the German cockroach, *Blattella germanica*

A thesis submitted for the Degree of Doctorate of Philosophy

by

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V. Summary

1. Epidemic disease outbreaks pose a significant risk to the stability and survival of many populations on earth. Current methods to understand how epidemic diseases transmit are often confounded by heterogeneity in infection rates amongst host populations. Endemic parasites, which are often less severe compared to epidemic diseases, may contribute to that variation by impacting on host biology and therefore altering the transmission of epidemic diseases.

2. The effects of an endemic, gastrointestinal infection on host fitness and host interactions with an epidemic parasite were explored in a novel invertebrate system developed for this study. The chosen host was the German cockroach, *Blattella germanica*, which was infected with an endemic protozoan parasite, *Gregarina blattarum* alone or in co-infection with an entomopathogenic nematode, *Steinernema carpocapsae,* which causes epidemic outbreaks in host populations.

3. There was evidence of density dependent regulation by the endemic parasite. Reductions in both host survival and fecundity during endemic infection both contributed to this regulation. The endemic parasite also had fitness costs for offspring from infected parents, who took longer to reach adulthood and were less likely to survive. Protozoan infected host populations had lower densities and showed less variance in population fluctuation compared to parasite free populations and the endemic parasite was generally found at high prevalence within the infected groups. When hosts infected with *G. blattarum* were exposed to *S. carpocapsa*e, the resulting co-infection led to reduced host survival but also reduced emergence of nematode transmission stages. Hosts infected with *G. blattarum* also had differential immune responses to macro and microparasites which could alter host susceptibility to different types of infections.

4. Infection with an endemic parasite caused substantial changes in the biology of the host, which may have important effects on host population ecology. Endemic parasites can also have considerable consequences for the transmission potential of more a virulent pathogen. These findings demonstrate the important of endemic

infections which should be given greater consideration in future host-parasite studies.

1. General Introduction

The term "parasite" is defined as any organism that is dependent on a host for resources (*i.e.* habitat, food, protection), and which does so to the detriment of the host (Anderson & May 1978). A wide variety of organisms can therefore be considered parasitic, from single celled microbes and viruses, to multicellular helminths, arthropods and even vertebrates (Viney & Cable 2011). Consequently, parasites are considered to be the most abundant form of life on earth (Hudson 2005), and few non-parasitic organisms are likely to evade infections or interactions with parasites during their lifespan. Parasites therefore feature strongly within all ecosystems, impacting on host abundance (Hudson, Dobson, & Newborn 1992; Albon *et al.* 2002), biodiversity (Hudson, Dobson & Lafferty 2006) and community structure (Mouritsen & Poulin 2002).

Host populations, both human and animal, are occasionally threatened by severe outbreaks of disease and there are several examples demonstrating that parasites can drive entire populations to extinction (Cunningham & Daszak 1998; Daszak, Cunningham & Hyatt 2000; Stuart *et al.* 2004; Schloegel *et al.* 2006; Pounds *et al.* 2006; Hawkins *et al.* 2006; Smith, Sax & Lafferty 2006; Skerratt *et al.* 2007; McCallum *et al.* 2009). Identifying how diseases will transmit through populations during an outbreak is essential for infection control strategies (Ferguson, Donnelly, & Anderson 2001). Yet disease transmission patterns may be confounded by several factors including heterogeneity in the susceptibility of hosts to infection (Dwyer, Elkinton, & Buonaccorsi 2007). Understanding the causes of heterogeneity in infection rates will improve the ability to monitor and predict disease transmission (Schleihauf, Watkins & Plant 2009).

Heterogeneity in host susceptibility to infection is intrinsically linked to host immune function, which can vary with host sex (Schmid-Hempel 2005), age (Stear *et al.* 2000) and environmental conditions (Krist *et al.* 2000; Ostfeld *et al.* 2006). The rate at which hosts acquire infection is also dependent on the contact rate between hosts (Clay *et al.* 2009), host density (Anderson & May 1978) and exposure risk (Woolhouse *et al.* 1997). Variation in infection rate is therefore related to patterns of host behaviour (Lafferty & Morris 1996) and heterogeneity in immune responses (Carius, Little & Ebert), as well as environmental stressors (Lafferty &

Kuris 2005). An important form of environmental stress that hosts frequently encounter is infection with endemic parasites. Recent studies have shown that co-infections, where the host is simultaneously infected with more than one parasite (Graham *et al.* 2008), can alter host responses to parasites (Graham 2008) and change the pattern of the infection dynamics (Fenton 2008). As outbreaks of epidemic disease often occur in the context of endemic parasite co-infections (Petney & Andrews 1998), understanding the effects of endemic infection on host fitness may improve the current methods in which to analyse disease transmission.

1.1 Endemic parasites and host fitness

In epidemiology, diseases are defined as endemic when they are maintained continuously within a population without the introduction of the disease from an external source (Guégan, Morand & Poulin 2005). In contrast, an epidemic occurs through the introduction of a novel agent or adaption of an existing disease which leads to the number of new cases exceeding those that would normally be expected in the population (Green *et al.* 2002). Some of the most common endemic diseases amongst host populations are gastrointestinal worms, such as cestodes, nematodes and trematodes (e.g. de Silva *et al.* 2003), which remain endemic due to the production of environmental transmission stages such as eggs and cysts which increases the circulation of the disease in the host population. It is estimated that a quarter of the global human population are infected with gastrointestinal nematodes including roundworm, tapeworm and pinworms, which can lead to malnutrition (e.g. Stephenson, Latham & Ottesen 2000), iron deficiency (e.g. Stoltzfus *et al.* 1996) and poor growth in children (Crompton & Neisheim 2002). Gastrointestinal infections are also highly significant in other host species, with nematodes such as *Haemonchus* sp., *Teladorsagia* sp. and *Trichostrongylus* sp. responsible for an estimated £1000 million cost to the livestock industry annually through loss of yield (Newton & Munn 1999). In wild animal populations, gastrointestinal infections can also change the dynamics of host population (Hudson, Dobson & Newborn 1992; Albon *et al.* 2002). Endemic gastrointestinal infections are therefore a considerable burden to human and animal populations around the world, and understanding the effects on the infections on host fitness is essential to improving disease control.

Gastrointestinal infections can bring about important consequences for host fitness, by altering the nutritional levels of the host in a variety of ways. A common consequence of gastrointestinal infection is host malaise and loss of appetite (e.g. Horbury, Mercer & Chappell 1995; Jones, Anderson & Pilkington 2006; Laurenson, Bishop & Kyriazakis 2011), which leads to decreased food intake and subsequent reductions in host fat and protein levels (e.g. Coop & Holmes 1996; Stephenson, Latham & Ottesen 2000; Sykes & Greer 2003). In the gut, the infection may also disrupt host digestion and absorption so that there is a decrease in the nutrient levels that are obtained from the consumed food (Fox 1997). Host nutritional levels are also influenced by the removal of nutrients from the gut or host tissue (Sykes & Greer 2003), by damage at the infection site, which can lead to loss of blood and iron deficiencies (Stoltzfus *et al.* 1996), as well as by the increased metabolism required to mount an immune response (Sheldon & Verhulst 1996).

As gastrointestinal infections can impact on the host energy budget, the resources available for life history processes such as growth, fecundity and survivorship, may also be impaired by the parasite. Farmed animals can have an increase in the rate of miscarriage when gastrointestinal parasites are present, which can have important economic costs for the livestock industry (Liu, Masters & Adams 2003). Gastrointestinal infections can also increase host mortalities, particularly in environmental extremes (Albon *et al.* 2002; Craig, Pilkington & Pemberton 2006). Given that both birth and death rates of host populations are important determinants of the dynamics of host populations (Anderson & May 1978), gastrointestinal infections, through indirect costs to host resources and life history, can also impact on the host at the population level.

Fluctuations in animal abundance can occur through density dependent feedbacks, where the maximum population density is limited by a decrease in host reproduction or an increase in host mortality (May 1981). Parasites can therefore impact on host density by affecting one or more of the host life history processes. For example, the parasitic nematode *Trichostrongylus tenuis* in the red grouse can lead to increased costs to host fecundity which can drive population cycles in the host density over time (Hudson, Dobson & Newborn 1992). Gastrointestinal infections in Arctic reindeer have also been shown to cause increased losses of offspring over the winter,

which may lead to seasonal dynamics in the host population (Albon *et al.* 2002). Yet with the exception of these two studies, there have been very few attempts to study the impact of gastrointestinal infections on host population dynamics. An increase in population level studies would therefore broaden our understanding of how parasites may regulate populations in other ecosystems, as well as how those dynamics might impact on the host interaction with other parasites.

The fitness costs to hosts from endemic gastrointestinal infections can also have implications for the host when epidemic outbreaks occur. Whilst very few studies have examined the effects of gastrointestinal infections on the infection rate of epidemic disease, it is known from co-infection surveys in the wild, that hosts with greater parasite diversity have more reduced energy levels (Lello *et al.* 2005), reduced fecundity (Lello *et al.* 2005) and an increased mortality rates (Jolles *et al.* 2008) compared to those hosts with single infections. Co-infections can also lead to an increase in the transmission rates of disease as hosts are more vulnerable to invasion by epidemic diseases, something that has been widely demonstrated in HIV-malarial co-infections (Ter Kuile *et al.* 2004; Gallagher *et al.* 2005; Abu-Raddad *et al.* 2006; Trott *et al.* 2011).

Yet the outcomes of the co-infection are not always negative for the host. Indeed, some parasites can increase the host immune function against invading parasite species (Bazzone *et al.* 2008). In a meta-analysis of different co-infections in mice, it was demonstrated that such variation in outcomes was dependent on the type of co-infection (Graham 2008). When gastrointestinal helminths which caused anaemia were present, microparasites that required red blood cells to replicate had reduced density, whereas helminths which supressed host inflammatory responses yielded higher numbers of microparasites during the co-infection (Graham 2008). The effects of helminth-microparasite co-infections are starting to be considered in theoretical models (Fenton, Lamb & Graham 2008; Fenton 2008). However, so far there have been few attempts to determine whether the differences in immunological or pathological effects during gastrointestinal infections will impact on the epidemiology of a parasite population, *i.e.* whether gastrointestinal infections increase or decrease the transmission rate of an epidemic in the host population. To date, this has been restricted by a lack of suitable empirical systems with which to

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explore parasite transmission. Accordingly, there is an urgent need to find novel systems in which to explore the effect of endemic infection on epidemic outbreaks, in order to improve treatment programmes for infectious disease.

1.2 Host-parasite model system

The host-parasite system that was used throughout this study comprised the German cockroach host *Blattella germanica* (Fig. 1.1a) infected with two parasite species, an endemic parasite, the gastrointestinal protozoan parasite *Gregarina blattarum* (Fig. 1.1b) and an epidemic species, the entomopathogenic nematode *Steinernema carpocapsae* (Fig. 1.1c).
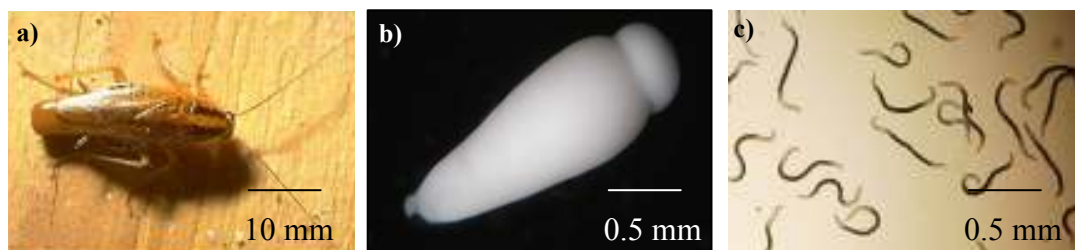
Figure 1.1: Model host-parasite co-infection system: (a) the German cockroach host, *Blattella germanica* (female with ootheca), (b) the gastrointestinal protozoan parasite, *Gregarina blattarum* (trophozoite), and (c) the entomopathogenic nematode *Steinernema carpocapsae* (infective juveniles).

The German cockroach, *B. germanica*, is a widespread pest species which mostly occur in close proximity to human and animal buildings (Rivault 1989). The close association with humans can lead to problems for human health including the onset of asthma in children (de Blay *et al.* 1997) and the distribution of diseases of clinical importance (Fotedar, Shriniwas & Verma 1991). As a result, the German cockroach has been widely used in scientific studies particularly with respect to control agents used to restrict cockroach lifespan and fecundity (e.g. Durier & Rivault 2000). A more detailed description of the German cockroach life cycle is presented in Chapter Three (p43). Briefly, adults emerge from final nymph instars and reach sexual maturity within three days of moulting. After mating, the female packages the eggs into an ootheca (egg case) which remains attached to the female until the nymphs are ready to hatch. Females continue to produce oothecae every 30 days for the duration of their lifespan (~240 days). The hatched offspring develop over a period of

approximately 60 days, and require six to seven moults to reach adulthood (Roth 1968).

Gregarines are gastrointestinal protozoan parasites which are endemic to many invertebrate species including annelids, arachnids, crustacean and insects (Clopton & Gold 1996). The gregarine species *G. blattarum* is a natural parasite of the German cockroach (Tsai & Cahill 1970). In a study conducted by Clopton & Gold (1996), it was shown that the parasite was also highly host specific, incapable of infecting four other species of cockroaches tested. There is no evidence (literature or personal observations) that *G. blattarum* is infective to juveniles, and the mechanism for this is currently unknown. As a result, all infected hosts described in this study refer to adult cockroaches only. The life cycle of the parasite is direct, with hosts becoming infected after the accidental ingestion of oocysts from contaminated faeces and food. Within the host intestinal tract, sporozoites contained within the oocyst emerge and migrate to the mid-gut of the host. The sporozoites attach to the intestinal epithelium between micro-villi and develop into trophozoites. When the trophozoites are fully developed, they detach from the mid-gut and pair up to form reproductive units (gametocysts). The gametocysts are passed out into the environment with cockroach faeces, where gametogeny and fertilisation occurs, eventually producing oocytes which are ingested by hosts to complete the cycle. To date, there has been little attempt to determine the effect of *G. blattarum* on the German cockroach host, with the main exception being a study by Lopes & Alves (2005) who found gregarines caused increased mortality of the German cockroach. As a gastrointestinal infection, there is the potential that this parasite will have important effects on the energy levels, life history and population dynamics of the host.

The entomopathogenic nematode *S. carpocapsae* is a highly virulent parasite with a broad host spectrum, which has led to its use as a biocontrol agent against a variety of important pests including corn borers (Ben-Yakir *et al*. 1998), leaf miners (Sher, Parella & Kaya 2000) and root weevil larvae (Booth, Tanigoshi & Shanks 2002), as well as the German cockroach (Koehler, Patterson & Martin 1992; Appel *et al*. 1993). The nematode life cycle is direct, whereby parasites are transmitted as non-feeding infective juveniles, which invade the host through natural openings such as host spiracles, mouth and anus (Adams & Nguyen 2001). Once inside the host

haemocoel, symbiotic bacteria (*Xenorhabdus nematophila*) are regurgitated by the nematodes, which cause septicaemia and host death within 72 hours (Adams & Nguyen 2001). Infective juveniles develop into feeding third stage juveniles, then fourth stage juveniles, and finally adult males and females (Grewel *et al.* 1997). The adults mate and produce eggs that hatch into first stage juveniles, and moult into second, third and fourth stages before emerging as second generation adults (Adams & Nguyen 2001). After two to three reproductive cycles, or when resources are limited, development ceases at the third juvenile stage, whereupon the juveniles re-ingest the bacteria and exit the host (Grewel *et al.* 1997). The induction of high host mortality and ability to cause serious and rapid depletions of invertebrate populations are important qualities for use of this parasite as an epidemic disease in epidemiological work.

1.3 Research aims

The objective of this thesis was to determine how endemic parasite infection can alter host fitness and whether those changes affect the interaction of the host with a more virulent disease.

Chapter Two: Host population dynamics

Parasite infections can regulate host population dynamics and previous studies have shown that gastrointestinal helminths can destabilise host populations. It is unknown whether endemic protozoan parasites can regulate host population dynamics, or whether endemic infections can regulate the dynamics of invertebrate populations. The aims of this chapter were to test the hypothesis that *G. blattarum* alters the regulatory processes of the German cockroach host population. German cockroach populations were monitored continuously for almost two years, and a series of host-parasite models were compared to the observed time series to assess density dependent regulation in the host population dynamics.

Chapter Three: Host life history

Host population dynamics are regulated by density dependence acting on the host birth or death rates. Further, these density dependent effects may be delayed, for example they may be mediated by fitness cost to offspring due to having infected

parents. *Gregarina blattarum* is known to reduce host survival, but it is unclear whether host survivorship changes with the age of the host, or what effects the parasite has on host fecundity and offspring fitness. The chapter tested the hypotheses that *G. blattarum* reduces German cockroach survival and fecundity, and increases the developmental rate of offspring.

Chapter Four: Epidemic transmission potential

Endemic parasites may compete with other parasites for host resources, which could potentially alter the transmission rate of epidemic parasites within the host population. One important component of parasite transmission is the reproduction of infective stages within the host. Host energy resources (lipid levels) were explored in hosts infected with *G. blattarum,* and the resource change was then used to determine whether resource costs imposed by the endemic infection altered the number of nematode transmission stages that emerged from cockroach hosts.

Chapter Five: Host immune responses

Heterogeneity in immune responses between individual hosts can alter the probability of infection within the host population. Such variation in immune response may be the result of parasite infections already present in the host. Gastrointestinal infections may alter host immune responses to parasites, which could impact on host susceptibility to more virulent diseases. Host immune responses to micro- and macroparasites were assessed in parasite free and *G. blattarum* infected hosts to test the hypothesis that gregarines alter host immune function.

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2. Do endemic infections in the German cockroach regulate host population dynamics?

2.1 Abstract

Endemic infections are costly to the host and can regulate host population dynamics. To date, this understanding is based on work conducted in mammalian and avian systems, whereas invertebrate populations may also be regulated by endemic parasite infections. The aims of this study were to determine whether endemic parasites are important influences on the regulatory processes in an invertebrate host population. Laboratory populations of the German cockroach, *Blattella germanica*, which either contained the gastrointestinal protozoan parasite *Gregarina blattarum* or were parasite-free controls, were monitored bi-monthly for almost two years by capture-mark-recapture. At each sampling time-point a sub-sample of the captured individuals was dissected to estimate the parasite load in infected groups. A series of host-parasite models were compared using a model fitting procedure. Infected colonies had lower mean densities and reduced variation around the mean compared to uninfected hosts, but there was no correlation between parasite infection levels and host density. Host populations in the absence of parasites were regulated by linear density dependence acting on death rate, and by a Poisson probability distribution, indicative of demographic stochasticity. Infected host population dynamics were described by several regulatory processes, including parasite-only host regulation, regulation from density dependence acting on births and density dependence acting on host death rate. The random error in the infected group also varied between environmental (Gaussian) and demographic (Poisson) stochasticity. These findings clearly demonstrate that endemic parasites affect the regulatory structure of the invertebrate host population, despite the fact that the dynamics of the parasites themselves are not correlated with host population density. The variability in the models that best fitted infected host colonies suggests that multiple regulatory processes may be occurring in this host-parasite system. Further work is needed to determine whether the regulation by the parasite is stabilising, and to differentiate the different levels of stochasticity that may influence the host dynamics.

2.2 Introduction

Animal population densities fluctuate in the environment. One of the most important factors governing such fluctuations are a result of density dependent regulation on population density (May 1981). Several density dependent factors have been proposed, which can lead to changes in host population regulation, including competition, predation and parasitism (Hudson, Dobson & Newborn 1992). Parasitism is particularly important because it can give rise to density dependent regulatory processes in the absence of predation or intra-specific competition (Anderson & May 1978; May & Anderson 1978). Yet in comparison to competition and predation studies, there are still relatively few empirical demonstrations of parasitic regulation of host populations.

Many studies to date have focused on the effect that acute microparasitic diseases can have on host population dynamics (Anderson & May 1981; Grosholz 1992; Sait, Begon & Thompson 1994; Bjornstad *et al.* 1998; Van Bressem, Van Waerebeek & Raga Esteve 1999; Boots & Norman 2000; Bonsall & Benmayor 2005; Kuenzi *et al.* 2007; Yoshida *et al.* 2007). Classic theoretical studies have demonstrated that endemic parasites can also have important regulatory effects on host populations (Anderson & May 1978; May & Anderson 1978). In general, endemic parasites can stabilise host population sizes when three conditions are met: high parasite aggregation amongst hosts; parasite-induced host mortalities; and density-dependent constraints on parasite reproduction (Anderson & May 1978). Under a different set of conditions, endemic parasites can lead to destabilising effects in the host population, through parasite-induced reduction in host fecundity, random or low levels of parasite aggregation among hosts and time delays between parasite reproduction and transmission (May and Anderson 1978). Hudson, Dobson & Newborn (1992) provided empirical support for the destabilising effects of host-parasite interactions in an avian host, the red grouse. After further analysis (Dobson and Hudson 1992) it was shown that the biological processes that led to the cycling dynamics in the host densities, were host fecundity costs of the parasite, low parasite aggregation and time delays in parasite transmission, providing clear evidence for the destabilising processes outlined by May & Anderson (1978). More recently, gastrointestinal nematode infections in the Artic Svalbard reindeer, a population with

no competitive herbivores or predators in the environment, has also been shown to cause cycling host population dynamics (Albon *et al*. 2002) due to parasite-induced fecundity costs.

Our understanding of how parasites regulate host population dynamics is extremely limited due to very few empirical studies. Further, to my knowledge, there have been no attempts to monitor endemic, gastrointestinal parasite regulatory processes in invertebrate populations. Several noticeable studies have considered the effects of parasitoids (Hassell, Comins & May 1991; Jones *et al.* 1993; Bonsall & Hassell 1998) and microparasite infections in invertebrate host populations (Anderson & May 1981; Boots & Norman 2000; Bonsall 2004; Bonsall & Benmayor 2005), demonstrating the potential for density dependent regulation in these host organisms. As endemic infections can cause substantial fecundity and mortality reductions in invertebrate hosts (e.g. Lafferty 1993; Jaenike, Benway & Stevens 1995), which may act to regulate host populations (Anderson & May 1978), it is important that the imbalance is addressed in entomological research.

Host-parasite dynamics have been previously described using a range of different models, including differential equations (Anderson & May 1978; May & Anderson 1978; Anderson & May 1981; Briggs & Godfray 1995; Milner & Patton 1999; Gaff & Gross 2007). These models can demonstrate a range of different processes for example the stage of the host life cycle (if any) when density dependence applies. Density dependence may consist of linear or non-linear negative feedbacks on the population density through either host birth or death rates (Bellows 1981). The density dependence is therefore vital in understanding population regulation (May 1981). There are also important density-independent processes which can cause populations to fluctuate. Ecological datasets are generally noisy, and the noise can occur due to random variation (stochasticity) in host numbers. This stochasticity may be a result of demographic variation in host birth and death rates or be due to variation in the environment (May 1973). Gaussian probability distributions are good representations of environmental stochasticity, whereas Poisson or negative binomial distributions generally give rise to demographic error (Melbourne & Hastings 2008). A maximum likelihood fitting approach can be a useful method with which to determine the most appropriate model structure, by finding the best fit between

empirical time series and population models (Bonsall & Hastings 2004; Bonsall & Benmayor 2005; Bateman, Coulson, & Clutton-Brock 2011; Smallegange, van der Meer & Fiedler 2011; Strevens & Bonsall 2011). This approach requires selection of a series of candidate models, followed by an estimation of the stochastic error, a likelihood fitting procedure and finally a goodness of fit test between the model predictions and observed data (Bonsall & Hastings 2004). For example, Bonsall & Benmayer (2005) used this approach to show that regulatory processes in the Indian meal moth, changed during different levels of parasitism. This approach may therefore provide an important first step in exploring the host and endemic parasite population dynamics and be particularly useful when there is stochasticity in the population dynamics.

Longitudinal studies of host density, parasite levels and distribution are also important in determining host population regulatory processes (Anderson & May 1978; May & Anderson 1978). Parasite infection dynamics are described by fluctuations in abundance (number of parasites per host), intensity (number of parasites per infected host) or prevalence (percentage of infected hosts). These levels may be synchronised or lag behind host densities if the host-parasite dynamics are linked by parasite regulation of host density (Dobson & Hudson 1992). The distribution of parasites can be estimated using Taylor's aggregation parameter b, which describes the slope of the regression of the log mean (x+1) parasite abundance on the log variance (Taylor 1970) and allows comparisons of different aggregations which are independent of the mean (Boag et $al.$ 2001). Parasites are considered aggregated when b > 1, with most parasite distributions in wild host populations having b values close to 1.55 (Shaw & Dobson 1995). Dobson & Taylor (1992) demonstrated that low levels of aggregation can led to destabilisation of the host dynamics. As parasite levels and distribution amongst hosts are important in determining host density regulation, measurements of parasite levels and distribution may also be important in understanding the regulation of invertebrate host populations.

