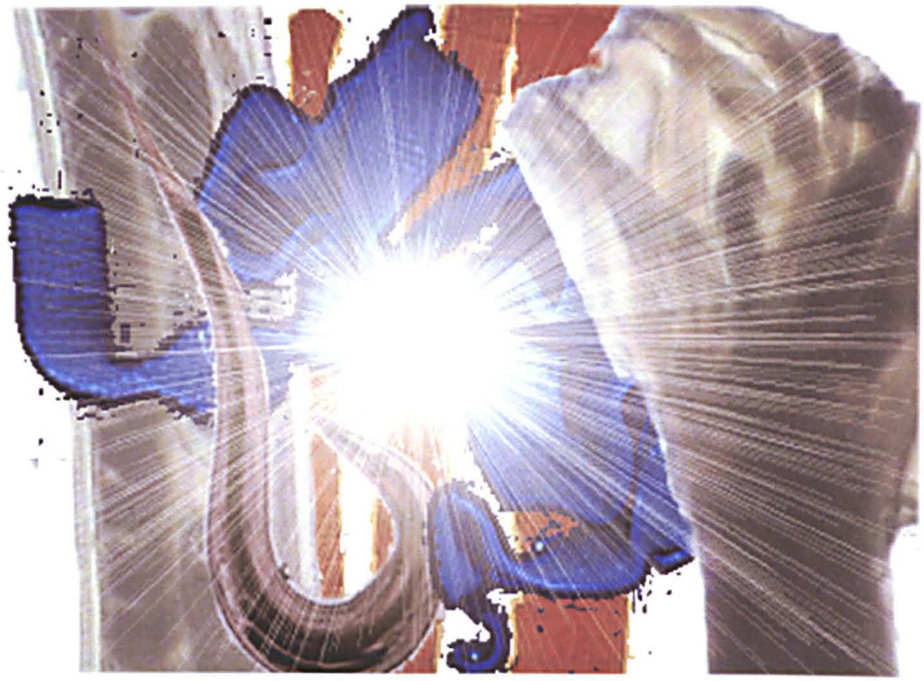
# The community ecology of rabbit (*Oryctolagus cuniculus*) parasites



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# Abstract

This thesis investigates aspects of the community ecology of rabbit parasites with particular emphasis upon the gut helminths, utilising a 23 (later extended to 26) year time series of rabbits and their parasites. A clearer understanding of parasite communities can lead to more effective biological control strategies. Rabbits are regarded as a serious pest species throughout Europe and the Antipodes and the use of the myxomatosis virus, as a biological control agent, has already been tried and failed. However, a clearer picture of the parasite community may offer future possibilities for control. Additionally, the rabbit is a good model for other grazing species, as it carries a similar gut helminth community. Drug resistance is an increasing problem in a wide range of parasites. A clearer appreciation of parasite communities could also aid in the search for effective and environmentally sound pathogen control strategies (e.g. via cross immunity or competition with benign species).

Theoretical models have revealed the importance of aggregation to the stability of the host-parasite relationship, to parasite evolution and to interspecific parasite interactions. A number of models have considered the effect of varying aggregation upon these dynamics with differing outcomes to those where aggregation was a fixed parameter. Here the stability of the distribution for each of the rabbit helminths was examined using Taylor's power law. The analyses revealed that aggregation was not a stable parameter but varied with month, year, host sex, host age, and host myxomatosis status.

Evidence for the existence of interspecific parasite interactions in natural systems has been

equivocal. Factors influencing parasite intensity were evaluated for the gut helminth. A network of potential interactions between the parasites was revealed. Only month was shown to be of greater influence on the community.

Following, from the above analyses, a community model was constructed which incorporated both seasonal forcing and interspecific parasite interactions, with interaction mediated via host immunity. One unexpected emergent property was an interaction between the seasonality and the immune decay rate with slower immune decay resulting in a shift of the immune response out of phase with the species against which it was produced. The model was also used to assess the potential effects of two control strategies, an anticestodal and a single species vaccine. The vaccine had greater effects on the whole community than the anticestodal because of the immune- mediated interactions.

The host is also an integral part of the community as the parasite dynamics are linked with that of their host. Therefore an assessment of the parasites' impact upon host condition and fecundity was also undertaken. This revealed a variety of positive and negative associations between the parasites and their host, with potential implications for future host control strategies.

This study has shown that ignoring parasite-parasite or parasite-host interactions and interactions of both the host and the parasite with the external environment, could result in a poor description of the community dynamics. Such complexities need to be considered and incorporated into theory if future control strategies for either host or parasites are to be effective.

## Acknowledgements

Well this thesis has been a real roller coaster ride and I'd like to thank all those who helped to make that ride less bumpy. First, of course I must thank Peter Hudson, primarily for giving me the opportunity to come to Stirling and take on this study, but also for giving me the space to experiment and take the project off into the areas in which I was most interested. Thanks must also go to Dan Tompkins, for getting me that all-important interview and for his help through the steep learning curve of my first year. Also of course to Brian Boag, without his data I wouldn't have a PhD, but also his constant enthusiasm and encouragement made working with him a great pleasure.

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If you are reading this in hard copy, then I guess it means I passed, so thanks also to my two examiners, Mark Viney and Mike Boots, in particular for hurrying along the viva.

I'd like to dedicate this thesis to my mum and dad, for supporting me so much and believing I'd get there one day.

This thesis was funded by a NERC studentship.

## Statement of Originality

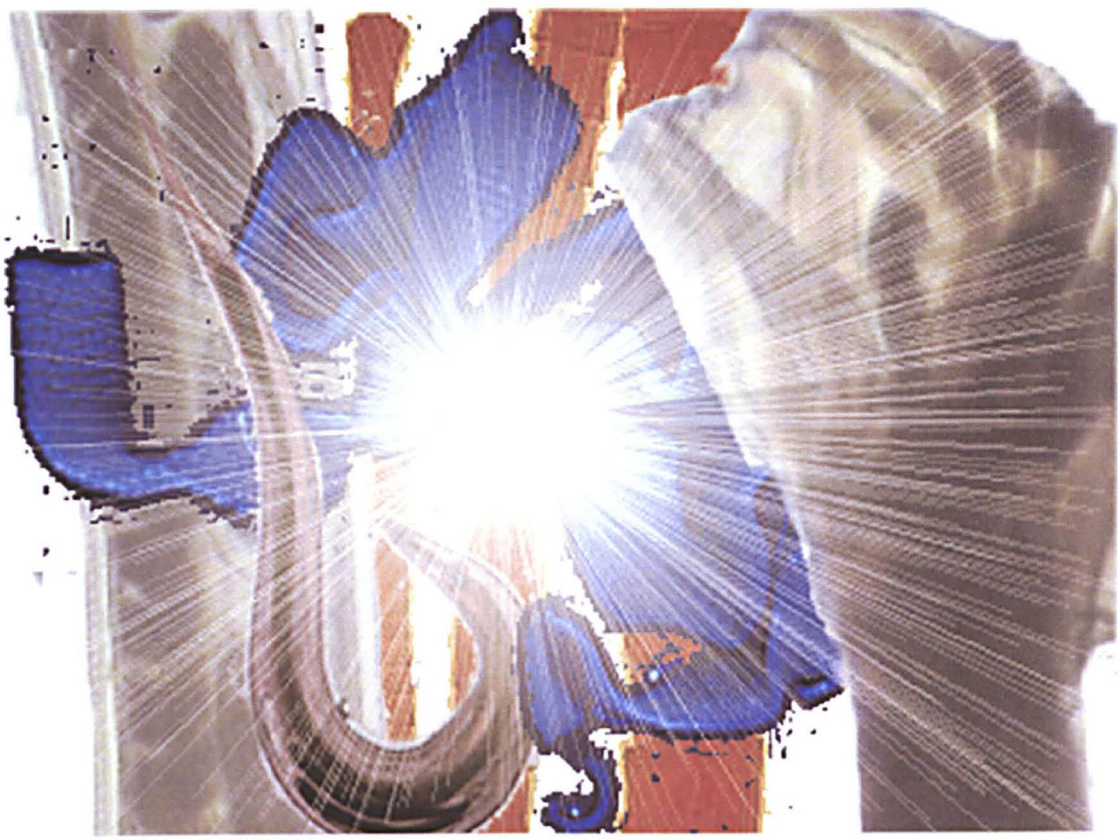
The work described in this thesis is original research carried out by myself and has not been submitted for consideration previously for a higher degree at this or any other university. Any references henceforth used have been appropriately acknowledged.

Joanne Lello

May 2003

# Contents

# GENERAL INTRODUCTION



# General Introduction

Over the past 30 years, the majority of research on the community ecology of parasites has tended to focus on the presence and relative abundance of their specific constituents (Bush & Holmes, 1986; Fiorillo & Font, 1996; Dove, 1999; Forbes, Alisauskas, McLaughlin, *et al.*, 1999; Alves & Luque, 2001; Behnke, Barnard, Bajer, *et al.*, 2001; Labriola & Suriano, 2001; Poulin & Valtonen, 2002). In contrast, relatively little attention has been focussed on the temporal dynamics of parasite communities (Holmes & Bartoli, 1993; Dobson & Roberts, 1994; Sousa, 1994; Gerard, 2001). Any community is made up of individuals from a range of species and the interactions between these species coupled with their interactions with their environment can result in the community exhibiting emergent properties, which are more than simply the sum of the parts of the individual species dynamics. As with free-living species, parasites live within a community and as such are likely to interact, either directly or indirectly. Determining the root of the emergent properties of parasite communities can provide insights into parasite dynamics, which will allow us to predict changes in the community and allow us to identify the means of controlling some species.

The wild rabbit (*Oryctolagus cuniculus*) is ubiquitous throughout the majority of Europe and the Antipodes. It is clearly a successful species but this success often brings it into conflict with humans as its propensity for crop consumption and its competition with livestock for forage, have led to its classification as a pest species. However, the rabbit has also been useful to humans and is commonly used as a laboratory model, a popular pet and, is still farmed for its meat and pelt (Cowan, Tapper & Hewson, 1991).

The rabbit is a grazer that behaves much like domestic grazing animals and in this respect is a good model herbivore that can be used to understand the parasitism of species of economic importance, such as the sheep and goat (Boag, 1999). It plays host to a diverse parasite community, particularly the gut helminths of which there are commonly five helminths found in UK rabbits (Boag, 1985, 1987a, 1993; Kinney, 1997; Allan, Craig, Sherington, *et al.*, 1999). Additionally there are a large number of common coccidian parasites of rabbits, *Eimeria* spp., most of which inhabit the rabbit gut (Bull, 1958; Stodart, 1968; Patterson, 1987; Hobbs & Twigg, 1998). However, only one coccidian, *Eimeria stiedae*, infects the rabbit liver and only this species is considered in this study (Dürr, 1972; Barriga, 1979). While rabbits may become infected by a number of notable viral diseases, myxomatosis is the most prevalent species and has clearly had a major impact on the rabbit populations throughout Britain (Thompson, 1955; Fenner & Ratcliffe, 1965; Ross, 1972; Flowerdew, Trout & Ross, 1992; Fenner, & Ross, 1994). At the current time, examination of samples, from our study population, have not found positive sera to the virus that causes rabbit haemorrhagic disease (RHD), which is the other important virus of UK rabbits (N. Forrester, pers. comm).

**The Gut Helminths**

The five gut helminth of the rabbit include two strongylid nematodes, *Graphidium strigosum* and *Trichostrongylus retortaeformis*; one oxyurid nematode, *Passalurus ambiguus*; and two cestodes *Mosgovoyia pectinata* (previously known as *Cittotaenia pectinata*) and *Cittotaenia denticulata*. These parasites are common throughout Europe (Mead-Briggs & Page, 1975; Rhodes, 1985; Boag, 1987a, 1993; Blasco, Torres, Feliu, *et al.*, 1996; Allan, Craig, Sherington, *et al.*, 1999; Haz, Alvarez, Freire, *et al.*, 2001) and the nematodes are also common in New Zealand and Australia, however the cestodes are

absent from the Antipodes (Bull, 1964; Dunsmore, 1980). The positions of these parasites relative to one another in the rabbit gut are presented in figure 1.

***The Strongylid Life Cycle***

The two strongylids have a simple direct life cycle, which includes free-living larval stages (figure 2). Parasite eggs pass in host faeces, the eggs hatch and the first stage larvae ($L_1$) develop within the faeces and feed on bacteria. They double in size before moulting to second stage larvae ($L_2$), which also feed on bacteria in the faeces (Anderson, 2000). The $L_2$s develop further before they undergo a final preparasitic moult to infective third stage larvae ($L_3$), maintaining the cuticle of $L_2$ as a protective sheath. The sheath helps prevent desiccation but also prevents the larvae from feeding and thus they are dependent on the internal energy reserves built up during the earlier stages of development (Anderson, 2000). Third stage larvae migrate from the faeces onto the vegetation and probably use capillary action to move to the tips of grass species and from there infect the rabbit (Crofton, 1954). If ingested by a host the sheath is lost in the host stomach and a moult to fourth stage larvae ($L_4$) occurs. $L_4$s migrate to the mucosa (in the stomach for *G. strigosum* and the small intestine for *T. retortaeformis*) where they may enter a state of hypobiosis (arrested development) within the mucosa, or undergo a final moult to fifth stage larvae ($L_5$). Stage five larvae leave the mucosa, and continue their development to adulthood. Adult parasites then mate and the females produce eggs (Anderson, 2000).

The period of development from egg to infective $L_3$s varies both between species and according to environmental conditions. Both temperature and humidity are important in larval development. Previous work has shown that *G. strigosum* eggs take between 8 and

10 hours to hatch at 20°C and a further 2 to 3 days to develop to infective larvae (Anderson, 2000).

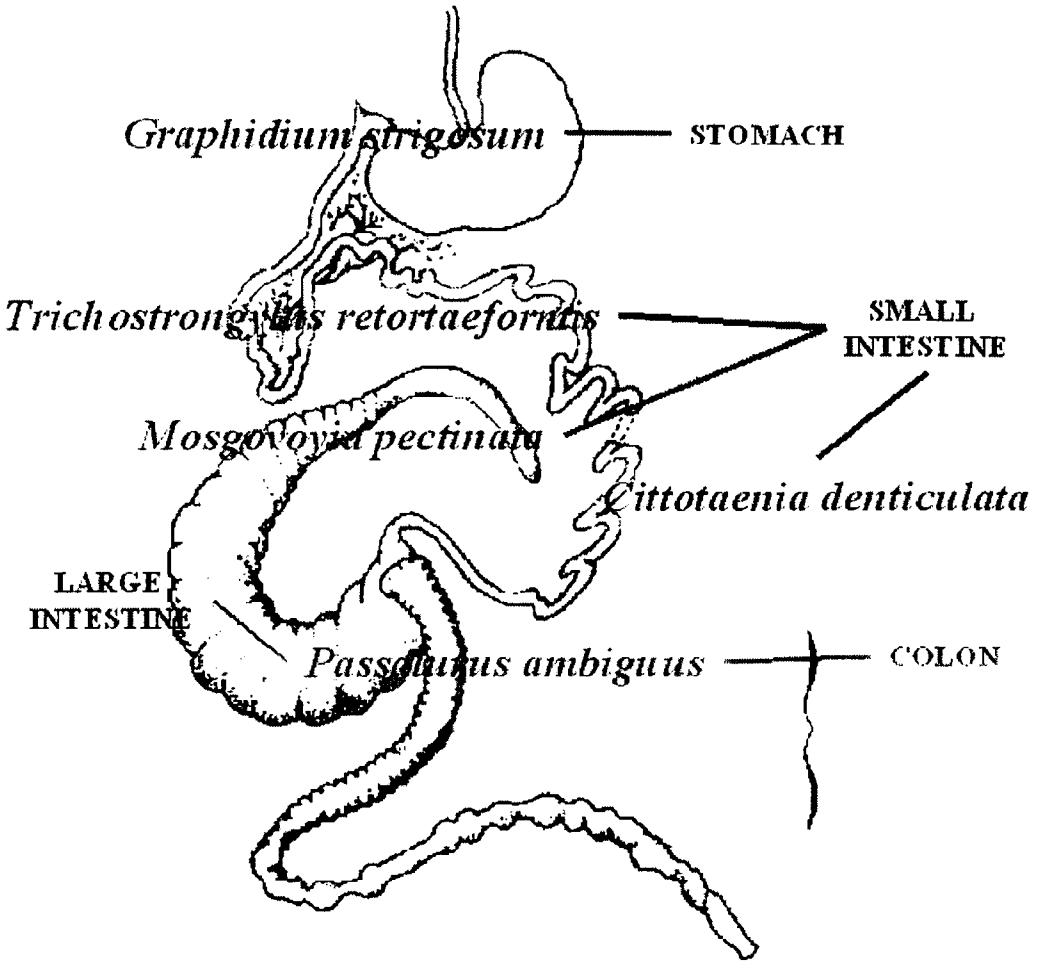



Figure 1: Representation of the positions of the helminths in the gut of the rabbit.

Dry conditions tend to damage the eggs and, even when larvae develop to $L_3$s under these circumstances, they are unlikely to establish within the host (Bull, 1964). *Trichostrongylus retortaeformis* took 19 hours to develop at 30°C and a further 3 days to reach the $L_3$ stage. However, at 5°C the eggs took 12 days to hatch and the development to $L_3$ took up to 73 days. Prior to development of the $L_1$s, the eggs of *T. retortaeformis* were killed by drying

but after this stage the eggs were found to be highly resistant to desiccation (Bull, 1964). Similarly $L_1$ and $L_2$ stages are killed by desiccation, but $L_3$s are resistant. Saunders, Tompkins & Hudson (2002) showed that variations in temperature were important in speeding the development of the larvae of the strongylid *Trichostrongylus tenuis* and this phenomenon is known to occur across strongylid species (Anderson, 2000). Therefore laboratory investigations of development times for the rabbit strongylids can only give us a broad estimate of development under natural conditions. Once within the host, the prepatent period, assuming that the parasites do not enter hypobiosis, for *G. strigosum* is 39 to 41 days (Dunn, 1978), compared with just 9 to 10 days for *T. retortaeformis* (Barker & Ford, 1975). Following maturation and mating, egg production by *G. strigosum* tends to increase for a few weeks and then declines (Bull, 1964), it is probable that this pattern is similar in *T. retortaeformis*. It is unclear whether this senescence is due to the helminth age or to the action of the host immune response. Viney (2002) found that the effect of the rat immune system on the rhabditid *S. ratti* resulted in oral plugs, which caused starvation and eventual reduction in worm fecundity.

***The Oxyurid Life Cycle***

The oxyurid *Passalurus ambiguus* also has a simple direct life cycle. However, there are no free-living larval stages as the entire development of the larvae from $L_1$s to $L_3$s occurs within the eggs of the parasite. According to most researchers, the gravid adult females make their way down to the anus of the rabbit where after extruding their anterior end they lay thousands of sticky eggs on the perineum (Anderson, 2000). Although some of these eggs may drop to the ground or become attached to the exterior of faecal pellets, the major route of infection in this species is likely to be via coprophagy and grooming. Following ingestion of the eggs the $L_3$s hatch in the small intestine, exsheath and make their way to

the mucosa where they develop and moult to fourth stage larvae (Anderson, 2000). The $L_4$'s are believed to feed by pinching off pieces of the mucosal epithelium. After further development they moult to the $L_5$ stage, which are only thought to scavenge on the gut contents (Skrjabin, Shikhobalova & Lagodovskaya, 1969; Dunn, 1978). The $L_5$s then develop through to adulthood and mate. Development through to $L_3$ takes 7 to 8 days at 35°–38°C (Anderson, 2000) and the prepatent period for this species is 8 to 9 weeks (Bull, 1964).

***The Cestode Life Cycle***

Details of the life histories of *M. pectinata* and *C. denticulata* are less well documented. In general these Anoplacephaloidae cestodes have an indirect life cycle requiring an intermediate host and this has made accurate recording of their life-cycles more difficult (Stunkard, 1941). Cestodes are hermaphroditic; their bodies are segmented into reproductive proglottids that are shed by the cestodes into the faecal material. The eggs develop within the faeces to the oncosphere stage (Bush, Fernandez, Esch, *et al.*, 2001).

According to Stunkard (1941), the orbatid soil mite is the intermediate host for the rabbit cestodes. The mites encounter the cestode eggs whilst foraging in the soil, they then break the shell and suck out the oncosphere. The oncosphere has pairs of hooks, which it uses to dig through the mite's gut wall. Once through the wall it develops into a thick walled cysticercoid. Mites are ingested incidentally with the vegetation as the rabbits forage and the cysticercoid hatches out in the intestine where it attaches to the gut wall with suckers and develops through to adulthood. Egg development through to the oncosphere stage probably occurs in passage through the rabbit gut and so the eggs are infective as soon as they are passed from the rabbit.

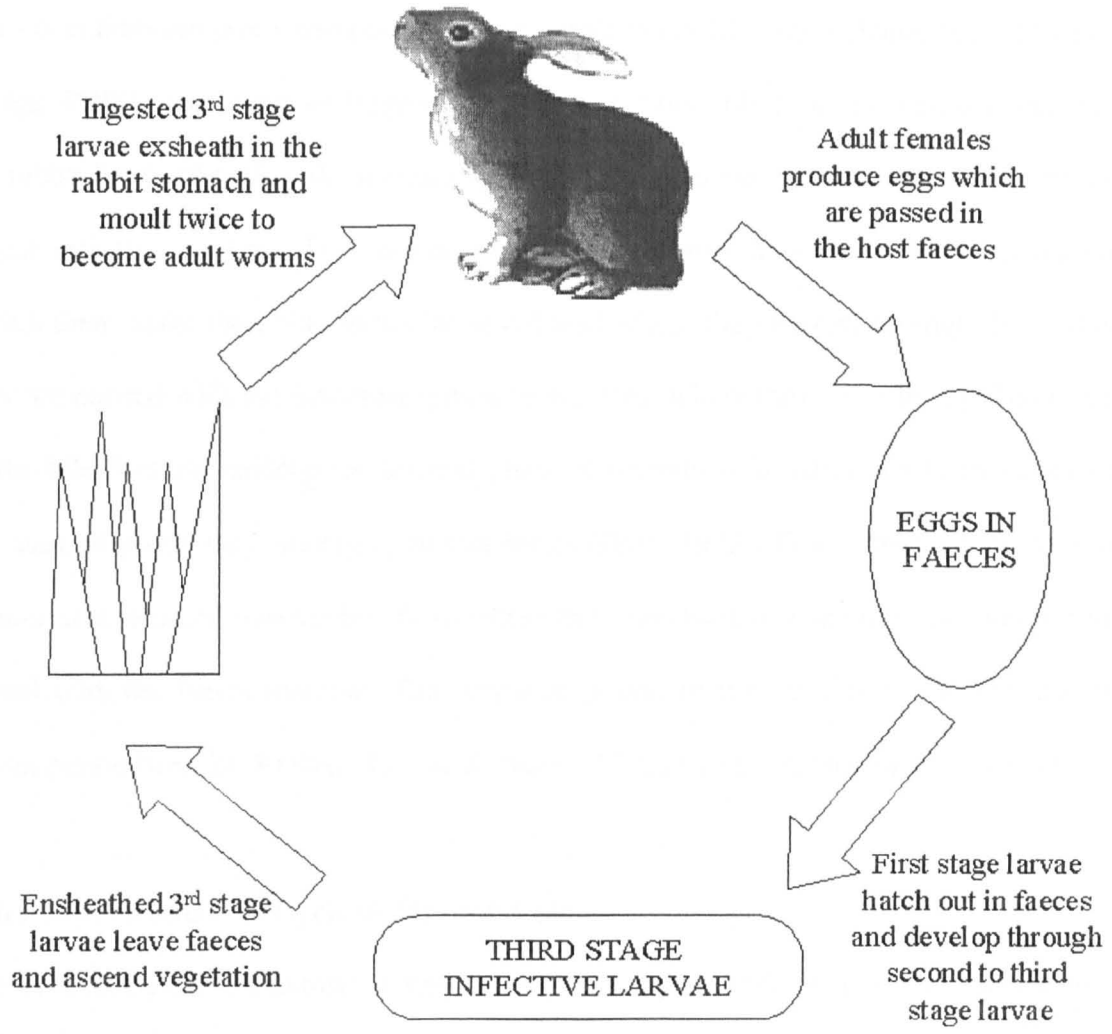



Figure 2: The life cycles of *Graphidium strigosum* and *Trichostrongylus retortaeformis*

The development time from oncosphere ingestion to development of an infective cysticercoid has only been considered by Stunkard (1941) and, because of the experimental protocol used, only an estimate of development time was possible. However, this estimate suggested a slow developmental period of about 3 to 4 months. The prepatent period for these species is estimated to be about 11 weeks.

## The Life Cycle of *Eimeria stiedae*

The coccidians are protozoan parasites with simple direct life cycles (Dürr, 1972; Hobbs & Twigg, 1998). All species undergo similar life stages but only *Eimeria stiedae* develops in the rabbit liver rather than the intestine (figure 3). Rabbits may become infected when they ingest infective oocysts. The oocysts excyst in the small intestine and the sporozoites within them make their way to the intestinal wall where they burrow through. From there they are carried with the intestinal lymph to the liver, where they enter the epithelial cells of the bile duct and undergo an asexual phase of reproduction. After one to two cycles of the asexual phase they undergo gametogenesis (Dürr, 1972). From this, new oocysts are formed and released into the bile from where they pass back into the intestine and become mixed with the faecal material. The prepatent period in this species is 14 days and the patent period from 21-30 days (Levine & Ivens, 1972; Darwish & Golemanski, 1991).

## Brief History and Life Cycle of Myxomatosis

The viral disease myxomatosis is a pox virus which was introduced to the European rabbit by cross infection from cottontail rabbits (*Sylvilagus brasiliensis*) in South America. Although the virus only causes a benign fibroma at the site of infection in the cottontail, its effects upon the European rabbit where originally devastating. In 1950 it was deliberately introduced, as a biological control agent, into rabbits in Australia devastating the infected population and reducing it by as much as 99%. In 1952 the virus was introduced to France and by 1953 to the UK with similar results (Cowan, Tapper & Hewson, 1991; Fenner & Fantini, 1999a, 1999b). However, the rabbit populations recovered and a combination of the evolution of attenuated viral strains and genetic resistance among the rabbit populations has reduced the impact of the virus in all areas of introduction. Nevertheless, the virus is

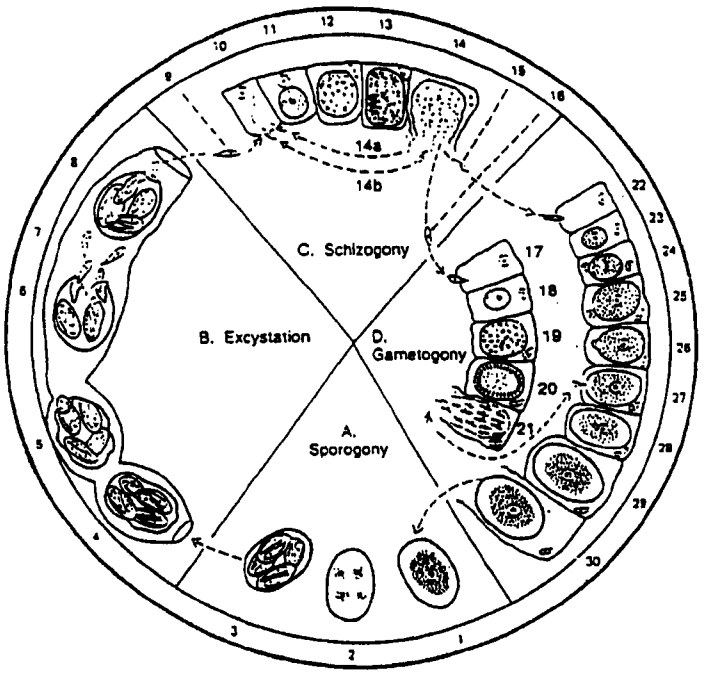



Figure 3. **The life cycle of *Eimeria stiedae*. A. Sporogony: development of oocysts in litter or soil with formation of sporozoites.** (1 - 3) oocysts develop and become infective - (contain 4 sporocysts each with 2 sporozoites). **B. Excystation of sporozoites in intestine.** (4 - 8) oocysts in intestine rupture, releasing sporozoites. **C. Schizogony-asexual reproduction with formation of merozoites in cells of the bile duct**; (9) sporozoite freed in intestine penetrates intestinal mucosa and passes in lymph to the liver; (10) sporozoite enter epithelial cells of the bile duct; (11) growth to form trophozoite; (12) nucleus undergoes multiple division to form schizont; (13) each nucleus forms a merozoite; (14) schizont and parasitized cell rupture releasing numerous merozoites; (14a) merozoites enter new epithelial cells to produce a second generation of schizonts (10-14); or become (15) third generation merozoite destined to produce female stage or (16) third generation merozoite destined to become male stage. **D. Gametogony-sexual reproduction with formation of gametes in epithelial cells of the bile duct**. (17- 21) male cycle: (17) merozoite enters epithelial cell. (18) growth and formation of microgametocyte. (19) multiple division of nucleus; (20) formation and alignment of microgametes (sperm) on periphery of cell; (21) rupture of cell and liberation of mature, microgametes; (22-30) female cyst: merozoite enters cell; (23) formation of macrogametocyte; (24) meiotic division of nucleus; (25) to (26) mature haploid macrogametes develop; (27) fertilization of macrogamete; (28) fusion of male and female nuclei to form zygote; (29 - 30) oocysts develop and mature and break out of epithelial cell then pass with bile into the lumen of the intestine and are voided with faeces (1). (Reproduced from http://www.cvm.uiuc.edu/courses/vp333/lab_notes/Laboratory%202.pdf - text edited).

still responsible for a considerable degree of mortality and morbidity, particularly amongst young rabbits, and localised epidemics still occur from time to time (Barnes & Tapper, 1986; Boag, 1988; Fenner & Ross, 1994; Fenner & Fantini, 1999b).

Despite the body of work that has been carried out on this species, the proximate cause of rabbit death is not known. The virus is transferred on the mouthparts of vectors and in the UK the usual vector is the rabbit flea (*Spilopsyllus cuniculi*). The virus initially causes a swelling at the site of infection but within 4 days the virus is found within all the rabbit body tissues. Within the following few days the characteristic pus filled lesions and swellings may be seen around the eyes, nose and mouth and on the genitalia. In highly susceptible hosts death generally occurs within 14 days (Fenner & Ross, 1994; Fenner & Fantini, 1999b).

## The Rabbit

Numerous texts provide detailed account of the history, biology, demography and behaviour of the European rabbit (Thompson & Worden, 1956; Martin, 1977; Mead-Briggs, 1977; Cowan, 1983; Gibb, White & Ward, 1985; Wheeler & King, 1985; Boag, 1987b; Cowan, 1987; McBride, 1988; Leach, 1989; Thompson & King, 1994; Fenner & Fantini, 1999a). This information will not be reiterated here, except where it is of potential relevance to the rabbits' parasite community or to the effect of that community upon their host.

### *Habitat*

Rabbits are extremely adaptable to different habitat types but prefer areas with slopes and/or tree cover (Cowan, Tapper, & Hewson, 1991). In both cases the preference arises

from the lands suitability for burrow production coupled with access to suitable food. Both slopes and woodland allow for drier conditions within the burrows and trees proffer the additional benefit of structural support, since their roots may lend strength to the walls and ceilings. Rabbits are adaptable in their choice of forage. They will take a wide variety of high-quality plant species, including arable crops, whenever they are available, but can survive, albeit at lower densities, in habitats as sparse as sand dunes or heather moorlands (Cowan, Tapper & Hewson, 1991; Thompson & King, 1994). These variations in habitat use have the potential to greatly impact upon the parasite communities of the rabbit. For example, rabbits choosing drier or more exposed habitats are likely to have different parasite fauna or different prevalences and intensities of parasite species than those choosing more sheltered and moist habitats.

Habitat is also important because a rabbit's choice of forage may affect aspects of the parasite life cycle and the resources the rabbits have to resist infection. Forage type may influence the gut parasites directly changing the chemical and nutritional environment within the gut. The forage may also have indirect effects upon all parasites by changing the level of host health and thereby its immune status.

***Rabbit Behaviour***

There are two aspects of rabbit behaviour, which are particularly pertinent to their parasites' population dynamics. Firstly, rabbits are social animals preferring, where forage and habitat permit, to live in social, matriarchal groups (Fenner & Fantini, 1999a). These groups live in warrens, which are a collection of interconnected burrows and rate of contact between rabbits will be dependent upon their warren structure, their burrow and their social status. Clearly this social system will have important implications for those

parasites, which are transmitted directly by social contact such as *P. ambiguus*. Myxomatosis will also be affected, though to a lesser extent by social contact because, whilst it is spread mainly by the rabbit flea (*Spilopsyllus cuniculi*), the spread of the flea is likely to be increased by increased social contact.

Also of relevance to the parasites is the rabbits' defecation behaviour. Rabbits produce two forms of faecal pellet. The first are hard faeces and are found in all areas that rabbits frequent. The pellets are small, dark brown, firm, oval pellets and are found both scattered (apparently at random; J. Lello pers obs.) wherever the rabbits forage and in distinct patches (often on bare earth) called latrines (Bang, & Dahlstrom, 2001), which are often found at the edges of warren territory and act as markers of territorial boundaries. Faeces may also be found in and around burrow entrances and may well act as markers here too. This form of pellet is the source by which the eggs of the intestinal parasites, and the oocysts of coccidial species, are released into the external environment. Whether or not the faeces are deposited in a suitable area for, contact with the host and where applicable for the development of the intermediate stages of the parasite, or for contact with intermediate hosts, has major implications for transmission and infection success of the relevant parasite species.

The second form of pellet are the soft faeces, which are mainly produced at night (Hirakawa, 2001). The rabbit is able to produce pellets from the partially digested contents of the caecum. This re-ingestion allows more nutrients to be gained than would be possible from single passage through the gut. These pellets are not deposited on the ground but are consumed directly from the animal's own anus or that of their offspring. As the majority of parasites, whose transmission depends on faecal deposition, require a period external to the

host before they are infective, soft pellets are effectively a dead end transmission route, but *P. ambiguus* eggs, which have developed to infective stage, may be transmitted as the rabbits feed on their faecal deposits. Rabbits may also feed soft faeces to their young and *P. ambiguus could* be transmitted to new hosts through this route.

***Life History of Rabbits***

The adult wild rabbit varies in mass from about 1200 to 2000 g with males typically heavier than females. Wild rabbits generally live no longer than 3 years but only about 10% live beyond the first year (Cowan, Tapper & Hewson, 1991). This life history will influence parasite dynamics because the death of a host also means the death of its parasites. Females reach sexual maturity as early as 3.5 months but in general will first mate when 6 to 10 months old; males reach maturity a little later, at about 4 months. The breeding season begins in January and extends into August with the peak being between March and June. A female is capable of having up to 4 litters a year with 3 to 7 kittens per litter (Cowan, Tapper & Hewson, 1991; Thompson & King, 1994; Fenner & Fantini, 1999a). This not only accounts for the rapid growth rate of rabbit populations but also has consequences for their parasites as it ensures a large influx of new susceptible hosts, in bursts of breeding; in effect a pulsed resource for the parasites. Young wean at around 21-25 days (approx. 150–200g) and maternal immunity seems to be, at least, partially protective, whilst the kittens are still taking milk (Cowan, Tapper & Hewson, 1991; Thompson & King, 1994; Fenner & Fantini, 1999a).

***Rabbit Immunology***

Despite the use of the rabbit for production of mono- and polyclonal antibodies and its use in the laboratory as a model for human and veterinary diseases, there are little data

available with respect to the rabbits' immune response to its natural parasites. Myxomatosis is known to suppress the immune system (Bull, 1964; Boag, 1988). Conversely, *T. retortaeformis* is known to stimulate an immune response, the strength of which appears to be dose dependent. The species attaining higher burdens if the parasite is given in trickle infection than when high doses are give, with the latter case resulting in expulsion of all worms shortly after establishment (Michel, 1952a, 1952b, 1952c). Hypobiotic larvae of both *T. retortaeformis* and *G. strigosum* were shown to continue their development following infection of the rabbit with myxomatosis, suggesting that hypobiosis is a method used by the parasites to evade host immune responses (Michel, 1952a, 1952b, 1952c; Bull, 1964; Boag, 1988).

**The Study Area and Data Set**

All the data used for the analyses in this thesis were collected by Dr. Brian Boag, whilst all data analysis was conducted by the thesis author. The data set consisted of a 23 year (later extended to 26 year) time series of rabbits and their parasites collected from a 400 ha site in Perthshire, Scotland (ordnance grid reference NO 280 340) from January 1977 to October 2003. The site varied between 180m-285m above sea level with the majority of the land being intensively farmed arable rotation. Higher areas were classed as heather moorland and there were patches of both deciduous and coniferous woodland at the site (figure 4). Host samples were collected, by shooting, and almost all (293 of the 310 possible) months are represented.

The data included the date the rabbit was shot, the warren number from which the rabbit originated, the vegetation class in which the warren was found and the vegetation on which the rabbits were feeding. These classes were, deciduous woodland, coniferous woodland,

permanent pasture, grass ley, arable land, heather, gorse, mixed (i.e. quarry and silage) and heathland (i.e. a mix of heather and permanent pasture). The direction in which the mouth of the rabbits' home burrow faced was also recorded. The rabbits' sex, and mass were recorded for all rabbits and, for a subset of the data, details of abdominal fat mass, ovary mass and testes mass. If kittens' stomachs were found to contain milk this was noted. Similarly lactation and number of foetuses if pregnant were recorded for female rabbits. Finally, any clinical signs of myxomatosis, spotting on the liver due to *E. stiedae* and full counts for all the gut helminths were recorded.

**Thesis Structure and Content**

The life histories of the rabbit parasites, the host biology and the external environmental conditions may all interact to alter the dynamics of the parasite community and of the host population. The main aims of the thesis were, first to determine the factors influencing the individual dynamics of the gut helminths of rabbit, including potential interspecific parasite interactions and second to consider the impact at the community level of the key influencing factors.


Figure 4. Section of ordnance survey map (grid reference NO 280 340) showing the study area. Double black and grey line denotes boundaries within which rabbits were shot.

*Chapter One*

If a parasite is aggregated within the environment, then it will be likely to display an aggregated distribution within the host. Similarly, differences in host foraging behaviours, and individual susceptibilities will also tend to produce aggregated distributions. Therefore, the distribution of a parasite species within its host is a result both of its distribution within the environment and the behaviour and biology of the host. However, those influences acting upon the parasite distributions may vary, temporally and between sub-groups of hosts, e.g. males and females.

In this chapter we examine the hypothesis that parasite aggregation is a variable rather than fixed characteristic. Most parasite species display aggregated distributions within their host and these are often well described by the negative binomial distribution. However, not all parasite distributions are a good fit to the negative binomial. Furthermore, the negative binomial measure of aggregation $k$ is sensitive to both sample size and sample mean, which makes it unsuitable for comparing distributions of samples with different means or with small sample sizes. An alternative measure is Taylor's power law, which regresses the log mean on the log variance with the gradient of the regression slope being the measure of aggregation. Here we utilised Taylor's law to compare the levels of aggregation of the gut helminths within different years, months, host age classes, host sexes, and in the presence and absence of myxomatosis.

*Chapter 2*

The dynamics of parasite populations are usually considered to be independent of one another and are not treated as a part of an interactive community. Here we utilised the powerful statistical analyses of generalised linear models (GLM) and restricted maximum

likelihood (REML) mixed models to examine the hypothesis that parasite interspecific interactions do occur. We restricted our analyses to adult rabbits and for each parasite considered the possible influences on the intensities only in infected rabbits.

*Chapter 3*

Interactive communities may exhibit emergent properties unpredictable from their component parts. Here we examined the influences of seasonality and interspecific interactions upon the dynamics of the community of the gut helminths. We extended a simple model previously used to predict the course of human filariasis infection. The extension included a seasonal sine wave function and those interactions between the helminths predicted in chapter 3. We assume parasite interaction to be mediated through host immunity. We also considered the effect on the community of perturbation of some of its members by simulated drug intervention and vaccination.

*Chapter 4*

Parasite damage to the host has implications for animal husbandry and for biological control of host populations. Whilst epidemic parasite infections in wild populations have been shown to have serious impacts on wild host populations (e.g. myxomatosis), endemic infections have been largely overlooked until recent years. The gut helminths in particular have often been considered to be relatively benign species. However, helminths of livestock are known to cause massive economic losses in many parts of the world. Here, again we utilise the statistical techniques of GLM and REML, to assess the association between parameters of rabbit health and fecundity and the parasites of the rabbit.

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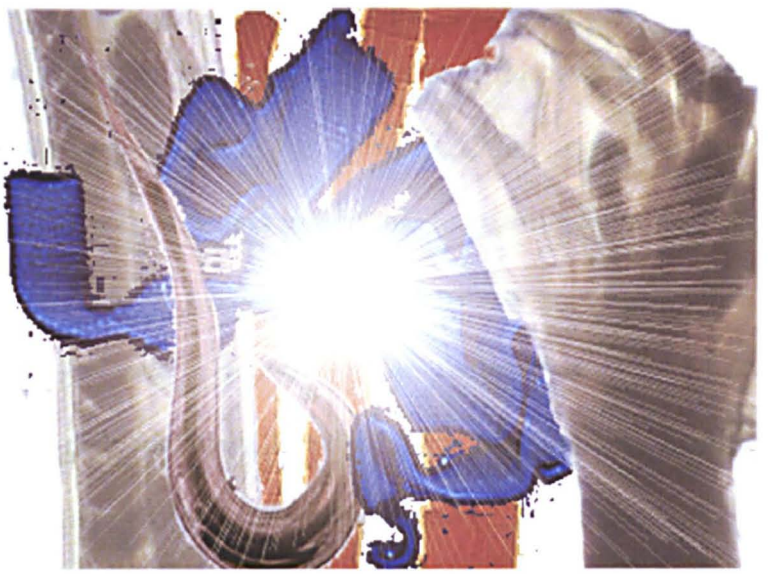
# CHAPTER 1

**Patterns of parasite aggregation** in the wild European rabbit (Oryctolagus cuniculus)

B. Boag, **J. Lello**, A. Fenton, D. M. Tompkins & P.J. Hudson (2001)

**International Journal for Parasitology, 31(13): 1421-1428**



# CHAPTER 1

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## Patterns of parasite aggregation in the wild European *rabbit (Oryctolagus cuniculus)*

Boag, B., **Lello, J.**, Fenton, A., Tompkins, D. M. & Hudson, P. J.

**Abstract**

Understanding the factors controlling the distribution of parasites within their host population is fundamental to the wider understanding of parasite epidemiology and ecology. To explore changes in parasite aggregation, Taylor's power law was used to examine the distributions of five gut helminths of the wild rabbit. Aggregation was found to be a dynamic process that varied with year, season, host sex, age class, and myxomatosis. Yearly and seasonal changes are thought, in the main, to be the result of variations in weather conditions acting upon infectious stages (or intermediate hosts). Evidence in support of this was the comparatively low degree of fluctuation in the aggregation of the pinworm, *Passalurus ambiguus*, as the infectious stage of this parasite is likely to be less susceptible to environmental variation. Host age had a marked effect on the level of aggregation of all parasites, but this effect varied between parasite species. *P. ambiguus*, *Trichostrongylus retortaeformis* and *Cittotaenia denticulata* aggregation were lower in adult than juvenile rabbits whilst *Graphidium strigosum* and *Mosgovoyia pectinata* aggregation tended to increase with age. Host immunity is thought to be responsible for these differences. Differences in aggregation for different parasites were also seen when the rabbit population was split into males and females. Myxomatosis had a

marked effect on helminth distribution with substantially less aggregation in rabbits showing clinical signs of the disease

## Introduction

Animal parasites generally exhibit an aggregated or overdispersed distribution within their host population (Barger, 1985; Boag, Hackett & Topham, 1992; Jaenike, 1996; Shaw, Grenfell & Dobson, 1998; Wilson, Bjørnstad, Dobson, *et al.*, 2001). Data from both domestic and wild animals indicate that parasite intensity and prevalence can vary with season (Boag & Thomas, 1977; Boag, 1985; Hudson, Newborn & Dobson, 1992), year (Hudson, Dobson & Newborn, 1998), host age (Boag & Kolb, 1989), sex (Michel, 1952a), immune status (Michel, 1952b), the presence of other parasitic organisms (Boag, 1988) and intra-specific competition (Keymer, A., 1982). Theoretical studies have identified the importance of aggregation to the stability and dynamics of the host parasite system (May & Anderson, 1978; Dobson & Hudson, 1992) and explored the consequences of having aggregation as a dynamical variable (Adler & Kretzschmar, 1992; Pugliese, Rosa & Damaggio, 1998). The importance of aggregation to our understanding of the parasite-host system is so fundamental that detailed temporal studies of how aggregation varies with changes in the host population, the environment and exposure to other parasites are needed. Laboratory experiments have been used to investigate temporal changes in aggregation (Scott, 1987) but there has been little investigation using long term data sets of wild animal populations. This paper is the first of a series that examines changes in the pattern of aggregation using data collected over a 23-year long-term study of rabbit parasites. Since many parasite populations are well described by the negative binomial distribution and the fundamental parasite-host models utilise the exponent

of this distribution (k), the majority of epidemiological studies estimate k with little critical assessment (Barger, 1985; Scott, 1987; Hudson & Dobson, 1995; Roberts, Smith & Grenfell, 1995; Fenton, Wall & French, 1999; Wilson, Bjørnstad, Dobson, *et al.*, 2001). However, while we do not refute the important role of k, we believe it does have certain limitations, which need to be borne in mind. For example, aggregation tends to be underestimated as sample size decreases, and k cannot be used to compare between species with different means (Taylor, Woiwod & Perry, 1979; Gregory & Woolhouse, 1993). Workers therefore need to evaluate the estimate of k carefully (Wilson, Bjørnstad, Dobson, *et al.*, 2001) and apply other estimates of aggregation where appropriate. Since the negative binomial k does not adequately describe the data explored here ($P<0.001$ for all parasites) and due to the requirement of comparing samples of different sizes and means, an alternative measure of aggregation was required. An alternative index, which is independent of sample size and sample mean, is Taylor's power law index of aggregation (b) (Ripley, 1981; Poulin & Morand, 2000). This is the linear relationship between the log variance and the log mean of counts described by the simple equation log variance = a + *b* log mean. The slope of the line, b, is the index of aggregation and its intercept on the ordinate, a, is the value sometimes referred to as the sampling coefficient (Boag, Hackett & Topham, 1992). It has been suggested that the *b* value for any given species should be stable (Taylor, 1970) and can be used to produce a 'normalising transformation' for individual species (Taylor, 1979).

The five most common helminths of the wild rabbit are considered in this study: the nematodes, *Trichostrongylus retortaeformis*, *Graphidium strigosum* and *Passalurus ambiguus* and the cestodes *Mosgovoyia pectinata* and *Cittotaenia denticulata*. The two cestodes have

similar lifecycles where eggs are passed within tapeworm proglottids in the rabbit faeces, they develop to an infective stage and must then be ingested by an orbatid mite (Stunkard, 1941), for further development to occur. Within the mite the tapeworm develops to the cysticercoid stage, which is infective to the definitive rabbit host upon accidental ingestion of the mites on vegetation. Eggs of the nematodes *G. strigosum* and *T. retortaeformis* are also passed in the rabbit faeces. These two parasites however do not have intermediate hosts. First stage larvae hatch out and develop through to the infective third stage larvae within the faeces and then migrate up the closest vegetation where they can be ingested by a new host. The sticky eggs of the third nematode, *P. ambiguus*, are laid by the adult female worms on the perianal skin of the rabbit. Here larvae develop to the infective third stage within the egg and infection occurs following ingestion during grooming or copraphagy. The aggregation of these parasites is thus considered in light of their differing life-cycles with the following questions being addressed: (1) Are there general differences between the cestodes and the nematodes? (2) Do distributions vary temporally with season and year? (3) Does host age and sex influence parasite distribution? (4) Does myxomatosis influence parasite distribution? (5) Is $b$ stable and can it be used to produce a 'normalising transformation' for individual species?