The aim of this work was to determine the effect of endemic parasites on host population regulatory processes. To this end, a time series dataset was collected using laboratory colonies of the German cockroaches (*Blattella germanica*) in

addition to the infection dynamics of its gastrointestinal parasite *Gregarina blattarum*. These data were explored using a model fitting procedure to determine the effect of the parasite on host density dependent and density independent processes. It was predicted that *G. blattarum* would cause regular cycles in the cockroach population through altered density dependent feedback. Further, the parasite dynamics were expected to be correlated (with or without time lag) with the host densities.

2.3 Materials and Methods

Host Culture

German cockroaches (ca. 100 individuals) were originally obtained from a laboratory supply company (Blades Biologicals Ltd.) in 2007 and were reared in 19 litre plastic boxes (Really Useful Box Co.) lined with Fluon® (Blades Biologicals Ltd.) to prevent cockroach escape. Boxes contained cardboard egg-box refugia (4 x sheets of 20cm^2), and colonies were provided with food (ground dog biscuits in the base of a 90 mm dia. Petri dish) and dechlorinated water (in 50 ml falcon tubes with a cotton wool bung). All cockroaches were maintained at $25 \pm 1°C$, $30 \pm 2\%$ relative humidity, with a 12:12 h LD photoperiod. A sub-sample of these cultures were dissected and found to contain *G. blattarum*, a pathogen specific to this cockroach species (Clopton & Gold 1996). As *G. blattarum* oocysts (transmission stages) are only transmitted horizontally, parasite free cultures were founded by collecting oothecae from gravid infected females, and rinsing with 10% ETOH and sterile distilled water to wash away any external oocysts (in accordance with Müller-Graf *et al.* 2001). The parasite free oothecae were then incubated on damp filter paper within 90 mm dia. Petri dishes until the offspring had hatched, upon which they were transferred to parasite-free containers and reared as above.

Population Sampling

The population sizes of uninfected and *G. blattarum* infected colonies of *B. germanica* were estimated using the mark-recapture technique. This method also allows for the removal of a sub-sample at each census point to determine the parasite load. Five colonies of *G. blattarum* infected (1-5) and five uninfected controls (A-E) were chosen randomly from the stock colonies and reared under standard laboratory

conditions as outlined above. Colonies were sampled fortnightly for up to 40 time-points (21 months) in all colonies except for two uninfected colonies (E and F), which were only sampled for 24 time-points (12 months) due to a gregarine contamination. Samples were collected by shaking the egg-box refugia over Fluon® lined plastic pots for 60 s. The sampling pots were then stored on ice (for up to 20 min) to allow individuals to be counted and marked. The adults were separated from any juveniles, sexed, and any previous marks noted. All adults within the sample were marked using a small dot of enamel paint (Revell®) on the pronotum, applied with an entomological pin. A different paint colour was used for each sampling occasion. A sub-sample of males and females (20% of each sex) at each time point was randomly collected and each host was dissected to determine the total gut-parasite content (or disposed of if from the uninfected colonies). The remaining adults were retained in recovery arenas with food, water and shelter for 24 h, and then released back into their respective colonies. The estimations of host population size were then derived from the stochastic model by Jolly (1965; see Appendix 7.1).

Parasite Sampling

At each sampling time-point, the sub-samples of cockroaches collected were anaesthetised with CO_2 and stored on ice for up to 30 minutes prior to dissection. Individuals were collected and wings, legs, head and oothecae removed and the cadaver transferred to a Petri dish (90 mm dia.) lined with black beeswax (Lassco®; to enable the cadaver to be fixed with entomological pins), and half-filled with 0.005 M phosphate buffered saline (PBS). Using a stereo-microscope (GX Optical XLT-101), the thorax was cut open and the mid-gut located which was pinned to the Petri dish. Parasites were subsequently counted by gently squeezing the gut with soft forceps to remove trophozoite parasite stages. Three measurements of parasite levels were then calculated from the parasite counts: abundance, the number of trophozoites per host, including uninfected hosts; intensity, the number of trophozoites per infected host; and prevalence, calculated as the percentage of infected hosts in each sample.

Statistical analysis

All statistical analyses were conducted within R version 2.13.2 (R Development Core Team 2011). The effect of *G. blattarum* on host population density was assessed using the bootstrapped mean density and coefficient of variation (10,000 resamples with replacement) and using these values in a subsequent two-sample T-test to compare between uninfected and infected host populations. Patterns of frequency in the dynamics of the uninfected and parasitized-host populations were compared visually using spectral analysis plots.

Parasite abundance, intensity and prevalence, normalised using a logarithmic transformation prior to the analysis, were compared between each of the five colonies using a General Linear Model. The aggregation levels of *G. blattarum* amongst hosts were assessed using Taylor's power law index of aggregation *b* (Taylor 1970), which describes the linear relationship between log mean and log variance of parasite counts, using the equation:

$$\text{log variance} = a + b \text{ log mean}$$

where *a* is the sampling coefficient (Boag, Neilson & Brown 1992). A bootstrapping technique was used to calculate the log mean and log variance, by random sampling (with replacement) of parasite counts within each time-point (n = 37). The sampling process was repeated 10000 times, and a linear model was used to calculate the value of Taylor's *b* for each sub-sample. The change in parasite aggregation over time, between colonies and between adult sexual stages (males, females or females with oothecae) was conducted using a general linear model. The relationship between parasite aggregation and the three measures of parasite number (prevalence, abundance, intensity) was determined using simple linear regression analysis. Correlations between log host and log parasite data were assessed using Pearson's correlation test and any time lags in the correlation were assessed using a cross-correlation plot.

Model Selection

A series of host and host-parasite candidate models was chosen based on derivations of the well-established host-parasite model devised by Anderson & May (1978),

adapted with different descriptions of host density dependence, parasite distribution and stochastic error using functions described by Bellow (1981) and previously used in host-parasite analysis by Bonsall & Benmayor (2005) (Table 2.1). These models only represent adult host populations, as *G. blattarum* does not infect juveniles in the German cockroach population (see Chapter 1, page 6).

Table 2.1: Candidate model list for German cockroach host (N_1) and *Gregarina blattarum* parasite (N_2) populations. Terms or equations in italics are exclusive to host-parasite models and are absent from host only (parasite-free) equations. The two parasite models (A and B) were each paired with the host models for host-parasite analyses (1-5). Other terms in the models are given as follows: t = time (days); r = instantaneous host birth rate; d = parasite independent host death rate; K = host population carrying capacity; θ = linearity of density dependence (where $\theta = 1$ is linear); D = density dependent host death rate; a & v = associated density dependent functions; b = parasite population growth rate; e = parasite dependent host death rate; g = parasite loss as a factor of host immune function; d = host independent parasite death rate; X = aggregation parameter. [†]*DD = density dependence*

Host Models	Uninfected populations	Description
1	$dN_1/dt = rN_1 - dN_1 - e*N_1*N_2$	Exponential growth
2	$dN_1/dt = rN_1*(1-(N_1/K)) - dN_1 - e*N_1*N_2$	Linear DD[†] on births
3	$dN_1/dt = rN_1*(1-N_1/K)^\theta - dN_1 - e*N_1*N_2$	Non-linear DD on births
4	$dN_1/dt = rN_1 - (d+(D*log(N_1)))*N_1 - e*N_1*N_2$	Linear DD on deaths
5	$dN_1/dt = rN_1 - (d+(log(1+(a*N_1)^v)))*N_1 - e*N_1*N_2$	Non-linear DD on deaths
Parasite Models		
A	$dN_2/dt = b*N_2*N_1 - (e+g+d)*N_2 - (e*N_2^2/N_1)$	Random distribution
B	$dN_2/dt = b*N_2*N_1 - (e+g+d)*N_2 - e*(X+1)N_2^2/(X*N_1)$	Aggregated distribution

The density dependent mechanisms present in the uninfected populations was assessed by fitting four models, each of which differed in the relationship between

host density and density dependence (linear/non-linear) and/or in the process upon which it acted (births and deaths). As a baseline control, the population dynamics were modelled using a simple exponential growth equation with no density dependence (Model 1). Each model included the instantaneous host birth rate (r) and parasite independent host death rate (d). Models 2 and 3 also included host population carrying capacity (K), and in Model 3 a descriptor (θ) was used to vary the linearity in the density dependence. Model 4 contained a density dependent parameter (D) on host death rate, which in Model 5 was described by two parameters for non-linear density dependence on host death rate (a & v). Infected host dynamics were assessed with the same five models but were also linked, via the parasite-induced host death rate (e), to a second equation, describing the parasite intensity dynamics. The infected host populations also included the rate of parasite growth (b) and two other descriptors of parasite death rate: death caused by interactions with the host immune system (g) and loss of hosts through natural host death rate (d). Further, the infected host populations were linked to two parasite models, each describing different levels of parasite distribution (random and aggregated, A and B respectively) and which were fitted to the parasite intensity data. For this, an aggregation parameter (X) was used to transform the distribution of the parasite population.

Model fitting and goodness of fit

The regulatory processes as described by the models were examined using a maximum likelihood-based approach to determine the best fitting model or models for each replicate colony. This approach used a model fitting procedure to compare the integrated models of the host and host-parasite dynamics with that of the observed data. This was implemented using numerical integration and optimization algorithms in the C programming language (Bonsall & Hastings 2004). This process generated a series of parameter estimates for each model with a likelihood value that described the relationship between the model and the observed data. The likelihood values were obtained from each model in turn and the fitting procedure was repeated for the uninfected (n = 5) and infected (n = 5) populations, for both random and aggregated parasite distributions and for three measures of host and parasite stochastic error: Gaussian, Poisson and negative binomial. An AIC score was

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subsequently obtained from each likelihood value (L) given the relationship: AIC = 2k − 2 ln (L), where k is the number of parameters in the model. Within each host colony, the AIC scores were weighted to determine the most appropriate model or series of candidate models and error distributions per colony. One-step-ahead predictions were then generated using the best fitting model or set of models for each colony. In the final step a goodness of fit test was performed to examine the similarities between the predicted (expected) and observed data for each candidate model using a Chi-squared test.

2.4 Results

Gregarina blattarum had a significant effect on the population density of its host *B. germanica*. The mean density of colonies containing the parasite were reduced by 71.6% from an estimated bootstrapped mean density of 4619 to 1309 per colony (t_4 = 3.07, p = 0.037; Table 2.2).

Table 2.2: Summary of uninfected German cockroach population densities (A-E) an infected colonies (1-5). Mean = bootstrapped mean with 2.5% and 97.5% confidence intervals (CI); the variation between samples over the time series for each colony is given as the coefficient of variation (C of V). . *CI = confidence intervals; .*

	Colony	Mean	2.5% CI	97.5% CI	C of V
Uninfected hosts (control)	A	2807	1640	4492	0.26
	B	3030	1543	5618	0.37
	C	2849	1623	4577	0.27
	D	6498	4699	9110	0.18
	E	7888	4370	13516	0.31
G. blattarum-infected hosts	1	1351	1064	1688	0.12
	2	1336	964	1901	0.19
	3	1356	1025	1746	0.14
	4	1095	849	1399	0.13
	5	1408	949	2009	0.19
Mean control	-	**4615**	**2775**	**7463**	**0.3**
Mean infected	-	**1309**	**970**	**1748**	**0.2**

Host population densities were considerably varied (Fig. 2.1), but the bootstrapped variation in colony density was substantially lower in infected (CV = 0.15) compared to uninfected colonies (CV = 0.28) (t_8 = 3.58, p = 0.007).
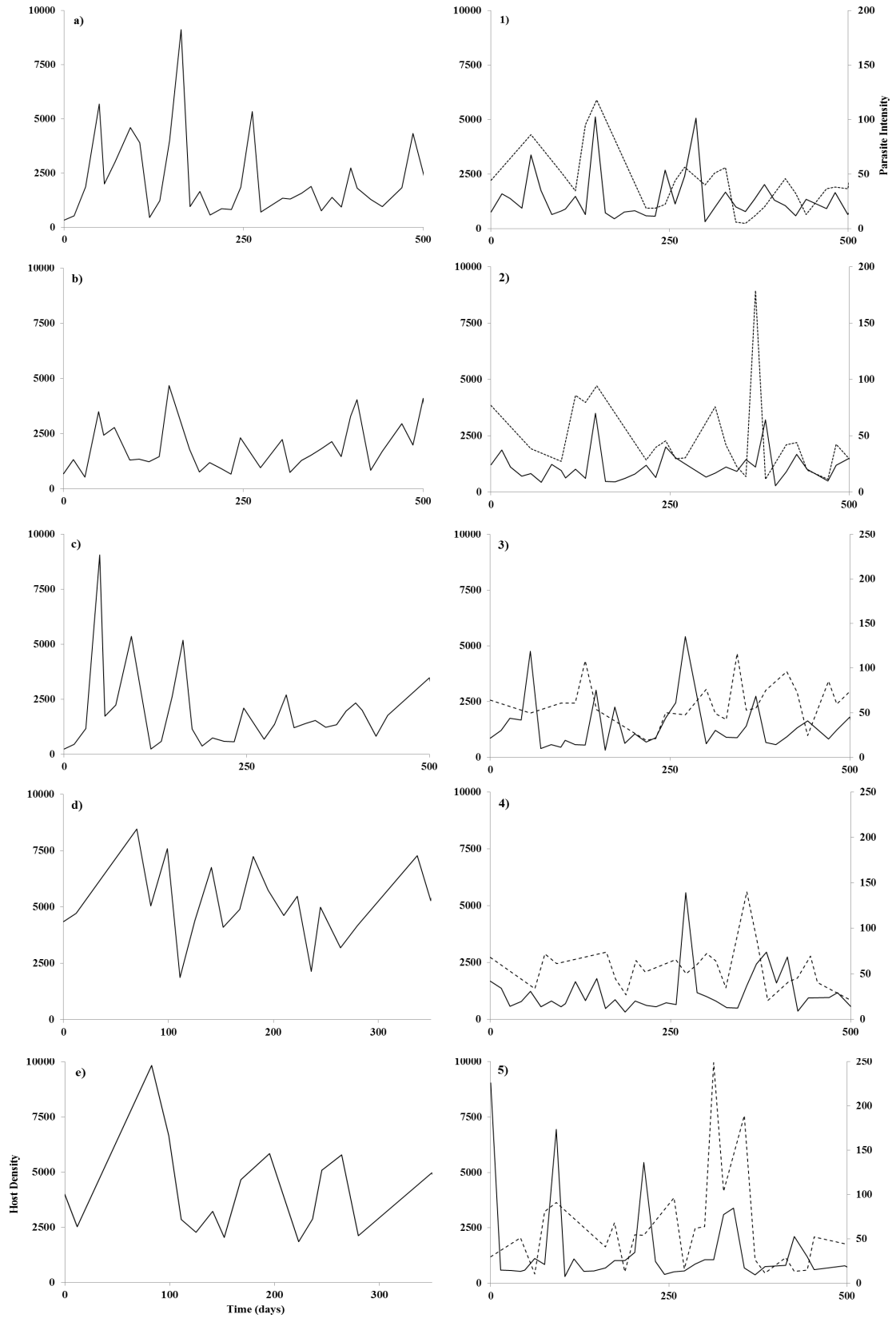
Figure 2.1: Time series of uninfected German cockroach populations (a-e, left panel), *Gregarina blattarum* infected populations (1-5, right panel), and *G. blattarum* intensity (dashed lines).

Host population densities varied over time between periods of high and low frequency fluctuations (Fig. 2.2). However, the spectral analyses did not show any consistent patterns (indicative of population cycles), either in uninfected colonies or infected colonies (see Appendix 7.2).
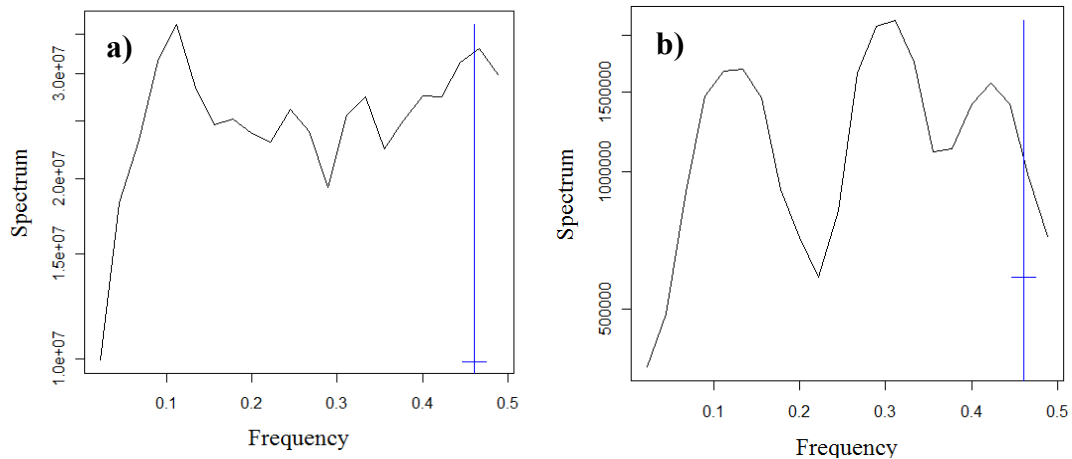
Figure 2.2: Example spectral analysis plot of an uninfected German cockroach population (a) and an infected host population (b). Blue line denotes 95% confidence intervals for the power spectrum; blue bar is mean power spectrum level. *All host and parasite spectral analyses are shown in Appendix 7.2.*

Parasite abundance and intensity were substantially different amongst the five tested colonies (Table 2.3; $F_{4,151} = 6.12$, $p < 0.001$ and $F_{4,151} = 5.84$, $p < 0.001$ respectively). The bootstrapped mean abundance of parasites 33.4 trophozoites in the host population and the bootstrapped mean parasite intensity was 48.4 amongst infected hosts. The bootstrapped mean prevalence of parasite infection varied between 10-95% through time with a mean prevalence of 65.3%, but there were no statistically significant differences in the prevalence levels between the five colonies ($F_{4,151} = 174$, $p = 0.144$).

Table 2.3: Summary of *Gregarina blattarum* parasite population dynamics (bootstrapped mean host density with the coefficient of variation), presented as mean parasite abundance, intensity and prevalence (%). *CI = confidence intervals.*

	Colony	Mean	2.5% CI	97.5% CI	Coefficient of variance
G. blattarum abundance	1	22.5	15.7	30.4	0.16
	2	27.6	21.0	34.8	0.13
	3	39.6	32.2	46.8	0.10
	4	37.3	30.1	44.8	0.10
	5	40.2	27.0	56.0	0.18
Mean abundance	-	**33.4**	**25.2**	**42.6**	**0.13**
G. blattarum intensity	1	31.8	23.3	41.1	0.14
	2	42.5	32.2	54.4	0.13
	3	55.8	45.9	65.8	0.09
	4	53.7	43.6	64.4	0.10
	5	58.0	39.9	80.4	0.18
Mean intensity	-	**48.4**	**37.0**	**61.2**	**0.13**
G. blattarum prevalence (%)	1	60.2	53.3	66.9	0.06
	2	63.2	57.6	68.7	0.05
	3	69.6	65.1	74.1	0.03
	4	68.2	62.9	73.5	0.04
	5	67.6	61.3	73.6	0.05
Mean prevalence (%)	-	**65.7**	**60.0**	**71.3**	**0.04**

Parasite distribution amongst hosts was generally aggregated (Fig. 2.3; b > 1) but the levels of aggregation varied with time, with an interaction between time and the status of the adult ($F_{2,102}$ = 3.26, p = 0.042). The predicted mean aggregations in males (1.58 ± 0.07 SE) and females with oothecae (1.59 ± 0.08 SE) were higher compared to females that were not carrying oothecae (1.39 ± 0.06 SE). Parasite aggregation was also negatively correlated with parasite abundance (R^2 = 0.15, t_{154} = -5.25, p < 0.001), intensity (R^2 = 0.19, t_{154} = -6.20, p <0.001) and prevalence (R^2 = 0.03, t_{154} = 2.35, p = 0.02).
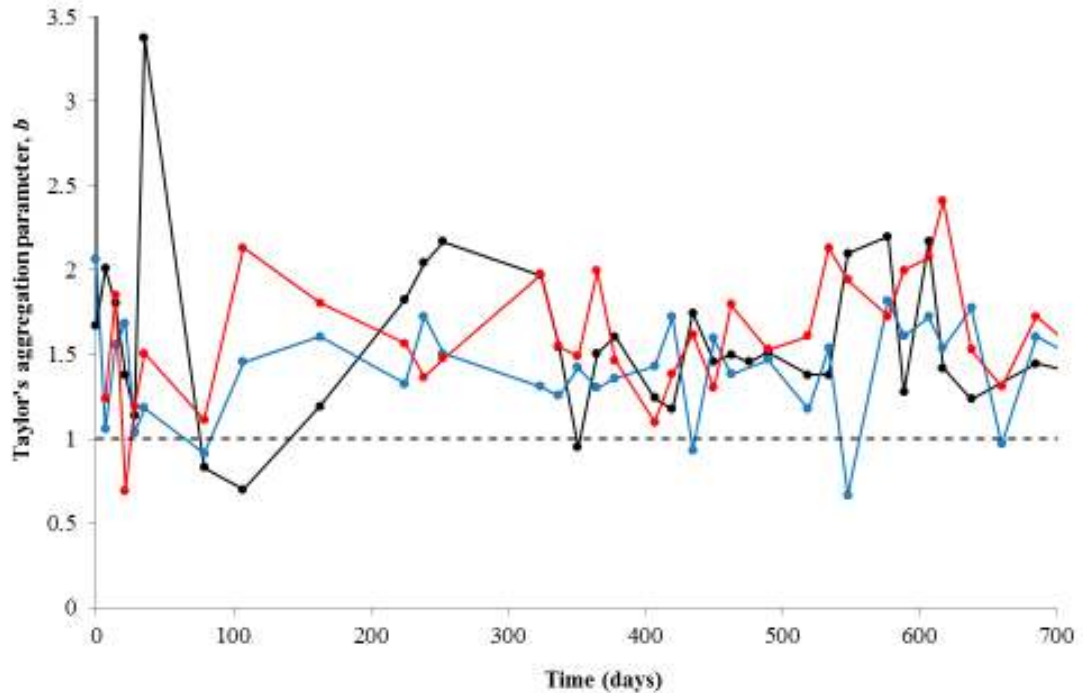
Figure 2.3: Time series of *Gregarina blattarum* parasite aggregation according to Taylor's aggregation parameter, *b* for males (black line), females (blue line) and females with oothecae (red line). Dashed line represents the random distribution level (b = 1).

There was no correlation in any of the five infected colonies ($p > 0.05$) between the host density and the parasite population measures of abundance, intensity or prevalence (e.g. Fig. 2.4a; see Appendices 7.3-5 for all correlation plots). There was also no evidence of time lags in host-parasite correlations in any of the colonies from the cross-correlation plot (e.g. Fig. 2.4b; see Appendices 7.3-5 for all cross-correlation plots).
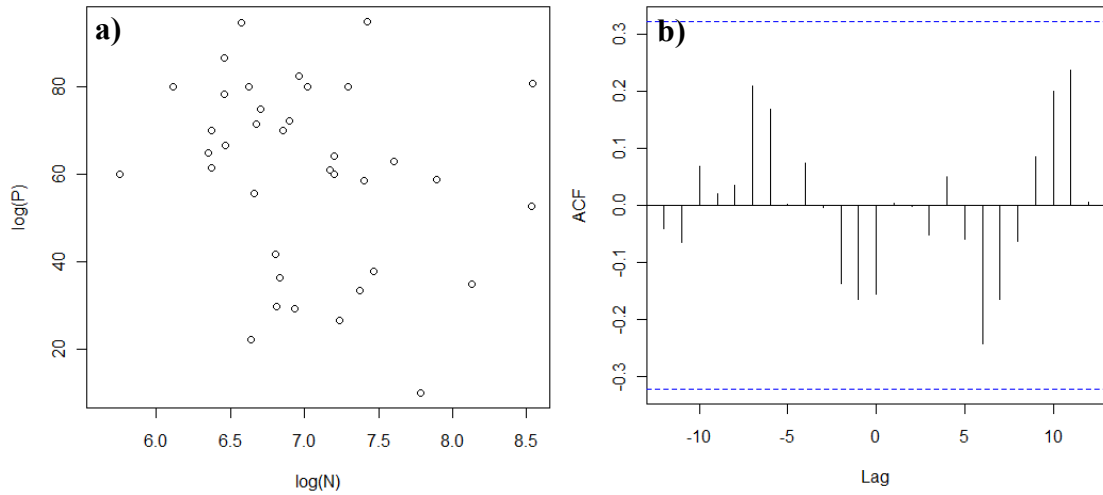
Figure 2.4: Example correlation plot of log German cockroach host density (N) against log *Gregarina blattarum* parasite prevalence (P) (a), and cross-correlation analysis of log host density with log parasite prevalence (b). Blue lines are 95% CI for cross-correlation significance level. *Colonies 2-5 and comparisons with parasite abundance and prevalence are shown in Appendix 7.3-5.*

Model Selection

The best model selected for each host and parasite population is shown in Table 2.4. Model 4, which describes host regulation by linear density dependence acting on host death rate (see Table 2.1 for model descriptions), most closely described the uninfected host colonies (A-E). These uninfected host populations were also associated with a substantial amount of Poisson stochastic error, as shown by irregular population fluctuations (Fig. 2.1) and poor goodness of fit between the deterministic model and the observed data (Fig. 2.5a, $p < 0.001$).