**Methods**

The rabbits were collected from a 400 ha site in Perthshire, Scotland (ordnance grid reference NO 280 340). The altitude varied between 180 and 285 m above sea level, with much of the lower lying land being in an intensively farmed arable rotation while the higher land was heather moorland. Management during the late 1970s and early 1980s became more intensive in some areas leading to a reduction in the rabbit population, (Boag, 1987). Samples were

collected, by shooting, in most months between January 1977 and December 1999. The dates, locations, vegetation, and topography of where the rabbits were shot were recorded. In the laboratory the rabbits were weighed, sexed and examined for signs of myxomatosis and other diseases such as coccidiosis. A rabbit was classified as having myxomatosis if it had typical sores around its eyes, nose, base of ears, reproductive or excretory orifices and was in poor condition. To quantify helminth burdens, the abdominal cavity of each rabbit was opened and the contents removed. The alimentary tract was separated into three regions (stomach, small intestine and large intestine), with the contents of each sieved through a 100 mesh (125 mm) sieve. The residues were collected and either examined fresh within 24 h or occasionally stored in 5 % formalin (2 % formaldehyde). Unless nematode numbers were very low no fewer than 20 worms were counted, the dilution never exceeding one part in 25. All cestodes were counted and identified using the key of Arnold (1938). For statistical analysis the rabbits were placed in the following mass categories to reflect ages: kittens, 200–749 g, juveniles, 750–1249 g and adults, 1250 g and above. Mass has been shown to be a good indicator of age in wild rabbits (Cowan, 1983). To investigate correlates of aggregation, the dataset was split by month (sample size range from 111 to 503), year (n=39 to 276), host myxomatosis status (myxomatosis negative n=2710, myxomatosis positive n=253), age group (adult n=480, juvenile n=411, kitten n=629) and sex (male n=831, female n=696). Data were further subdivided for bootstrapping as detailed below, however subsamples were excluded from the analyses if the number of rabbits was less than 11 (although samples were generally considerably in excess of this number). Previous work has shown that rabbits at this study site exhibiting the characteristic lesions of myxomatosis had higher numbers of certain helminths. Since myxomatosis occurred mainly between July and December in the majority of years

(Boag, 1988), and rabbit numbers peak from May to September at this site, the investigation of the effect of myxomatosis was restricted to data collected between July and September. Host age group comparisons were restricted to myxomatosis negative animals from months May to August since this was the period when all age groups were most abundant.

A bootstrapping technique was developed (as a Visual Basic programme for Microsoft Excel) to calculate Taylor's power law parameter b. This technique compensated for outliers and enabled accurate estimates of *b* to be calculated for each subsample of the data examined. The programme randomly sampled (with replacement) 50 parasite counts and calculated the log (mean + 1) and log (variance + 1) of each subsample. This was repeated 50 times with an estimate of *b* calculated as the slope from the linear regression of log (variance + 1) onto log (mean + 1). The whole process was then repeated 100 times to allow means and S.E. for both a and *b* to be calculated. To control for uneven sampling among months the overall *b* value for each parasite was calculated as the average of the monthly *b* values. Statistical comparisons between groups were achieved through general linear models (GLM) in the MINITAB statistical package. The formula used to produce the 'normalising transformation' is:

$$Z = x^{1-\frac{1}{2b}}$$

where Z is the transformed value, x the untransformed value and *b* is Taylor's power law index of aggregation (Taylor, 1970). The average of the monthly *b* values obtained for each species were used in the transformations. The resulting distributions were then tested for normality using the Kolmogorov–Smirnov test in the Minitab statistical package. These distributions were compared with a log transformation using the same test.

## Results

A total of 2963 rabbits were included in the analysis, in which the averaged monthly *b* values for the parasites were 2.34 (S.E. 0.02) for *G. strigosum*, 2.46 (S.E. 0.01) for *T. retortaeformis*, 2.05 (S.E. 0.01) for *P. ambiguus*, 1.82 (S.E. 0.01) for *M. pectinata* and 1.59 (S.E. 0.02) for *C. denticulata*, with GLM regression coefficients being significantly ($p<0.001$) positive for the nematodes (0.14 for *G. strigosum*, 0.20 for *T. retortaeformis* and 0.03 for *P. ambiguus*) and significantly ($p<0.001$) negative for the cestodes (-0.09 for *M. pectinata* and -0.27 for *C. denticulata*). The cestodes thus exhibited a lower degree of aggregation than the nematodes.

There were no clear monthly trends in the data (figure 1). Variation in the aggregation of *G. strigosum* and *T. retortaeformis* throughout the year was considerable (variance=0.35; 0.21, respectively) although both species did display higher levels of aggregation through the winter months. Aggregation of *C. denticulata* also varied substantially (variance=0.31), although in this case the degree of aggregation was low between December and April. Both *P. ambiguus* and *M. pectinata* showed much less seasonal variability than the other species (variance=0.03; 0.04, respectively), with no obvious seasonal trend. Considerable variation in aggregation was observed among years for all parasites, although there were no overall consistent trends (figure 2). As for the monthly split, less variation was seen in *P. ambiguus* and *M. pectinata* (variance=0.06 and 0.11, respectively), compared with the other three species (*G. strigosum* variance=0.20, *T. retortaeformis* variance=0.25, *C. denticulata* variance=0.26).

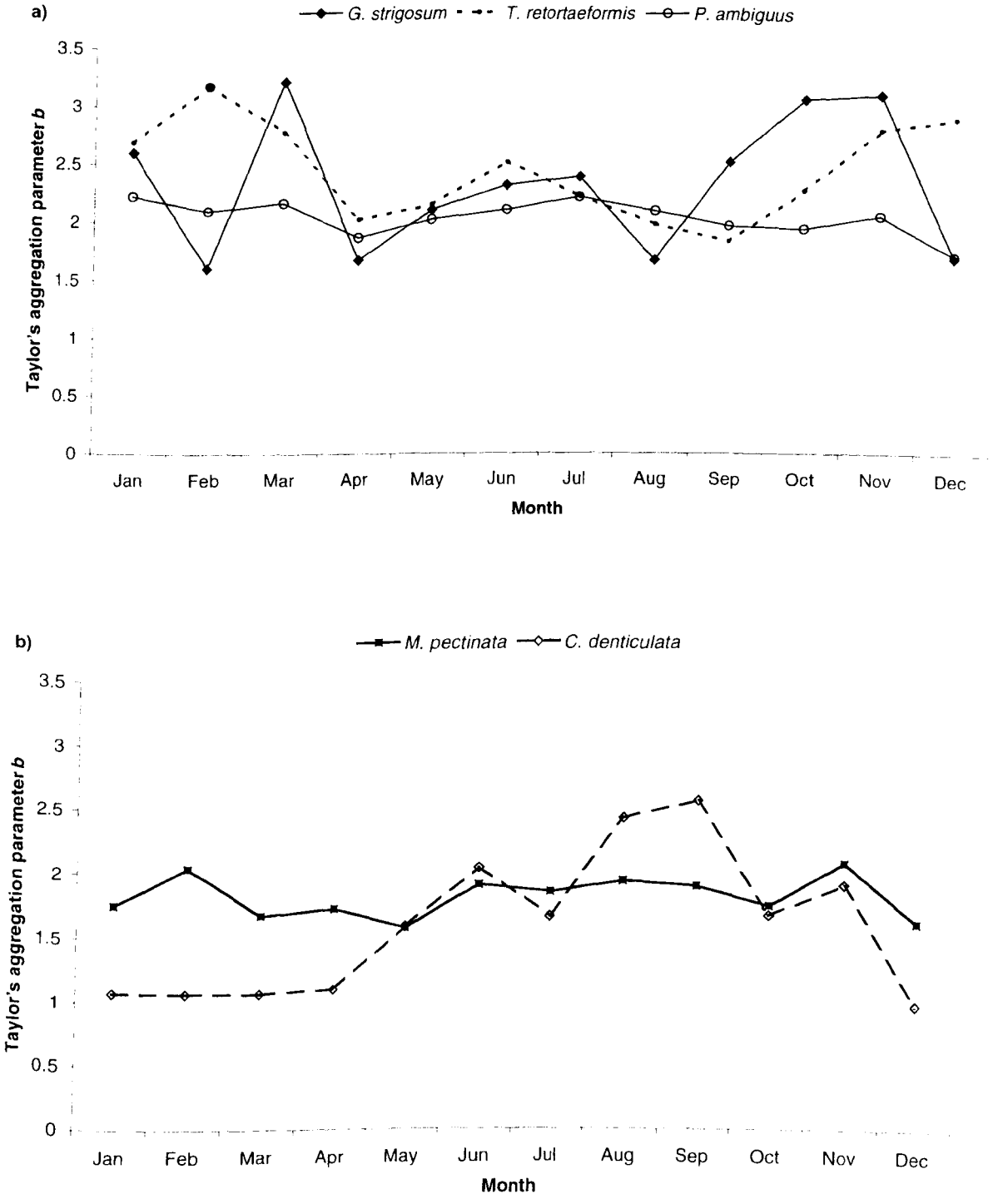


Figure 1. Monthly variation in aggregation of a) nematode and b) cestode parasites of the wild rabbit. (S.E.'s never exceed 0.09)

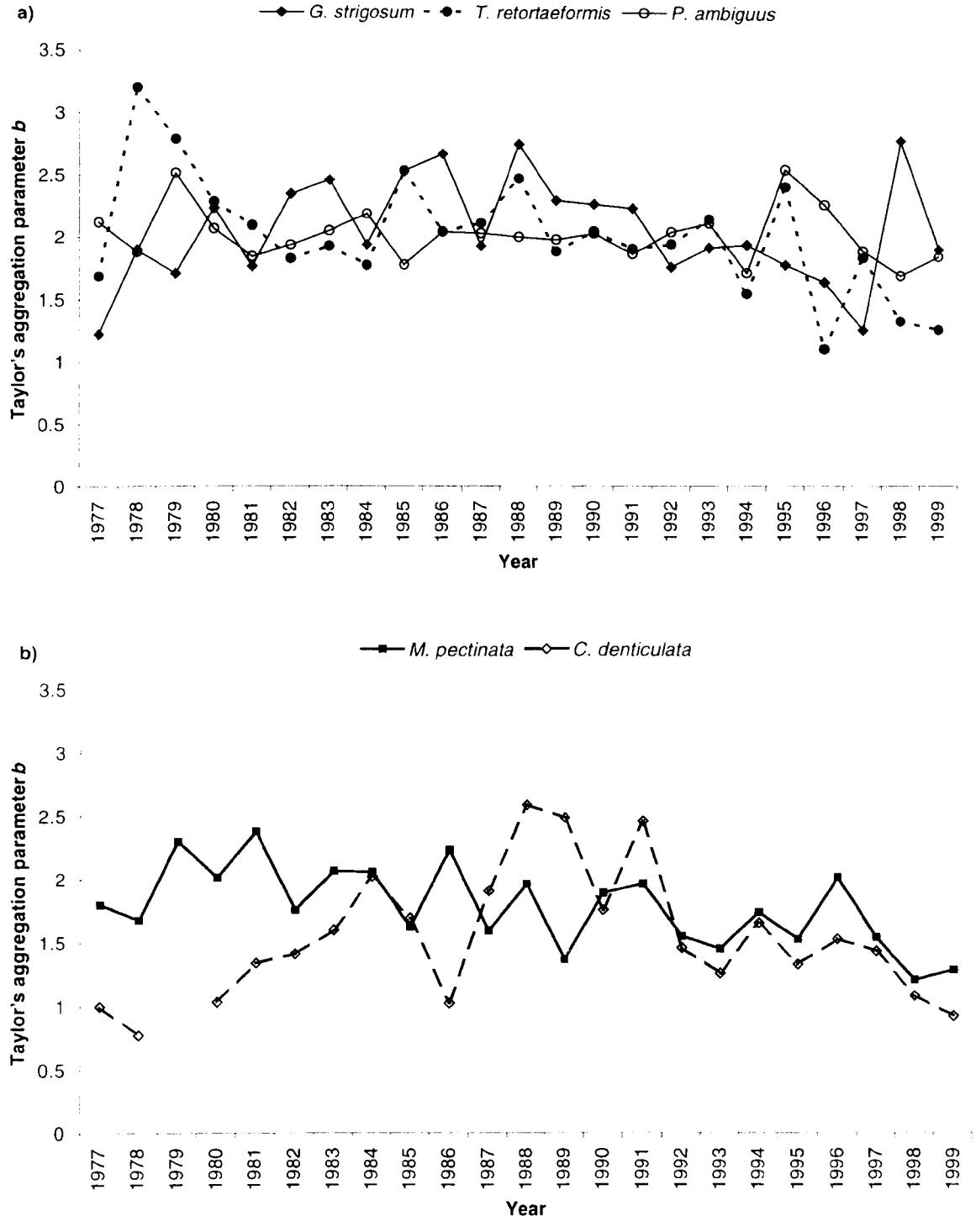


Figure 2. Yearly variation in aggregation of the a) nematode and b) cestode parasites of the wild rabbit. (S.E.s never exceed 0.11)

In the dataset of myxomatosis negative individuals there were no consistent trends in aggregation of parasites in adult rabbits versus juveniles versus kittens (table 1). However, all parasites, except *M. pectinata*,were less aggregated in kittens than in juveniles ($p<0.001$). Furthermore, parasite aggregation in adult compared with juvenile rabbits was greater for *G. strigosum* and *M. pectinata* but less for all other species ($p<0.001$). When parasite aggregation was examined in relation to host sex, *G. strigosum*, *C. denticulata*, and *M. pectinata* were seen to be more aggregated in adult male than in adult female rabbits ($p<0.001$, $p<0.005$ for *C. denticulata*), *T. retortaeformis* was more aggregated in adult female than in adult male rabbits ($p<0.001$), while there were no significant differences between the sexes for *P. ambiguus* (table 2).

For all parasites studied, in all age classes (except for *C. denticulata* in adult rabbits), aggregation was significantly lower (approaching a random distribution in some instances) in rabbits exhibiting characteristics of myxomatosis than those with no symptoms (all $p<0.001$; table 3). All attempted transformations were carried out on data with zeros removed as initial transformations with zeros included were poor for both log and Taylor's transformations. The untransformed data and both the transformed datasets were significantly different from normal ($p<0.01$) according to the Kolmogorov–Smirnov test. The log transformation actually performed better, as seen by the tests D values (range 0.22–0.33 for untransformed data, 0.06–0.12 for Taylor's transformation and 0.03–0.07 for the log transformation).

Table 1. Effect of host developmental stage Taylor's power law index of aggregation *b* for nematode and cestode parasites of rabbits uninfected with myxomatosis (figures in brackets are S.E.)[◊].

| | *Graphidium strigosum* | *Trichostrongylus retortaeformis* | * *Passalurus ambiguus* | *Mosgovoyia pectinata* | *Cittotaenia denticulata* |
|---|---|---|---|---|---|
| **Adult rabbits** | 2.03 | 1.93 | 1.89 | 1.78 | 1.51 |
| | (0.03) | (0.01) | (0.01) | (0.01) | (0.02) |
| **Juvenile rabbits** | 1.78 | 2.35 | 3.18 | 1.50 | 2.02 |
| | (0.02) | (0.02) | (0.04) | (0.02) | (0.04) |
| **Kittens** | 1.75 | 2.12 | 1.98 | 1.56 | 1.53 |
| | (0.02) | (0.02) | (0.02) | (0.02) | (0.02) |

[◊] Significant differences between all groups ($p<0.001$)

* *Passalurus ambiguus* was not present in kittens in months 7 and 8. All values for this species pertain to months 5 and 6 only.

Table 2. Effect of host sex on Taylor's power law index of aggregation *b* for nematode and cestode parasites of adult rabbits (figures in brackets are S.E.) [◊].

| | *Graphidium strigosum* | *Trichostrongylus retortaeformis* | * *Passalurus ambiguus* | *Mosgovoyia pectinata* | ** *Cittotaenia denticulata* |
|---|---|---|---|---|---|
| **Males** | 2.57 | 2.22 | 2.05 | 2.28 | 1.76 |
| | (0.02) | (0.02) | (0.01) | (0.01) | (0.02) |
| **Females** | 2.2 | 2.41 | 2.06 | 1.91 | 1.68 |
| | (0.02) | (0.02) | (0.01) | (0.01) | (0.01) |

[◊] Significant differences between all groups ($p<0.001$, except * N.S.; ** $p<0.005$)

Table 3. Effect of myxomatosis on Taylor's power law index of aggregation *b* for nematode and cestode parasites of rabbits (figures in brackets are S.E.) [◊].

| | *Graphidium strigosum* | *Trichostrongylus retortaeformis* | *Passalurus ambiguus* | *Mosgovoyia pectinata* | *Cittotaenia denticulata* |
|---|---|---|---|---|---|
| **Infected adult rabbits** | 1.07 (0.01) | 1.54 (0.03) | 1.48 (0.03) | 1.07 (0.02) | 1.01 (0.02) |
| **Uninfected adult rabbits** | 2.08 (0.05) | 1.84 (0.02) | 1.99 (0.01) | 1.54 (0.01) | 1.00 (0.01) |
| **Infected juvenile rabbits** | 1.38 (0.01) | 1.69 (0.03) | 2.11 (0.04) | 1.47 (0.01) | 1.30 (0.04) |
| **Uninfected juvenile rabbits** | 1.47 (0.02) | 2.07 (0.01) | 3.13 (0.07) | 1.89 (0.01) | 1.78 (0.05) |
| **Infected kittens** | 0.79 (0.02) | 1.02 (0.05) | NA NA | 0.91 (0.01) | 0.77 (0.02) |
| **Uninfected kittens** | 1.27 (0.02) | 1.85 (0.05) | NA NA | 2.06 (0.01) | 1.22 (0.03) |

[◊] Significant differences between all groups ($p<0.01$) except for *C. denticulata* in adult rabbits (N.S.).

NB. Adult values calculated from months 8 and 9, juveniles months 7, 8 and 9 (*P. ambiguus* months 7 and 8 only), kittens month 7 and 9 only (*M. pectinata* month 7 only).

## Discussion

The extent of this time series has provided a unique opportunity to study the phenomenon of aggregation using a range of parasite species in a single wild host species. There were significant differences in aggregation between all species of parasite investigated, with the underlying trend being lower aggregation of the cestodes than of the nematodes. This may be a consequence of differing modes of transmission. High spatial aggregation of infective stages can potentially lead to highly aggregated distributions within infected hosts (Keymer, A. E. & Anderson, 1979). Like other strongylid nematodes, *Graphidium strigosum* and *T. retortaeformis* infective third stages are unlikely to disperse very far from the faeces in which they develop (Michel, 1969; Saunders, Tompkins & Hudson, 2000), and the contact dependent

dispersal of *P. ambiguus* is also likely to be highly limited. However, the cestodes' reliance on the orbatid mite to distribute their infective stages may result in wider dispersal. Whilst the microscopic size of the mites precludes their travelling large distances from their point of infection, they are still likely to disperse further than the nematode larvae/eggs. An alternative or perhaps additional possibility is the effect of crowding. Nematodes are small and space limitation in the host is not generally a problem, except at very high densities. Tapeworms, on the other hand, can take up a great deal of space in the gut possibly resulting in density-dependent, intra-specific competition (Read, 1951). Such competition would result in a shortening of the tail of the distribution thus reducing the aggregation of the parasites. This explanation is supported by the larger of the two cestodes in this study *C. denticulata*, which occurs at a lower average intensity, (two worms per host compared with seven for *M. pectinata*; (Mead-Briggs & Page, 1975)), having the lowest level of aggregation of all five helminths studied.

There were temporal changes in aggregation, clearly indicating that *b* is not a stable species characteristic in this dataset. However, *b* was less variable for certain species than for others, differences which may be accounted for by the different parasite life-histories. For example, *P. ambiguus*, with the lowest degree of temporal variation, is the species least likely to be affected by external environmental conditions since its infective larvae do not leave the egg until the egg is ingested by the host (Grice & Prociv, 1993; Hugot, Reinhard, Gardner, *et al.*, 1999). *Mosgovoyia pectinata* also has a low level of variation. Preliminary examination of prevalence and intensity data for both *M. pectinata* and *P. ambiguus* (unpublished) has indicated a positive association between these two species, which may account for the low

level of variability of *M. pectinata*.

While previous studies have shown that the intensity of rabbit worm burdens varies with age (Boag & Kolb, 1989), the present study shows that such variation is also true for parasite aggregation. For all species, except for *M. pectinata*, there was a rise in aggregation from kitten to juvenile rabbits. Mechanisms that could account for such a rise are, first, the development of acquired immunity (Grenfell, Wilson, Isham, *et al.*, 1995) and, second, differences in the behaviour of the young rabbits. Both the intensity and prevalence of worm infection tends to be low in kittens, probably due to their minimal contact with the infective parasite stages (Boag, 1985). However, as rabbits develop, they begin to range further from their burrows and this could lead to certain individuals feeding on more heavily infected areas than others. While a similar rise in aggregation was observed between juvenile and adult rabbits for some parasites (*G. strigosum* and *M. pectinata*), the others showed significant declines. Such differences may be caused by host immunity. Evidence suggests that the strength and form of the rabbit immune response and thus the manner by which immunity influences aggregation, is parasite species dependent (Boag & Kolb, 1989). Whilst heterogeneity/variation in the immune response is generally considered to be a potential cause of parasite aggregation (Anderson & Gordon, 1982), there are circumstances in which it may also lower aggregation. For example, *T. retortaeformis* prevalence and intensity declines in older rabbits, a decline which may be explained by density-dependence in the host immune response. If very high levels of infection elicit large immune responses, such a mechanism can act to limit worm burdens and reduce aggregation (Anderson & Gordon, 1982; Grenfell, Wilson, Isham, *et al.*, 1995). The observation that rabbits given a large challenge of *T.*

*retortaeformis* larvae quickly reduce the worm burden via immunity, while those having a smaller challenge are less likely to rid themselves of the burden, is evidence in support of this mechanism (Michel, 1952a). Parasite-induced host mortality may also be acting to reduce the highest worm burdens, as the higher the worm burden the more likely a rabbit is to die as a consequence. This will effectively 'pull in' the tail of the distribution. Older rabbits may thus have lower parasite aggregation not only due to the fact that worm burden generally increases with age, flattening out the distribution curve, but also through an increase in such parasite-induced mortality.

Another factor examined was the influence of host sex on parasite aggregation. The data show that, for three out of the five parasite species, aggregation is higher in male rabbits than in female rabbits. The higher aggregations in males may be explained, by males tending to have larger home ranges than females (Cowan, 1987), and thus being liable to graze areas with a greater variation in parasitic contamination. An alternative is that variation in testosterone between males may produce greater variation via the immuno-suppressive effects of the hormone (Folstad & Karter, 1992). Interestingly *T. retortaeformis* appears to be less aggregated in males than females. Previous studies suggest that this may reflect changes which can occur in the immune status of female rabbits where the stress associated with pregnancy can allow arrested larvae in the gut wall to begin developing (Michel, 1952b).

Of all the factors investigated myxomatosis had the most consistent impact, lowering the degree of aggregation for all parasites in all age groups (except for *C. denticulata* in adult rabbits). Myxomatosis has been associated with increases in the prevalence and intensity of *G.*

*strigosum* (Mykytowycz, 1959) *T. retortaeformis*, *P. ambiguus* and *M. pectinata* (Boag, 1988). Mykytowycz (1959) proposed that the reason myxomatosis increased prevalence and intensity of *G. strigosum* was because it suppressed the infected rabbits immune response and allowed the arrested stages of the parasites residing in the gut wall to develop. If, as proposed by Anderson and Gordon (1982) and Grenfell *et al.* (1995), host immunity to nematode infection varies between individuals, then the breakdown in immunity of rabbits due to myxomatosis could explain the decreases in aggregation observed.

An important conclusion to come out of this work is that Taylor's power law index of aggregation *b* does not appear to be stable and therefore cannot be considered a species characteristic. This supports the view that aggregation is a dynamic phenomenon (Anderson & Gordon, 1982). It was thus not possible to use *b* to produce a transformation, which would 'normalise' the data for statistical analysis; indeed, the log transformation was found to be better in all cases. Perry (1987) states that *b* provides only a first approximation for transformation and that an iterative approach should be used to achieve the 'normalising' equation. However, such a process is beyond the scope of the current paper.

Taylor's power law did prove to be a very useful tool for examining the dynamics of parasite aggregation changes within infected hosts. Understanding the factors controlling aggregation is fundamental to the wider understanding of parasite epidemiology and ecology and should give another insight into the relationship between the parasites and their host. This study has raised several further questions about parasite aggregation in rabbits, such as: (1) are seasonal variations linked to weather conditions? (2) Is crowding in the rabbit or distribution on the

field responsible for the lower aggregation of cestodes? (3) To what extent do inter-species interactions affect aggregation? These issues will be explored in more detail in future papers.

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# CHAPTER 2

## "War of the worms": Competition and mutualism amongst the gut helminths of a mammalian host

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**In preparation for submission to Nature**

# Chapter 2

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**"War of the Worms": Competition and mutualism amongst the gut helminths of a mammalian host.**

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## Abstract

The role of direct and indirect interspecific parasite interactions in influencing parasite dynamics and shaping parasite communities is far from clear. Indeed there has been a dichotomy of views: laboratory studies have found evidence of cross immunity, immuno-suppression and competition but analyses of hosts sampled in field studies have generally found that parasite communities are little more than random assemblages. We report here on an analysis of a 23 year time series of parasites from a free ranging rabbit population and provide evidence suggesting that interspecific interactions have an important influence on parasites dynamics and therefore shape community dynamics. Rabbit parasites provide a useful model for natural and domestic herbivore systems and these findings are important for the development of environmentally acceptable means of parasite control.

## Introduction

A central tenet of community ecology is that species groupings exhibit emergent properties through their direct and indirect interactions, which are not apparent from a simple

investigation of individual species. Laboratory studies of concomitant infections of parasites have provided good evidence for interactions, which would be expected to influence parasite abundance and shape the dynamics of parasite communities (Behnke, Wakelin & Wilson, 1978; Silver, Dick & Welch, 1980; Christensen, Nansen, Fagbemi, *et al.*, 1987; Adams, Anderson & Windon, 1989; Behnke, Barnard, Bajer, *et al.*, 2001; Cox, 2001). In contrast, field studies of parasitic infections (particularly of mammals), have found that parasite communities are little more than random assemblages. This has been taken to imply that interactions do not affect the dynamics of parasite communities and may be artefacts of laboratory infections (Bush & Holmes, 1986; Haukisalmi & Henttonen, 1993a, 1993b; Forbes, Weatherhead & Bennett, 1994; Poulin, 1996; Nilssen, Haugerud & Folstad, 1998; Forbes, Alisauskas, McLaughlin, *et al.*, 1999).

In seeking an explanation for the discrepancy between the laboratory and field studies, some workers have argued that, under natural conditions, parasites are unlikely to interact where they are temporally or spatially separated in the host (Cabaret & Hoste, 1998; Poulin, 2001; Poulin & Valtonen, 2002). However, while this would be true if competition was direct, it would not necessarily be the case where interactions were indirect and mediated via host biology. Here we present evidence from an examination of parasite intensity data collected over a period of 23 years, which reveals that interspecific parasite interactions may have a significant and substantial impact on parasite dynamics.

Within parasite systems, individual hosts provide discrete and replicated infracommunities that provide good power for the examination of parasite dynamics. The wild rabbit

(*Oryctolagus cuniculus*) is an ideal host species with which to examine interspecific interactions between parasites since much is known about its ecology and biology from both field observations and laboratory experiments (Thompson & Worden, 1956; Cowan, Tapper & Hewson, 1991). Furthermore, it plays host to a diverse gut helminth community (Mead-Briggs & Vaughan, 1973; Mead-Briggs & Page, 1975; Boag, Lello, Fenton, *et al.*, 2001) that reflects well the community seen in several other mammalian herbivores (Crofton, 1954; Boag, 1999). Consequently, the rabbit parasite community is an ideal model system to assess the dynamics and control of parasites of economically important domestic herbivores.

We sampled a wild rabbit population monthly for 23 years and recorded the prevalence and intensity of the gastrointestinal helminths. In the UK, the wild rabbit population is dominated by five gut helminths: the strongylid nematodes, *Graphidium strigosum* (living in the stomach) and *Trichostrongylus retortaeformis* (small intestine); the anoplocephaloid cestodes, *Mosgovoyia pectinata* (small intestine) and *Cittotaenia denticulata* (small intestine); and the oxyurid nematode *Passalurus ambiguus* (large intestine and colon). The relative positions of these species in the host gut are important in terms of the potential mechanisms of interaction (Boag, Lello, Fenton, *et al.*, 2001). Our objective was to identify how important interspecific interactions are to the dynamics of a whole community of parasites and to identify the putative mechanisms by which interactions are mediated.

**Methods**

Rabbits were collected from a 400 ha site in Perthshire, Scotland (ordnance grid reference NO 280 340) between the months of January 1977 and December 1999. Total gut helminth counts,

presence of myxomatosis and presence of *Eimeria stiedae* were recorded along with details of the external environment and aspects of host biology. Further details of the study site, collection protocols and data may be found in Boag *et al.* (2001).

We used a combination of generalised linear modelling (GLM) and residual maximum likelihood (REML) linear mixed model analyses of parasite count data, to test the null hypothesis that parasite interactions do not influence parasite dynamics. All analyses were conducted using the GENSTAT statistical package. All models were initially run as REMLs with the random terms of warren code and year of capture. Fixed terms included all recorded measures of host biology, the external environment and other parasite species. Interactions between all model terms were initially included up to the third order. Only adult rabbits (mass>1249g; n=1526) were used for this analysis since they were likely to have been exposed to infective stages of all parasites by this age. In addition, the distributions of the gut helminth parasites in different age classes of the rabbit have been shown to have very different distributions and must therefore be analysed separately (Boag, Lello, Fenton, *et al.*, 2001). The data for the gut helminths do not conform to the negative binomial distribution (Boag, Lello, Fenton, *et al.*, 2001). Attempts to fit the data to this distribution in the GENSTAT statistical package, also revealed that this non-conformance resulted from a skew to the right in the distributions which was greater than expected. Previous work has also shown that in order to obtain a 'near' normal distribution for the rabbit helminth data, zeros must be removed from the data prior to a log transformation being applied (Boag, Lello, Fenton, *et al.*, 2001). Therefore, the intensity data for the parasite species treated as the dependant variable was log transformed (zeros removed) prior to analysis. Each helminth was treated as the dependent

variable in one of the analyses. The helminths not being treated as the dependant variable were included in the models (as determined from submodels) as independent factors (presence absence) or as independent variables (log (x+1) intensity data) as appropriate. Non-significant terms were removed from the models in a stepwise manner until a minimal model was produced. This model was further refined following bootstrapping of the model effects and removal of any terms that were not significantly different from zero. Once the bootstrapping refinements were complete each model was run once again in its final form to obtain effect sizes and significance levels.

**Results**

A regression of parasite A on parasite B, is not the same as a regression of B on A since the spread of the residuals from the fitted line will differ depending which species is regressed on the other. Therefore it is possible for parasite B, when treated as the independent variable to be predicted to have a significant effect on A but when treated as the dependent variable to not be significantly affected by A. The models include many aspects of the host biology and the external environmental, which would be likely to create the appearance of interspecific interactions when none exist. We do however acknowledge that we may not have recorded all of the potentially important influences. Infection experiments will be necessary to confirm the predictions of this study, but we believe that we provide compelling preliminary evidence for interspecific interactions in this host species. For this reason we shall refer to the effects as the models predict them with respect to their direction in the gut of the rabbit.

All predictions are presented as, the percentage difference between the predicted level of the dependent variable (after back-transformation) with no parasites of the interacting species, compared with the predicted level of the dependent variable (after back-transformation) with the geometric mean (in concomitant infection) of the interacting species. According to the model predictions, same-locale, downstream interactions and upstream interactions occurred between the helminths. One significant ($p<0.001$) interaction was seen between species in the same location in the gut, the model predicting a positive effect of *C. denticulata* upon the intensity of *T. retortaeformis* infection (figure 1a and table 1c). The geometric mean of *C. denticulata* when in a concomitant infection with *T. retortaeformis* was 1.54 worms. The model predicts that the presence of one *C. denticulata* would be associated with an increase of 30%, and two worms with an increase of 51%, in the number of *T. retortaeformis*. However, there is some heteroscedasticity in the scatter of residuals for this interaction and as a more appropriate transformation could not be found, this particular interaction should be viewed with caution.

*Trichostrongylus retortaeformis* was also subject to a downstream influence with *G. strigosum* appearing to have a positive effect ($p<0.001$) upon its strongylid relative (figure 1b and table 1c). At the geometric mean (in concomitant infection) of 18 worms, *G. strigosum* was predicted to increase the numbers of *T. retortaeformis* by 46% relative to a rabbit harbouring no *G. strigosum*. A second downstream interaction was more complicated, with *M. pectinata* in the small intestine, predicted to have a positive effect in female, but not male hosts ($p=0.003$), upon the intensity of *P. ambiguus* in the large intestine/colon (figure 1c and table

1e). The geometric mean of three *M. pectinata* (in concomitant infection) was predicted to increase the intensity of *P. ambiguus* by 163% relative to a host containing no *M. pectinata*.

The analysis of *M. pectinata* data revealed that the association between these species also appears to operate in the opposite, upstream, direction, with *P. ambiguus* having a positive effect upon *M. pectinata*. Again, this association was complicated by host sex such that it increased the intensity of *M. pectinata* in male but not female rabbits (p=0.008; figure 1d and table 1b). The geometric mean of *P. ambiguus* (in concomitant infection) in male rabbits, is 127 and this was predicted to cause a 67% increase in *M. pectinata* relative to the numbers in hosts not infected with *P. ambiguus*. *Mosgovoyia pectinata* also had an upstream effect, apparently reducing the intensity of *G. strigosum* in the stomach (p=0.011; figure 1e and table 1a). In this case, the geometric mean of *M. pectinata* (in concomitant infection) was 3 and this was predicted to cause a decrease in *G. strigosum* of 19%. Finally *T. retortaeformis* was also predicted to affect the upstream *G. strigosum* (p=0.018; Fig 1f and table 1a) but in this case the effect was negative and complicated by host mass (also a proxy for age; Cowan, 1983). Furthermore, it was the presence not the intensity of *T. retortaeformis* that was important and whilst the level of the effect decreased as rabbit mass increased the effect in the majority of rabbits was negative. The predicted effect of *T. retortaeformis* in a rabbit of average mass (1590g in concomitantly infected rabbits) was to reduce *G. strigosum* numbers by 29%.

No parasites were predicted to affect the intensity of *C. denticulata* (table 1d).

**Table 1: Minimal restricted maximum likelihood (REML) and generalised linear models (GLM) of factors affecting intensity of the five gut helminths (30). All minimal models effect sizes were significant after bootstrapping (iterations=1000) with p=0.05.**

a) Log *Graphidium strigosum* intensity. Model Type: REML (Random Terms = warren number + year)

| Model Term | Wald Statistic ($\chi^2$) | d.f. | p |
|---|---|---|---|
| Month | 182.50 | 11 | <0.001 |
| Host Food Type | 25.18 | 1 | <0.001 |
| Presence / Absence *Trichostrongylus retortaeformis* * Host Mass | 8.84 | 1 | 0.018 |
| Log (x+1) *Mosgovoyia pectinata* | 6.6 | 1 | 0.011 |

b) Log *Mosgovoyia pectinata* intensity. Model Type: REML (Random Term = warren number)

| Model Term | Wald Statistic ($\chi^2$) | d.f. | p |
|---|---|---|---|
| Month | 62.49 | 11 | <0.001 |
| Sex | 0.00 | 1 | 0.951 |
| Sex * Log (x + 1) *Passalurus ambiguus* | 7.14 | 1 | 0.008 |

c) Log *Trichostrongylus retortaeformis* intensity. Model Type: GLM

| Model Term | F Statistic | d.f. | p |
|---|---|---|---|
| Month * Host Sex | 31.5 | 11,1195 | <0.001 |
| Host Sex * Host Mass | 2.8 | 1,1195 | 0.003 |
| Presence / Absence of myxomatosis | 12.8 | 1,1195 | <0.001 |
| Log (x +1) *Cittotaenia denticulata* | 23.98 | 1,1195 | <0.001 |
| Log (x+1) *Graphidium strigosum* | 31.94 | 1,1195 | <0.001 |

d) Log *Cittotaenia denticulata* intensity. Model Type: GLM

| Model Term | F Statistic | d.f. | p |
|---|---|---|---|
| Month | 1.41 | 11,288 | <0.001 |

e) Log *Passalurus ambiguus* intensity. Model Type: GLM

| Model Term | F Statistic | d.f. | p |
|---|---|---|---|
| Month | 26.4 | 11,280 | 0.011 |
| Host Sex | 1.6 | 1,280 | 0.223 |
| Host Sex * Log (x+1) *Mosgovoyia pectinata* | 9.3 | 1,280 | 0.003 |
| Warren vegetation class | 16.2 | 1,280 | <0.001 |
| Presence / absence of myxomatosis | 8.1 | 1,280 | 0.011 |

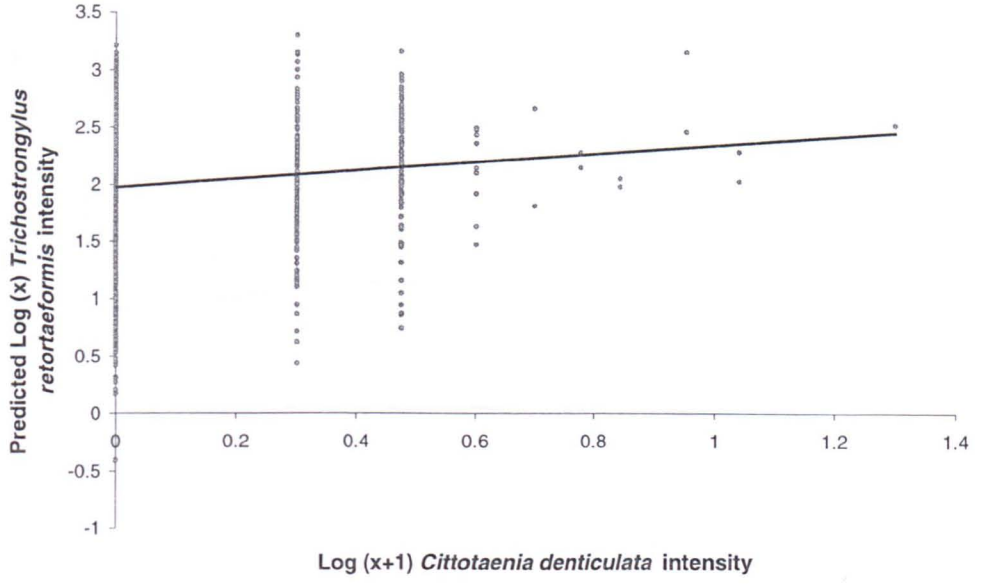



Fig 1 a) Relationship with residual fits of log (x+1) intensity of *Cittotaenia denticulata* and *Trichostrongylus retortaeformis* log intensity (zeros removed) predicted from the general linearised model.

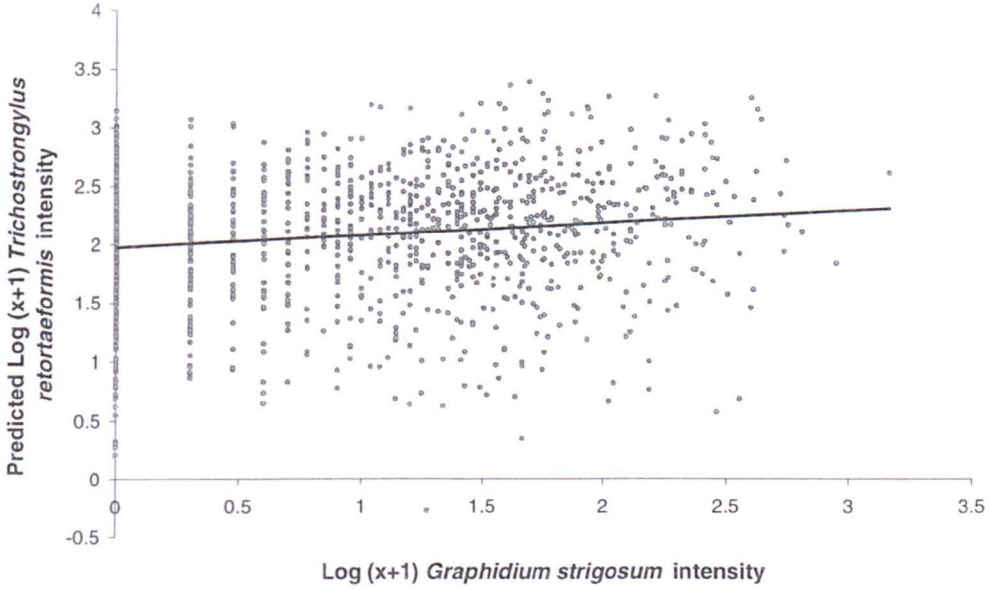



Fig 1 b) Relationship with residual fits of log (x+1) intensity of *Graphidium strigosum* and *Trichostrongylus retortaeformis* log intensity (zeros removed) predicted from the general linearised model.

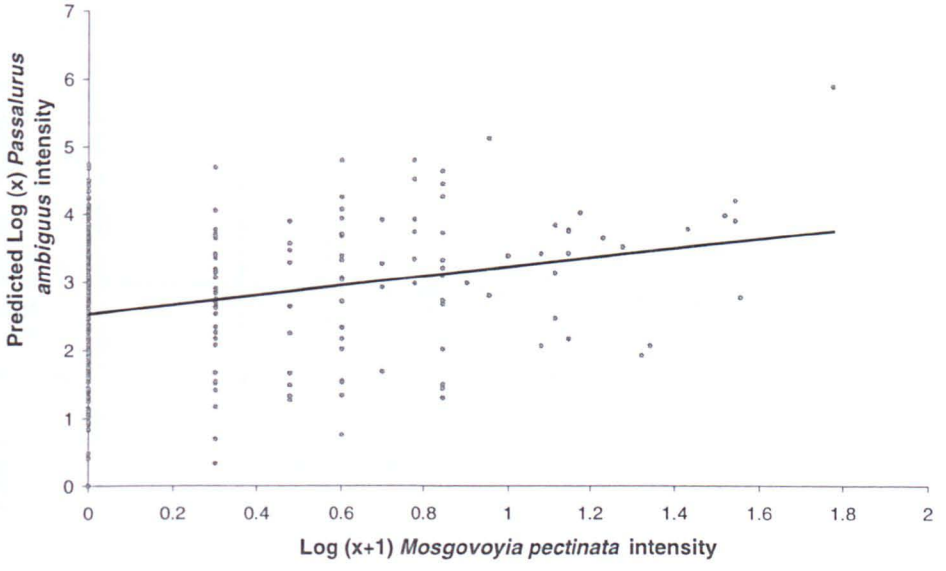



Fig 1 c) Relationship with residual fits of log (x+1) intensity of *Mosgovoyia pectinata* and *Passalurus ambiguus* log intensity (zeros removed) predicted from the general linearised model.

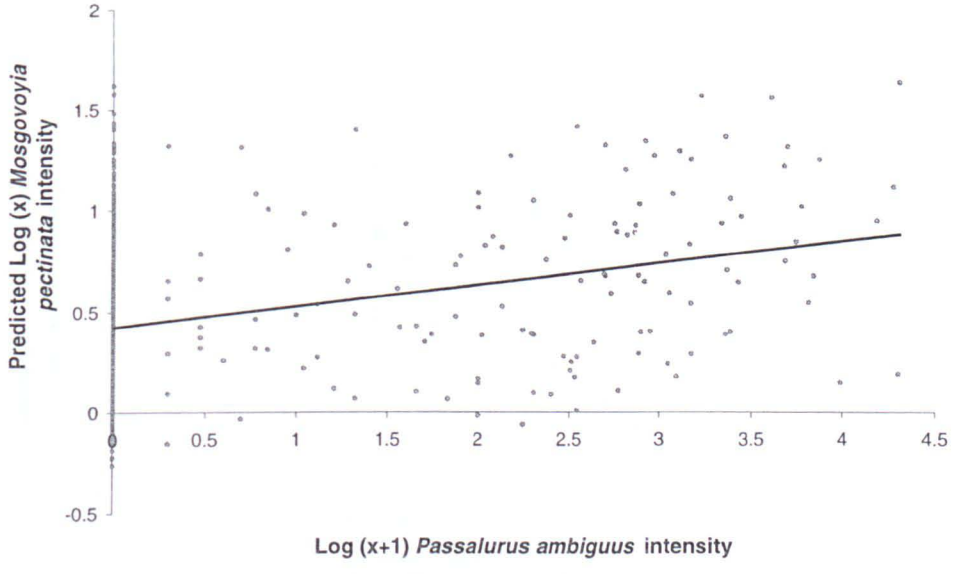



Fig 1 d) Relationship with residual fits of log (x+1) intensity of *Passalurus ambiguus* and *Mosgovoyia pectinata* log intensity (zeros removed) predicted from the restricted maximum likelihood model.

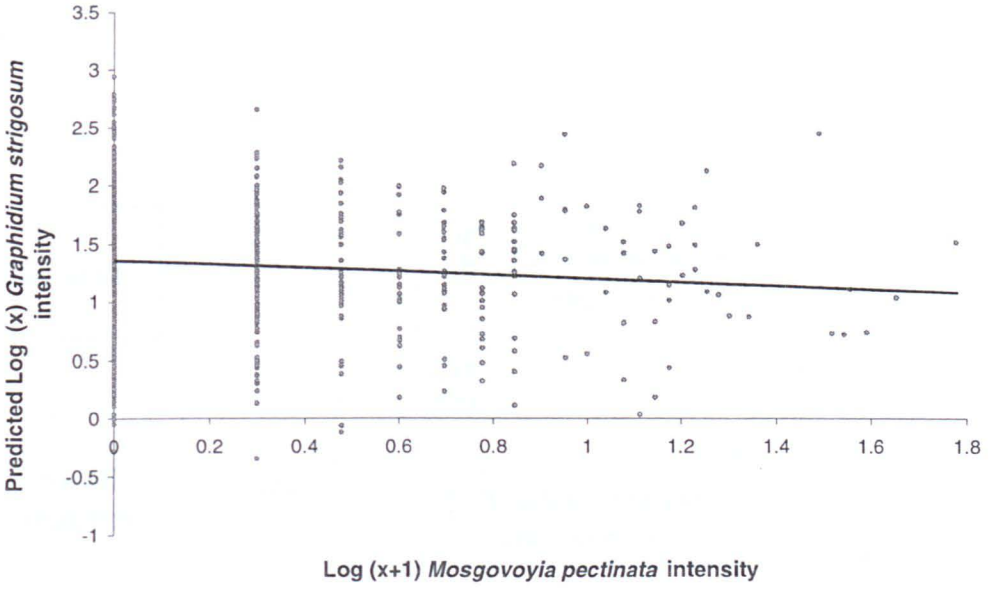



Fig 1 e) Relationship with residual fits of log (x+1) intensity of *Mosgovoyia pectinata* upon *Graphidium strigosum* log intensity (zeros removed) predicted from the restricted maximum likelihood model.