Table 2.4: Goodness of fit test (Chi-squared) between the closest fitting model predictions and observed time-series data for uninfected German cockroach hosts (A-E), infected hosts (1-5a) and *Gregarina blattarum* parasites from infected host populations (1-5b). *Models not significantly different to observed data (p > 0.05). Neg-bin = negative binomial*

	Colony	Host error	Parasite error	Parasite distribution	Model	AIC	Chi-sq	P
Uninfected hosts	A	Poisson			4	78.65	1590.26	< 0.0001
	B	Poisson			4	84.51	27403.90	< 0.0001
	C	Poisson			4	85.64	16430.46	< 0.0001
	D	Poisson			4	45.52	712.79	< 0.0001
	E	Poisson			4	70.34	579.33	< 0.0001
Infected hosts	1a	Gaussian	Poisson	Random	2	156.97	125.55	< 0.0001
	2 a	Gaussian	Neg-bin	Random	3	147.24	30.59	0.288*
	3a	Poisson	Gaussian	Aggregated	4	125.18	20.19	0.821*
	4a	Poisson	Gaussian	Random	1	119.58	626.18	< 0.0001
	5a	Poisson	Gaussian	Random	1	130.50	653.40	< 0.0001
Parasites	1b	Gaussian	Poisson	Random	2	156.97	423.66	< 0.0001
	2 b	Gaussian	Neg-bin	Random	3	147.24	76.19	< 0.0001
	3b	Poisson	Gaussian	Aggregated	4	125.18	89277.05	< 0.0001
	4b	Poisson	Gaussian	Random	1	119.58	268.10	< 0.0001
	5b	Poisson	Gaussian	Random	1	130.50	311.23	< 0.0001

The candidate model selection was more varied in the infected host populations (colonies 1-5) where almost every candidate model was used as the best descriptor for one or other of the infected colony dynamics. Only the non-linear density dependence model (model 5) gained no support by comparison of the weighted AIC. There was a better goodness of fit of infected models to the observed data compared to parasite-free models (Fig. 2.5b), with non-significant differences between predictions and observed data for colonies 2 (Chi-sq$_1$ = 30.59, p = 0.288) and 3 (Chi-sq$_1$ = 20.19, p = 0.821). However, more than half the host population deterministic models (Table 2.4; colonies 1, 4 and 5) and all of the parasite deterministic models were poor fits to the data as determined by a goodness of fit test. The stochastic error description for the infected host populations was either Gaussian or Poisson distributed. The parasite populations were most frequently (4/5) described by a random distribution amongst hosts and were associated more often (3/5) with a Gaussian error structure.
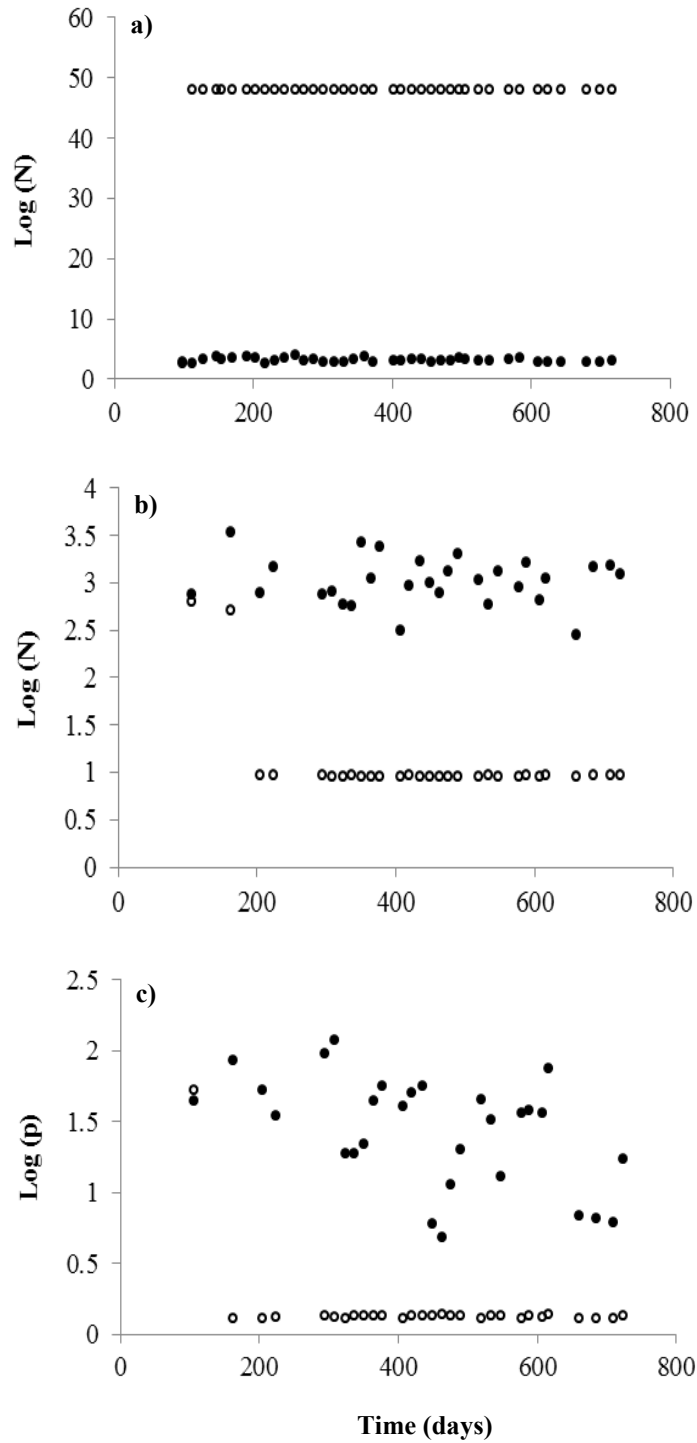
Figure 2.5: One-step-ahead predictions for log German cockroach host numbers (N) in an uninfected sample population "A" (a) and infected population "1" (b), and parasite (P) intensity from colony "1" (c). Closed circles are observed data and open circles are model predictions. *All one-step-ahead predictions are shown in Appendix 7.6.*

2.5 Discussion

This study has demonstrated that endemic parasites can alter the regulatory structure of an invertebrate host population. In the absence of parasites, cockroach populations were most frequently described with models that included linear density dependence acting on host mortality (Table 2.1, equation 4). However, where parasites were present, the dynamics of host populations were not described by any single model form. Instead, models describing each colony contained different density dependent functions and descriptions of the host stochasticity. In a study by Bonsall & Benmayor (2005), the density dependent processes were shown to be shifted from density dependence on host death rates to host birth rates in the presence of multiple microparasite infections compared to single infections. The current work demonstrates that a shift can also occur in host density dependence in the presence of endemic, gastrointestinal infections. However, it is not clear from these data whether host birth or death rates are more important or whether the effects are linear or non-linear, due to the various outcomes from the fitting procedure. It is likely that multiple processes may be responsible for the changes in host regulatory processes which needs further investigation, for example by fitting models that allow density dependence on both processes.

A goodness of fit test was used to assess the suitability of the deterministic model selection (*i.e.* in the absence of stochasticity) when compared to the observed data. There was good support for two infected host population models (colonies 2 and 3), although there were no similarities in the density dependent function of the two models, which described non-linear density dependence on births and linear density dependence on deaths repetitively (Table 2.4). The remaining infected host population deterministic models (colonies 1, 4 and 5), the parasite population models and the parasite free population models (a-e) all had poor fits to the observed data (Fig. 2.5). This suggests that the stochasticity in the models were extremely important in the initial model fitting procedure. In parasite free models this was best described with a Poisson distribution. This particular stochastic distribution is generally used in modelling demographic stochasticity (Melbourne & Hastings 2008) and has been demonstrated to be important in other invertebrates such as stoneflies (Engen *et al.* 2011), mayflies (Engen, Aagaard & Bongard 2011),

mosquitoes (Otero, Solari, & Schweigmann 2006) and moths (Bonsall & Benmayor 2005). However, in parasite infected host populations, the stochasticity shifted towards a Gaussian error distribution which is normally indicative of environmental variation. Endemic parasites are therefore impacting not only on the deterministic processes in the host population dynamics, but also the stochastic processes which may explain the selection of multiple models for the infected populations, and the poor goodness of fit.

Stabilising effects of host populations by parasites are indicated by reduced fluctuations, parasite-induced host mortality rate and over-dispersion of parasites within hosts (Anderon & May 1978). *Gregarina blattarum* reduced the mean density of host populations by 71% compared to populations where the parasite was absent. Reduction in mean host density is likely to be a consequence of reductions in host fecundity or survival and given the stabilising effects of *G. blattarum* it would be expected that survivorship costs in the hosts are more influential in the regulatory processes (addressed in Chapter Three). The time series were also extremely varied (Fig. 2.1) but the variation in host population fluctuations was reduced in infected hosts compared to parasite-free populations. However, rather surprisingly, there was no relationship between the levels of the parasite infection (abundance, intensity or prevalence) and the dynamics of the host population which is also noticeable when parasites regulate host populations (Dobson & Hudson 1992).

From the model fitting procedure, the parasite dynamics were best explained by a model containing random parasite distribution. This was surprising as an aggregated distribution was most frequently measured in the observed samples (Fig. 2.3; b > 1). Boag *et al*. (2001) demonstrated that parasite data does not always conform to standard error descriptions, such as the negative binomial distribution. Therefore the selected parasite distributions in this current study may require further investigation to capture the aggregated distribution in the population models. Overall, the aggregation levels that were measured in the host time series dataset, is indicative of stabilising effects on the host population (in accordance with Anderson & May 1978), and combined with the reduced variation in host density this suggests that the parasites are stabilising rather than destabilising host population dynamics in this system.

This study also provided evidence of sex bias and female reproductive stage variation in parasite distributions, where infections in females without oothecae were more randomly distributed than males or females that carried oothecae. Variation in the levels amongst hosts may be caused by environmental, behavioural or social factors (Boag *et al.* 2001). Female cockroaches for example, may reduce food consumption and spend longer periods inside refugia when incubating oothecae (Lee & Wu 1994), thereby altering their parasite exposure risk. This can have an important impact on the transmission rate of the parasites as well as on the regulatory processes that govern the host population dynamics.

The endemic parasite, *G. blattarum* is clearly having an effect on the overall host population density, the structure of the deterministic models and the model stochasticity terms. There is good support that the endemic parasites are stabilising the dynamics of this invertebrate host population, but further work is needed to test this hypothesis. Now that the general host and parasite population structures and stochastic error distributions associated with this host-macroparasite system have been established, future work can explore the effect of different types of stochasticity (demographic, environmental and process errors) that are clearly important in influencing the host-parasite dynamics. Overall, this study provides a novel insight into how endemic parasites may regulate invertebrate host populations.

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3. *Gregarina blattarum* infection reduces both survival and fecundity in the German cockroach

3.1 Abstract

Endemic infections can cause changes to the regulatory processes which drive host population dynamics. Finding which life history traits are most affected by parasitism can help establish which regulatory processes are the most important in governing host dynamics. The key aim of this study was to determine whether observed population level effects of an endemic infection with *Gregarina blattarum* in German cockroach hosts, *Blattella germanica,* were more likely to be due to parasite induced changes in host survivorship or host fecundity. The effect of *G. blattarum* on four life-history traits (adult survivorship, female maturation time, fecundity and nymph developmental rate) was explored. Infection reduced the mean lifespan of females by 42.6%, and by 35.7% in males. There were no differences in the female maturation period or the fertilisation rate of oothecae between parasite-free or infected females, but uninfected females produced almost twice as many oothecae during their lifespan as parasitized females. The number of nymphs produced by infected females was reduced by 36% from 127.73 ($\pm$ 49.57) to 82.86 ($\pm$ 35.67). The developmental rate of nymphs from infected parents was approximately two weeks longer compared to parasite-free cohorts, and there was a significant reduction in nymph survivorship to adulthood. *Gregarina G. blattarum* infection resulted in a substantial change in most measures of host life history. The main exception was female maturation time, but the energy required to maintain this trait could lead to trade-offs with future reproductive effort and survival. As survival and fecundity were strongly affected by *G. blattarum*, it is likely that both processes are playing a role in the regulation of host populations.

3.2 Introduction

Parasite infections have important consequences for the dynamics of host populations (Anderson & May 1978, 1982; May & Anderson 1978; Hudson, Dobson & Newborn 1992). Population dynamics are regulated by density dependent feedbacks that result in reduced survival or birth rates as population density reaches a carrying capacity (May 1981). When parasitism leads to substantial reductions in host survival, host populations are more likely to be stabilised by the parasite (Anderson & May 1978). On the other hand, when host fecundity is reduced by infection, the parasite can destabilise the host population, leading to regular cycles in the host population dynamics (May & Anderson 1978; Hudson, Dobson & Newborn 1998; Albon *et al.* 2002). Determining which life history traits are most affected by parasitism can therefore be vital in understanding how parasites regulate host populations.

Whilst several prominent studies have examined the population level effects of epidemic microparasite infections (e.g. Anderson & May 1981; Boots & Norman 2000; Bonsall 2004; Bonsall & Benmayor 2005) and parasitoids, in invertebrate populations (e.g. Hassell, Comins & May 1991; Jones *et al.* 1993; Bonsall & Hassell 1998), relatively few studies have considered the effects of endemic disease in invertebrates. In previous work, the regulatory effects of the gastrointestinal parasite *Gregarina blattarum* on population dynamics of the German cockroach, *Blattella germanica* were explored (Chapter Two). However, there was evidence for the regulation of cockroach populations by both birth and death dependent processes (Table 2.4, p33). It is therefore essential to determine whether birth or death rates are more strongly influenced by the parasite in order to fully explain the regulatory processes occurring in this invertebrate system.

The life history consequences of *G. blattarum* infection have not been extensively studied previously in the German cockroach, although a study by Lopes & Alves (2005) briefly mentioned that *G. blattarum* infection is associated with increased mortality of cockroaches within laboratory reared colonies. In other invertebrate species, gregarine infection is associated with altered host mating behaviour (Simmons 1990; Tsubaki & Hopper 2004), fecundity (Córboda-Aguilar, Salamanca-

Ocaña & Lopezaraiza 2003; Canales-Lazcano, Contreras-Garduno & Cordoba-Aguilar 2005; Kumano *et al.* 2010) and can lead to the development of smaller offspring (Bouwma, Howard, & Jeanne 2005; Er & Gökce 2005). It is therefore likely that *G. blattarum* infection can impact on other components of the cockroach life cycle as well as host survival.

The life cycle of the German cockroach can be divided into four key stages: female maturation, ootheca (eggs case) development, nymph emergence and nymph development to adulthood (Ross & Mullins 1995). Female maturation is defined here as the time from eclosion of last instar female nymphs into adulthood, to when the first ootheca is visible protruding from the female abdomen. This period therefore includes the time taken for females to reach sexual maturity, for eggs to be fertilised by the male, and for the eggs to develop and begin to be packaged into an ootheca (Roth 1968). When all the eggs have been assembled, the ootheca remains attached to the female until the nymphs are fully formed. Nymphs then emerge from the ootheca whilst it is still attached to the female after which the egg case is discarded (Ross & Mullins 1995). The mean number of offspring per ootheca varies with strain and temperature, but is approximately 47 nymphs at $25^{o}C$ from the first two oothecae with reductions thereafter (Ross & Mullins 1995). Females produce up to nine oothecae throughout their lifespan continuously every 30 days (Roth 1965), and can occur by both single as well as multiple copulations (Ross & Mullins 1995). The offspring develop over a period of approximately 60 days, and moult several times before they reach adulthood (Roth 1968). The number of instars can vary depending on environmental conditions and can increase from six to seven under stressors such as malnutrition (Roth 1968).

The aim of this study was to determine the effect of *G. blattarum* on the life history of the German cockroach. There has been no evidence from previous work (personal observations and literature) that nymphs are infected with *G. blattarum*. Therefore this study has focused on the effect of infection on adult life history and the effect of parental infection on offspring fitness. An additional component to this study was to explore potential trade-offs between life history traits, and therefore female maturation time and the time that oothecae (egg cases) were incubated for were also measured, and were expected to be traded-off against other host fitness costs (*i.e.*

fecundity and survivorship). It was expected that *G. blattarum* would reduce host survivorship and fecundity of the German cockroach host. Further, it was expected that offspring survival and developmental rate will be negatively affected by infection, with nymphs requiring a longer developmental time to reach adulthood. An alternate hypothesis, is that trade-offs would exist between host life history traits, so that a decrease in host survivorship would be associated with an increase in host fecundity or that adult fitness may be traded off against the fitness of offspring.

3.3 Materials & Methods

Host-parasite cultures

German cockroaches, *B. germanica*, were reared in colonies under standard laboratory conditions (see Chapter Two, p21) and all were provided with ground dog food (Tesco® Complete) and de-chlorinated water *ad libitum*. These rearing conditions were also maintained for all the life-history experiments described below. A sub-set of three parasite-free colonies and three colonies infected with the gastrointestinal parasite *G. blattarum* were chosen at random from the stock supplies and used for the life history experiments to control for variation between populations.

Experimental set-up for life-history evaluation

Female (n = 300) and male (n = 300) final instar nymphs, removed from the three uninfected and three infected colonies, were reared in isolation in 90 cm diameter Petri dishes, until they reached their adult moult. The first 50 females and 50 males emerging from their final moult from the same colony, were paired and transferred to Fluon®-lined plastic rearing pots with lids (Cater For You Ltd; 275 ml vol., 11.5 cm dia. x 7.5 cm height). To maintain infection levels, frass (faeces and debris) was collected each week from all three infected colonies and mixed to ensure the uniform distribution of gametocysts (gregarine infective stages). A sample of 0.1 g of frass was then added to each experimental container. Frass was also collected from uninfected colonies, mixed, and 0.1 g added to the uninfected containers as a control for any nutritional input or pheromonal influences that might be coming from the frass.

Life history measurements

i. *Host survivorship*

The survival of adult cockroaches was monitored daily until both the male and female had died. The time (in days) that adults were alive was calculated from the day the female and male first emerged into adulthood to the day on which they died, and used to calculate both mean lifespan and survival probability. All cadavers were removed from the experimental arena on the day of death. Due to rapid desiccation of host tissue, it was not possible to determine *G. blattarum* parasite load in dead hosts.

ii. *Female maturation time*

Female maturation time was calculated from the day the female reached adulthood to the day when the first ootheca was observed protruding from the female. Females were monitored daily to determine whether oothecae had hatched. Any oothecae that did not hatch were assumed to be unfertilised and were subsequently used to calculate the percentage of fertilised oothecae.

iii. *Oothecae, Egg and Nymph counts*

Females were monitored daily for the presence of oothecae, the length of time the oothecae was attached to the female (protrusion time, days) and the number of nymphs that hatched per female. The total number of oothecae produced per female was then calculated for both fertilised and unfertilised oothecae. The length of time females spent carrying oothecae (protrusion time, days) was estimated for each ootheca, and these data were also summarised as the total protrusion time per female lifespan. The discarded cases from the first oothecae produced per female (fertilised only) were collected and the number of un-hatched eggs were counted using a stereo microscope (GX Optical XLT-101). The hatch rate was then estimated from the total number of eggs that were produced per oothecae. Newly hatched nymphs were counted by anaesthetising the contents of each experimental arena with CO_2 and removing all nymphs with soft forceps. The first nymph cohort from each cockroach pair was placed into new pots and reared in sibling groups under the same conditions

as the adults. All other nymphs were returned to the colonies from which their parents had been sourced.

iv. Nymph development and survival

Nymphs from the first cohort were monitored daily for progression through their different instars and survival to adulthood. The developmental rate of the offspring was calculated as the number of days spent in each instar and the total number of days to adulthood.

Statistical Analysis

The effect of *G. blattarum* on life history measurements of the German cockroach was assessed using a series of generalised linear models (GLMs) and generalised linear mixed models (GLMMs), using the statistical packages R version 2.13.2 (R Development Core Team 2011) and ASReml-R version 3.0 (VSN International, 2009). Details of the structure of all the initial models and any interactions between model variables are presented in Table 3.1. In general, the models contained the life history measurement being assessed as the dependent variable and all models included host infection status as an independent variable. All models were initially assessed using GLMMs where the random model contained the colony identification code (colony ID), to control for variation between the laboratory stock populations from which the adults were originally obtained. Where colony ID was found to be non-significant (via assessment of the Log-Likelihood), a GLM replaced the GLMM. Where the dependent variable was a repeated measure a GLMM was always used with the unique identification number for each individual placed in the random model to control for pseudoreplication. In all repeated measure models a splinic term for time was also initially incorporated in the random model. Each set of model residuals were checked for normality and the models were refined in a stepwise manner using the Log-likelihood (for the random model) and Wald test statistic (for the fixed model) for the GLMMS, and the ANOVA table and associated F statistic for the GLMs.

Table 3.1: Initial generalised linear mixed model (GLMM) and Generalised linear model (GLM) structures for the effects of *Gregarina blattarum* infection on German cockroach life history components. v denotes a covariate; f denotes a categorical variable. Nb. All second order interactions between fixed model terms were also included. † *First oothecae data only.* ‡ *Fertilised oothecae data only.*

Cockroach life-history measurement	**Generalised linear mixed models (GLMMs)**			**Generalised linear models (GLMs)**	
	Dependent variable *Error structure (link function)*	**Fixed model variables**	**Random model variables** (after removal of insignificant values)	**Dependent variable** *Error structure (link function)*	**Independent variables**
Adult lifespan	Adult lifespan (days) *Gaussian (identity)*	Infection status (parasite-free or *G. blattarum* infected) (f) ; Sex (f)	Colony (f)		
Female maturation	Female maturation time†‡(days) *Gaussian (identity)*	Infection status(f)	Colony (f)	Proportion of all oothecae fertilised (*Gaussian (identity)*)	Infection status / Female maturation time (days) (v)
	Proportion of first ootheca fertilised *Binomial (logit)*	Infection status (f) / Female maturation time† (days) (v) / Ootheca protrusion time† (days) (v)	Colony (f)		
Oothecae, eggs & nymphs				Number of eggs† *Gaussian (inverse)*	Infection status (f) / Female maturation time†‡ (days) (v) / Ootheca protrusion time†‡ (days) (v)
				Nymph hatch rate† *Inverse Gaussian ($1/\mu^2$)*	Infection status (f) / Female maturation time (log days) (v) / Infection status (f)
	Number of oothecae‡ *Gaussian (identity)*	Infection status (f)		Number of oothecae *Gaussian (identity)*	
				Total oothecae protrusion time as proportion of female lifespan (maturation time removed) *Gaussian (identity)*	Infection status (f)
	Number of nymphs per ootheca *Gaussian (identity)*	Infection status (f) / Female maturation time† (days) (v) / Ootheca number (v)	ID code for experimental arena (f) spline (Oothecae number) (v)	Number of nymphs† *Gaussian (inverse)*	Infection status (f) / Female maturation time†‡ (days) (v) / Protrusion time†‡ (days) (v) / Number of eggs†(v)
				Total number of nymphs *Gaussian (identity)*	Infection status (f) / Female lifespan (days) (v) / Female maturation time (days) (v) / Total oothecae protrusion time (days) (v)
Instar development & survival to adulthood	Development time per instar (days) *Poisson (log)*	Infection status (f) / Ootheca protrusion time†‡ (days) (v) / Number of nymphs† (v) / Female maturation time‡ (days) (v) / Nymph instar number (v)	ID code for experimental arena spline (Nymph instar number) (v)	Total instar development time (days) *Gaussian (identity)*	Infection status (f) / Female maturation time‡ (days) (v) / Ootheca protrusion time†‡ (days) (v) / Number of nymphs† (v)
				Proportion of nymphs surviving to adulthood *Gaussian (identity)*	Infection status / Female maturation time‡ (days) (v) / Ootheca protrusion time†‡ (days) / Number of nymphs† (v) / Nymph development time to adulthood (days) (v)

3.4 Results

i.   Host survivorship

The lifespan of *B. germanica* was significantly lowered by infection with *G. blattarum* (Table 3.2) and the relationship between lifespan and infection status varied between the sexes (infection status:sex, $F_{1,557} = 31.14$, $p < 0.001$). Infected female lifespan was reduced by 42.6% compared to uninfected females, whereas infected male lifespan was reduced by 35.7%. There was a significant effect of *G. blattarum* infection on the probability of survival of both females ($Chisq_1 = 80.9$, $p < 0.001$) and males ($Chisq_1 = 145$, $p < 0.001$) over time.

Table 3.2: Summary of the main effects of *Gregarina blattarum* on German cockroach life history. *p <0.05, p**< 0.01, ***p <0.001. *NA* = all nymphs reached adulthood in 6 instars.

	Control (±1 SD)	Infected (±1 SD)	Test (F)	d.f	p value
ADULT SURVIVORSHIP					
Adult female lifespan (days)	263.02 (±87.83)	151.13 (±53.53)	31.14	1,557	<0.001***
Adult male lifespan (days)	159.67 (±50.78)	102.72 (±26.03)	31.14	1,557	<0.001***
FEMALE MATURATION					
Female maturation time (days)	13.95 (±5.26)	14.63 (±4.15)	182.7	1,198	0.834
Proportion of 1st ootheca fertilised (%)	64.66	69.53	0.851	1,4	0.105
Proportion of all oothecae fertilised (%)	66.9	39.6	42.35	1,289	<0.001***
OOTHECAE, EGGS & NYMPHS					
Number of oothecae (fertilised)	3.79 (±1.35)	2.58 (±1.05)	5.46	1,4	0.078
Number of oothecae (all)	6.53 (±2.29)	3.14 (±1.19)	146.8	1,299	<0.001***
Total ootheca protrusion time (% of female lifespan)	61.79	57.94	3.52	1,277	0.062
Number of eggs in 1st oothecae (fertilised only)	36.64 (±0.42)	35.72 (±0.35)	4.73	1,158	0.031*
Hatch rate of 1st ootheca (%)	93.07 (±8.24)	85.41 (±13.51)	7.30	1,152	<0.01**
Number of nymphs in 1st oothecae	34.15 (±5.07)	30.37 (±5.03)	11.17	1,158	<0.01**
Total number of offspring per female	127.73 (±49.57)	82.86 (±35.67)	7.00	1,4	<0.01**
NYMPH DEVELOPMENT & SURVIVAL					
Nymph development tine to adulthood (days)	73.16 (±9.22)	86.66 (±4.50)	140.9	1,8731	<0.001***
Number of instars	6	6	*NA*	*NA*	*NA*
Nymph survival to adulthood (%)	80.65	78.36	7.98	1,98	<0.01**

Survivorship plots show the uninfected females are most at risk of death after 120 days (Fig. 3.1a) whereas infected females have a significantly higher and constant risk of death at an early stage (after only 90 days). Male survivorship probability declines rapidly after 90 days (Fig. 3.1b) whereas in infected males, a high and constant risk of death commenced the day they emerged to adulthood.
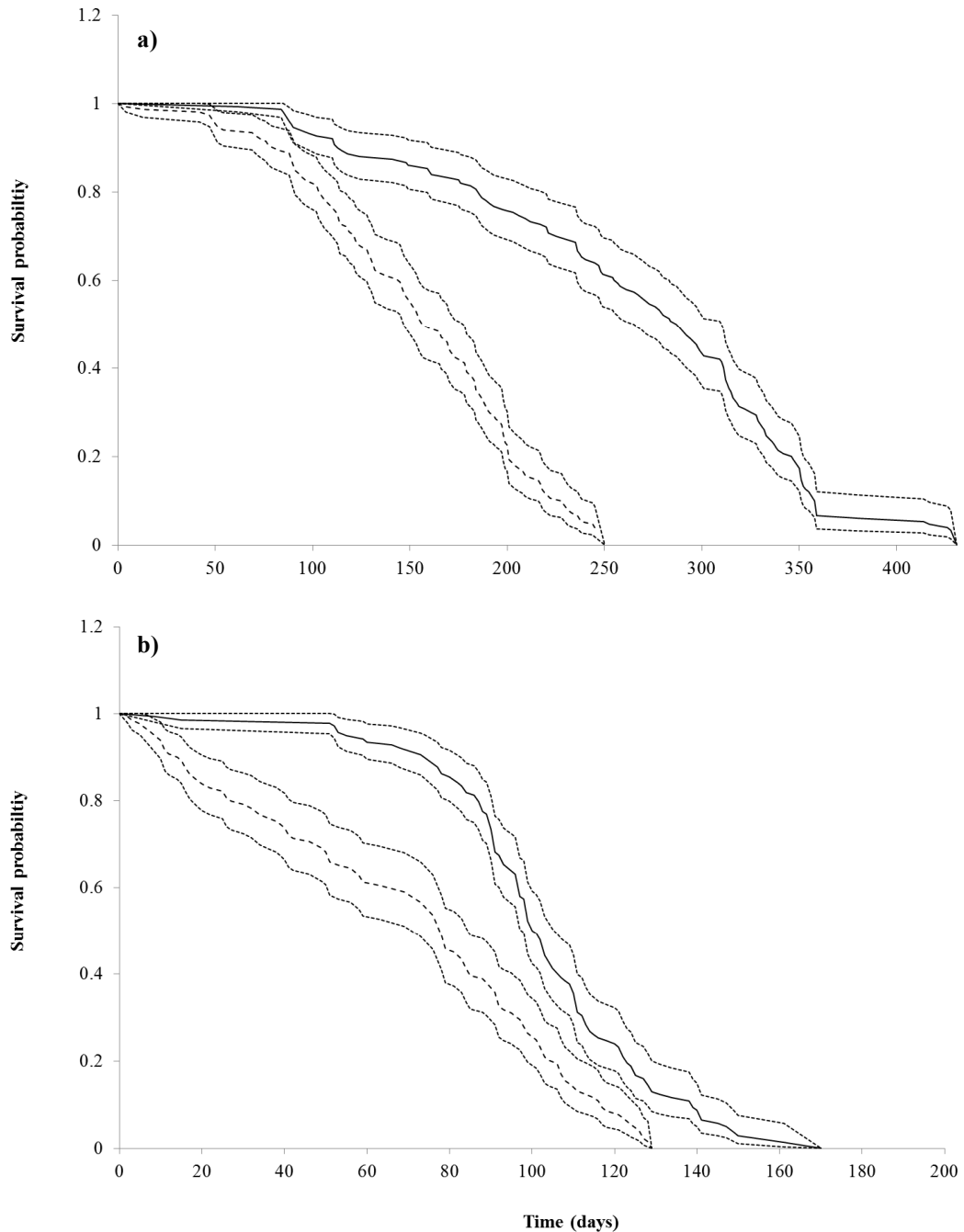

Figure 3.1: Kaplan-Meier probability of survival of female (a) and male German cockroaches (b). Each sex is divided between parasite-free hosts (black lines) and those infected with *Gregarina blattarum* infection (dashed lines). Dotted lines show ±1 SE.

ii. *Female maturation*

Infection did not affect the mean maturation time of female cockroaches (Table 3.2; $p > 0.05$). The proportion of oothecae that were fertilised was significantly higher in uninfected females (Table 3.2; $F_{1,289} = 42.35$, $p < 0.001$), although this was not significant for the first oothecae ($p > 0.05$). In addition, the proportion of oothecae that were fertilised was negatively associated with female maturation time ($F_{1,286} = 14.93$, $p < 0.001$) and positively associated with oothecae protrusion time ($F_{1,286} = 58.59$, $p < 0.001$).

iii. *Oothecae & nymph counts*

There was a highly significant effect of parasite infection on the number of oothecae produced by each female over the course of their lifespan ($F_{1,299} = 146.84$, $p < 0.001$). Females without an infection produced on average more than twice as many oothecae (6.53 ± 2.29 SD) compared to those that were infected (3.14 ± 1.19 SD). However, the total number of fertilised oothecae (those that produced viable offspring only) was not statistically different ($F_{1,299} = 5.46$, $p = 0.078$) between uninfected and infected females (3.79 ± 1.35 SD and 2.58 ± 1.05 SD respectively). Therefore, two thirds of the oothecae produced by uninfected females (66.9%) were unfertilised, compared to just over a third of those produced by infected females (39.6%) ($F_{1,289} = 42.24$, $p < 0.001$).

All female cockroaches spent over half their lifespan carrying oothecae (Table 3.2). When controlling for adult lifespan and maturation rate, uninfected females carried oothecae for a slightly greater proportion of their lives (61.7% $\pm$ 0.01 SD) compared to infected females (57.9% $\pm$ 0.01 SD), but this result was not statistically significant ($F_{1,277} = 3.52$, $p = 0.062$).

Infection significantly reduced the mean egg number in the first ootheca (Table 3.2). The effect of infection on female egg number was also affected by the time taken for females to mature (infection status:maturation time, $F_{1,158} = 4.73$, $p = 0.031$). The number of nymphs emerging from the first oothecae was more strongly associated with egg number in uninfected groups (Fig. 3.2; egg number:infection status, $F_{1,158} = 11.17$, $p < 0.01$). The proportion of nymphs that hatched from the available eggs in

the first oothecae was therefore lower in infected hosts (Table 3.2; $F_{1,152} = 7.30$ p $<$ 0.01).
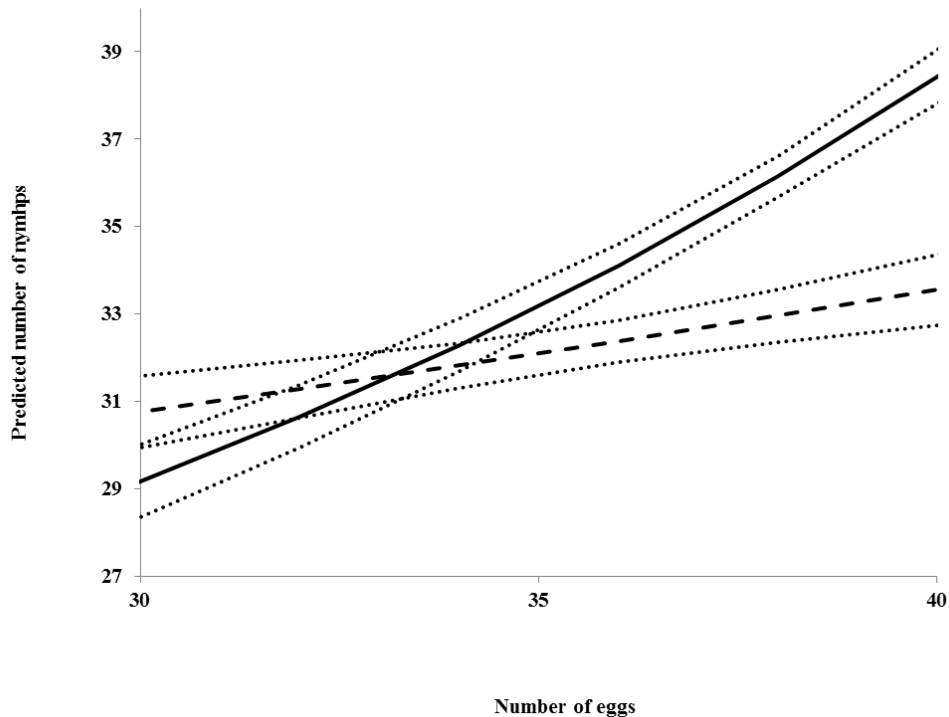
Figure 3.2: Predicted number of German cockroach nymphs in the first oothecae from uninfected (black line) and infected (dashed line) females with increasing egg number. Dotted lines show ±1 SE.

Infection reduced the total number of nymphs produced by females over their lifetime by 36% (Table. 3.2; $F_{1,4} = 7.00$, p $<$ 0.01). The nymph cohort size also varied depending on which oothecae the female had produced ($F_{1,228} = 55.06$, p $<$ 0.001), cohorts were generally smaller in the first oothecae compared to the second and declined thereafter (Fig. 3.3). Infection lowered the number of nymphs produced in all oothecae, although this was only significant in the first five oothecae ($F_{1,259} = 13.56$, p $<$ 0.001). Nymph numbers were also associated with male lifespan, so that longer lived males fathered more nymphs ($F_{1,268} = 24.23$, p $<$ 0.001).
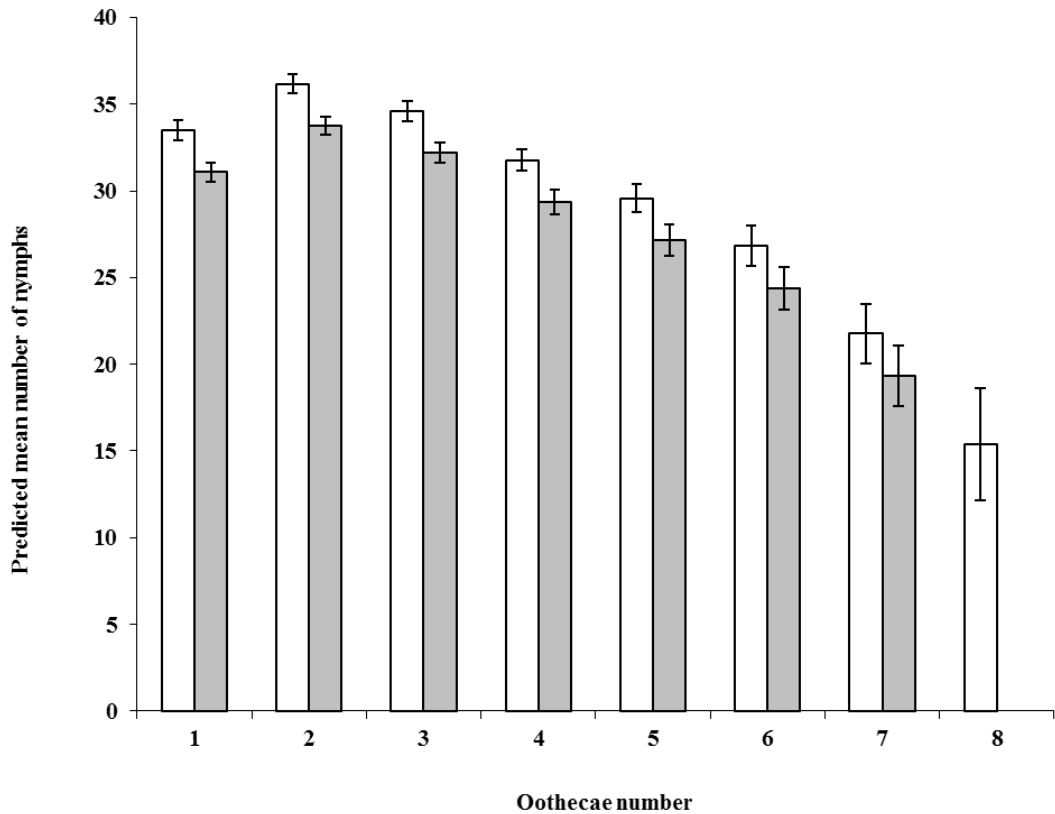
Figure 3.3: Predicted mean number of German cockroach nymphs for each ootheca produced by uninfected females (white bars ±1 SE) and females infected with *Gregarina blattarum* (grey bars ±1 SE).

iv. Nymph development and survival

All nymphs undertook six instars to reach adulthood (Fig. 3.4). Nymphs from infected parents took on average 14 days longer to reach adulthood than nymphs from infected parents ($F_{1,8731}$ = 140.94, p < 0.001). There was also a positive association between the number of nymphs that were present in the cohort and the time taken to reach adulthood where larger groups took longer to develop ($F_{1,4246}$ = 8.47, p = 0.004). Infection status was also highly significant in affecting the time between instars ($F_{1,1044}$ = 98.61, p < 0.001). The number of days that the offspring spent in each instar varied with the instar stage, and the relationship between instar number and length of time spent in that instar also varied with the maternal maturation time (instar:female maturation time, $F_{1,1040}$ = 14.57, p < 0.001).
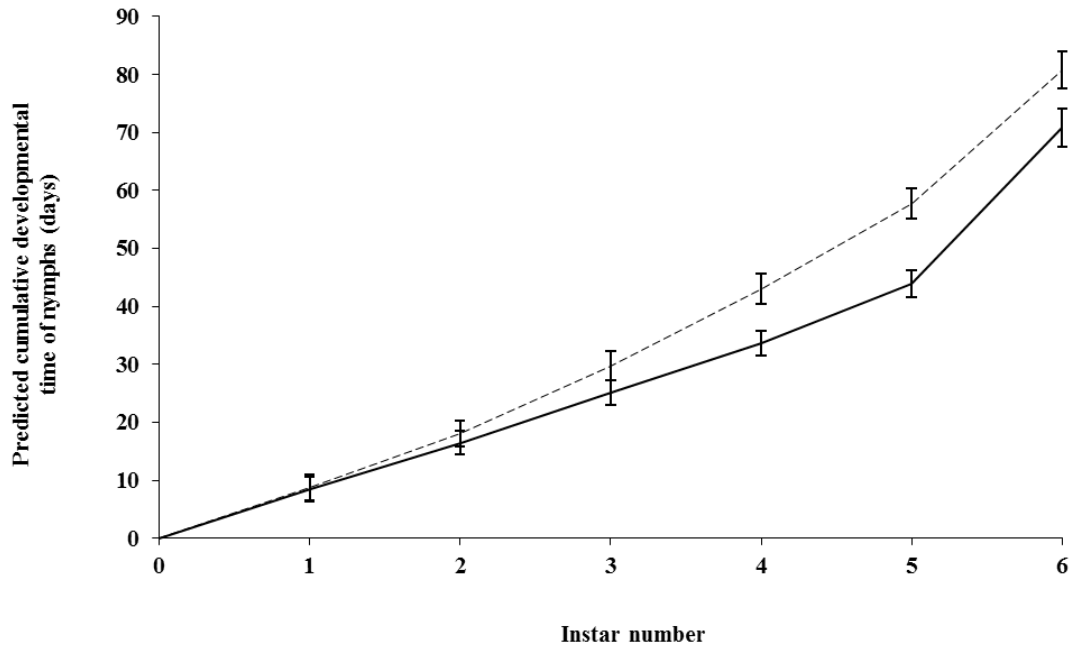
Figure 3.4: Predicted developmental rate per instar of nymphs from uninfected parents (bold line ±1 SE) and infected parents (dashed line ±1 SE).

Offspring survival rate was significantly affected by parental infection status, and the relationship between parental infection status and offspring survival was also affected by the maternal maturation time (maternal maturation:infection status $F_{1,98}$ = 7.984, p < 0.01). Uninfected females that took longer to mature produced nymphs with a greater likelihood of surviving to adulthood (coefficient = 0.02) whereas there was a negative relationship between maternal maturation time and nymph survival in infected groups (coefficient = -0.04).

3.5 Discussion

The effect of *G. blattarum* infection on a range of host life history measurements in the German cockroach was assessed to determine the most important effects driving changes at the host population level. Specifically, four questions were addressed in this study:

1) Does G. blattarum affect host lifespan and survival risk?

The adult lifespan of *B. germanica* adults is substantially reduced when hosts are infected with *G. blattarum*. Infected hosts were also at greater risk of dying at an earlier age compared to uninfected hosts which was particularly true of males, whose increased risk of death began from the day they moulted into adulthood. Other studies have also shown that gregarine infection can reduce host lifespan (Lantova *et al.* 2011; Kumano *et al.* 2010; Thomas & Rudolf 2010; Canales-Lazcano *et al.* 2005; Tsubaki & Hopper 2004) but by assessing survivorship risk here it was possible to identify the period of their lifespan in which the hosts were most likely to die. However, the current study did not observe the same degree of host damage (pathogenicity) as described by Lopes & Alves (2005), whose work found that the mortality of German cockroaches infected with *G. blattarum* was associated with a putrid smell and septicaemia. All dead hosts in this study desiccated quickly with no evidence of putrification. As gregarines cannot invade host tissue other than the host gastrointestinal tract (Harry 1970), previously reported septicaemia may be a result of other infectious diseases which were not specified (Lopes & Alves 2005). This highlights the possibility that *G. blattarum* may increase host susceptibility to other infections. Whilst the mechanism of increased host death in infected hosts is unknown, it is possible that the lower lifespan is a product of fewer host resources during infection, as shown in other host-parasite systems (Sorci *et al.* 1996; Sorensen & Minchella 1998; Brown, Loosli & Schmid-Hempel 2000). In Chapter Four evidence is provided for *G. blattarum* host resource costs which may explain the reductions in host lifespan and other parasite related costs discussed in this study.

2) Is female maturation affected by parasitism?

Infection with *G. blattarum* did not alter the time taken for females to mature. This 14 day period consisted of the time taken for females to reach sexual maturity after final instar eclosion, the mating ritual, whereby males present the female with a food packet, copulation, and the time until the first oothecae is observed protruding from the female (Ross & Mullins 1995). Each of these processes are in their own right, costly to maintain and as a result would be expected to be traded-off against other life history processes, particularly in times of stress such as parasitism. Yet an interesting characteristic of German cockroach females is that they continue to develop oothecae regardless of whether fertilisation has occurred (Ross & Mullins 1995). In further work, infection is shown to reduce host energy levels (Chapter Four). With that in mind, it was surprising to find that the fertilisation rate (*i.e.* the proportion of oothecae that produced viable offspring) was similar between parasite-free and infected hosts (approx. 66 %). As host resources are lower during *G. blattarum* infection, conserving the maturation period and fertilisation rate must therefore require additional investment by females or males, or incur costs in future life history traits. Engvist & Sauer (2001) demonstrated that males of the scorpionfly, *Panorpa cognata* that are in poor condition can increase the quantity of a food packet presented to the female, thereby trading-off current versus future reproductive success. Male cockroaches also provide a nuptial gift to the female to initiate copulation (Ross & Mullins 1995). Examination of the male and female investment in reproductive fitness could therefore determine whether trade-offs in the allocation of resources occur during parasite infection which could be important in determining the life history changes during parasitism in this species.

3) Do infected females produce fewer ootheca, eggs and nymphs?

The current study has shown for the first time that *G. blattarum* has substantial reproductive costs for the German cockroach. Overall, 36% fewer nymphs hatched from eggs produced by infected compared to uninfected females. The reduction in nymphs was due to both the reduced number of eggs that were packaged into the oothecae and the reduced hatch rate of these eggs. Fecundity has been shown to be limited by gregarine infection in other systems (Kumano *et al.* 2010; Canales-Lazcano *et al.* 2005; Cordoba-Aguilar *et al.* 2003) but the mechanism is not

currently known. Fan *et al.* (2002) demonstrated that large amounts of maternal resources are required for egg development, and water soluble nutrients are transferred to oothecae as they develop during the protrusion of oothecae (Mullins *et al.* 2002). Reduced host resources could therefore limit host egg quality as well as quantity. As there is evidence that *G. blattarum* infection is associated with reduced host lipid levels (examined in Chapter Four) this may explain the reduction in host fecundity.

Whilst infection reduced the overall nymph number produced by each female, there were no differences in the percentage of oothecae that were fertilised (*i.e.* produced viable offspring) between parasite-free and infected females. However, uninfected females produced twice as many unfertilised oothecae as infected females. Male lifespan was always considerable shorter than females (Fig. 3.1), and a lack of males in later life could have resulted in this excess of unviable oothecae in parasite-free females. This point is emphasised by the fact that longer lived males fathered more nymphs. The females in the current study were housed individually, whereas in the colonies, females would have access to multiple partners and this may raise the levels of fecundity above that observed for the singly-paired females used in this study. Therefore, fecundity costs may be underestimated here when compared to within colonies and fecundity could therefore be much more greatly affected by parasitism than was determined here.

4) Do nymphs that emerge from infected parents have altered developmental rate or survival?