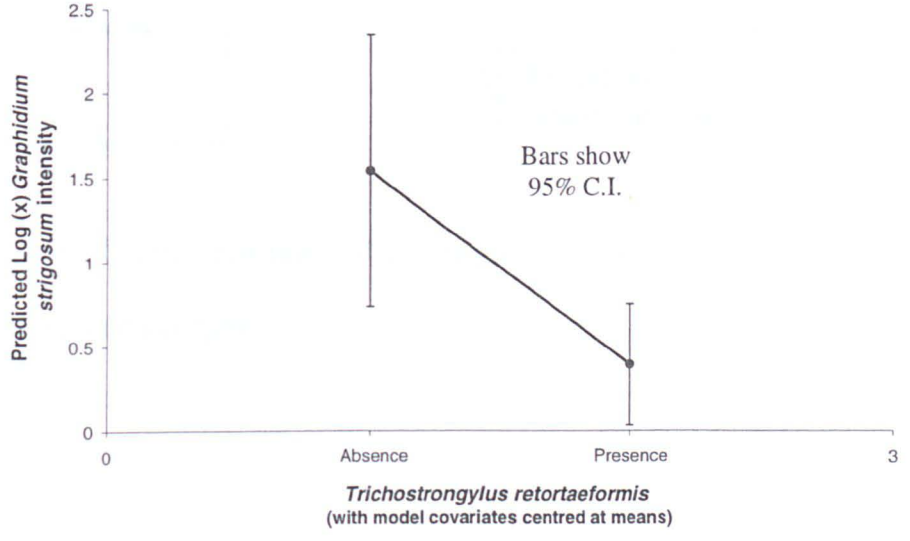



Fig 1 f) Relationship between the presence/absence of *Trichostrongylus retortaeformis* and *Graphidium strigosum* log intensity (zeros removed) in a rabbit of average mass (1590g) predicted from the restricted maximum likelihood model.

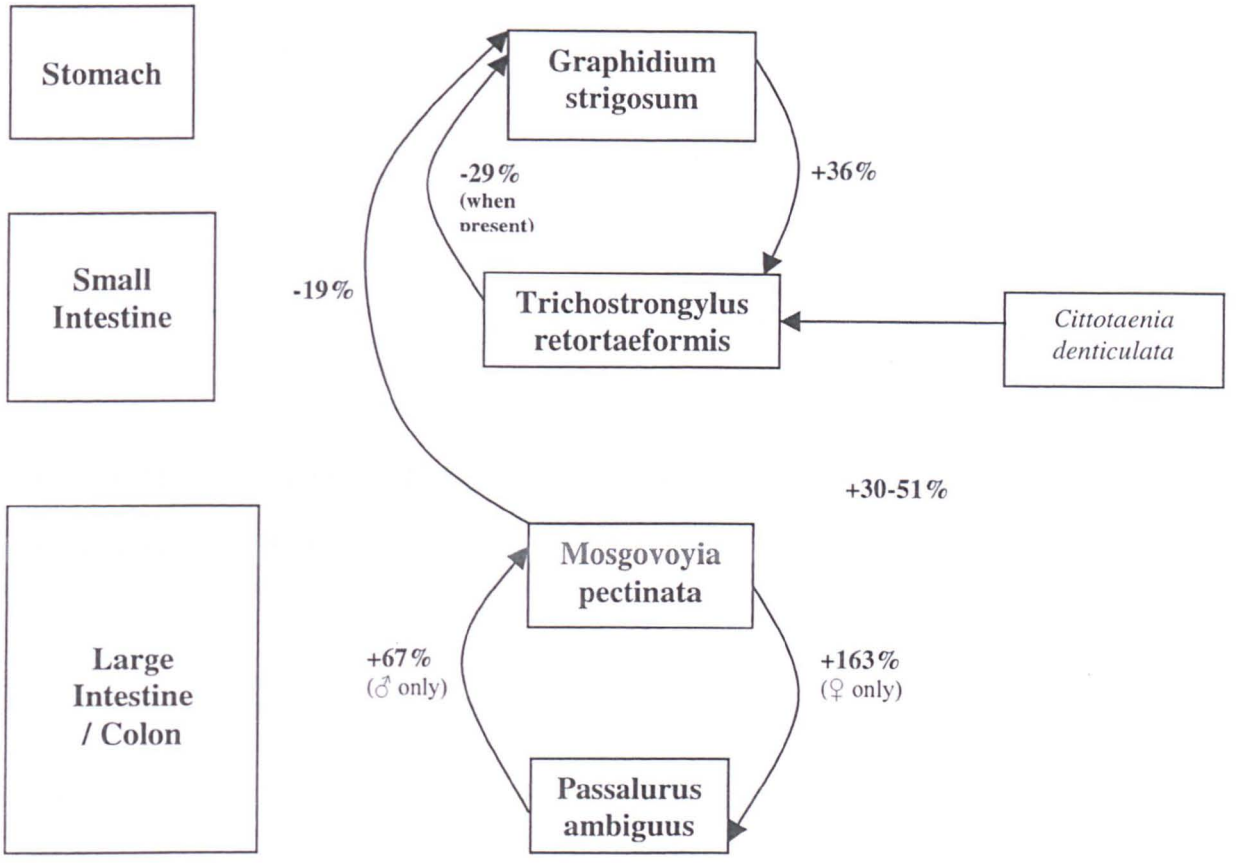
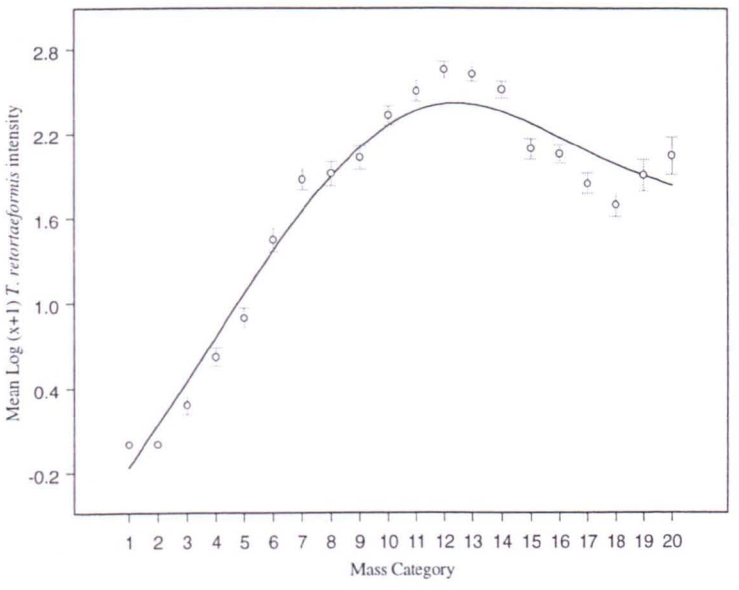



Figure 2. Relative positions of the rabbit gut helminths in the host gut, with interaction directions and strengths.




| Category | Rabbit Mass (g) |
|---|---|
| 1 | 0 to 100 |
| 2 | 101 to 200 |
| 3 | 201 to 300 |
| 4 | 301 to 400 |
| 5 | 401 to 500 |
| 6 | 501 to 600 |
| 7 | 601 to 700 |
| 8 | 701 to 800 |
| 9 | 801 to 900 |
| 10 | 901 to 1000 |
| 11 | 1001 to 1100 |
| 12 | 1101 to 1200 |
| 13 | 1201 to 1300 |
| 14 | 1301 to 1400 |
| 15 | 1401 to 1500 |
| 16 | 1501 to 1600 |
| 17 | 1601 to 1700 |
| 18 | 1701 to 1800 |
| 19 | 1801 to 1900 |
| 20 | 1901 + |

Key to figure 3

Figure 3 a) Spline fit of *Trichostrongylus retortaeformis* log (x+1) intensity regressed on host mass category, with SE bars.

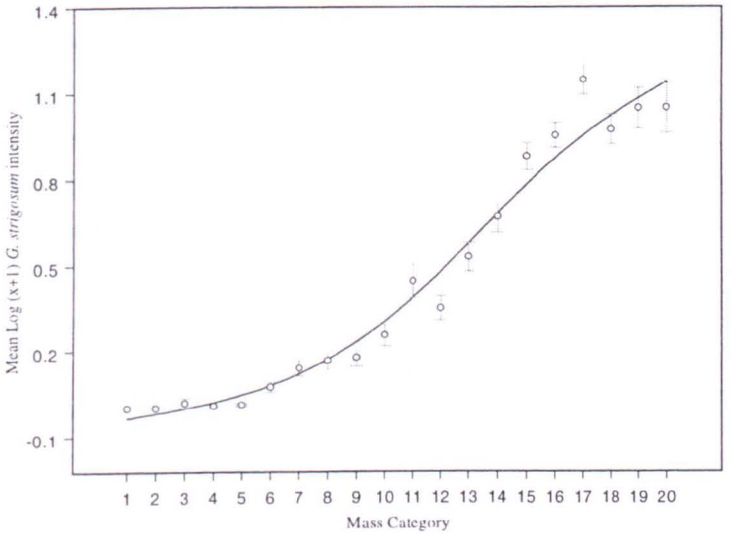



Figure 3 b) Spline fit of *Graphidium strigosum* log (x+1) intensity regressed on host mass category, with SE bars.

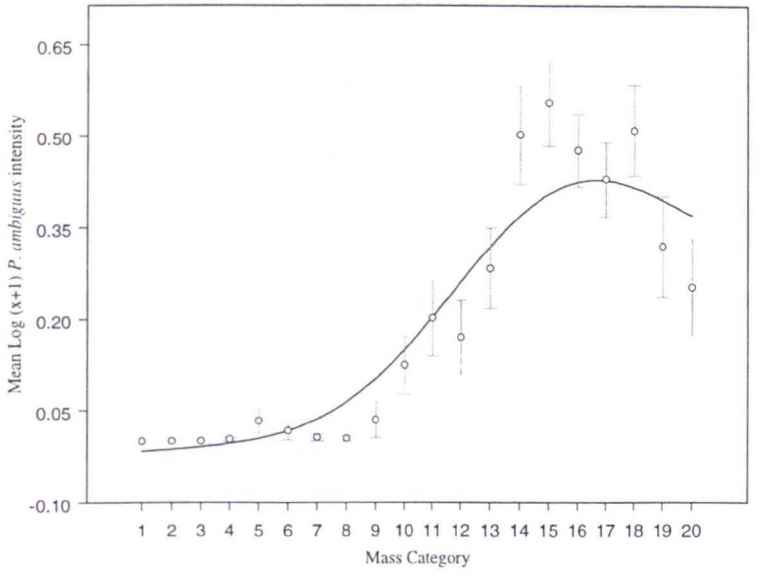



Figure 3 c) Spline fit of *Passalurus ambiguus* log (x+1) intensity regressed on host mass category, with SE bars.

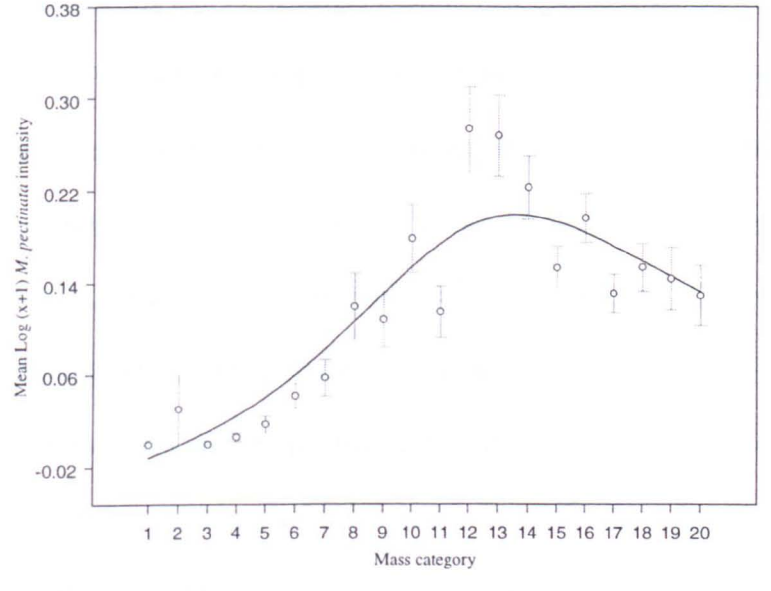



Figure 3 d) Spline fit of *Mosgovoyia pectinata* log (x+1) regressed on host mass category, with SE bars.

## Discussion

This study has established that there were statistically significant associations between the gut helminths and that the predicted effects are substantial, (figure 2) we now consider the most likely mechanisms underlying those interactions based on our understanding of the biology of the parasite species and the data. Where the same-locale or downstream interactions were observed, the parasites may be having a direct influence on one another via by-products, competition for a resource, or physical crowding. However, where upstream interactions occur, the most likely route is via the host immune system.

The lifetime reproductive success of most parasites is heavily influenced by the host's susceptibility to infection and the development of acquired immunity. Hence the putative explanation for the observed interactions between *G. strigosum* and *T. retortaeformis* is that there is some degree of cross immunity between the two species. Age-intensity curves for *T. retortaeformis* and *G. strigosum* support previous findings (Michel, 1952; Ford, 1971; Boag & Kolb, 1989) that *T. retortaeformis* stimulates an acquired immune response, since the fitted spline turns over (figure 3a; 3$^{rd}$ order GLM spline fit in GENSTAT $p<0.001$), whilst the *G. strigosum* age-intensity curve does not (Fig 3b; 3$^{rd}$ order GLM spline fit in GENSTAT $p<0.001$), indicating no, or little, immune response to the latter species. As *G. strigosum* appears to evoke little immune response, it must either be averting or suppressing the host immune system and the positive effect this species is predicted to have upon *T. retortaeformis* may well be a consequence of this reduced immune response. Conversely *T. retortaeformis* stimulates acquired immunity and this marked response may explain why the presence, rather than the intensity, of this species negatively affects *G. strigosum*. It is possible that the

presence of *T. retortaeformis* may 'switch on' the immune response, which *G. strigosum* is either suppressing or averting.

The two-way interaction of *P. ambiguus* and *M. pectinata* is affected in both directions by host sex (*P. ambiguus* = positive effect only in male hosts, *M. pectinata* = positive effect only in female hosts), once again implying mediation through host biology. Age-intensity curves of both *P. ambiguus* (figure 3c; 3rd order GLM spline fit in GENSTAT $p<0.001$) and *M. pectinata* (Fig 3d; 3rd order GLM spline fit in GENSTAT $p<0.001$) show declines in heavier (i.e. older rabbits) suggesting that both parasite species stimulate an immune response in the rabbit. The negative effect of *M. pectinata* on the upstream *G. strigosum* must be mediated through host biology, and most likely through host immunity. *Mosgovoyia pectinata*'s stimulation of the host's immune system may make it more difficult for *G. strigosum* to evade any non-specific immune response. In contrast to the other interactions, the mechanism behind the strong positive relationship between the cestode *C. denticulata* and the nematode *T. retortaeformis* is difficult to ascertain since they both inhabit the same location in the gut and therefore the interaction between them may be either direct or indirect. However, the relationship between *M. pectinata* and *G. strigosum* may suggest that indirect interaction through host immunity is also a likely mechanism for *C. denticulata* and *T. retortaeformis*. These analyses indicate both the potential importance of interspecific parasite interactions and the nature of how the immune response may shape the parasite community.

In addition to the inclusion in the model of factors that are usually used to explain away apparent interactions between parasites (e.g. season, vegetation class at warren, host food

type) the biology of the helminths offers further support that the interactions are genuine. There is, for example, no obvious reason why the directly transmitted oxyurid, *P. ambiguus*, whose sticky eggs are mainly transmitted by grooming and copraphagy, should be found in the same group of rabbits as *M. pectinata*, which is indirectly transmitted through the ingestion of the soil mite intermediate host. The use of GLM and REML analyses have revealed an array of potential interactions between parasites in this host species, which would not have been elucidated by less powerful techniques.

Since concomitant infections are the norm rather than the exception (Petney & Andrews, 1998) and given the increasing incidence of anthelmintic resistance amongst gut helminths (Cornell & Grenfell, 2000), alternative control strategies are required and a clearer understanding of parasite community ecology may provide such an alternative. One implication is that parasites could be used to control secondary infections. For example, artificial infection with one relatively benign species could be used to stimulate cross immunity and prevent subsequent infection with a deleterious species without resorting to anthelmintics. However, knowledge of the interactions between species is also essential as uninformed treatment against one deleterious species could allow an increase in the intensity of a second harmful species. These findings also highlight the importance of including such interactions in the dynamical studies of parasite communities where (with few exceptions (Dobson & Roberts, 1994) parasite interspecific conflicts and mutualisms are ignored in the null models.

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# CHAPTER 3

## Modelling the consequences of interspecific parasite interactions on parasite dynamics in a seasonally cycling system.

**J. Lello**, A. Fenton, R. Norman, B. Boag, & P.J. Hudson

**In preparation for submission to Journal of Animal Ecology**

# Chapter 3

## Modelling the consequences of interspecific parasite interactions on parasite dynamics in a seasonally cycling system

**Lello, J.,** Fenton, A., Norman, R., Boag, B. & Hudson, P. J.

### Abstract

Using a simple model of parasite dynamics in which the host population is not explicit in the model but is included only as an immune response term, we have examined the effects of seasonality and interspecific parasite interactions as mediated through immunity. The seasonal model is used, firstly to examine the consequences of interactions between season and immune mediated interspecific parasite interactions and secondly to examine the effects of parasite control in a dynamic system. These factors were examined using a five species parasite model based on data from a 23 year time series of wild rabbit gut helminths. We found that the interaction between seasonality, immunity and parasite uptake rates, all combine to produce complex dynamics. These dynamics can change not only the degree of interaction between the parasites but can potentially alter the seasonal cycles of the parasite species and therefore have implications for both the theoretical understanding of species interactions and also for parasite control programmes.

### Introduction

Multi-species models of parasite dynamics are rare and those that do exist have mainly utilised the basic form of the classic Anderson and May parasite models (Anderson & May, 1979; May & Anderson, 1979). Typically the only explicit form of parasite

interaction in these models is through an increase in host death rate, where combined parasite burdens lead to the death of the host (Anderson & May, 1979; May & Anderson, 1979). Using such model structure, workers have found that high levels of aggregation are necessary to attain coexistence, as the mechanism within the model promotes parasite species isolation in different hosts (Dobson, 1985; Dobson & Roberts, 1994; Pugliese, 2000). Whilst these models have been a great theoretical step forward, they fall short of reflecting the true biology of many parasite communities where concomitant infections are the norm (Christensen, Nansen, Fagbemi, *et al.*, 1987; Cox, 2001). Janovy *et al* (1990) did model interactions explicitly in a simulation model of community structure. However, the model was not designed to consider the dynamics of the parasite community and only considered parasite prevalence at fixed time points.

The models detailed above cannot be used in the forms developed to date, to describe interactions, where those interactions are more complex than a simple increase in host death rate. For this reason we have chosen to step away from the Anderson and May format and instead adopt a much simpler model formulation which is also well established in the literature (Woolhouse, 1992; Chan, Srividya, Norman, *et al.*, 1998; Norman, Chan, Srividya, *et al.*, 2000). This model form has a constant uptake rate, and there is no explicit modelling of the host but rather the model describes either parasites in a single host or the mean parasite burden in the average host.

Evidence from field data for the existence of interspecific parasite interactions, particularly in mammalian hosts, is equivocal (Dash, 1981; Bush & Holmes, 1986; Lotz & Font, 1991; Haukisalmi & Henttonen, 1993; Holmes & Bartoli, 1993; Forbes, Weatherhead & Bennett, 1994; Nilssen, Haugerud & Folstad, 1998; Forbes, Alisauskas, McLaughlin, *et al.*, 1999;

Behnke, Bajer, Sinski, *et al.*, 2001; Poulin, 2001; Dezfuli, Volponi, Beltrami, *et al.*, 2002). Temporal separation of parasites within hosts has been given as one explanation for a lack of observed interaction between parasites in natural systems, (Christensen, Nansen, Fagbemi, *et al.*, 1987; Haukisalmi & Henttonen, 1993). For example, Christensen *et al* (1987) states that even under laboratory conditions, inappropriate timing of infection may result in infections with two potentially interactive species developing independently of one another. Seasonal differences in parasite intensity peaks also reflect differences in order and timing of infection. Therefore seasonality may have major implications for the course of parasite interspecific interactions. Additionally in chapter 2 generalised linear models (GLM) and restricted maximum likelihood models (REML) were used to examine interspecific interactions amongst five gut helminths of the wild rabbit (*Oryctolagus cuniculus*), month (season) was shown to be a highly significant factor in all models. Clearly the interplay between the interspecific parasite interactions and the seasonal dynamics may be extremely important.

The aim of the present paper is to address two questions pertinent to parasite community ecology and to practical parasite control. First, how does seasonal forcing and / or differing seasonal peak numbers between parasite species, affect interspecific parasite interactions when they are mediated through host immunity? Secondly, what are the consequences of such effects upon the efficacy of different parasite control methods? For this latter question we model the rabbit gut helminth system and parameterise the model with intensity data (parasite numbers in infected hosts only) from a 23-year time series of gut helminths from a wild rabbit host population.

**Basic Model Structure**

The model in its simplest form consists of pairs of coupled differential equations, one for the adult parasites ($P_i$) of each species *i* [1], and one for the dynamics of the host immune response ($I_i$) to that species [2]. Note that the dynamics of the host population are not described explicitly and so we model the mean parasite burden of an 'average' host. Whilst macroparasite models generally involve a separate equation(s) for the transmission stage(s) of the parasite, this information is often lacking in the biological data. For this reason the model incorporates a simple constant uptake rate ($\Lambda_i$) in which parasite fecundity, larval survival and establishment within the host are all implicit within the term. The natural death rate of parasites within their host under field conditions is difficult if not impossible to assess. As the population dynamics of the rabbit gut helminths show seasonal cycles, we have assumed that their maximum life span is 1 year. Therefore, the value of the death rate (d) is set to produce an exponential decay to virtually zero by month 12 therefore the rate of change of the average parasite burden over time, for parasite i, is given, in the first instance, by:

$$dP_i/dt = \Lambda_i - d_i P_i \qquad [1]$$

Whilst parasite numbers are controlled by their natural death rate, they may also be affected by the host immunity. Immunity can act upon various stages of the parasite life cycle, i.e. fecundity or larval establishment (incorporated through $\Lambda_i$) or upon adult survival (incorporated in $d_i$). A separate immune response is created to each parasite. By varying the parameters this can be made to mimic either a specific antibody response or a broader based immune response, as will be discussed in more detail later. For each

parasite, immune response is produced at some rate ($\alpha_i$) per parasite and decays at some rate ($\delta_i$); setting $\delta_i$ to zero simulates life long immunity [2].

$$dI_i/dt = \alpha_i P_i - \delta_i I_i \qquad [2]$$

The immune response acts upon the parasite via an exponential term incorporated into the basic parasite model. This exponential term may be associated with the parasite uptake rate, the parasite death rate or both but in this study we have only allowed it to alter uptake rate [3]. The equation for the parasite burden now becomes

$$dP_i/dt = \Lambda_i e^{c_i I_i} - d_i P_i \qquad [3]$$

The strength of the effect of this immune response is determined by a constant ($c_i$).