The developmental time required by nymphs to reach adulthood was up to two weeks slower in infected nymph cohorts, and nymphs from infected parents were less likely to survive to adulthood. The delayed development was not a result of increased instar number (as found by Roth 1968), as all the nymphs monitored required six instars to reach adulthood. Delayed nymph development has been previously associated with gregarine infection in the flour beetle *Tribolium confusum* (see Thomas & Rudolf 2010). To the best of my knowledge, gregarine infection in the German cockroach does not occur until the nymphs reach adulthood (personal observations and literature). Therefore, fitness costs associated with longer development and reduced survival must be attributed to parental resource

provisioning. Indeed, in Chapter Four it is shown that parasitized adults have fewer storage energy reserves. Extending the nymph study here to explore reproduction and survival of the nymphs as adults would identify whether there are future costs of parental parasitism for offspring. As the infected adults chosen in this study were taken from infected colonies, it is likely that their parents were also infected, and therefore the costs associated with the life history traits measured in this study may also be related to the adults' own parental resource provisions. Such stage-dependent costs of parasitism, for example, the delayed development rate in juveniles and reduced offspring survival, can also be incorporated into population models to explain host population dynamics (Briggs & Godfray 1995; Bjornstad et al. 1998). Nymph fitness may therefore not only incur costs for future reproductive and survival, but impact on the processes that regulate the population as a whole.

The results presented here demonstrate that *G. blattarum* infection affects both the life-span and fecundity of the host. It is recognised that parasites which affect host survival are more likely to stabilise host population dynamics (Anderson & May 1978) whereas reductions in host fecundity during parasite infection are more likely to destabilise the host population (May & Anderson 1978; Hudson, Dobson & Newborn 1992; Albon *et al*. 2002). Chapter Two revealed that both birth and death rates were important in regulating German cockroach population dynamics in colonies containing *G. blattarum*, and the current study demonstrates that it is possible for *G. blattarum* to impact on both processes. Future modelling attempts might therefore be improved by incorporating the effects of parasitism on host birth and death rate as well as considering the reduced developmental rate of the offspring.

In conclusion, *G. blattarum* imposes significant fitness costs for both fecundity and survival on its German cockroach host indicating that both processes may be important in regulating host population dynamics. There are also substantial costs of parental infection to offspring fitness which may contribute to the life history effects observed in the adults. Resource investment at the start of the female reproductive cycle may also explain future reproductive and survival costs of infection and by exploring host resource availability during infection would enable further understanding of how endemic parasites may affect invertebrate life history.

Endemic infections are therefore extremely important in invertebrate life history and should be considered in future invertebrate studies.

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4. Endemic infection in the German cockroach reduces parasite transmission potential during co-infection

4.1 Abstract

Parasite transmission can be dependent on the level of resources within the host. Endemic infections can decrease host resources which could indirectly affect the transmission rate of other parasites during co-infection. This study aimed to determine whether the reproduction, and therefore transmission potential of an epidemic infection, is limited by energy costs imposed on the host by an endemic infection. The amount of stored energy resources (lipids) within the German cockroach were measured in hosts that were starved and compared to hosts that were infected with the endemic parasite *Gregarina blattarum* and parasite-free controls. The effect of infection on host feeding rates was also monitored to assess if any resource changes were due to altered host feeding behaviour. The reproduction of an epidemic parasitic infection of the nematode *Steinernema carpocapsae* was assessed by counting the emergence of nematode infective stages from control hosts fed *ad libitum,* starved hosts and hosts infected with *G. blattarum*. Host lipid levels were reduced by 48.1% in starved female hosts when compared to hosts fed *ad libitum*. Infection reduced lipid levels in females by between 37.1% and 69.9% (where difference were due to reproductive status) and by 49.4% in males. There were no differences in the food consumption rate between infected and uninfected hosts. Infection with *G. blattarum* reduced the emergence of nematode infective stages by 60.5% which was comparable to host starvation. Endemic infection with *G. blattarum* is associated with a substantial reduction in host energy resources and those resources are likely to be the cause of the reduced reproductive capacity of *S. carpocapsae*. As the number of infective stages produced by the parasite is a large component of parasite transmission, the rate of transmission is likely to be reduced by the substantial losses in infective stages emerging from co-infected hosts. Therefore, future studies of disease transmission should consider the effects of other diseases in the host population.

4.2 Introduction

The transmission rate of a parasite is fundamentally linked to parasite reproduction (Heffernan, Smith & Wahl 2005), where the reproductive capacity of parasites is dependent on the availability of resources within the host (Poulin & George-Nascimento 2006; Seppala *et al.* 2008). Thus, if hosts are deprived of energy, resource restrictions can limit the reproductive rate of the parasite (Lafferty & Kuris 2005; Seppala *et al.* 2008). As parasites must acquire host resources in order to reproduce, infection can also lead to a reduction in host energy levels (Tocque 1993; Tocque & Tinsley 1994). More specifically, host condition can be reduced directly by parasite acquisition of host resources used for parasite life history processes including reproduction (Booth, Clayton & Block 1993) or indirectly because of an increase in host metabolism (through increased immunity and/or repair) (Sheldon & Verhulst 1996). Reduced host condition may also occur through decreased host food intake due to a loss of appetite during infection (Stein *et al.* 2002). Therefore outcomes of the host-parasite interaction is integrally linked to the levels of resources within the host, both in terms of the resources that are available to the host and those resources that are utilised and available to the parasite.

As parasite infection can lead to altered host resource levels, the presence of one infection could potentially create a limitation for the reproduction of a second parasite species. Co-infections, where more than one parasite is present in the same host, are extremely common in nature (Petney & Andrew 1998) and can limit host resources further than single infection alone (Lello, Boag & Hudson 2005). In particular, endemic parasites, such as gastrointestinal helminths and protozoa, which often have direct costs to host energy levels (Booth, Clayton & Block 1993), are commonly present when the host encounters more severe infections such as microparasites or other species causing epidemic outbreaks (Lello *et al.* 2004; Fenton 2008). Co-infections can also lead to altered host susceptibility to diseases (Lello & Hussell 2007; Cattadori *et al.* 2008) and modify the host responses to different infections (Graham 2008). The reproduction and transmission rate of acute infections could therefore be confounded by resource costs associated with infections.

This study explores the relationship between an endemic parasite and an acute infection in a model host, the German cockroach, *Blattella germanica*, and examines

whether host resources play a role in directing this relationship. In addition to the cockroach host, the study system compromised an endemic gastrointestinal protozoan parasite *Gregarina blattarum* which persists at relatively stable levels in the host population (see Chapter Two) and an entomopathogenic nematode, *Steinernema carpocapsae* which causes acute infection leading to host death within 72 hours (Adams & Nguyen 2001).

The German cockroach is an omnivorous consumer, with the majority of energy resources stored as lipids in the fat body (Ross & Mullins 1995). Infection by the parasite *G. blattarum* occurs after accidental consumption of host faeces, whereupon infective stages (sporozoites) migrate and attach to the host cell wall (Clopton & Gold 1996). Invasion of gregarine species in other hosts can damage the host cell walls, where the parasite forms an attachment during infection (Takahashi, Kawaguchi & Toda 2009) and this may lead to a reduced capacity of the host to absorb nutrients. The second parasite species, *S. carpocapsae* invades the host through openings in the host cuticle (mouth, spiracles and anus). In the host, the nematodes release symbiotic bacteria (*Xenorhabdus nematophila*) that colonise and metabolise stored host energy reserves (Adams & Nguyen 2001). The nematodes are able to grow and reproduce, utilising both host energy reserves directly and those that have been digested by the bacteria. When host resources are depleted, the nematode life cycle switches to the production of non-feeding transmission stages (infective juveniles), which re-ingest the bacteria into a dedicated vesicle in their gut, and then they exit the host (Adams & Nguyen 2001).

The main aims of this study were to determine whether an endemic infection could alter the reproduction, and therefore transmission potential of a second parasite species during an acute infection. Four questions were specifically addressed in this study: (i) Do host starvation and *G. blattarum* infection reduce host lipid levels? (ii) Are any resource reductions explained by behavioural changes in host food consumption? (iii) Does host starvation and *G. blattarum* infection reduce the reproductive rate of *S. carpocapsae*? (iv) Is host mortality due to *S. carpocapsae* infection greater in hosts co-infected with *G. blattarum*?

4.3 Materials & Methods

Host cultures

German cockroaches, *B. germanica*, were obtained for this study from laboratory stock colonies reared under standard laboratory conditions (see Chapter Two, p. 22). The two groups of hosts used in this study were removed from colonies that were parasite free or those that contained high prevalence of infection (> 80%) with the endemic parasite *G. blattarum*.

Nematode cultures

Stock nematode cultures of the nematode *S. carpocapsae* (original stock obtained from Becker Underwood Ltd.), were maintained in cockroach hosts within infection arenas comprising a 275 ml plastic pot (Cater For You Ltd.; 275 ml vol.; 11.5 cm dia. x 7.5 cm height), lined with Fluon$^®$ and with a sterile sand substrate base, inoculated with nematodes (50 nematodes/cm^2). The sand was moistened weekly with distilled water to prevent desiccation of the nematodes. Cockroaches were added to the infection arenas (n = 20) and died within 72 hours. For the purpose of maintaining stock levels, the cadavers were replaced every seven days to allow for infective juveniles to replenish the sand substrate. The removed cockroaches were placed onto White's traps containing 30 ml sterile distilled water (White 1927) to isolate infective juveniles. For experimental work, nematodes were collected from the White's traps after 14 days, transferred to 50 ml falcon tubes, topped up to 50 ml using sterile distilled water and stored at 5°C for up to 10 days. The nematode concentration per ml was estimated using a Sedgewick-Rafter counting cell under a compound microscope (Olympus UCC/BY 501 at x40 magnification).

Experiment One: Host lipid levels in starved and G. blattarum infected hosts

The effect of starvation on the lipid levels of the cockroach was assessed over a 28 day period in female cockroaches collected from uninfected colonies only. A total of fifty females without oothecae (to control for the effects of oviposition on lipid levels; Rust *et al.* 1995) was randomly selected and maintained in ten groups of five in circular plastic container (275 ml; Cater4You Ltd.) lined with Fluon$^®$. The first five groups were fed on ground dog food *ad libitum* and distilled water (fed group) as

a control, and the remaining five groups were provided with distilled water only (starved group). At 0, 7, 14, 21 and 28 days, a group of five females from the starved and fed groups respectively were collected for lipid analysis. Lipid levels were obtained using a simple chloroform extraction technique (in accordance with Marden 1989). Cockroaches were anaesthetised with CO_2 and placed in a glass vial (5 ml), dried in an oven for 24 hours, weighed (accurate to 10 μg) and then immersed in 1 ml of 95% chloroform. After 24 hours, the chloroform was removed and the samples were gently rinsed in 1 ml of distilled water, repeated three times, and dried for 72 hours. The amount of lipid in each host fat body was calculated from the difference in mass between dry cockroach weight and the amount lost after the extraction.

The effect of parasitism on host energy reserves (lipids) was measured in hosts infected with *G. blattarum*. Hosts were taken directly from uninfected (n = 30 females; n = 30 males) and infected colonies (n = 30 females; n = 30 males). All individuals were anesthetised with CO_2, weighed, female sexual status noted (no eggs present, eggs inside the body, oothecae present) and dissected to assess parasite load in the mid-gut (as described in Chapter Two p22). The lipids were subsequently extracted using the same procedure as for the starvation experiment (above).

Experiment Two: Host food consumption

Cockroach food consumption during infection was measured to determine the effect of *G. blattarum* on host feeding rate. To ensure all hosts collected from infected colonies were parasitized, a hundred female cockroaches, which were collected from infected colonies, were maintained separately for 24 hours in arenas comprising of Petri dishes (90 mm dia.) with 5g ground dog food (provided in a 50 mm dia. Petri dish lid), and a 5 ml specimen tube containing distilled water sealed with a cotton wool bung. At the same time, a further thirty females from parasite-free colonies were collected and maintained for 24 hours under the same conditions (control group). After 24 hours, host frass (faeces and debris) in the infected cockroaches was examined to determine the presence of any gregarine infective stages (gametocysts), which has been previously found to correlate positively with trophozoite intensity in the host gut (see Appendix 7.7). The thirty cockroaches with the highest gametocyst levels in their frass were retained and the others were returned to the colony. Immediately after gametocyst assessment, each food dish from the uninfected and

infected female arenas was weighed (accurate to 10μg) and weighed weekly thereafter for six weeks to monitor the mean food consumption rate.

Experiment Three: Nematode reproduction in starved and G. blattarum infected hosts

The effect of host condition on nematode reproduction was determined by measuring the emergence of infective juvenile nematodes from hosts that were starved and fed. Uninfected female cockroaches (n = 50) were collected and divided into groups of five females and incubated under the same conditions as the previous starvation experiment (see above), with the exception that at each time interval, a group of five cockroaches from the starvation and fed group was transferred to a nematode infection arena. The infection arenas were prepared by pipetting 5 ml of a solution of infective juveniles prepared in distilled water (concentration = 6.3 x 10^4) onto a piece of filter paper (90mm dia.; Whatmann®) within a Fluon® lined pot. Hosts were exposed to nematodes for 72 hours, during which time all the hosts died. At 72 hours, the cockroaches were removed from the infection arenas, weighed and then checked for the presence of an ootheca. Each cockroach was then transferred to a separate White's trap (White 1927). The total amount of nematodes that emerged from each host were collected from the White's Traps after 14 days and counted using a Sedgewick-Rafter counting cell under a compound microscope (Olympus UCC/BY 501).

The emergence of nematodes from *G. blattarum* infected hosts was measured to determine the effect of endemic infection on nematode reproduction. Infection arenas were prepared as for the starvation experiment (see above), but all cockroaches were supplied with *ad libitum* food and water, and the two treatment groups comprised of females (without oothecae) taken from colonies with or without *G. blattarum* infection. After 72 hours, cockroach cadavers were transferred from the infection arenas to White's trap. Nematodes were collected into 50 ml falcon tubes at three day intervals and counted using a Sedgewick Rafter counting cell. The White's traps were then refilled with distilled water and this process was repeated every three days for 28 days, until the nematodes ceased emerging from the cadaver.

Experiment Four: Host mortality rate during single (S. carpocapsae) and co-infection (S. carpocapsae and G. blattarum)

The effect of *G. blattarum* infection on the survival rates of hosts when infected with *S. carpocapsae* was assessed by monitoring the time to host death in infection arenas. Infection arenas (n = 40) were prepared by placing a single piece of 90 mm diameter filter paper onto the base of a 275 ml plastic pot (Cater For You Ltd.). Infective juveniles were collected from stock White's traps after 10 days, counted with a Sedgewick-Rafter counting cell under a compound microscope (Olympus UCC/BY 501), and the concentration adjusted with distilled water to give four working solutions: 0, 120, 1200, 12000 nematodes/ml. The infection arenas were then prepared by pipetting 1 ml of nematode solution (10 arenas per concentration) directly onto the centre of the filter paper. Immediately after the nematodes were added, 25 female cockroaches (without oothecae to reduce variability between samples) were collected from parasite free colonies and 25 from *G. blattarum* infected colonies, and each cockroach was added to a separate infection arena. Hosts were monitored every 8 hours for 30 days or until death. The time to host death was recorded and used to calculate the probability of survival.

Statistical Analysis

The effect of starvation on host lipid levels, the association between parasite intensity and host lipid levels and the number of nematode infective stages emerging from host cadavers were assessed using three General Linear Models (GLM) in the R statistical package version 2.10.1 (R Development Core Team; 2009). The dependent variables in each model were transformed prior to analysis using a square root, natural log and base ten log transformation respectively, to normalise the standardised residuals for each model. In the starvation analysis, the variables included in the model were host treatment (starved or fed) and treatment time. Host lipid levels were assessed for correlation with parasite intensity (normalised using a natural log transformation (x+1)), and the reproductive status of the host (males, females, females with eggs, females with oothecae). For the nematode emergence model the effect of feeding status (starved or fed hosts), and exposure time were assessed. All second order interactions were included in the models, and each model was subsequently refined by stepwise deletions.

The effects of *G. blattarum* on host food consumption and the emergence of nematodes over time were assessed using a repeated measures General Linear Mixed Model (GLMM) using a restricted maximum likelihood method in the package ASReml-R (v2; VSN International Ltd.) within the R interface. Host ID number and a splinic fit to time were included in the random models to account for repeated measures in the experimental design and non-linearities in the relationship between time and the dependent variables respectively. The fixed model included the variables host mass (mg), presence/absence of *G. blattarum* and time. The food consumption model also contained a variable describing whether the female had produced an ootheca during the experimental period. Residuals from both models were checked for normality before refinement by removal of the nonsignificant terms in a stepwise manner using the using the Log-likelihood for the random model and the Wald test statistic for the fixed model.

The effect of *G. blattarum* infection on survival of hosts during *S. carpocapsae* infection were assessed using a Generalised Linear Model in R version 2.10.1 (R Developmental Core Team 2009) with an inverse Gaussian error distribution and log link function. The standardised residuals were checked for normality and the model was refined using stepwise deletions. The probability of survival in parasite-free and *G. blattarum* infected hosts was then assessed using a Kaplein-Meier survival curve for each of the four nematode concentrations (0, 120, 1200 and 12000 nematodes/ml) and the differences between uninfected and infected groups was determined using a log rank test.

4.4 Results

i. Does host starvation and G. blattarum infection reduce host lipid levels?

Starvation of *B. germanica* adult females (not carrying oothecae) resulted in a substantial decline in lipid levels of 48.1% over the 28 day observation period (Fig. 4.1; $F_{1,80} = 15.20$, $p < 0.001$). Females that were fed an unlimited food source during the same period accumulated 73.2% more lipids compared to the start of the experiment.
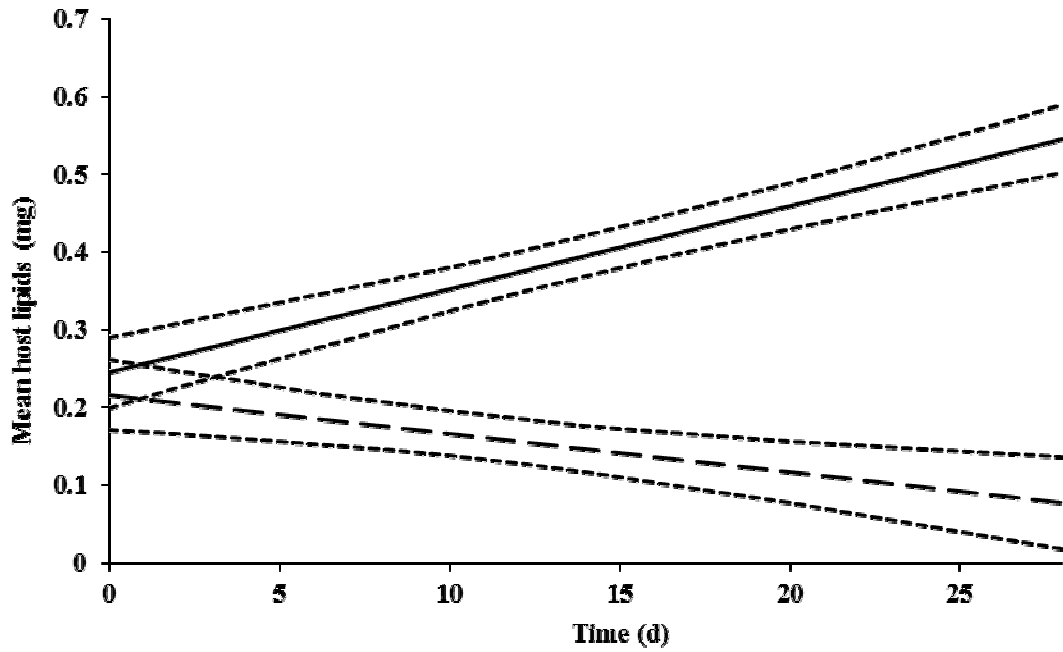
Figure 4.1: Predicted change in host lipid levels with time (days) in German cockroach females fed *ad libitum* (bold line ±SE) or starved (dashed line ±SE).

All cockroaches infected with *G. blattarum* had significantly lower lipid levels than uninfected cockroaches (Table 4.1; $F_{1,180}$ = 63.18, p < 0.001). Females carrying oothecae suffered the greatest percentage decline in lipid levels during infection. Males lost similar amounts of lipids (49.4%) as starved hosts. Females carrying oothecae also had fewer lipids than uninfected females but this loss was not as high as those females that were starved.

Table 4.1: The mean lipid levels of German cockroach females with or without *Gregarina blattarum* infection for females without eggs, with eggs, with oothecae and in adult males. * Significance level p < 0.05*

Sex/status	Mean lipids in uninfected hosts (mg ± SE)	Mean lipids in infected hosts (mg ± SE)	Infection induced lipid loss (%)
Females (no eggs/ootheca)	0.32 (±0.09)	0.13 (±0.07)	58.7*
Females with eggs	0.64 (±0.15)	0.40 (±0.09)	37.1*
Females with oothecae	0.57 (±0.26)	0.18 (±0.18)	68.9*
Males	0.15 (±0.06)	0.07 (±0.03)	49.4*

Host lipid levels were negatively associated with the intensity of the parasite infection (Fig. 4.2) and an interaction between parasite intensity and with the reproductive status of the host (females, females with eggs, females with oothecae, males) (infection intensity:status, $F_{3,175}$ = 3.81, p < 0.05). Lipid levels of females carrying oothecae were decreased more sharply with increasing parasite intensity than the other three reproductive status groups.
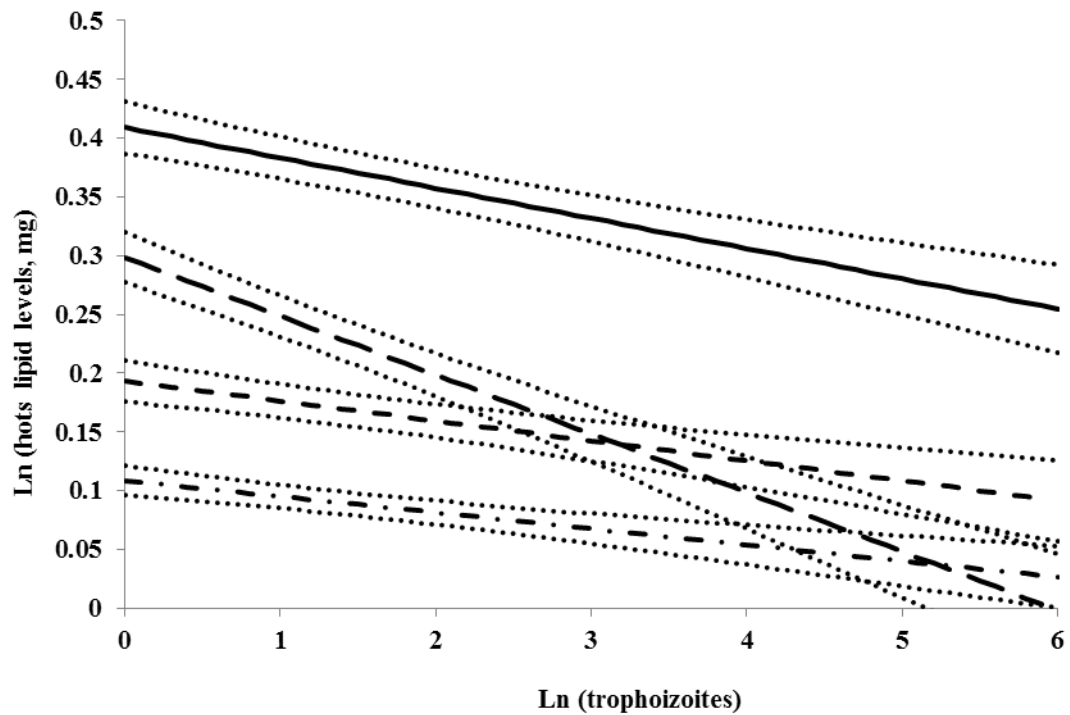
Figure 4.2: Predicted mean host lipid levels (ln) with increasing *G. blattarum* trophozoite intensities (ln(x+1)) in *Blattella germanica*. Four lines show (i) females with eggs (bold line ±1 SE), (ii) females with oothecae (long dashed line ±1 SE), (iii) females without eggs/oothecae (small dashed line ±1 SE) and (iv) males (dot-dash line ±1 SE).

> ii.   *Are any resource reductions explained by behavioural changes in host food consumption?*

There was no effect of infection on host food consumption ($F_{1, 1022} = 0.04$, $p = 0.851$). However, daily food consumption was affected by time ($F_{1, 1023} = 4.59$, $p = 0.033$), host size ($F_{1, 1023} = 7.22$, $p = 0.008$) and whether or not females carried an ootheca ($F_{1, 1023} = 126.4$, $p < 0.001$). Food consumption generally declined with time and larger females consumed on average a higher mass of food. Females without oothecae consumed significantly more food per day (0.89 mg) than those that were carrying an ootheca (0.65 mg).

Nematode reproduction was 62.0% lower in hosts that were starved prior to nematode infection, compared to hosts that had been fed *ad libitum* over the 28 day treatment period ($F_{1,16}$ = 6.41, p = 0.022). Nematode emergence in *G. blattarum* infected hosts, changed substantially with time ($F_{1,329}$ = 11.35, p < 0.001) and the mass of the female ($F_{1,44}$ = 0.01, p < 0.001) where females that were lighter yielded fewer nematodes. However, no shift in the pattern of emergence was detected, with the emergence peaking at 10 days in both groups (Fig. 4.3), but there was a substantially lower output of nematodes from *G. blattarum* infected hosts between days 5 and 12 ($F_{1,44}$ = 0.35, p < 0.001). Overall, there was a 60.5% reduction in the nematode output from hosts infected with *G. blattarum* compared to those that were infected with nematodes alone.
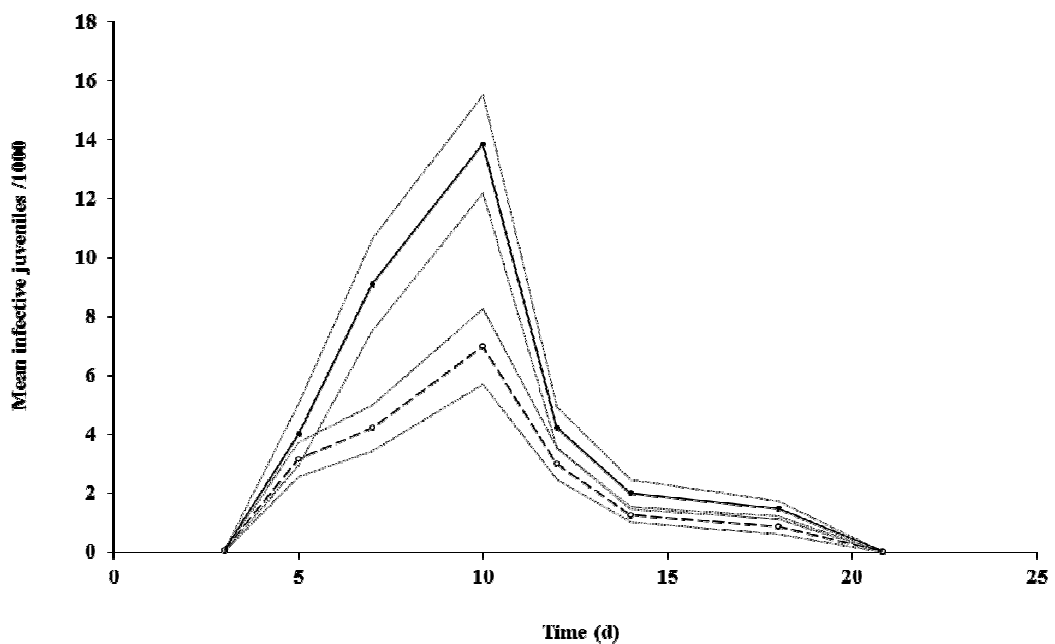
Figure 4.3: Predicted number of *Steinernema carpocapsae* infective juveniles emerging from uninfected female cockroaches (bold line ±1 SE), and from females infected with *Gregarina blattarum* (dashed line ±1 SE) over a 28 day period. Model predictions were generated at the mean weight of females.

iv.   Is host mortality due to S. carpocapsae infection greater in hosts co-infected with G. blattarum?

Cockroach survival risk when hosts were infected with nematodes, was significantly affected by the *G. blattarum* infection status of the host ($F_{1, 197} = 5.60$, $p = 0.018$) and the concentration of nematodes that the host was exposed to ($F_{1, 197} = 22114$, $p < 0.001$). All cockroaches died from a dose of 12000 nematodes/ml over the 30 day monitoring period, whereas only 12% and 72% died from doses of 120 and 1200 nematodes/ml respectively. The mortality rate was significantly different between uninfected and infected cockroaches at a concentration of 120 nematodes/ml (Fig. 4.4; $Chi\text{-}sq_1 = 4.1$, $p = 0.042$), which resulted in an overall mortality over the 30 day monitoring period of 4% and 24% respectively. Although there was a slight increase in the mortality rate of co-infected hosts at 1200 nematodes/ml, the mortality rates were not significantly different at 1200 or 12000 nematodes/ml concentrations ($p > 0.05$).
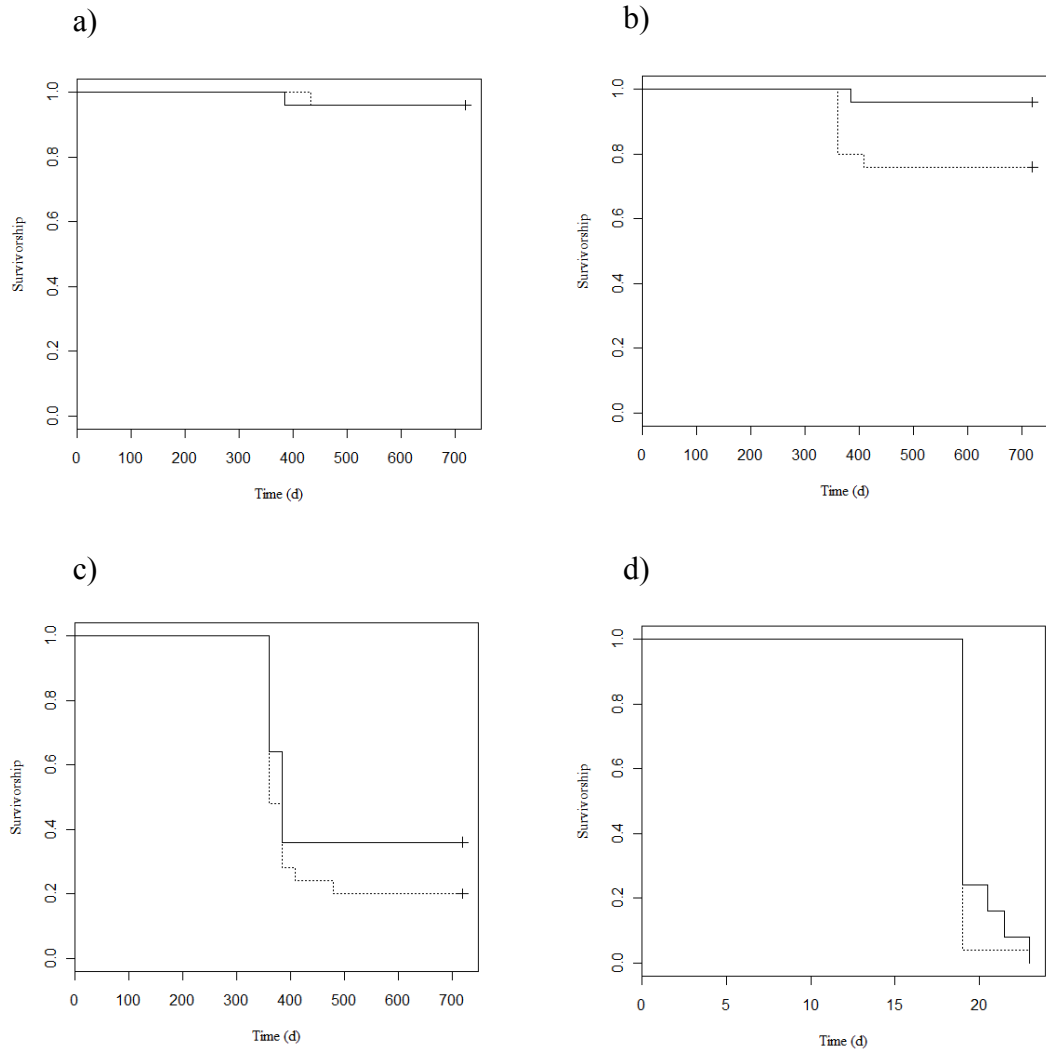
Figure 4.4: Kaplein Meier survival probability of German cockroaches singly infected with *Steinernema carpocapsae* (black lines) and those co-infected with *Gregarina blattarum* and *S. carpocapsae* (dashed lines). Four concentrations of nematodes were tested: a) 0, b) 120, c) 1200 and d) 12000 nematodes/ml. Only for cockroaches at 120 nematodes/ml (b) was there a statistically significant difference in the survival probability (p < 0.05).

4.5 Discussion

The aim of this study was to determine whether an endemic parasite species could alter the transmission potential of an acute parasite through the indirect cost of host resource depletion. The endemic parasite *G. blattarum* caused substantial reduction in host lipid levels, which for most of the females tested (those not bearing eggs/oothecae and those with oothecae) was greater than the effect of 28 days starvation (Table 4.1). Gregarine infection was subsequently associated with a substantial reduction in the emergence of infective larvae of the entomopathogenic nematode *S. carpocapsae* as well as decreased survivorship of hosts exposed to nematodes. Gregarine infection, through resource depletion, can therefore have a significant effect on the transmission potential of an acute parasitic infection.

Host nutritional status may be limited during parasite infection through changes in host feeding behaviour. For instance, parasitism in the gut of ruminants can lead to malaise and general lack of appetite (Stein *et al.* 2002). However, hosts did not alter food consumption rates during infection with *G. blattarum*, and hence the reduction in host lipid levels cannot be the result of decreased food intake. There was also no evidence from this study that hosts were able to increase their feeding rate, a mechanism that can mediate the effects of parasitism in other invertebrates (Lee *et al.* 2006; Ponton *et al.* 2010), as hosts were provided with food *ad libitum* and did not over-feed. The reduction in host lipid levels could therefore occur due to parasite acquisition of the host resources, as resources are required for parasite reproduction (Poulin & George-Nascimento 2006; Seppala *et al.* 2008) or be the consequence of increased host metabolism as energy may be required to mount an immune response (Lafferty & Kuris 2005; Chapter Five) or repair damage (Takahashi, Kawaguchi & Toda 2009). Indeed, Takahashi, Kawaguchi & Toda (2009) were able to demonstrate that gregarine infection damaged micro-villi in the epithelial wall. Therefore, the infection may have prevented the host from absorbing lipids from the gastrointestinal tract, leading to a decline in measured host lipid levels.

The effect of parasitism was found to be most profound in females carrying oothecae (Fig. 4.2). Although no difference was found between the food consumption rate of infected and uninfected hosts overall, during the time females were carrying oothecae they were found to consume less food than at any other time during their

reproductive cycle. This may explain why lipid loss is greatest in these females, because both the pressure of reduced food intake and parasite infection is acting to lower host resource levels. Reduction of food intake in females carrying oothecae has been previously reported (Lee & Wu 1994), and which may be a strategy to protect the host from parasitic uptake during oviposition. Females carrying oothecae are therefore potentially allocating more resources to rearing their offspring at this stage in their reproductive cycle than to their own metabolic needs.

Although lipids were lower in infected hosts than in starved hosts, the output of nematodes was similarly reduced by infection (60.5%) and starvation (62.5%). This indicates that the transmission stages are either being provisioned differently or have obtained energy from another source to maintain the similar reproductive levels. One possible source would be the gregarines themselves, which are digested during *S. carpocapsae* infection but which were removed before lipid analysis. Ciancio, Scippa & Cammarano (2001) demonstrated that rich lipid deposits are present in the cytoplasm of the gregarine species, *Lankesteria ascidiae* which could therefore provide an energy resource. However, if the nematodes are not able to compensate for lipid reductions by obtaining resources from gregarines, there may be consequences for the resource provisioning of the infective larvae. The transmission stages are dependent on what can be acquired within the host as the infective larvae themselves are non-feeding (Qui, Lacey & Bedding 2000). Therefore the infective larvae may harbour fewer energy reserves from hosts where *G. blattarum* infection is present. Whilst it has previously been reported that nematode lipid levels do not alter immediate infectivity (Patel, Stolinski & Wright 1997), the lipids in the larvae are finite and so there must ultimately be a cost of limiting this resource. As the nematodes may have to survive long periods in the environment (Adams & Nguyen 2001; O'Leary *et al.* 2001), their survival probability may be limited by the lack of resources, which could impact on the rate of transmission. Nematode transmission in hosts that are infected with the endemic infection could therefore be altered both by the substantial reduction in the number of transmission stages as well as fitness consequences for the individual larvae.

The survivorship of hosts was decreased in cockroaches that were co-infected with *G. blattarum* and *S. carpocapsae*. Poor host condition can lead to increased host

mortality rates during infection (Brown, Loosli & Schmid-Hempel 2000). This decrease in survival may therefore be a result of limited resources during *G. blattarum* infection. Resource reduction by *G. blattarum* may diminish the host's ability to mount an immune response, which may also explain the increased mortality rates of co-infected hosts. In Chapter Five, it is demonstrated that the anti-microbial defence is depleted in hosts when *G. blattarum* is present. Nematodes release a symbiotic bacterium which enables degradation of host tissue in order for nematodes to acquire resources and reproduce (Adams & Nguyen 2001). Therefore, host immune activity against the bacterial symbiont may also be limited by resource depletion, which could allow for increased colonisation of the bacteria during infection, and account for the faster host death rate. Therefore whilst nematodes cannot reproduce at the same intensity in hosts where *G. blattarum* are present, they may be capable of recruiting host resources faster through reduced host immune responses. This could potentially allow for selection of faster transmission rates of parasites from hosts where the endemic parasite is present.

By exploring host resources during parasite invasion, this study has identified how an endemic parasite species can alter the reproductive rate of an acute infection. The reproductive rate of the parasites is directly linked to the number of parasite stages, therefore endemic infections could have important consequences for parasite transmission. As endemic infections are extremely common in nature, the ability to predict and model disease transmission of infectious diseases is likely to be improved by considering endemic diseases in the host population.

4.6 References

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5. Immune response to micro- and macroparasites in the German cockroach, *Blattella germanica* is altered by gastrointestinal infection

5.1 Abstract

Host responses to parasite infections can vary between individuals across a population. Endemic infections in vertebrates contribute to this variation by changing host responses to the invading parasites, but so far this has not been extensively studied in an invertebrate host species. This study aimed to determine whether the presence of an endemic infection influenced host investment in different host immune response components. The laboratory system was comprised of German cockroaches infected with a gastrointestinal parasite, *Gregarina blattarum* and parasite free control hosts. Three measures of host immune function were explored which together cover both macro- and microparasite immune function. First, general immune fitness (active against both micro- and macroparasites) was assessed by measuring the levels of phenoloxidase in haemolymph extracted from both *G. blattarum* infected and parasite free hosts. Second, a measure of anti-microparasite response was determined by measuring the lytic activity of the cockroach haemolymph, by assessment of the size of zones of growth inhibition of *Micrococcus lysodeikticus* on culture plates. Third, host response to macroparasites was measured by challenging the host with an artificial parasite (a nylon filament) and recording the amount of melanisation and cellular debris that formed around the nylon. The generalised immune response (measured phenoloxidase levels) did not differ between *G. blattarum* and uninfected hosts, although there was a substantial reduction in haemolymph protein levels in infected hosts. However, there was a substantial reduction in lytic activity, the anti-microparasite response in the infected hosts. Conversely, the anti-macroparasite response, encapsulation, was greater in hosts infected with *G. blattarum*. Infection with a gastrointestinal parasite therefore changed the balance of immune response against micro- and macroparasites, compared to gregarine uninfected hosts. This variation may be caused by trade-offs between different forms of immunity. These findings demonstrate the potential for co-infections to alter the susceptibility of invertebrate hosts to different parasite infections, as has been shown previously in vertebrate systems.

5.2 Introduction

Parasites can pose a significant threat to the health and fitness of many living organisms. As such, animals have evolved a set of innate and/or acquired immune functions in order to respond to the potential harm posed by disease (Goldsby *et al.* 2006; Koella 2009). However, even within a single host population there can be large variation in the ability to respond to the same parasitic disease (Sheldon & Verhulst 1999; Norris & Evans 2000; Rolff & Siva-Jothy 2003; Wilson 2005). This can lead to heterogeneity in host resistance, susceptibility and variation in the longevity and severity of the disease between different hosts (Henter & Via 1995; Paterson, Wilson & Pemberton 1998). Understanding the causes of such variation can not only improve treatment strategies, but also help develop a better understanding of the ecological processes that govern disease virulence evolution and the evolution of host immune function (Wilson 2005).

The causes of variation in host responses to parasite infections can be attributed to several factors including host and parasite genetic heterogeneity (Tella *et al.* 2000; Kaslow, Dorak & Tang 2005; Tinsley, Blanford & Jiggins 2006), sex of the host (Schmid-Hempel 2005), the age of the individual (Stear *et al.* 2000), resource availability (Schmid-Hempel 2005), seasonality (Dowell 2001; Faustino *et al.* 2004) and stochasticity in the environment (Krist *et al.* 2000; Ostfeld *et al.* 2006). Another important factor that affects host susceptibility is the presence of other infections (Lello *et al.* 2004; Cattadori, Albert & Boag 2007). Hosts encounter many different parasites during their lifetime (Petney & Andrews 2008) and either previous infections or concomitant infections may impact on which individuals become infected, as well as the severity and longevity of disease (Lello & Hussell 2008; Cattadori, Albert & Boag 2007).

In mammals, the immune cells involved in the adaptive immune response against microparasites are type 1 T helper cells (Th1), whereas another class of T helper cells (Th2) are important in the response against macroparasites (Romagnani 1991a, b). The production of these two immune cell groups is governed by trade-offs, so that up-regulation of one process is associated with down-regulation of another (Kidd 2003; Fenton, Lamb & Graham 2008). Therefore, hosts that are infected with parasites which lead to the induction of Th2, have been shown to have increased

susceptibility to microparasites (Chakkalath & Titius 1994) whereas those that induce Th1 responses can occasionally increase host susceptibility to macroparasites (Graham 2008). Understanding these processes can be useful in determining how endemic parasites can alter variation in host immune responses. Yet most co-infection studies to date have focused on mammalian systems. Invertebrates have simple immune systems that are easy to monitor and manipulate experimentally (Wilson 2005). Exploring host immunity during co-infections in invertebrate hosts may improve our understanding of the processes that determine variation in immune response as a whole.

Like vertebrates, invertebrates have an immune system that is capable of responding to different types of parasites. Most of the immune functions are innate, however there is also increasing evidence of long term up-regulation and memory within that innate framework (Moret & Siva-Jothy 2003; Kurtz & Franz 2003; Korner *&* Schmid-Hempel 2004; Kurtz 2005; Schulenburg, Boehnisch & Michiels 2007). General response against both micro- and macroparasites are determined by humoral responses, particularly the action of the enzyme phenoloxidase which is induced during parasitic invasion with a variety of organisms including fungi, parasitoids, bacteria and nematodes (Bogos *et al.* 2007). The responses that are active against microparasites include the action of lytic enzymes, particularly lysozymes which causes breakages in the peptidoglycan wall in gram positive and gram negative bacteria (Koella 2009). The ability to respond to macroparasites such as nematodes is controlled by cellular responses and in particular, the encapsulation response (Peters & Ehlers 1997; Lavine & Strand 2002; Crossan *et al.* 2007). Encapsulation is the aggregation of haemocytes around the outside of a foreign body such as a parasite, which is reinforced with a layer of melanin and initiated by the action of phenoloxidase (Levin *et al.* 2005). The production of melanin is also used to strengthen the host cuticle against parasitic invasions (Wilson 2005). There is also increasing evidence that a similar paradigm to the Th1/Th2 in vertebrates may also be present in invertebrates due to the specific responses to different parasites (Cotter, Kruuk & Wilson 2004). The invertebrate model may therefore not only improve assessments of disease transmission by invertebrate vectors, but may also improve the understanding of immune processes in other organisms.

The host organism used in this study was the German cockroach host, *Blattella germanica*, parasitized with a gastrointestinal macroparasite, *Gregarina blattarum*. The infection cycle of *G. blattarum* is described more detail in Chapter One. Briefly, hosts are infected with *G. blattarum* through the accidental ingestion of parasite transmission stages (oocysts) by the host (Clopton & Gold 1996). The oocysts migrate to the mid-gut of the host, the main site of infection, where sporulation occurs and the emerging sporozoites attach to the mid-gut wall (Clopton & Gold 1996). The cells grow to form trophozoites which eventually fuse in pairs to form reproductive units (gametocysts) which are passed into the environment where cellular division takes place (Clopton & Gold 1996).

The aim of the current study was to determine whether an endemic parasite infection could alter different components of the invertebrate immune response. Three specific measurements of host immune function were assessed: general immune function (humoral phenoloxidase and haemolymph protein levels), anti-microparasitic response (lysozyme activity) and anti-macroparasitic response (encapsulation). It was expected that immune function would be decreased in hosts that were infected with the endemic infection, as hosts have been demonstrated to have fewer resources (lipids, Chapter Four) with which to mount a response to parasitic invasion.

5.3 Materials & Methods

Host cultures

German cockroaches, *B. germanica* were collected from parasite free colonies or colonies containing the parasite species *G. blattarum*. The rearing conditions of these hosts are provided in detail in Chapter Two (p. 21).

Haemolymph sampling

Cockroaches were randomly collected from uninfected (n = 50) and infected colonies (n = 50) and were retained in small plastic containers with *ad libitum* food and water for one hour prior to sampling. Each host was anaesthetised with CO_2 and an area below the left posterior limb was swabbed with 70% ethanol followed by PBS to remove any external bacterial contaminants. The haemolymph was collected using a sterilised syringe with 100 μl of EDTA to prevent coagulation. Haemolymph

was collected from five cockroaches at a time and the sample pooled into a 1.5 ml eppendorf tube and immediately placed on ice. The samples were stored at -20°C until required.

Protein and Phenoloxidase Assays

Host protein levels in the presence and absence of *G. blattarum* were determined using the BioRad® protein assay kit (Sigma-Aldrich) with bovine serum albumin (BSA) as the protein standard. Two replicates from each haemolymph sample (n = 5 uninfected; n = 5 infected) were added to a 96 well plate in 10 μl quantities with 200 μl of filtered dye reagent. After five minutes absorbance measurements were made using a UV spectrophotometer with a micro-plate reader (VERSAmax®) at 600 nm. The protein content was then estimated from the BSA standard curve. Phenoloxidase activity was measured by adding 6 μl of haemolymph to 300 μl of PBS in a 1.5 ml eppendorf tube. Two 100 μl replicate aliquots of each sample were dispensed into a 96 well plate with 100 μl of 4 mM L-Dopamine (Sigma Aldrich) in each well, and immediately placed under a spectrophotometer with a plate reader following which a reading was taken every 30 seconds for 45 minutes. The levels of phenolodixase activity was calculated as the amount of activity required (in PO units per mg protein) to change the absorbance by 0.001 nm per min (Cotter, Kruuk & Wilson 2004).

Lytic activity

The lytic activity of cockroach haemolymph against *Micrococcus lysodeikticus* was determined using a lysozyme assay. Plates were prepared with a 10 ml layer of 1% Agar (Sigma-Aldrich) inoculated with 5 mg per ml of freeze-dried *M. lysodeikticus*. These agar plates were perforated 14 times at equal spacing with a glass capillary tube (2 mm dia.) and 1 μl of haemolymph was placed in each well with 2 replicates for each sample (n = 10 uninfected; n = 10 infected). A standard curve of hen egg-white lysozyme (Sigma-Aldrich) was prepared in distilled water and added in duplicates to the agar plates with a distilled water control. The plates were stored upside down for 20 minutes in a laminar flow cabinet and then transferred to an incubator at 33°C. After 24 hours the diameter of inhibition of bacterial growth

surrounding the inlet-wells was measured using a calliper (accurate to 0.5 mm) and the lysozyme concentration equivalent was calculated from the standard curve.

Encapsulation response

The anti-macroparasitic responses of hosts with *G. blattarum* were determined by measuring the levels of melanisation and cell deposition surrounding a piece of nylon filament (in accordance with Cotter *et al.* 2008). Nylon filaments (2mm lengths of fishing wire) were UV sterilised for 15 minutes. Female cockroaches (with oothecae absent to control for variation in resources associated with oviposition, see Chapter Four) were randomly selected from uninfected (n = 20) and infected colonies (n = 20) and anaesthetised with CO_2. The implant area between the 5th and 6th sternite was swabbed with a cotton wool bud soaked in 70% ethanol followed by a swab with phosphate buffered saline (PBS). The nylon filament was implanted by piercing the cuticle with the nylon, using sterilised forceps. Cockroaches were left in 275 ml plastic containers with lids (Cater For You Ltd.) with *ad libitum* food and water and incubated at $25^{o}C$. The implants were extracted by dissection after 24 hours. Each implant was gently rinsed with PBS and placed on a microscope slide. The level of melanisation was captured using a digital micro-imaging camera (Olympus Advanced DP72) affixed to a stereo microscope (GX Optical XLT-101). Two images were taken from the implant within 180° rotation of each other. The area of melanisation was then analysed using the image analysis software package Image J® (see Cotter, Kruuk & Wilson 2004).