Equation [3] models the mean number of parasites in an average host. In natural systems however, parasites often fluctuate in a seasonal cycle. Seasonality is included in our model via the addition of a simple sine wave function [4], which is again incorporated into the parasite uptake rate. Its inclusion on uptake rate is intuitively sensible, as parasite fecundity and larval survival (implicit in $\Lambda_i$) are the stages of the parasite life cycles that are most likely to be affected by seasonal variation. We now have:

$$dP_i/dt = \Lambda_i \left(1+\lambda_i \sin(2\pi((t+g_i)/\tau))\right) e^{c_i I} - d_i P_i \qquad [4]$$

In equation 4, $\lambda_i$ is the amplitude of the wave, t is the time step, $g_i$ allows us to determine when the peaks in species intensity occur (this can be different for the different parasite species) and $\tau$ is the wavelength. As the model time step is one month, $\tau$ is set at 12 to create an annual cycle.

We now incorporate interactions between species into the model. One of the main assumptions of the model is that interspecies interactions occur via host immune response. The reasoning behind the decision to incorporate interactions in this manner is two-fold. Firstly, laboratory investigations of parasite interaction, which have identified a mechanism, have mainly found that the interaction is mediated through host immunity (Christensen, Nansen, Fagbemi, *et al.*, 1987; Behnke, Bajer, Sinski, *et al.*, 2001; Cox, 2001); secondly in the latter part of this paper we describe the parameterisation of this model using a 23-year time series of five rabbit gut helminths, most, if not all of which, we believe to interact through host immunity (see chapter 2).

Parasite interspecific interaction is incorporated in the model by allowing the immune response produced by one parasite to have an effect upon other parasites [5], the strength of which is moderated by a constant $\gamma_{ij}$.

$$dP_i/dt = \Lambda_i (1+\lambda_i \sin(2\pi t/\tau)) e^{(c_i I_i + \gamma_{ij} I_j)} - d_i P_i \qquad [5]$$

If the value of $\gamma_{ij}$ (where $j$ is the code of the affecting species) is the same as the $c_i$ value for the parasite against which the immune response is produced, then the system is a closer mimic of a non-specific immune response. However, if the $\gamma_{ij}$ is lower than the appropriate

$c_i$ value then this more closely mimics a specific antibody response, where there is only partial cross reactivity with the second parasite species.

Clearly, in equation [5], where the immune response is incorporated on the uptake term, if $\gamma_{ij}$ is negative, the immune response of parasite *j* will have a negative effect upon parasite *i*. In some systems, such as rats and mice (which are frequently used in laboratory experiments) those model parameters relating to immunity, i.e. $\alpha_i$, $c_i$ and $\gamma_{ij}$, could be parameterised with real data on immune responses (Paterson & Viney, 2002). However, for most parasite-host interactions little, if anything, is known about the specifics of the immune response.

**The Effect of Seasonal Forces on Interspecific Interactions**

In order to examine the effect of seasonal forces on parasite interactions we parameterised a two species model where parasite 2 had an effect upon parasite 1 ($\gamma_{12}<0>\gamma_{12}$) parasite 1 had no effect on parasite 2 ($\gamma_{21}=0$). We first ran the model without the seasonal terms and determined the mean number of parasites (after the initial transient period) at a range of values for $\gamma_{12}$ (-2 to 2) and two values of $\delta$ (1.343 and 0.25; set the same for both parasites). We then added in the seasonality function and repeated the model run. Whilst there was a difference between the seasonal and non-seasonal models, this difference was small. At $\delta$=1.34 and $\gamma_{12}$=2, the difference between the mean number of parasite 1 in the two models was just 0.067 (which was the largest difference over the ranges of $\gamma_{12}$ and $\delta$ used).

When $\gamma$ was negative, the addition of the seasonal function always resulted in a reduction in the mean parasite numbers, but these differences tended also to be small. For example, the non-seasonal model means were $P_1$=0.54 and $P_2$=0.59; whilst the seasonal model

means were $P_1$=0.51 and $P_2$=0.56 (with parameter values: $\Lambda_i$=1, $c_i$=-0.05, $\gamma_{12}$=-0.25, $\lambda_i$=1, $\delta_i$=1.343, $\alpha_i$=1, $d_i$=1.343). Whilst seasonality did affect the mean values for both parasites, the difference between the means of the two parasites (before and after the addition of the seasonal function) is negligible. Therefore, seasonality alone appears to have little effect upon the strength of the parasite interaction.

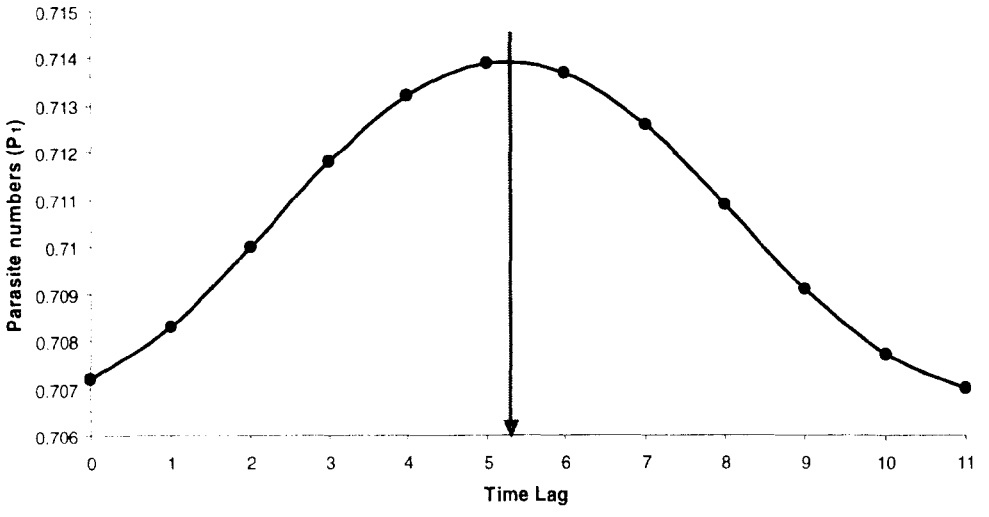
**Seasonal Asynchrony and Host Immune Response**

Intrinsically, seasonality may be of little consequence to interspecific parasite interactions, however differences in seasonal intensity / abundance peaks between parasite species may play a greater role. We might expect that when parasites are completely out of phase with one another there will be a much-reduced effect of $P_2$ on $P_1$. However, in a system where interaction is mediated through host immunity the effect of a time lag could be reduced or even negated if the immune response is long lived. For example if two parasites are six months out of phase but the immune decay takes several years, then the difference in timing of the intensity peaks might be irrelevant, as immunity from previous months would still be acting upon the parasites.

Model simulations have shown that at high immune decay rates (e.g. exponential decay within one month) the least effect of $P_2$ on $P_1$ was approximately the point at which the two parasites were most out of phase (6 months; figure 1a). However, closer inspection of figure 1a reveals that the peak of $P_1$ occurs slightly away from the midpoint of the seasonal lags. When the immune decay rate ($\delta$) was reduced (i.e. exponential decay in 5 years) the overall effect of $P_2$ on $P_1$ increased, because the total immunity level was then higher, as it was made up not only of immune response produced in that month but also in previous months. However, an additional and unexpected result also occurred. Figure 1b reveals that

at very low rates of immune decay, the point of least interaction between the parasites occurs when they are only partially out of phase.

a)




b)

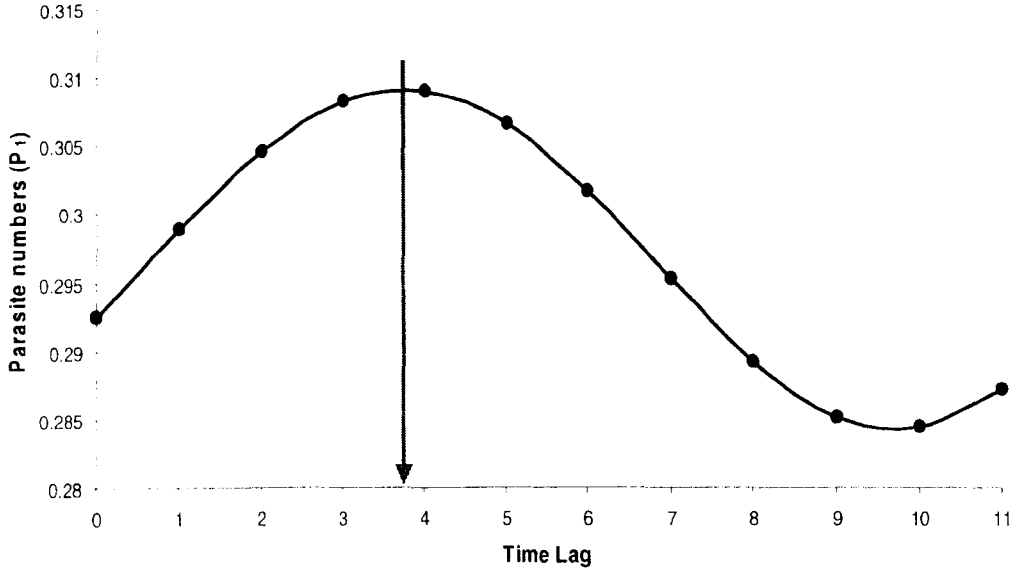



Figure 1. The effect of time lags between $P_1$ and $P_2$ (where $P_2$ is negatively affecting $P_1$) upon the numbers of $P_1$ with immune decay rates of, a) one month and b) five years. The grey arrow denotes the point of least effect of $P_2$ upon $P_1$.

The explanation for this phenomenon may be observed more clearly by examining the numbers of and immune response to parasite 2 (i.e. the species unaffected by the interspecific interaction). Figure 2a, reveals that when immune decay was rapid, the parasite and its immune response cycled in phase with one another. However, when immune decay rate was slower (figure 2b) the immune response to parasite 2 became shifted out of phase. Decreasing $\delta$ destabilised the dynamics of both parasite and immune response. Woolhouse (1992) used a form of the basic model presented here and found that changing values of parasite uptake rate ($\Lambda$) produced phase shifts in the age intensity curves of modelled parasites. We found that these phase shifts also occurred in our model when seasonality was removed, and that similar shifts occurred when any of the immune parameters were altered. However, the phase shifts are complicated by the seasonal function because no shift can be greater than 6 months, otherwise the form of the sine waves means that the shift will then be bringing the parasites back into phase with one another.

Table 1a shows the effect of changing $\delta$, whilst holding all other parameters constant, upon the month of the seasonal peak (with t+0 for both species) for $P_1$, $P_2$, $I_1$ and $I_2$. Table 1b shows the same effect when all parameters except $\Lambda$ are constant. Clearly such changes have significant implications for the strength of the interactions between parasites and consequently for the development of suitable control. For example if the combination of the model parameters results in the peak immune response to $P_2$ occurring 3 months after the peak intensity of $P_2$ and $P_1$ intensity also peaks 3 months after $P_2$ then the action of $P_2$ on $P_1$ will be greater than if the two parasites were cycling in phase.

Table 1a. The effect of changing values of $\delta$ upon the month in which $P_1$, $P_2$, $I_1$ and $I_2$ reach peak values, where $P_1$ has a negative effect upon $P_2$. Parameter values are: $\Lambda$=1, c=-0.05, $\lambda$= , $\alpha$=1, d=1.343, (values apply to both parasites) and $\gamma_{1,2}$=-0.25.

| | | Month in which parasite/immune values peak | | | |
|---|---|---|---|---|---|
| $\delta$ | **Decay Period** | $P_1$ | $P_2$ | $I_1$ | $I_2$ |
| 0.00 | Life long immunity | 4 | 4 | 12 | 12 |
| 0.01 | 134.3years | 4 | 4 | 7 | 7 |
| 0.10 | 13.4 years | 3 | 3 | 6 | 6 |
| 0.20 | 6.7 years | 2 | 3 | 5 | 5 |
| 0.50 | 2.7 years | 2 | 3 | 4 | 5 |
| 0.60 | 2.2 years | 3 | 3 | 4 | 5 |
| 0.70 | 1.9 years | 3 | 3 | 4 | 4 |
| 3.00 | 5.3 months | 3 | 4 | 4 | 4 |
| 3.50 | 4.6 months | 4 | 4 | 4 | 4 |
| 50.0 | 0.3 months | 4 | 4 | 4 | 4 |

Table 1b. The effect of changing values of $\Lambda$ upon the month in which $P_1$, $P_2$, $I_1$ and $I_2$ reach peak values, where $P_1$ has a negative effect upon $P_2$. Parameter values are: $\delta$=0.5, c=-0.05, $\lambda$=1, $\alpha$=1, d=1.343, (values apply to both parasites) and $\gamma_{1,2}$=-0.25.

| | Month in which parasite/immune values peak | | | |
|---|---|---|---|---|
| $\Lambda$ | $P_1$ | $P_2$ | $I_1$ | $I_2$ |
| 0.01 | 4 | 4 | 5 | 5 |
| 0.50 | 3 | 3 | 5 | 5 |
| 1.00 | 2 | 3 | 4 | 5 |
| 1.50 | 2 | 3 | 4 | 4 |
| 2.00 | 2 | 2 | 4 | 4 |
| 5.00 | 1 | 2 | 4 | 4 |
| 10.0 | 1 | 1 | 4 | 4 |
| 20.0 | 12 | 1 | 4 | 4 |
| 30.5 | 12 | 12 | 4 | 4 |
| 50.0 | 12 | 12 | 4 | 4 |
| 100.0 | 11 | 12 | 4 | 4 |
| 250.0 | 11 | 11 | 4 | 4 |
| 1000 | 11 | 11 | 4 | 4 |

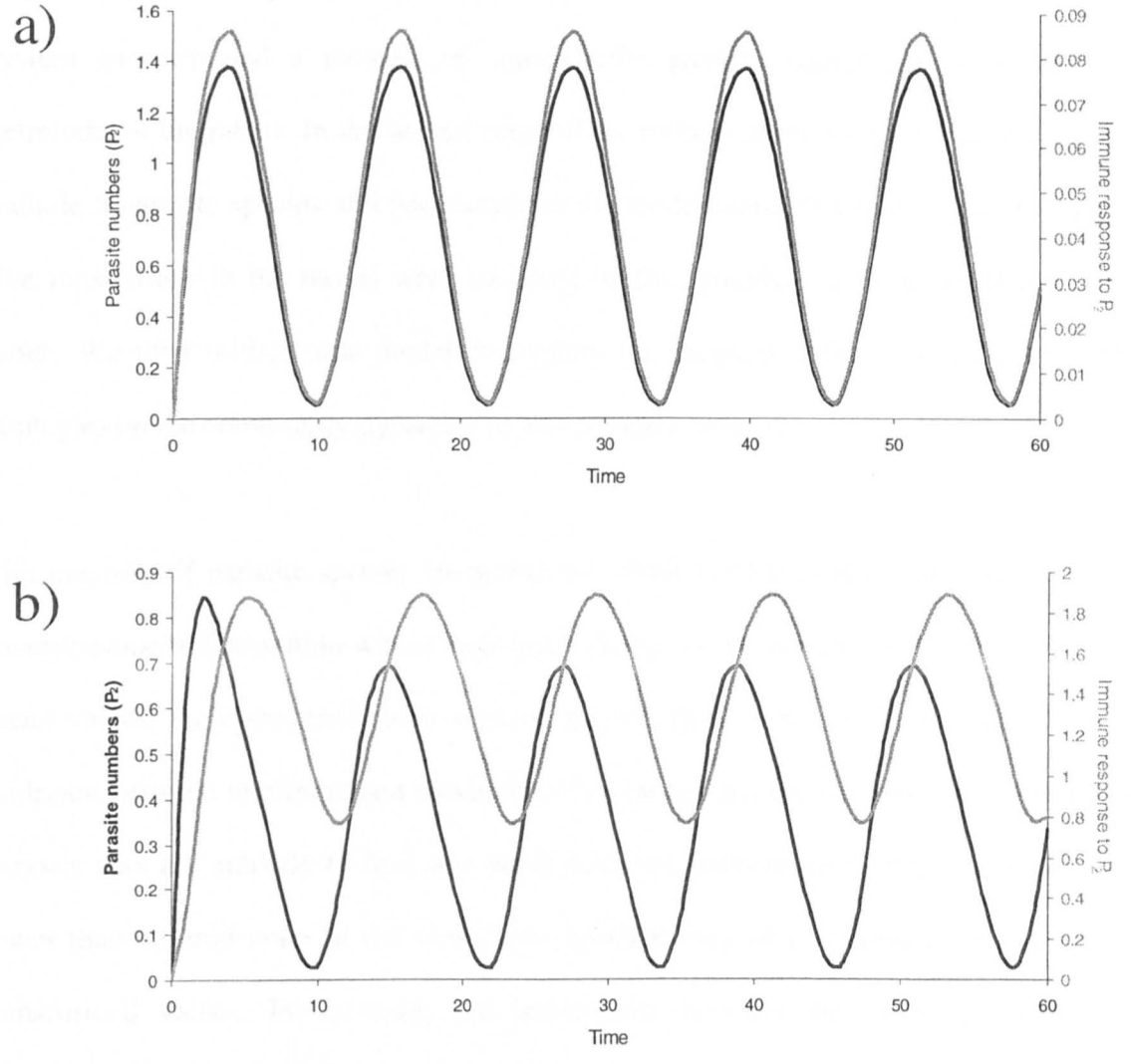



Figure 2. The effect of immune decay rates on timing of immunity. In a) with 1 month immune decay, parasite and immune response cycle in phase. In b) with 5 year immune decay, the immune response cycles out of phase with the parasite. Black line denotes parasite numbers and grey line denotes immune response.

## Applying the Model to a Specific Parasite Community

The rabbit (*Oryctolagus cuniculus*) is an ideal model host species for grazers of economic importance, and sheep in particular. In chapter 2 we used the 23 year time series of this system and revealed a network of interspecific parasite interactions between 5 gut helminths of the rabbit. In the second stage of the present study we extended the model to include 5 parasite species and parameterised the model using this rabbit parasite data set. The interactions in the model were informed by the statistical analyses from this earlier work. We then utilised this model to explore the potential effects of different control strategies on the community dynamics of this complex system.

The majority of parasite species, including the rabbit gut helminths, show an aggregated (overdispersed) distribution within their hosts (Boag, Lello, Fenton, *et al.*, 2001) and their mean values, therefore, tend to be skewed towards their minimum value rather than the midpoint between minimum and maximum. This means that the seasonal cycles of the raw parasite data are unlikely to fit a sine wave since the parasite mean will tend to be much lower than the mid point of the wave. This problem may be overcome by using the log-transformed values. Furthermore, the interactions between the rabbit gut helminths, revealed in chapter 2, were elucidated using the log intensities (i.e. the log numbers of parasites in infected animals only) of the parasites. We therefore parameterised this model with the log intensity data of the five parasites in infected hosts only. The five helminths were numbered as 1=*Graphidium strigosum*, 2=*Trichostrongylus retortaeformis*, 3=*Mosgovoyia pectinata*, 4=*Cittotaenia denticulata* and 5=*Passalurus ambiguus*. Species 1 and 2 are strongylid nematodes, 3 and 4 are cestodes and 5 is an oxyurid nematode. Their relative positions in the rabbit gut and the direction and form of interactions between them are shown in figure 3.

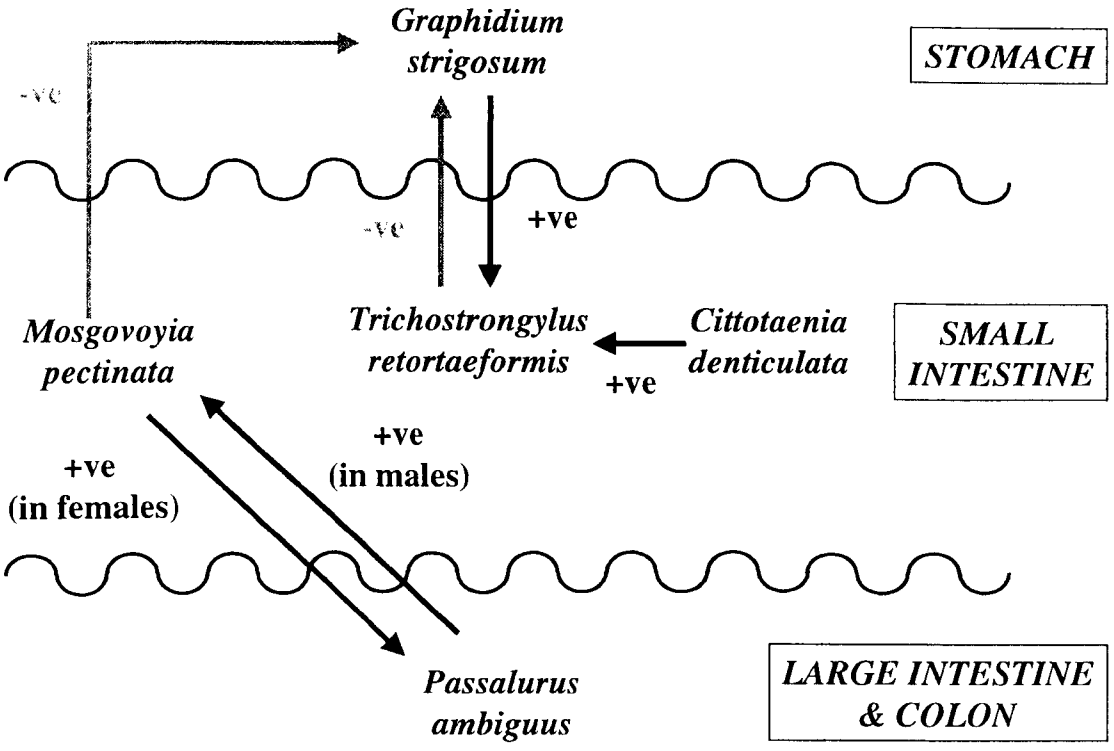



Figure 3. Interactions between the gut helminths of the wild rabbit as determined by generalised linear models and restricted maximum likelihood models (chapter 2).

In this study the parameterisation of the model took a very simple form, the minimum and maximum values for each parasite are calculated from the log intensity data. For this purpose, a separate subset of the data was taken for each parasite, where any interacting parasites were removed from the data set (e.g. for *G. strigosum* the rabbits positive for *T. retortaeformis* and *M. pectinata* were taken out), all zero values were removed, and the data was log transformed. This subset of the data was then used to calculate the bounds of the data (i.e. minima and maxima) within the upper and lower 10% quantiles, as calculated by bootstrapping (1000 iterations). The model was parameterised by varying the $\Lambda_i$ and $\lambda_i$ (where *i* is the code of the parasite species) whilst maintaining $\alpha_i$ and $c_i$ at given constants,

until the maxima and minima values matched the values calculated from the real data. The next step was to recalculate the upper and lower 10% quantiles for each parasite, with each interacting parasite (separately) added back to the data set. The value of $\gamma_{ij}$ (where *j* is the code for the interacting species) was then varied until the maxima for each parasite (with the exception of *P. ambiguus* where the minima was used, as the maxima does not change in the real data) matched the real data. Once each individual interaction had been calculated the $\gamma_{ij}$ value for each parasite interaction was multiplied by the prevalence (p) of parasite *j* to give the overall effect of that species in the whole data set (table 2).

To assess the model fit, the upper and lower 10% quantiles were then calculated as before but for the whole data set (no zeros) and these data were compared with the final model predictions (table 3). It can be seen that the model with interactions was a reasonable match to the real data except that it overestimated the upper bound of *M. pectinata* and, to a smaller extent, that of *P. ambiguus* and the lower bound of *T. retortaeformis*. This is a flaw in the model's ability to alter the amplitude of the parasite cycles. Shortfalls between the real data and the model output might also be due to interactions between parasites that were not established in the previous statistical analyses of the rabbit data. The strict criteria used in those analyses might have excluded some interactions which were genuine but for which the statistical power was not great enough to consider them significant. Despite these shortfalls, we consider the model a reasonable fit to the data and hence useful for the purpose of examining the relative merits of parasitic disease treatment programmes.