Statistical analysis

The effect of *G. blattarum* on three measurements of host immune function, phenoloxidase activity, lysozyme-like response and encapsulation response, were assessed using three Generalised Linear Models in R version 2.13.2 (R Development Core Team 2011). The model family error structure and link functions for the three respective models were: gamma with identity link; Gaussian with log link and inverse-Gaussian with link $1/mu^2$. The overall protein concentration in host haemolymph was assessed with a General Linear Model, with protein levels log transformed prior to analysis. The standardised residuals from each model were

checked for normality prior to analyses, and the effect of infection on immune response was assessed using the F test statistic.

5.4 Results

Overall immune function

Infection with *G. blattarum* was associated with a 57% reduction in mean haemolymph protein (Fig. 5.1a; $F_{1,16} = 4.85$, $p = 0.043$). The mean concentration of protein was 0.46 mg/ml ($\pm$ 0.02 SE) in parasite free hosts and 0.19 mg/ml ($\pm$ 0.01 SE) in *G. blattarum* infected hosts. The humoral levels of phenoloxidase activity were higher in infected hosts (16.35 PO units/mg protein $\pm$ 6.08 SE) compared to parasite free hosts (4.66 PO units/mg protein $\pm$ 1.74 SE) but the result was not statistically significant (Fig. 5.1b; $F_{1,8} = 4.43$, $p = 0.069$).

Lysozyme activity (anti-bacterial)

The response of hosts to a microparasitic challenge, measured as levels of haemolymph lytic activity, were significantly lower in infected hosts compared to hosts that were parasite free (Fig. 5.1c; $F_{1,38} = 20.44$, $p < 0.001$). Parasite-free lytic activity was 1.12 µg/ml ($\pm$ 0.09 SE), but infected hosts only produced half the lytic response (0.61 µg/ml 0.08).

Encapsulation response (anti-helminthic)

Infection with *G. blattarum* led to a significant increase in the encapsulation response of hosts to an artificial parasite (nylon filament) *in vivo* (Fig. 5.1d; $F_{1,48} = 6.738$ $p = 0.013$). The mean area of melanin on the nylon filament was 0.32 mm^2 ($\pm$ 0.15 SE) in parasites free hosts, and was 2.6 times higher in infected host, *i.e.* 0.86 mm^2 ($\pm$ 0.16 SE).
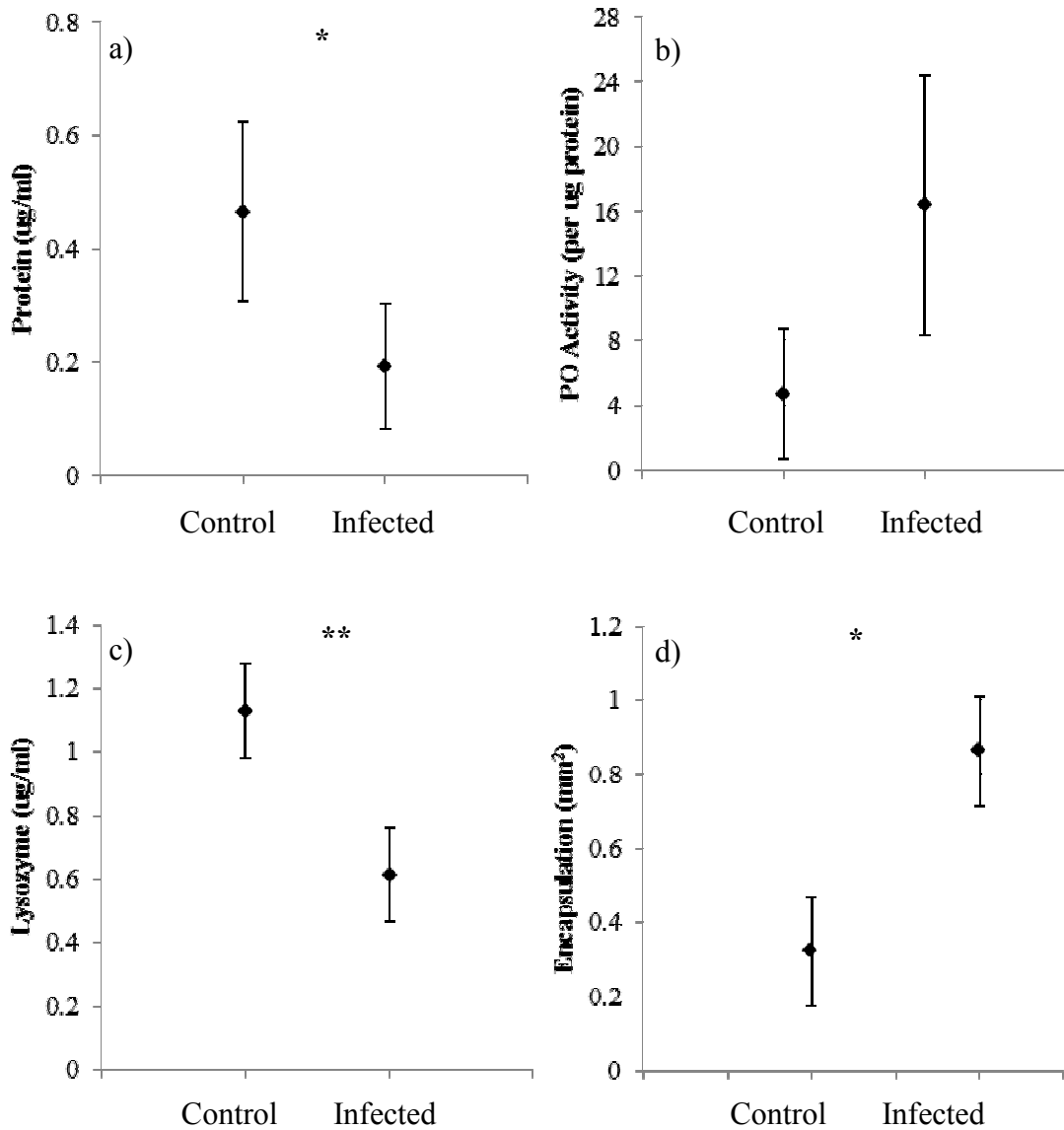
Figure 5.1: Predicted mean immune response levels in parasite free hosts (control) and hosts parasitized with *Gregarina blattarum* (infected) for a) haemolymph protein (mg/ml), b) phenoloxidase activity (µg/mg protein), c) lysozyme-like lytic activity (µg/ml), and d) nylon filament encapsulation (mm^2). Error bars show ±1 SE. *p < 0.05; ** p < 0.001.

5.5 Discussion

Gregarina blattarum infection was found to cause changes in the German cockroach response to both micro- and macroparasite challenges. The levels of humoral lytic activity (lysozyme) and protein levels were, as predicted, reduced by *G. blattarum* infection. However, there was slightly greater activity of phenoloxidase (a general measure of anti-macro- and microparasite immune function; Adamo 2004) within the available protein, although this was not statistically significant. Further, the levels of encapsulation response to macroparasites were enhanced in the presence of *G. blattarum*. The presence of the gregarine infection is therefore likely to increase the susceptibility of the host to other microbial infections, particularly bacteria, but at the same time offer some protection to the host against infection with multi-cellular macroparasites.

The levels of haemolymph lytic activity and protein were expected to decline in *G. blattarum* infected hosts, as previous investigations have found that host energy resources are reduced during infection (Chapter Four). Proteins are an important resource for the invertebrate immune system required for humoral and cellular responses (Koella 2009). It is therefore likely that energy costs from *G. blattarum* infection are constraining proteins levels required during the immune response. Although protein levels were lower, the amount of phenoloxidase that was active within that protein pool was not significantly affected by *G. blattarum*. One possible explanation for this is the role that phenoloxidase has in melanin production during an encapsulation response, which is substantially increased in *G. blattarum* infected hosts (discussed below). Another possibility is that the phenoloxidase levels are maintained in an effort to respond to damage caused at the infection site. Infection by gregarines can increase host damage to the epithelial cell wall (Takahashi, Kawaguchi & Toda 2009; Valigurova *et al.* 2007) and therefore slightly raised levels of phenoloxidase activity, which is also used in host repair processes (Adamo 2004) may be present as part of a reparative process.

The encapsulation response was increased in hosts where *G. blattarum* was present. The mechanism for the increase in encapsulation response is unknown, but evidence has previously been reported that both lytic activity (anti-microparasitic) and encapsulation activity (anti-macroparasitic) are subject to trade-offs so that hosts

which can mount high levels of one response are limited in their ability to mount the other response (Cotter *et al.* 2004). Such trade-offs have been likened to the Th1/Th2 paradigm in vertebrates, demonstrating the importance of invertebrate immunity models for our understanding of vertebrate immunity (discussed below; Koella 2009). The evidence provided in this current study is also indicative of immune response trade-offs, however it is not clear how *G. blattarum* infection affects the differential response to micro- and macroparasites. Host resources are clearly depleted in *G. blattarum* infected hosts (Chapters Four and Five); therefore to mount a higher immune response to macroparasites, hosts must divert resources from other life processes. This may explain previous findings that gregarine infected hosts have reduced fecundity and lifespan (Chapter Three). Further exploration of the resources available in different host tissue (*i.e.* cuticle, mid-gut and haemolymph) is required to determine whether trade-offs occur in resource allocation during *G. blattarum* infection.

Overall, there is strong evidence from these findings to suggest that gregarines may increase host protection to macroparasitic diseases and decrease the host response to microparasites. In order to draw comparisons with the vertebrate Th1/Th2 paradigm, it should be evident that gregarine infection up-regulates protection against similar parasites and down-regulates immunity to more distantly related species (Graham 2008). Gregarines are eukaryotes, which are resistant to lytic enzymes, but as single celled organisms it is unlikely that they will induce an encapsulation response. However, gregarines do form large reproductive units of fused cells (gametocytes), visible to the naked eye, which could be large enough to induce encapsulation responses. Therefore, it is possible that gregarines may enhance the host ability to respond to other macroparasites, and such a reaction could be traded-off against the host's ability to respond to microparasites. One way to test this would be to explore the immune responses to the gregarines themselves. Gregarines are extremely common to invertebrates; however there have been surprisingly few studies undertaken to examine the immune response within the host when gregarines are present, and none (to my knowledge) of the response to gregarines at the infection site. Further work will therefore explore immune function to the gregarines in the mid-gut to assess trade-offs and potential comparisons to the vertebrate Th1/Th2 paradigm.

In conclusion, the presence of an endemic infection can alter the immune function to different parasites in this invertebrate system. Energy costs of infection to the host are likely to explain reductions in anti-microparasitic response. However, further work is needed to determine how the encapsulation response is increased and indeed whether or not there are trade-offs with immune functional responses. Extensions of this work may reveal parallels with vertebrate immunity, particularly the Th1/Th2 paradigm, and as such this study system should prove an asset for future immunological research. Overall, gastrointestinal infections are likely to impact on the susceptibility of the host to other infections and future studies should consider the effect of gastrointestinal infections in the host population when exploring the variation in host susceptibility to disease.

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6. General Discussion

6.1 Endemic infections and host fitness

Parasites are an important threat to life on Earth, both with respect to population decline and the threat of population extinction (Cunningham & Daszak 1998; Daszak, Cunningham & Hyatt 2000; Stuart *et al.* 2004; Schloegel *et al.* 2006; Pounds *et al.* 2006; Hawkins *et al.* 2006; Smith, Sax & Lafferty 2006; Skerratt *et al.* 2007; McCallum *et al.* 2009). As increasing population density, loss of habitat and drug resistance are likely to lead to an increase in the emergence of diseases over the next century, predicting where and when new diseases will emerge from will be important for targeted prevention treatment strategies (Daszak *et al.* 1999). Outbreaks of epidemic infection are likely to occur in areas of the world where gastrointestinal infections are endemic (Petney & Andrews 1998). However, endemic infections are rarely considered when modelling disease outbreaks. Identifying how endemic parasite infections affect host biology and their interaction with other diseases will therefore improve our understanding of disease emergence and evolution for improved treatment strategies in the future.

Endemic parasites have long term associations with their host population, and as a result can bring about substantial changes to the host energy budget, life history and population dynamics (Hudson, Dobson, & Newborn 1992; Zinsstag *et al.* 1997; Albon *et al.* 2002; Lello *et al.* 2005). This study provided further evidence that gastrointestinal protozoans can lead to host resource decline (Chapters Four and Five), with important consequences for host fecundity, life span and population dynamics (Chapters Two and Three). Very few empirical examples exist that demonstrate endemic parasites regulate host population dynamics, and most field-based examples are also influenced by external factors such as climate or predation (Dobson & Hudson 1992; Albon *et al.* 2002). Laboratory populations of invertebrates in the absence of environmental variation and inter-specific competition can be regulated by parasites (e.g. Bonsall & Benmayor 2005) and parasitoids (e.g. Hassell, Comins & May 1991). The findings from this study provide new evidence that endemic parasites of invertebrates mediate important changes in the regulatory processes of their hosts (Chapter Two). Indeed, endemic infection

alone altered host population regulation, which led to the stabilisation of the host population, as infected host densities were less varied than parasite-free colonies. Stabilising effects of the endemic parasite was also demonstrated by increased host mortality during *G. blattarum* infection (Chapter Three) and aggregated parasite distributions amongst hosts (Chapter Three), both of which are understood to stabilise host populations (Anderson & May 1978). There was also some support for parasite stabilisation from the model fitting process. Endemic infections can therefore cause regulatory changes in host dynamics, which could have important effects on the host population in natural systems.

Endemic infections can also have an important impact on the interaction of the host with other parasites. Co-infections may compete for host resources and this may lead to an increase in reproduction and transmission rate (May & Nowak 1995; Mosquera & Adler 1998; Sharomi *et al.* 2008). In Chapter Four, it was demonstrated that resource costs imposed by an endemic infection can impact on the transmission potential of a more severe parasite species. The reduction in resources by *G. blattarum* infection was associated with a subsequent decline in the output of infective larvae. Whilst this work did not assess transmission rate, there is strong support that co-infection would alter nematode transmission in this system both by the substantial reduction in nematode numbers and also by potential nutrient costs to the emerging nematodes. Endemic infection was also found to affect the survival rate of hosts during co-infection (Chapter Four). A decrease in host survival was thought to be a result of reduced host resources which were necessary for host defence against the nematodes. One of the most important invertebrate defence strategies against macroparasites is the encapsulation response, which has previously been shown to limit the mortality of wax moth larvae when infected with *S. carpocapsae* (Crossan *et al.* 2007). Whilst there was evidence in this study to show that both proteins and lipids were reduced during *G. blattarum* infection, the encapsulation response was in fact enhanced by gregarine infection in the gut (Chapter Five). However, on entry to the host, infective juveniles release a bacterial symbiont which enables host digestion and parasite reproduction (Adams & Nguyen 2001). As the host anti-bacterial response was lower in those hosts that were infected with *G. blattarum*, the reduction in host survival during co-infection could be explained by increased bacterial growth in hosts were lytic activity is reduced. Therefore, whilst

co-infection alters host-parasite interactions, there may also be consequences for the symbiont-nematode interaction as higher bacterial digestion of host resources could potentially select for increased pathogenicity.

There is a considerable amount of variation in immune responses amongst host populations which has been previously linked to a range of different biological factors including co-infection (Graham 2008). Immune responses are also an important factor in understanding disease transmission and host susceptibility to disease (Henter & Via 1995; Paterson, Wilson & Pemberton 1998). Gastrointestinal infections are endemic in many parts of the world where newly emerging disease outbreaks occur, which could affect host interaction with acute infections in both positive and negative ways. In humans for instance, helminths infections, can sometimes increase host susceptibility to HIV (Maggi *et al.* 1994) and malaria (Spiegel *et al.* 2003), but helminths can also decrease the inflammatory responses produced in malarial infections (Nacher *et al.* 2000). Here it was demonstrated that the presence a gastrointestinal gregarine infection can also lead to both enhancement and reduction in host responses to different pathogens. Whilst eradication of gastrointestinal infections has been the focus of treatment efforts by the World Health Organisation in an attempt to remove diseases from the world in general (Bentwich *et al.* 1999), caution should be given to complete removal of gastrointestinal helminths as this could lead to an increase in the host susceptibility to other infections. It is therefore recommended that infection control programmes establish the parasite infections that are endemic in host populations in order to develop the most appropriate control strategies.

6.2 Limitations and strengths of the study

The invertebrate system used in this study was an established laboratory model species, the German cockroach, *Blattella germanica*. German cockroaches are extremely useful study organisms for population level work as well as individual based studies, due to large population densities, ability to rear all stages in the same container and culture isolated individuals in a simple experimental set-up. The two parasite species that were used in this study, *G. blattarum* and *S. carpocapsae*, have also been studied extensively and as a result have established modes of culture and maintenance. Combining the three chosen species into the same study therefore

provided an opportunity to use established laboratory methods with a novel co-infection system.

The mark-recapture method applied to assess cockroach population dynamics was based on technique previously used to determine German cockroach abundance in the wild (Rivault 1989; Tee, Saad & Lee 2011). The method developed for this laboratory study was adapted to include sub-sampling of hosts which allowed simultaneous monitoring of parasite levels during the longitudinal study. Many previous studies of host populations have only collected dead individuals, to monitor host and parasite loads (e.g. Bonsall & Benmayor 2005); therefore the methods here were an improvement on previous studies by reducing the possibility of skewed parasite loads from only collecting dead samples. The method of mark-recapture presented in this report could also be adapted to take haemolymph samples from individuals if required which could be of use for future ecological and immunological work.

As with other population level studies of animals, identifying the dynamics of the population density is laborious, and requires monitoring over a substantial time period in order to capture any population cycles. The study here was conducted for approximately two years, but in that time no clear cycles were observed which may be a result of the time scale monitored. Overall, this approach did yield substantial rewards with respect to the detailed dataset on host and parasite numbers, the number of census time-points of host numbers and parasite distributions. Indeed, a substantial amount of data remains to be analysed including sex difference in population dynamics and the ratio of adults to offspring in the population over time (described below).

Ecological datasets, particularly time-series studies are inherently noisy (e.g. Grenfell *et al.* 1998), and the dataset collected here was no exception. An advanced level of analysis using complex modelling approaches was therefore required to assess the parasite effects on the host population dynamics. As no previous attempts have been made to monitor cockroach population dynamics, there are no examples with which to begin a model selection process. The selection of candidate models was therefore based on assumptions of general host-parasite systems (Anderson & May 1978; Bellows 1981; Bonsall & Benmayor 2005). It was therefore necessary to

explore a wide candidate model selection for each of the infected and parasite-free colonies monitored. The levels of analysis allowed for assessment of the host-parasite dataset that would not have otherwise been possible with correlation or simple time series analysis.

The use of a novel co-infection system (*G. blattarum* and *S. carpocapsae)* provided an interesting opportunity to explore disease dynamics, which has generated considerable scope for future work (discussed below). The main limitation to working with *G. blattarum* is one that is problematic to infectious disease work in general, *i.e.* that careful laboratory management is required to prevent contamination of parasite free stocks. Whilst true epidemic studies could not be investigated with the parasite *S. carpocapsae*, as the nematodes are obligate killers (*i.e.* no host recovery from disease), preliminary work (not shown here) found that continuous exposure of hosts to *S. carpocapsae* can select for resistant hosts. Therefore, there is the potential for this co-infection system to be used as an endemic-epidemic system in future work.

6.3 Future work

The effect of endemic infections on host population dynamics is still an understudied area of host population ecology. Here, the population level work could not fully determine the biological effects on host population dynamics, as parasite-free hosts and the parasite dynamics were not well explained by the goodness of fit test. There are several possibilities for this which could be explored in future work. For example, there is clear evidence from the life history assessment, of sex and stage (juveniles) differences in the effects of parasitism on host fitness. The dataset that was collected for the host densities will therefore be separated into males and females to determine whether the dynamics differ between each of the sexes. Incorporating juvenile developmental into the population models could also improve the population models as there is a delay in offspring survival. To date, the biological estimates to start the model fitting procedures were also based on previously published work (Müller-Graf *et al.* 2001) as well as some parts of the life history data when they were available at the start of the modelling process. Fixing the parameters with measured life history data collected here, rather than allowing the model to be adjusted throughout the fitting process (through several thousand

iterations), would be an important next step in assessing the model suitability. A final development of the modelling process would be to distinguish the different types of stochasticity which may contribute to the large fluctuations in the host density using various stochastic models.

The effect of endemic parasites has been shown to cause trade-offs in life history processes, which may be driven by changes in the allocation of host resources (e.g. Lafferty 1993). In order to fully understand whether resource trade-offs were responsible for the resource and life history changes in the German cockroach, it will be necessary to examine the levels of nutrients in host eggs and offspring to determine whether resources are partitioned differently towards reproduction in infected hosts. *Gregarina blattarum* infected hosts were capable of increasing their production of an encapsulation response, despite having fewer resources. Therefore, assessing resources within the haemolymph and at the infection site (mid-gut) would enable me to determine whether resources are partitioned differently during *G. blattarum* infection. Preliminary attempts were also made to explore the composition of lipids within the cuticle and fat body of the host, using chromatography to separate the lipids into the constitutive lipids (polar and neutral lipids) and determine the fatty acid composition of those separate lipid classes (not presented here). However, no differences were found between the composition and the quantity of lipids between infected and uninfected hosts. This information was not congruent with the findings from the simple lipid extractions (Chapter Four) but this was probably due to extremely low infection levels in the sampled cockroaches. Therefore, a repeat of the experiment with higher infection levels within the sampled cockroaches would be necessary to truly assess whether lipid composition, rather than just quantity, is altered under infection conditions.

The infectivity and transmission of parasites is not solely dependent on the number of reproductive stages that are produced but also the nutrient levels within those transmission stages (Medica & Sukhdeo 1997). There was evidence that nematodes may utilise gregarines as a nutrient supply in order to maintain the same levels of reproduction in infected hosts as those hosts that were starved (Chapter Four). Assessing lipid levels of the gregarines would therefore determine whether the gregarines in this study are a sufficient supply for the levels of nematode

reproduction that were measured. Separating the lipids in gregarines as well as the host diet using chromatographic techniques (as outlined above) would also determine whether the gregarines utilise food from the cockroach diet in the mid-gut lumen or remove nutrients directly from the host. Further identification of the lipid levels in the nematodes themselves would then allow any costs for nematode fitness to be determined, as the infective stages are non-feeding and therefore dependent on the levels of nutrients obtained within the host for transmission.

Despite the estimated millions of species of gregarines that may exist (Clopton & Gold 1996), there have been surprisingly few studies (<10) to assess immune function of hosts to gregarines, none in the German cockroach and no direct measurements in the gastrointestinal tract. The immune results presented in this report have so far demonstrated the general functional responses to different types of parasite of cockroaches infected with *G. blattarum* infection, but there is considerable scope for expansion. For example, exploring the levels of haemocytes in the haemolymph, which are essential in the encapsulation response (e.g. Cotter, Kruuk & Wilson 2004), could help determine whether the increased encapsulation response in the presence of *G. blattarum* is facilitated by a higher abundance of immune cells. This process may also be improved by fluorescent labelling of immune cells to visualise the phagocytic response (Mortensen & Glette 1996). Future work will also explore differential immune responses in different host tissue. This could enable trade-offs to be determined between immune responses used at the site of *G. blattarum* infection (mid-gut) and infection sites required to mount a defence to nematodes (cuticle and haemolymph).

Co-infections are extremely widespread and varied, and hosts are likely to be exposed to not only one or two parasites but potentially hundreds of parasites during their lifespan (Petney & Andrews 1998). Indeed, as parasite heterogeneity in the host increases, so are the constraints on host resources and host life history increased (Lello *et al.* 2005). Therefore, just as the host-parasite system presented here has been used to demonstrate clear effects of endemic infection on the host fitness and interaction with other parasites, it would also provide a platform for work with other parasites. For example, the pinworm, *Blatticola blattae* (see Muller-Graf *et al.* 2001)

has been shown to alter the life history of the German cockroach and could therefore be used with the gregarines as a gastrointestinal co-infection model.

6.4 Conclusions

This study has revealed the important consequences that endemic infections can have, creating resource constraints in the host which in turn alter host life history traits and host population dynamics. Resource reductions and immune function variations caused by endemic infection also impact on other invading parasite species, reducing the fecundity and hence transmission potential of the second parasite infection. The development of the German cockroach infection model has therefore provided a novel approach to exploring the effects of endemic infections on host biology and interactions with acute parasite infections. There is considerable scope for further work in this study system particularly regarding co-infection and host immunity. Accordingly, this new model will provide a platform for future host-parasite investigations.