Table 2. Full parameter list for the five species model of the rabbit gut helminth system. Values in brackets for γ, represent the code number of the interacting species.

| | *G. strigosum* | *T. retortaeformis* | *M. pectinata* | *C. denticulata* | *P. ambiguus* |
|---|---|---|---|---|---|
| Λ | 1.838 | 3.23 | 0.84 | 0.899 | 3.46 |
| λ | 0.568 | 0.41 | 0.55 | 0.6 | .52 |
| c | 0 | .41 | -0.05 | -0.05 | -0.05 |
| g | +2 | -0.05 | -8 | -4 | -8 |
| d | 1.343 | -6 | 1.343 | 1.343 | 1.343 |
| α | 1 | 1.343 | 1 | 1 | 1 |
| δ | 1.343 | 1 | 1.343 | 1.343 | 1.343 |
| γ | (2) -0.0235<br>(3) -0.8740 | (1) 0.1350<br>(4) 0.1080 | (5) 0.0820 | | (4) 0.3200 |
| p | 0.47 | 0.78 | 0.22 | 0.18 | 0.12 |

Table 3. Model predictions and bootstrapped upper and lower 10% quantiles of the actual parasite species' intensity (in infected animals only) data, for the five common gut helminths of the wild rabbit.

| | **Actual** | | **Model** | |
|---|---|---|---|---|
| **Species** | **Minimum** | **Maximum** | **Minimum** | **maximum** |
| *G. strigosum* | 0.6 | 1.9 | 0.6 | 1.8 |
| *T. retortaeformis* | 2.0 | 3.0 | 1.5 | 3.1 |
| *M. pectinata* | 0.3 | 0.7 | 0.3 | 0.9 |
| *C. denticulata* | 0.3 | 1.0 | 0.3 | 1.0 |
| *P. ambiguus* | 1.4 | 3.4 | 1.3 | 3.5 |

## Exploring Treatment Strategies

Here we examine two possible treatment methods. In the first instance the model was made to mimic a once yearly application of an anticestodal drug, timed to have the greatest effect upon the sum of the *C. denticulata* numbers over a 24 month period. In the second case a biennial vaccination against this single species was considered, again timed to have the greatest effect upon the sum of the *C. denticulata* numbers over the 24 month period. Different intervals between treatments for the anticestodal and the vaccination were

employed as the vaccination effect remains in the system for a longer period. In the anticestodal treatment, both *C. denticulata* and *M. pectinata* were reduced to zero every year at month seven.

In the case of the vaccination, only *C. denticulata* is affected, the vaccination being delivered every second year in month four. Vaccination programmes aim to increase the immune response against a parasite and also to decrease the immune decay rate (i.e. decrease $\delta$). For this reason, in the vaccination model the immune response to the vaccination for *C. denticulata* was modelled as being ten times higher than the response against the parasite itself and the immune decay rate was slowed to 10 years. Nevertheless, it can be noted from figure 4 that the vast majority of this response has decayed by the second year, which is the reason for the biennial vaccinations. The difference in the best time to treat, between the anticestodal and vaccination models, is probably due to the peak shifts, as observed in the simple models when immune decay rate was altered.

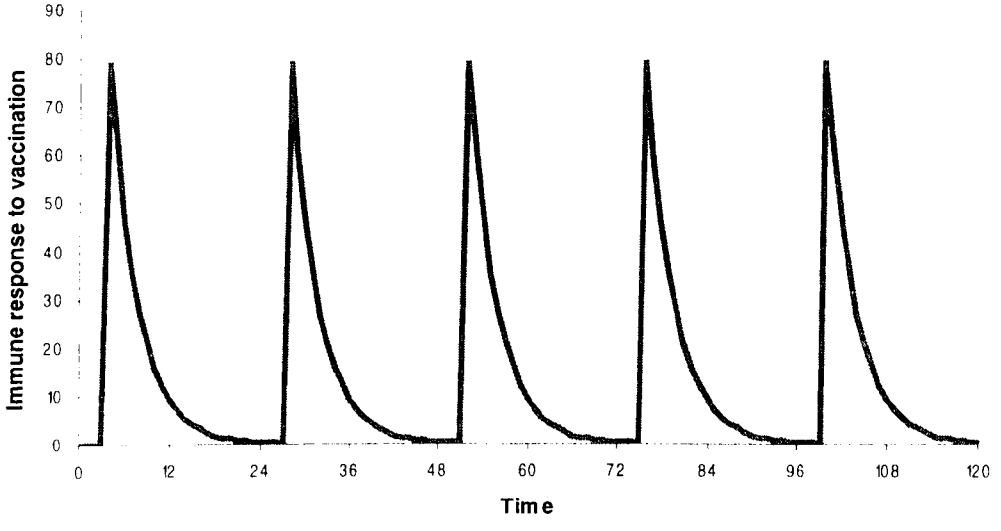



Figure 4. Immune response to vaccination with vaccination administered biennially in the fourth month. Immune decay tending to zero by the time of each administration.

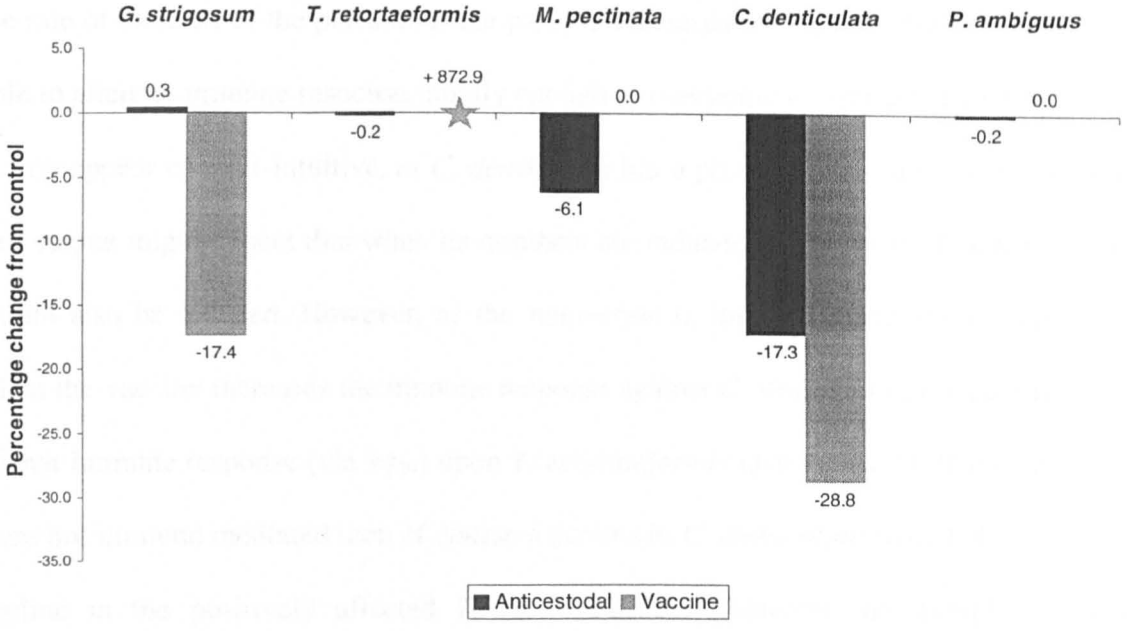



Figure 5. The effect of anticestodal treatment and vaccination upon the predicted mean log intensities (zeros removed) of the five gut helminths. Bars represent the percentage change from the null model. As the *T. retortaeformis* mean with the vaccination treatment is extremely high, its bar is represented by a grey star.

The 24 month, cumulative parasite totals revealed that both treatments had wider reaching effects than simply a reduction in numbers of the treated species (figure 5). However, it is clear that the vaccination had rather more substantial secondary effects (upon others species) than did the anticestodal. This makes sense, as the interspecific interactions in the model are mediated through immunity, and so increasing the immune response to one parasite has a proportional effect upon other interacting species. An important thing to note in this example is that *T. retortaeformis* numbers are extremely high suggesting that a vaccination of this form would be likely to result in a rise in *T. retortaeformis* numbers that would cause serious health problems for the rabbit. This would either result in a strong immune response against *T. retortaeformis*, akin to the vaccination, or alternatively in the death of the host. Which result occurs will depend both upon the parasite species and upon

the rate of increase of the parasite. If the parasite increases too rapidly, the host may not be able to elicit an immune response rapidly enough to overcome it. This positive effect might at first appear counter-intuitive, as *C. denticulata* has a positive effect on *T. retortaeformis* and so one might expect that when its numbers are reduced the effect on *T. retortaeformis* would also be reduced. However, as the interaction is immune mediated and not direct, when the vaccine increases the immune response against *C. denticulata*, the positive effect of that immune response (via $+\gamma_{24}$) upon *T. retortaeformis* also increases. If the interaction were not immune mediated then of course a decline in *C. denticulata* would also result in a decline in the positively affected *T. retortaeformis*. Therefore the interplay between parasite interactions, host immunity and treatment strategy can be complex, leading to non-intuitive and potentially harmful results.

**Discussion**

In chapter 2 we postulated the direction and quantified the degree of effect of the interspecific parasite interactions in this parasite system. These findings have major implications for both animal husbandry and for human health issues as the design of parasitic disease treatment programs have, to date, taken little account of the potential effects of interactions in parasite communities. In the example we have presented in this study, vaccination against a relatively benign species, *C. denticulata*, was shown to have severe consequences in terms of a rise in numbers of *T. retortaeformis*, a species considered to be detrimental to rabbit health (Boag, Neilson, Robinson, *et al.*, 1998). However, if *C. denticulata* had negatively affected *T. retortaeformis*, then a vaccination of *C. denticulata* could potentially have reduced *T. retortaeformis* numbers without any serious harm to the rabbit. Therefore, both the sign of the interaction and the type of

interaction, i.e. whether it is direct or mediated via host immunity, should be a critical consideration in any treatment program.

The interplay between the seasonal forcing and the immune mediated interactions is also important. The peak shifts resulting from various parameter changes could force parasites, which would normally cycle with one another, to be pushed apart effectively producing completely different seasonal cycles for each parasite. An interesting next step would be to examine the seasonal dynamics of parasites with similar life history traits, to see if they differ when both the species are in the system together and when one or other is not. A shift in the seasonal peaks of one in the presence of another could be an indication of interspecific interactions. Finally the shift of immunity out of phase with the parasite to which it is responding is important because of the implication to any interactions with out of phase species, as discussed earlier.

This study has made three main assumptions, that interaction is via immunity and that the immune function and the seasonal sine wave function are reasonable and realistic representations of what is occurring in nature. In view of the important implications of this study, future work will be require to assess how robust the model predictions are to different immune and seasonal functions. Additionally, studies to ascertain the mechanism of immune function and to estimate specific parameter values for the immune system, would be a sensible next step.

These findings in conjunction with our previous paper suggest that workers, whether they be theoreticians or practitioners, need to be careful to establish whether interactions are

important in their study systems and to consider what effect manipulating species might have upon the population dynamics of other species within that community.

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# CHAPTER 4

## Evidence of parasite induced effects on the condition and fecundity of the wild rabbit, *Oryctolagus cuniculus*

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**In preparation for submission to International Journal for parasitology**

# Chapter 4

**Evidence of parasite induced effects on the condition and fecundity of the wild rabbit, *Oryctolagus cuniculus***

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## ABSTRACT

The rabbit is a good parasite-host model for domesticated grazing species since it harbours a similar gut helminth community, particularly to that found in sheep and goats. Few studies have shown evidence of the natural effect of parasites on wild vertebrate hosts and it is also difficult to obtain evidence for domesticated animals in natural situations. Here we examined the potential for five gut helminths of the rabbit, the viral disease myxomatosis and the coccidian parasite *Eimeria stiedae* to alter the condition and fecundity of their host. Adult host body mass was negatively associated with the number of gut helminth species harboured and by the presence of both *E. stiedae* and myxomatosis. Additionally, both myxomatosis and the number of gut helminths species were predicted to have a negative affect upon the mass of abdominal fat. No parasites were found to be associated with a reduction in the number of foetuses in pregnant rabbits, but the strongylid nematode, *Graphidium strigosum* had a strong positive association with ovary mass, and the hypotheses which may explain this counterintuitive finding are discussed. Two cestodes, *Mosgovoyia pectinata* and *Cittotaenia denticulata* were both associated (negatively and positively, respectively) with testes mass. The potential consequences of this for the social status of rabbits are also considered.

## INTRODUCTION

The potential for both macro- and micro-parasites to regulate their host population, through a density dependent reduction in host survival and / or fecundity is an important component in the theoretical models of Anderson & May (1978) and May & Anderson (1978). Models using this form require that the parasite population grow faster than the host population. If the parasites are Poisson distributed, this regulation is unstable (May & Anderson, 1979; Hudson, Dobson, Cattadori, *et al.*, 2002). Stable regulation requires aggregation of the parasite within the host population, although if the aggregation becomes too high, the parasites fail to control the host population, as too many parasites are lost when infected hosts die. These models and subsequent advances (Scott & Dobson, 1989; Dobson & Hudson, 1992; Grenfell & Gulland, 1995; Greenman & Hudson, 1999) revealed that parasite affects upon host fecundity could have major, and often destabilising, affects upon host population dynamics.

A first step in determining whether a parasite species has the capability of regulating its host population is to determine whether the parasite species exerts any density dependent detrimental effects upon the host (Scott & Dobson, 1989). The clearest evidence for the regulation of a wild host population by a parasite comes from detailed experimental manipulations of red grouse parasites (Hudson, 1986; Hudson, Dobson & Newborn, 1992). In these studies, the experimental reduction of natural *Trichostrongylus tenuis* infections with an anthelmintic, produced a strong and significant increase in the fecundity of the grouse (Hudson, Newborn & Dobson, 1992). Further work on the grouse system also revealed that parasites could have indirect effects upon survival, by making the infected host more vulnerable to predation (Hudson, Dobson & Newborn, 1992). In another study, in which groups of wild

Svalbard reindeer were treated with anthelmintics, both pregnancy and back fat depth were seen to be negatively affected by the gut helminths (Albon, Stien, Irvine, *et al.*, 2002; Stien, Irvine, Ropstad, *et al.*, 2002). Murray, Cary & Keith (1997) and Ives & Murray (1997), also found that there were interactions between parasitism, predation and food quality that could lead to population regulation of snowshoe hare hosts. The grouse and hare systems differ in that the parasites alone were not believed to cause significant mortality of hares. Another environment-parasite interactions is found in the Soay sheep system. Gulland & Fox (1992) and Grenfell & Gulland (1995) described how the interaction between parasites, climate and forage destabilised the Soay sheep populations on St Kilda.

In this study we examined the relationship between seven species of parasites that inhabit wild rabbits and estimates of host health and fecundity. Further, we discuss what the consequences of these effects could mean in terms of host population regulation. We concentrate upon the effects of the five most common gut helminths of the rabbit in the UK. These five species include two strongylid nematodes, *Graphidium strigosum* (stomach) and *Trichostrongylus retortaeformis* (small intestine), one oxyurid nematode, *Passalurus ambiguus* (caecum and colon) and two cestodes, *Mosgovoyia pectinata* and *Cittotaenia denticulata* (both in the small intestine). Following the snowshoe hare system we postulated that these helminths tend to have sublethal effects, which may reduce condition and thereby influence host survival and fecundity.

In addition to the helminths, we also considered two microparasites: the virus myxomatosis and the coccidian parasite *Eimeria stiedae*. Previous work has revealed the effects of *E. stiedae* on mortality but suggested that these effects were generally

restricted to young rabbits (Gomez-Bautista, Rojo-Vazquez & Alunda, 1987; Hobbs & Twigg, 1998). Similarly myxomatosis has been shown to cause major mortality in rabbit populations (Barnes & Tapper, 1986; Ross & Tittensor, 1986; Fenner & Fantini, 1999). However, with the attenuation of virus strains and the build up of genetic resistance in rabbit populations, it is believed that the majority of mortality is now found amongst young rabbits (Villafuerte & Vinuela, 1999). Nevertheless adult rabbits are found with the disease and whilst many animals recover from the infection, they are seriously weakened whilst the infection takes its course (Fenner & Fantini, 1999). In this study there was no evidence of infection with the other significant viral disease of rabbits, viral haemorrhagic disease (RHD), in any of the rabbits collected during the course of the study (N. Forrester, pers. comm.).

To examine the host-parasite relationship, we utilised a 26 year time series of rabbits and their parasite fauna from a single site in Scotland. These data are unusual in several respects; first, the length of the time series; second, the regularity and frequency of collections; third, the volume of data from a single site and finally, the quality of the data collected. The data included full helminth counts, details of the host biology and details of the hosts' environment.

## METHODS

The 23 year time series of rabbits collected from a 400 ha site in Perthshire, Scotland (ordnance grid reference NO 280 340) between the months of January 1977 and December 1999, is described elsewhere (Boag, Lello, Fenton, *et al.*, 2001; chapter 2 and chapter 3). Here the data set was extended to include a further three years from 2000 to 2003. Post mortems were conducted on all rabbits and the rabbits' sex and total body

mass were recorded in all instances. Additionally mass of abdominal fat, ovary mass and testes mass, were recorded from a subset of the data. Full helminth counts were carried out on all rabbits and the animal was assessed for clinical signs of myxomatosis and *E. stiedae* (scored as present if ρ5 liver spots). Further details of the data and collection protocols may be found in (Boag, Lello, Fenton, *et al.*, 2001).

Mass is a good proxy for age in the rabbit (Cowan, 1983). Only adult rabbits (>1249g, i.e. approx. 130 days; n=1526) were used for this analysis since they were likely to have been exposed to infective stages of all parasites and were also available to breed. Additionally, previous work on the gut helminths of the rabbit showed that these parasites, in different rabbit age classes (kitten, juvenile and adult), have very different distributions and would need to be analysed separately (Boag, Lello, Fenton, *et al.*, 2001).

We used a combination of generalised linear modelling (GLM) and residual maximum likelihood (REML) linear mixed model analyses of parasite intensity data, to test the null hypothesis that parasites do not influence host condition or fecundity measures. For the rabbit mass and abdominal fat analyses, an index of parasitism, rather than individual species intensities or presence, were included as a factor in the analyses. The histograms of the data for host total body mass and for abdominal fat showed a slightly skewed distribution and so these data were normalised by log transformation (log (x) for total body mass and log (x+1) in the case of abdominal fat) prior to analysis. All data predictions from the models are presented as the back-transformed values.

In the fecundity analyses either the log (x+1) intensity or species presence were included in the model while host body mass was included as a covariate. The decision to use either a parasites' log (x+1) intensity or its presence was assessed with submodels. Aspects of both host biology and the external environment were also included in all analyses and the details of all terms in the initial models and their interactions are described in table 1.

***Measures of Host Health and Fecundity***

While examining the relationship between parasite intensity (and prevalence) with mass we also examined the relationship between age and parasite intensity. Since mass is used as an estimate of age there is a confounding age-mass relationship in any regression of host mass on parasite intensity or prevalence. We reduced the effect of this confounding relationship by using an index of parasitism (species richness), for the gut helminths, rather than the individual species' intensity or prevalence, since this measure is less age dependent, (median=2 for all adult mass categories).

Mass of abdominal fat has been used as an estimate of recent host condition in previous studies (Martin, 1977) and as such is likely to be a better gauge of the effect of the current parasite burden than total host mass (host mass was initially included as a covariate in this model).

Table 1: Terms initially included in the host health and fecundity models. (v denotes variables and f denotes a factor). All reasonable interactions were included and were only excluded if insufficient information was available, e.g. a month * vegetation class interaction would have resulted in many missing interaction levels.

| Host Health / Fecundity Measure | Dependent variable | Independent variables / factors |
|---|---|---|
| Total Rabbit Mass | Log transformed rabbit mass | • Month (f)<br>• Direction of burrow entrance (f)<br>• Vegetation type around burrow (f)<br>• Vegetation type which rabbits eat (f)<br>• Sex of the host (f)<br>• Presence / absence of myxomatosis (f)<br>• Presence / absence of *E. stiedae* (f)<br>• Index of parasitism (number of gut helminths) (f)<br>• Month * Host Sex<br>• Month * Index of parasitism<br>• Host Sex * Presence / absence of myxomatosis<br>• Host Sex * Presence / absence of *E. stiedae*<br>• Host Sex * Index of parasitism |
| Mass of Abdominal Fat | Log transformed abdominal fat | • Month (f)<br>• Direction of burrow entrance (f)<br>• Vegetation type around burrow (f)<br>• Vegetation type which rabbits eat (f)<br>• Sex of the host (f)<br>• Host total body mass (v)<br>• Presence / absence of myxomatosis (f)<br>• Index of parasitism (number of gut helminths) (f)<br>• Month * Host Sex<br>• Month * Host total body mass<br>• Month * Index of parasitism<br>• Host Sex * Host total body mass<br>• Host Sex * Presence / absence of myxomatosis<br>• Host Sex * Index of parasitism<br>• Host total body mass * Presence / absence of myxomatosis<br>• Host total body mass * Index of parasitism |

Table 1 (cont)

| | | |
|---|---|---|
| Foetal number in utero | Number of foetuses (no zeros) from March to June. No transformation | • Month (f)<br>• Direction of burrow entrance (f)<br>• Vegetation type around burrow (f)<br>• Vegetation type which rabbits eat (f)<br>• Host total body mass (v)<br>• Lactating / non-lactating (f)<br>• Log (x+1) *G. strigosum* (v)<br>• Log (x+1) *T. retortaeformis* (v)<br>• Log (x+1) *P. ambiguus* (v)<br>• Log (x+1) *M. pectinata* (v)<br>• Presence / absence of *C. denticulata* (f)<br>• Month * Host total body mass<br>• Month * Lactating / non-lactating<br>• Month. * Log (x+1) *G. strigosum*<br>• Month * Log (x+1) *T. retortaeformis*<br>• Month * Log (x+1) *P. ambiguus*<br>• Month * Log (x+1) *M. pectinata*<br>• Host total body mass * Lactating / non-lactating<br>• Host total body mass * Log (x+1) *G. strigosum*<br>• Host total body mass * Log (x+1) *T. retortaeformis*<br>• Host total body mass * Log (x+1) *P. ambiguus*<br>• Host total body mass * Log (x+1) *M. pectinata* |
| Ovary mass | Untransformed combined mass of both ovaries | • Month (f)<br>• Host total body mass (v)<br>• Lactating / non-lactating (f)<br>• Number of foetuses in utero (v)<br>• Log (x+1) *G. strigosum* (v)<br>• Log (x+1) *T. retortaeformis* (v)<br>• Presence / absence of *P. ambiguus* (f)<br>• Presence / absence of *M. pectinata* (f)<br>• Log (x+1) *C. denticulata* (v)<br>• Host total body mass * Number of foetuses in utero<br>• Host total body mass * Lactating / non-lactating<br>• Host total body mass * Log (x+1) *G. strigosum*<br>• Host total body mass * Log (x+1) *T. retortaeformis*<br>• Host total body mass * Presence / absence of *P. ambiguus*<br>• Host total body mass * Presence / absence of *M. pectinata*<br>• Host total body mass * Log (x+1) *C. denticulata* |
| Testes mass | Untransformed combined mass of both testes | • Month (f)<br>• Host total body mass (v)<br>• Log (x+1) *G. strigosum* (v)<br>• Presence / absence of *T. retortaeformis* (f)<br>• Log (x+1) *P. ambiguus* (v)<br>• Log (x+1) *M. pectinata* (v)<br>• Presence / absence of *C. denticulata* (f)<br>• Host total body mass * Log (x+1) *G. strigosum*<br>• Presence / absence of *T. retortaeformis*<br>• Host total body mass * Log (x+1) *P. ambiguus*<br>• Host total body mass * Log (x+1) *M. pectinata*<br>• Host total body * Presence / absence of *C. denticulata* |

***Host Fecundity***

Foetal number is a direct measure of current host fecundity but provides no direct measure of lifetime reproductive effort. Examining the number of foetuses in rabbits that are pregnant, during the main reproductive months of March through June, minimises the degree of variation in the data set and therefore enables a clearer assessment of the potential effects of the parasite species upon their hosts' fecundity. Ovary mass provides an indirect measure of fecundity since mass is positively associated with higher numbers of follicles (Giuliano, Lutz & Patino, 1996; Hasumi, 1996; Radovanovic, StosicBogdanovic & Gledic, 1997). Furthermore, we have evidence from our data that larger rabbit ovaries are associated with larger follicles (B. Boag, pers. comm.) and previous work has shown that larger follicles result in larger young, faster growth leading to a earlier asymptote of mass at puberty (Fox, 1974).