6.5 References

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7. Appendices

7.1 Jolly's Stochastic Method

Jolly's stochastic method (Jolly 1965), like many other mark-capture-recapture methods, is a mathematical framework with which to estimate animal abundance (Begon 1979). A sub-sample of the population is first obtained, whereupon the individuals are counted and marked, and then released back into the population. When the population is resampled, the number of marks that are recorded from the previous time-point can be used to estimate the total population size, using a series of mathematical formulae. Jolly's method is based on three general assumptions: (i) there are several marking occasions, (ii) several recaptures, (iii) only the most recent marks are noted (all previous marks are ignored). The population size, N, on a given day i, is then estimated from the following equation:

$$N_i = \frac{M_i(n_i + 1)}{(m_i + 1)}$$

Where M_i is the number of marked individuals at risk of being recaptured on the ith occasion, n_i is the number of individuals captured and m_i is the total number of individuals that had a mark on the ith occasion. M_i is obtained from the following equation:

$$M_i = m_i + \frac{z_i r_i}{y_i}$$

Where z_i is the number of individuals that were marked before day i, but not caught on day i, and then caught after day i. Likewise, y_i is the number of individuals that were released at point i that were subsequently caught again. Therefore, the value of N will undergo a slight adjustment with every additional time point added to the series. r_i refers the number of individuals released at timepoint i.
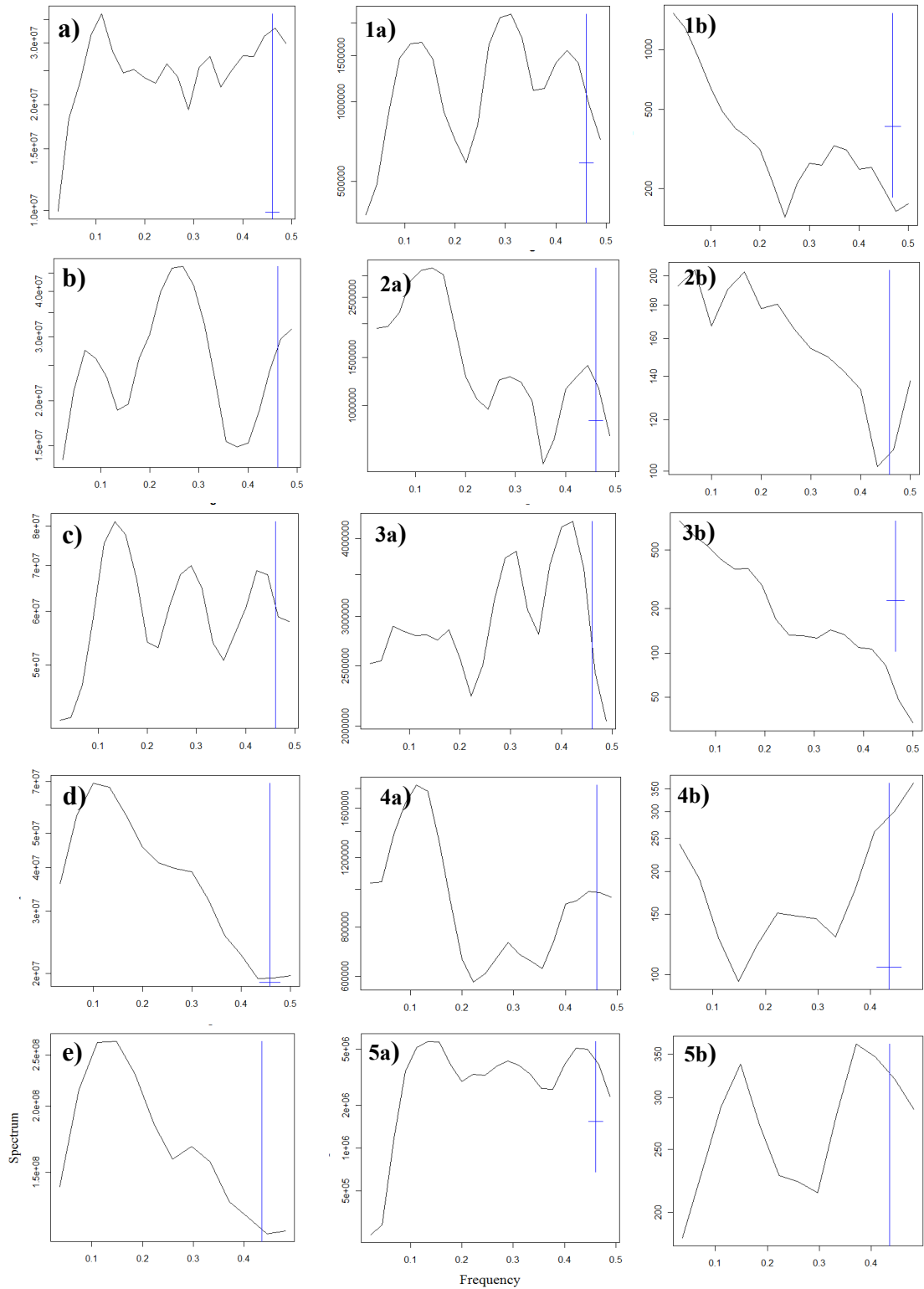
Appendix 7.2: Spectral analysis of an uninfected German cockroach population (a-e), *Gregarina blattarum* infected host populations (1-5a) and *Gregarina blattarum* infection intensity dynamics (1-5b).
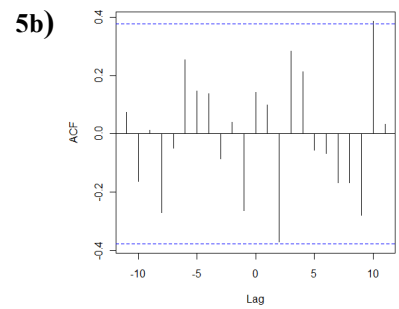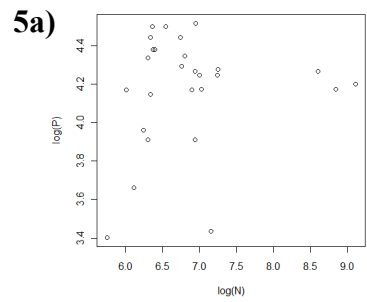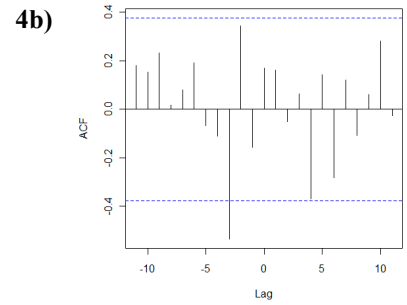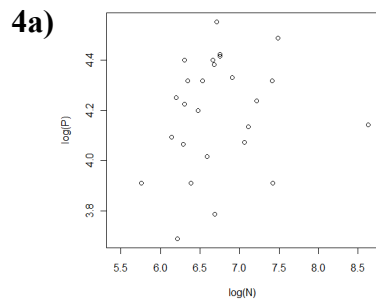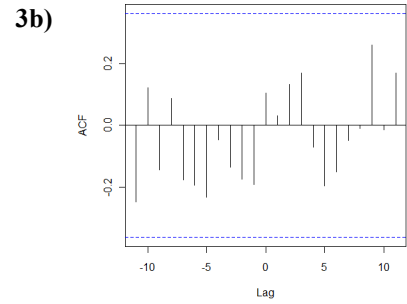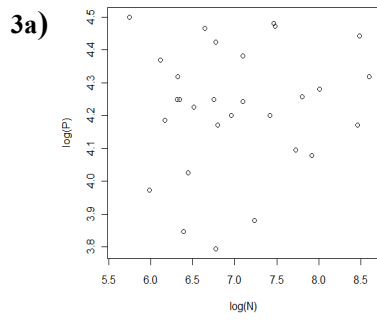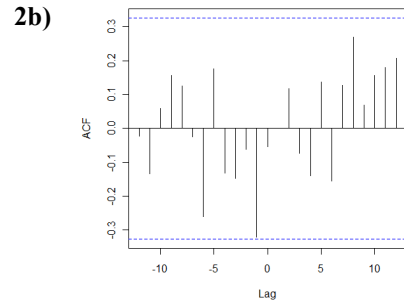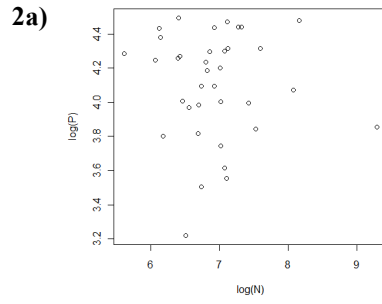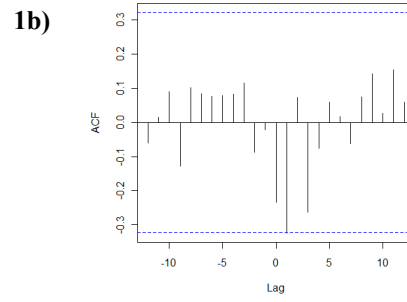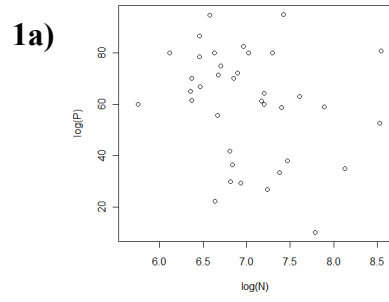
Appendix 7.3: Correlation of log host density (N) and log parasite prevalence (P) (1-5a) and cross-correlation of log host density with log parasite prevalence (1-5b).
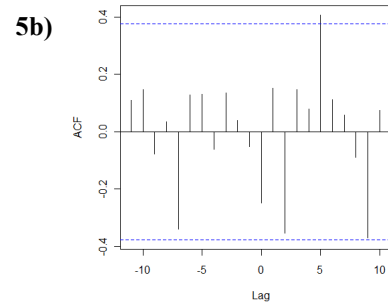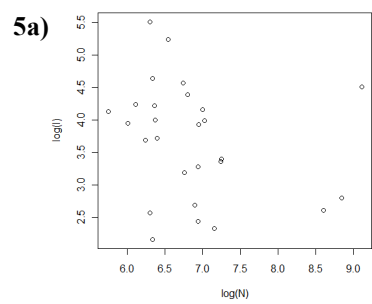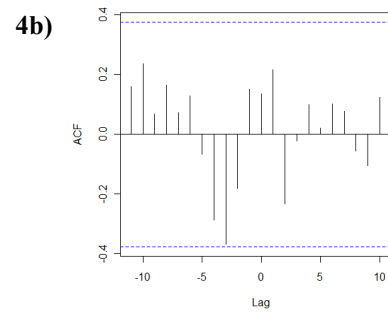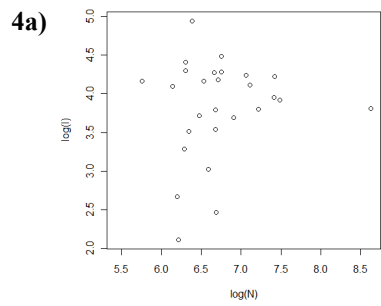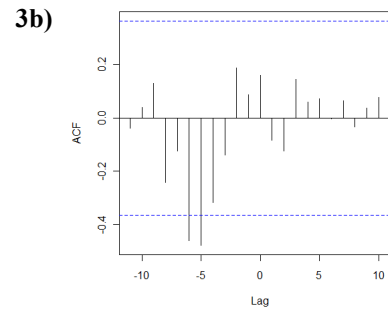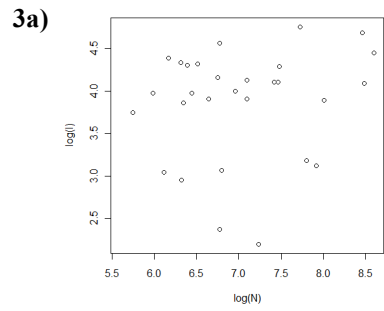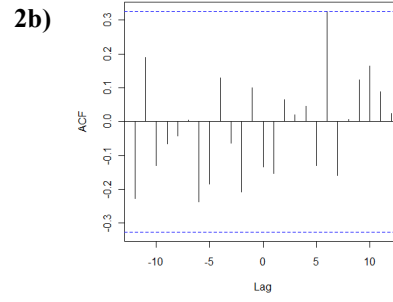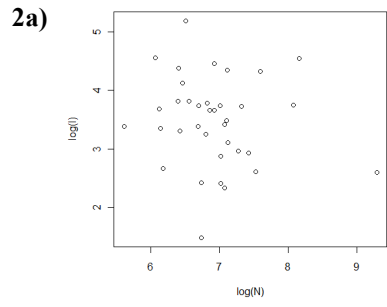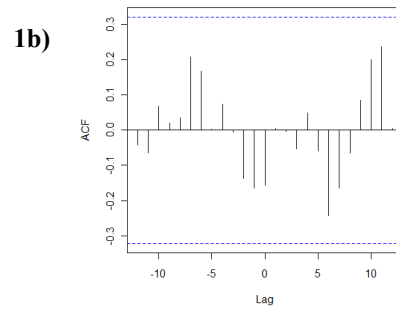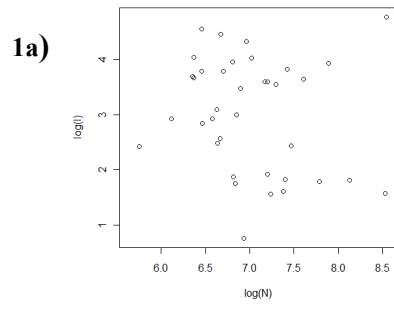
Appendix 7.4: Correlation of log host density (N) and log parasite intensity (I) (1-5a) and cross-correlation of log host density with log parasite intensity (1-5b).
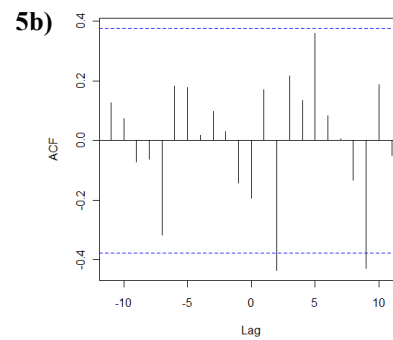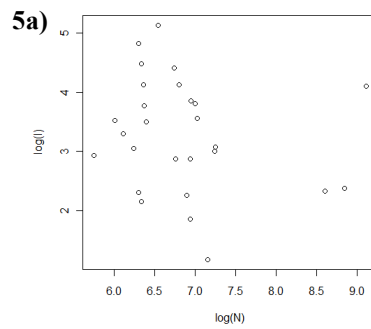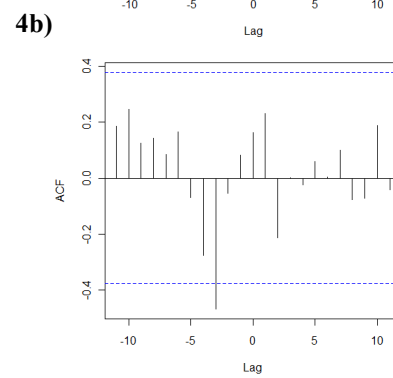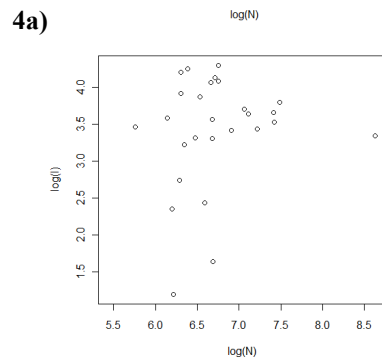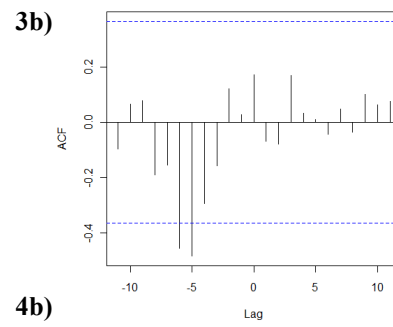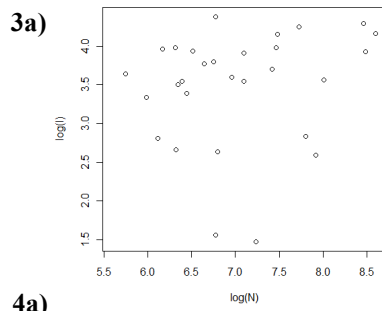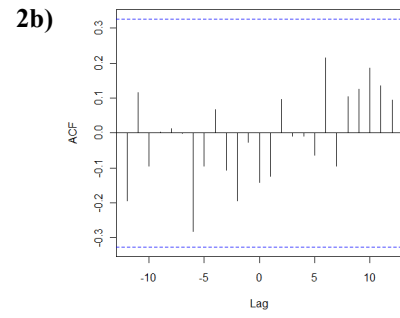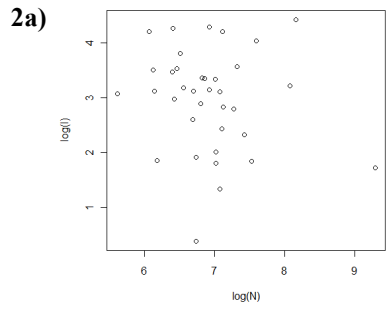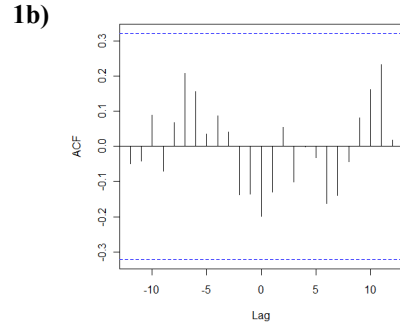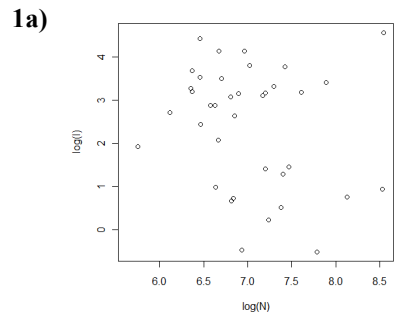
Appendix 7.5: Correlation of log host density (N) and log parasite abundance (A) (1-5a) and cross-correlation of log host density with log parasite abundance (1-5b).
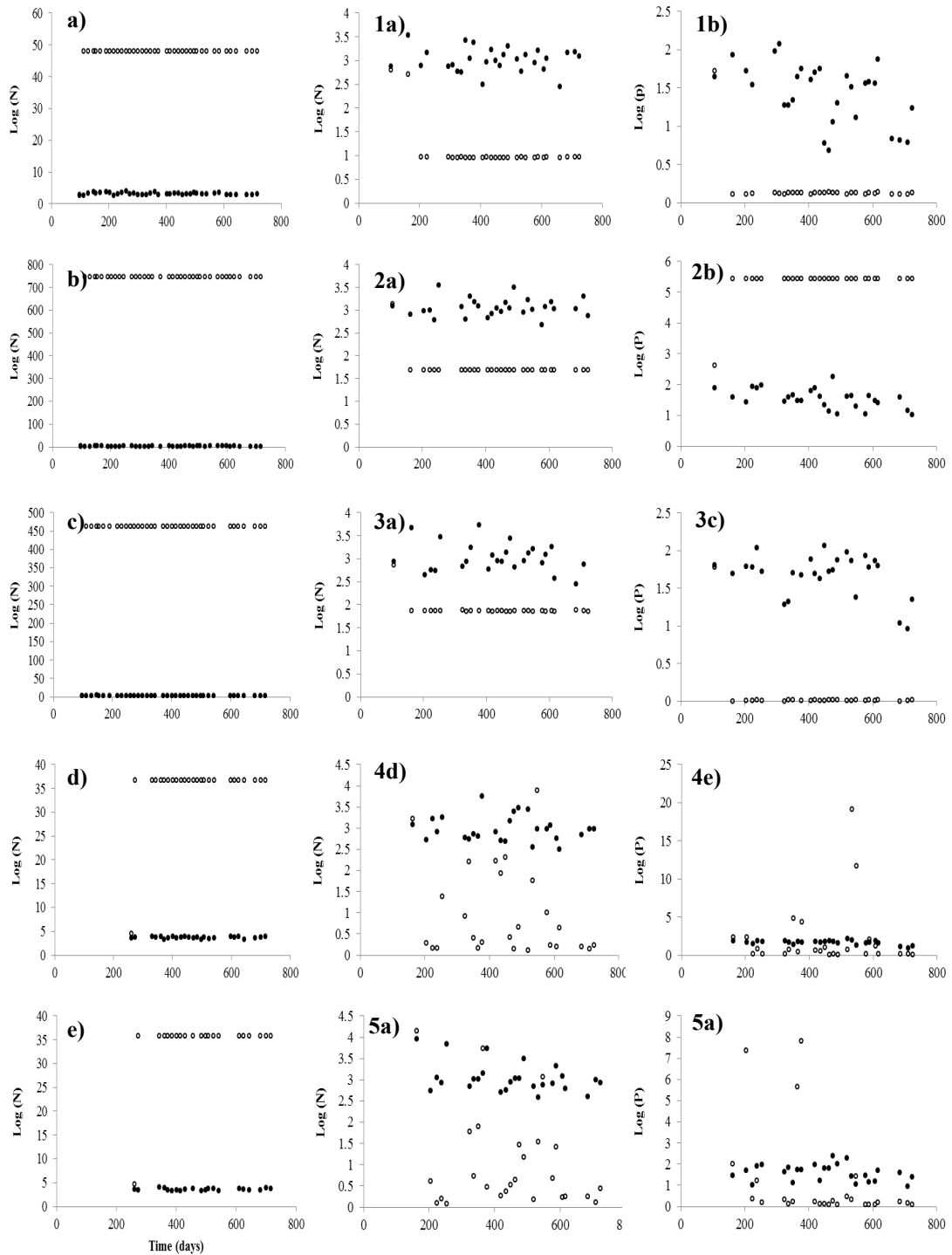
Appendix 7.6: One-step-ahead predictions for log host numbers (N) in uninfected population (a-e) and infected populations (1-5a), and parasite (P) intensity (1-5b). Black dots are observed data and empty dots are model predictions.
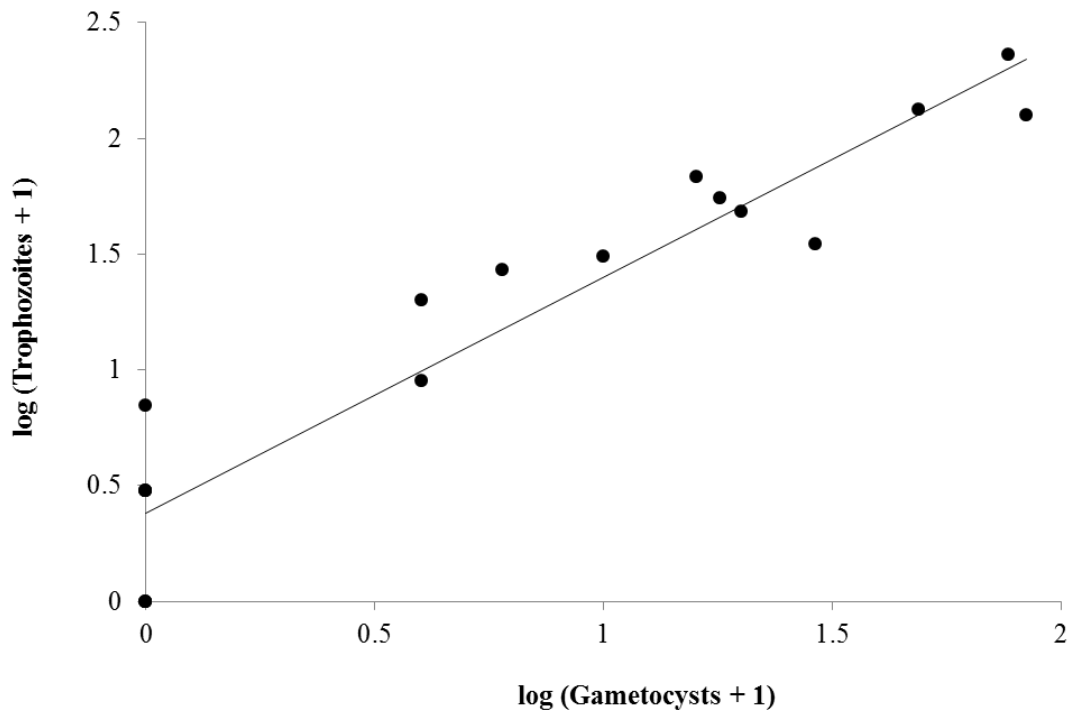
Appendix 7.7: Positive correlation (R^2 = 0.89, t_{16} = 11.68, p < 0.001) between log parasite levels in host frasse (gametocysts collected after 24 hour host incubation) and log parasite counts from host mid-gut (trophozoites dissected from host gut).