Testes mass may also be positively associated with fecundity since large testes are generally linked with high levels of testosterone and high production of sperm (Stockley, Searle, Macdonald, *et al.*, 1996; Moreira, Macdonald & Clarke, 1997; Brodowski, Jewgenow, Pielowski, *et al.*, 2001; Byrne, Roberts & Simmons, 2002; Coker, McKinney, Hays, *et al.*, 2002). High testosterone and sperm production have, in turn, been associated with more dominant animals and with a greater success in sperm competition. Moreover, the more dominant bucks in our sample population tended to have larger testes than their subordinates (Boag, pers. comm.).

## RESULTS

The possible effects of the parasites upon adult rabbit condition and fecundity were considered in terms of the parasite associations with host body mass, mass of abdominal fat, foetal numbers and ovary and testes mass. The minimal models for each dependent variable are presented in table 2. As all the data were correlational, we cannot state with absolute certainty whether the parasites are truly affecting the dependent variables (i.e. the measures of host condition and fecundity) or whether the converse is true. However, running the models with the parasites as the dependent variables with the measures of host fecundity as independent variables/factors, tended to produce predicted values outside of the bounds of the natural data, or the models proved unstable. Moreover, in a number of the examples we have further reasons to believe that the models are correct in predicting genuine affects of the parasites on their host and these will be discussed individually later.

### *Rabbit body mass*

The minimal model for total host body mass (table 2a) revealed that the presence of, myxomatosis, *E. stiedae* and a high index of parasitism (species richness), were all predicted to have a negative association with rabbit body mass. Myxomatosis was associated with body mass in interaction with host sex, such that the model predicted a negative effect in females but no significant effect in males (figure 1). However, even in female rabbits the effect of myxomatosis was predicted to be quite small, causing only a 4% (64.6g) reduction in mean body mass. The predicted effect of coccidiosis (figure 2) was only slightly larger and resulted in a reduction in body mass of approximately 6% (88.6g). Finally hosts that harboured between 1 and 3 species of parasite were predicted

to have 6% (94.0g) less mass and between 4 and 5 species 9.4% (147.0g) less, than species harbouring no parasites (figure 3).

***Abdominal Fat***

In a subset of individuals we also recorded (n=159; years 1988-89 & 200-02) the mass of the rabbits' abdominal fat. Both the presence of myxomatosis and the number of parasite species harboured by the host had negative associations with abdominal fat. Mass of the rabbit was also included in the models as a covariate (table 2b). In this subset of data, insufficient rabbits were positive for *E. stiedae*, for the parasite to be included in the analysis. The presence of myxomatosis appeared to reduce abdominal fat (figure 4) by 56% (3.4g). For rabbits harbouring 3 or more of the 5 gut helminths, the model predicted 46% less abdominal fat than for hosts harbouring fewer than 3 species (figure 5)

Table 2: Minimal restricted maximum likelihood (REML) and generalised linear models (GLM) revealing those factors and covariates of the rabbits' external environment, biology and parasitic infections which are predicted to be significantly ($p<0.05$) associated with aspects of the host condition and fecundity.

**a) Adult Rabbit Total Body Mass. Model Type: REML (Random Terms = year)**

| Model Term | Wald Statistic ($\chi^2$) | d.f. | p |
|---|---|---|---|
| Month | 145.26 | 11 | <0.001 |
| Presence / absence of *E. stiedae* | 23.99 | 1 | <0.001 |
| Presence / absence of myxomatosis * Host Sex | 4.80 | 1 | 0.028 |
| Index of parasitism (i.e. number of gut helminths) | 30.45 | 2 | <0.001 |

**b) Mass of Abdominal Fat. Model Type GLM**

| Model Term | F Statistic | d.f. | p |
|---|---|---|---|
| Month | 19.03 | 11,140 | <0.001 |
| Host total body mass | 2.09 | 1,140 | <0.001 |
| Vegetation type on which rabbits feed | 0.74 | 1,140 | 0.028 |
| Presence / absence of myxomatosis | 1.39 | 1,140 | 0.003 |
| Index of parasitism (i.e. number of gut helminths) | 2.54 | 1,140 | <0.001 |

**c) Adult Ovary Mass. Model Type: GLM**

| Model Term | F Statistic | d.f. | p |
|---|---|---|---|
| Number of foetuses in utero | 0.274 | 1,39 | <0.001 |
| Lactating or non-lactating | 0.2016 | 1,39 | 0.003 |
| Log (x+1) *G. strigosum* | 0.247 | 1,39 | 0.001 |

**d) Adult Testes Mass. Model Type: GLM**

| Model Term | F Statistic | d.f. | p |
|---|---|---|---|
| Month | 62.01 | 8,44 | <0.001 |
| Log (x+1) *M. pectinata* | 4.42 | 1,44 | 0.022 |
| Presence / absence of *C. denticulata* | 5.22 | 1,44 | 0.014 |

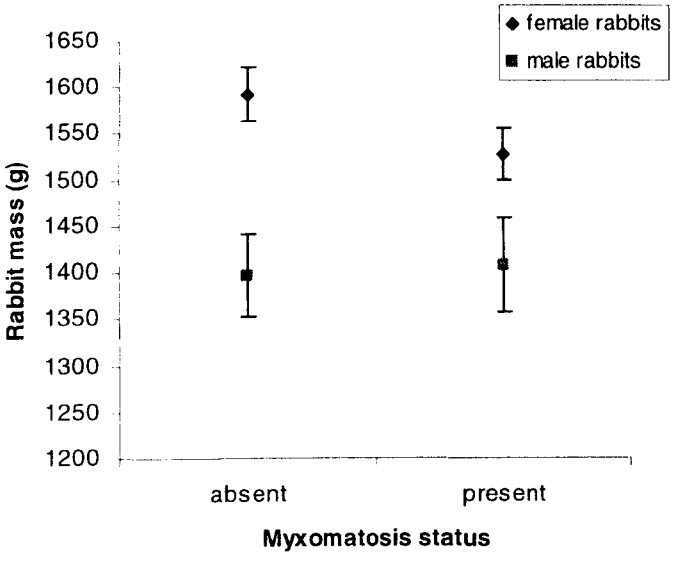


Figure 1. Predicted effect of myxomatosis on rabbit total body mass. Back-transformed values with 95% confidence intervals are shown.

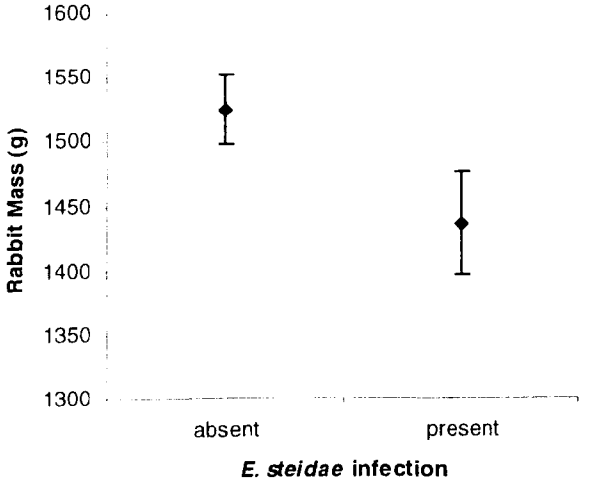


Figure 2. Predicted effect of *Eimeria stiedae* on rabbit total body mass. Back-transformed values with 95% confidence intervals are shown.

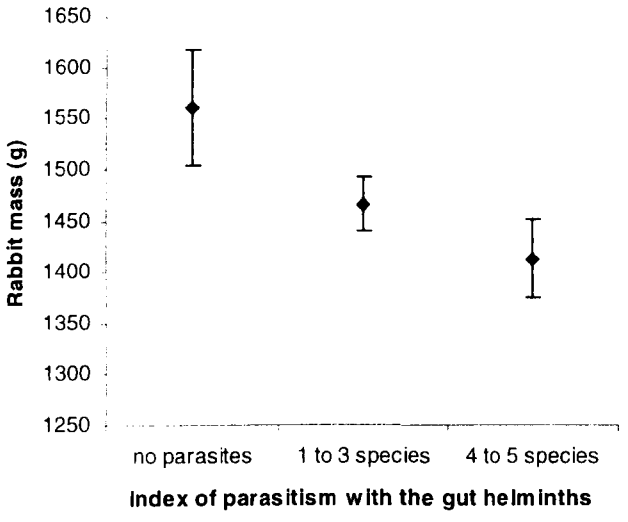


Figure 3. Predicted effect of the index of parasitism (number of gut helminth species) on rabbit host total body mass. Back-transformed values with 95% confidence intervals are shown.

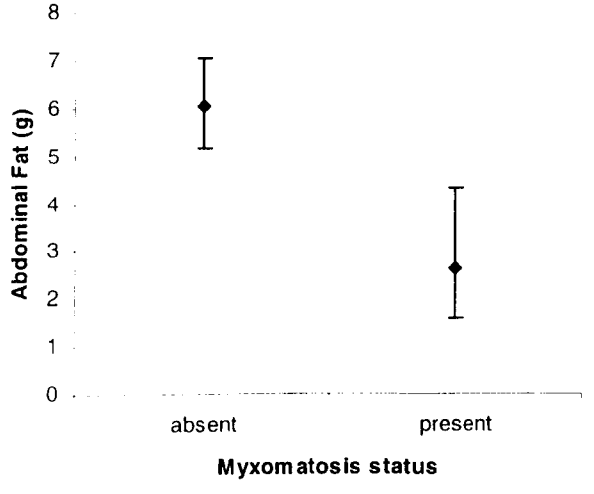


Figure 4. Predicted effect of myxomatosis on the mass of abdominal fat. Back-transformed values with 95% confidence intervals are shown

***Number of foetuses***

The majority of pregnancies occurred between March and June inclusive, and the analysis of foetal numbers were therefore restricted to pregnant adults in those months (n=136). No parasites were significantly associated with the number of foetuses.

***Ovary mass***

Only in a relatively small subset of data did we record ovary mass (n=43; years 1987, 1998 and 2001-02) in adult rabbits. *Graphidium strigosum* was positively associated with ovary mass (table 2c) and the effect was predicted to be strongly positive (figure 6). The geometric mean intensity of *G. strigosum* in these data (i.e. 24 worms) is associated with a 71% (0.2g) increase in ovary size compared to the predicted value with no *G. strigosum* present.

In order to further scrutinize this association of *G. strigosum* with ovary mass, we examined the correlation between, foetal number per adult female during the breeding season (March - June) each year and both the mean log (x+1) intensity (including zero counts) or prevalence of *G. strigosum* in juveniles (July - October), using a Spearman's rank correlation in the GENSTAT statistical package. The correlation was positive and when years 1977-79 were excluded, because the prevalence of *G. strigosum* in those years was exceptionally low (7% compared with 52% in the rest of the data) and years with less than 5 rabbits sampled, for either period, were also removed because of the potential bias inherent in such a small sample, this relationship becomes highly significant (intensity r=0.60, p=0.017; prevalence r=0.66, p=0.008; n=15). However, it should be noted that there was no significant effect of mean log (x+1) *G. strigosum* intensity or sample size in a GLM analysis in which all the years were included and

where sample size was included as a covariate but we feel that the inherent bias in the small samples is probably not overcome by this method. In other words, high ovary mass is associated with high intensity of infection in the breeding females and is also associated with high intensity and prevalence of *G. strigosum* in the cohort of juveniles for that year. This implies that plenty of infective larvae were available on pasture for the juveniles to be infected with. This relationship was not observed for any of the other helminth parasites, although there was a significant positive (r=0.50, p=0.048; n=16) correlation between *T. retortaeformis* mean log (x+1) intensity in kittens (sampled from April through September) and number of foetuses per female (from March through June) in the same year.

***Testes mass***

Testes mass was also recorded for a subset of individuals (n=57; years 1989 and 2001-02). *Mosgovoyia pectinata* log (x+1) intensity was predicted to have a negative effect upon the testes (figure 7; table 2d). The geometric mean intensity of *M. pectinata* (i.e. 3 worms) was predicted to result in an 18% (0.8g) reduction in testes mass. Finally, the presence of the other cestode, *C. denticulata* was predicted to have an overall positive effect, in association with rabbit mass, upon testes mass (figure 8, table 2d). At the average rabbit mass the presence of *C. denticulata* was predicted to increase testes mass by 17% (0.6g).

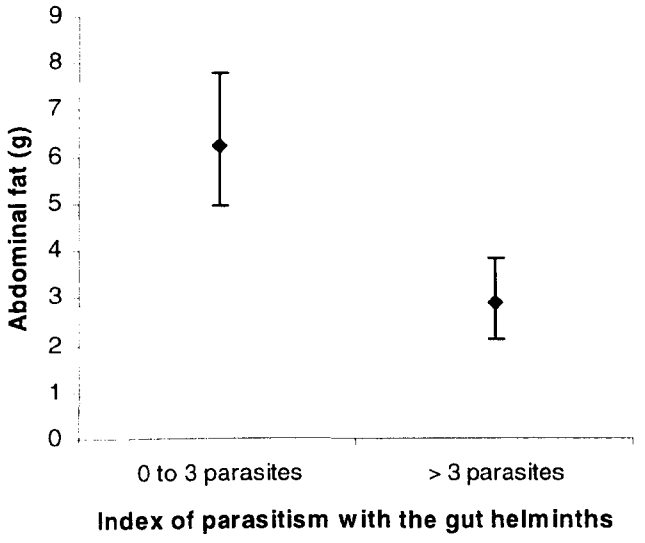


Figure 5. Predicted effect of the index of parasitism (number of gut helminth species harboured) on mass of abdominal fat. Back-transformed values with 95% confidence intervals are shown.

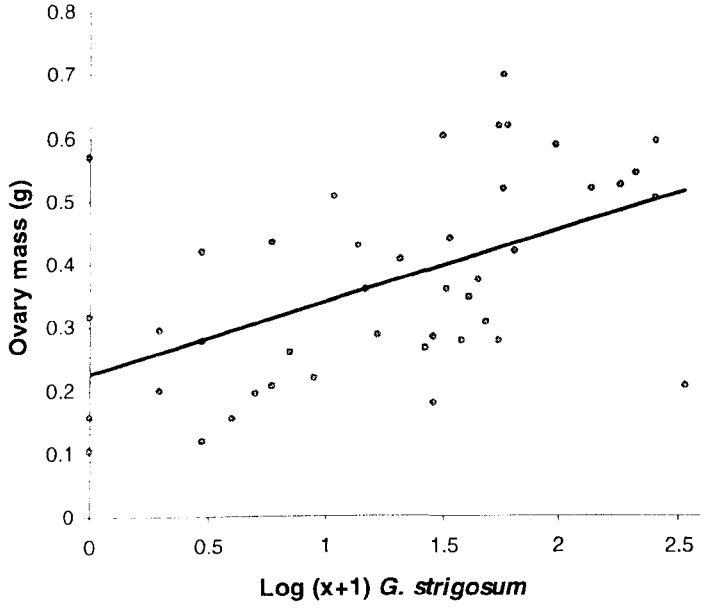


Figure 6. Predicted effect (with residuals) of Graphidium strigosum upon the mass of the adult rabbit ovaries.

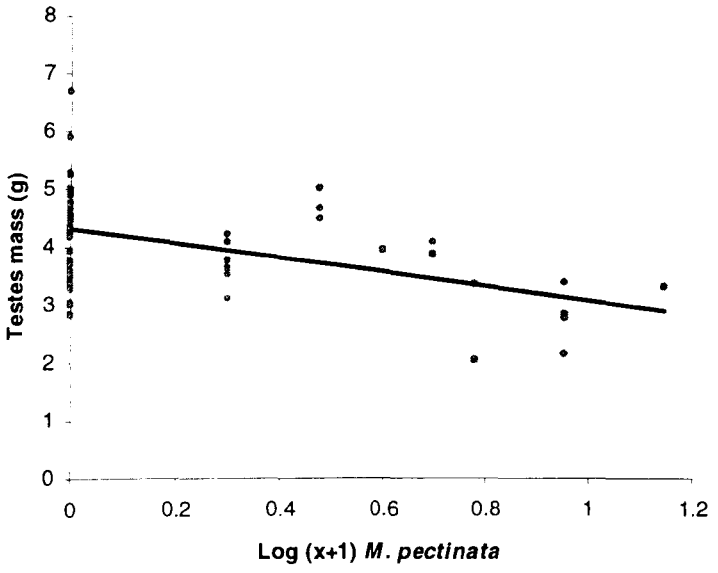


Figure 7. Predicted effects (with residuals) of Mosgovoyia pectinata on the mass of the adult rabbit testes.

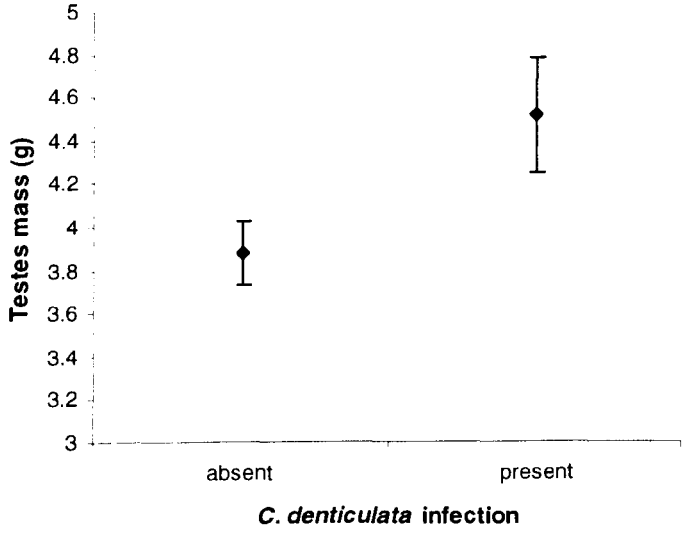


Figure 8. Predicted effect of C. denticulata on the mass of the adult rabbit testes. Back-transformed values with 95% confidence intervals.

## DISCUSSION

The experimental manipulations of the red grouse parasites have unequivocally demonstrated that parasites can reduce host fecundity and survival and that such effects can destabilise populations (Hudson, 1986; Dobson & Hudson, 1992; Hudson, Dobson & Newborn, 1992; Tompkins & Begon, 1999). Support that this same phenomenon occurs in mammalian hosts has come from studies on Svalbard reindeer, snowshoe hares, bank voles and wood mice, and Soay sheep (Grenfell, Wilson, Isham, *et al.*, 1995; Feore, Bennett, Chantrey, *et al.*, 1997; Ives & Murray, 1997; Murray, Cary & Keith, 1997; Albon, Stien, Irvine, *et al.*, 2002; Stien, Irvine, Ropstad, *et al.*, 2002; Telfer, Bennett, Bown, *et al.*, 2002). Only a few observational investigations provide enough power for elucidation of the parasite host relationships. However, the time span and quality of our data set did allow the elucidation of at least some of the potential associations between the wild rabbit host and its parasites.

### *Measures of Host Health*

The precise effect of rabbit body weight and abdominal fat upon survival is not known. However, whilst domestic animals and livestock may have excess body fat, it is generally accepted that wild animal populations tend not to (Clutton-Brock, 1999). Body fat is often stored to assist survival through winter months, when less food is available and so any reduction in fat is likely to reduce the probability of survival. In addition the level of fat a rabbit stores has been experimentally shown to have a positive effect on breeding success (Martin, 1977; Levai & Milisits, 2002).

The increasing index of parasitism has a clear negative association with total host body mass and with the mass of abdominal fat. However, it is possible that lighter and less

fatty hosts have lower species diversity because the host is a poor quality environment for the parasites and thus the host cannot support as many parasite species. Similarly the host may be in a poor quality habitat itself where rate of food intake and contact rate with infective larvae are reduced. Nevertheless, this would result in a reduction of intensity rather than of diversity so this explanation seems unlikely. Both myxomatosis and *E. stiedae* were also negatively associated with host mass and myxomatosis with abdominal fat. As experimental work has shown that myxomatosis and *E. stiedae* both negatively affect host body weight and fat levels respectively (Ross, 1972; Barriga & Arnoni, 1979; Fenner & Ross, 1994; Fenner & Fantini, 1999), the negative association in these cases can be assumed to be the result of effects of the parasite upon the host. If the model prediction is real and hosts which have greater parasite diversity do have less fat, those host are likely to be subject to reduced fecundity and increased morbidity and mortality. The effects of myxomatosis and of the index of parasitism on abdominal fat are predicted to be very substantial, although in this case it is only when the parasite diversity reaches 3 species that the effects are significant. Furthermore, the predicted effects of myxomatosis are a good match those predicted by Boag (1999), where he extrapolated from a study of sheep parasites.

***Host Fecundity***

Whilst there is no significant association of any of the parasites with the number of foetuses per pregnant female, there was a strong positive association between *G. strigosum* and ovary mass. We could only find evidence from one study that has suggested that there may be a positive effect of a parasite species in a vertebrate host population. In an observational study, of cowpox virus infection in small rodents, evidence was presented, which suggests that cowpox may have a positive effect upon

bank vole survival (Telfer, Bennett, Bown, *et al.*, 2002). An explanation for this was implied from an earlier laboratory experiment where cowpox infection was shown to delay fecundity in bank voles and wood mice (Feore, Bennett, Chantrey, *et al.*, 1997). As increased fecundity is often associated with decreased survival the reduction of fecundity by the virus was suggested to result in an increase in host survivorship during the breeding season.

The relationship between ovary and testes mass and fecundity may not be linear. However, increased mass of both ovaries and testes, have been associated with increased fecundity in some studies and testes mass with dominance. As dominance itself is associated with mating success, high testes mass, whilst the least direct measure, may still be positively correlated with fecundity. Changes in ovary mass and the prevalence of pregnancies (from the raw data) by month revealed that they have the same seasonal pattern (figure 9). This provides circumstantial evidence for the role of increased ovary size as a proxy for reproductive success, which is complimentary to studies conducted in other host-parasite systems (Giuliano, Lutz & Patino, 1996; Hasumi, 1996; Radovanovic, StosicBogdanovic & Gledic, 1997).

Observational studies in Australia suggested that host hormones positively affected the numbers of both *G. strigosum* and *T. retortaeformis* in pregnant and lactating rabbits (Bull, 1959; Dudzinski & Mykytowycz, 1963; Dunsmore, 1965, 1966a). High intensities of *G. strigosum* in these studies were associated with the breeding season of the host and the parasite intensities were higher in females than males. An experimental study, in which ovaries and / or fallopian tubes of the rabbits were removed, initially appeared to corroborate this view (Dunsmore, 1966b).

However, closer examination of these results reveals that, whilst there was no apparent difference in uptake of larvae in the *G. strigosum* infected rabbits, there was a significant difference in the *T. retortaeformis* infected hosts with the same treatment. The larval uptake in the ovariectomised females was the same as that in males, suggesting a possible change of behaviour in these animals rather than a direct effect of the hormones upon the worms. Male rabbits tend to roam further (B. Boag, pers comm.) and are therefore likely to uptake fewer larvae than females, which remain closer to the burrow and whose forage will therefore be more contaminated. Furthermore, in a follow-up study where hormone levels were manipulated (no organ oblation) the host hormones affected only *T. retortaeformis* numbers (Dunsmore & Dudzinski, 1968).

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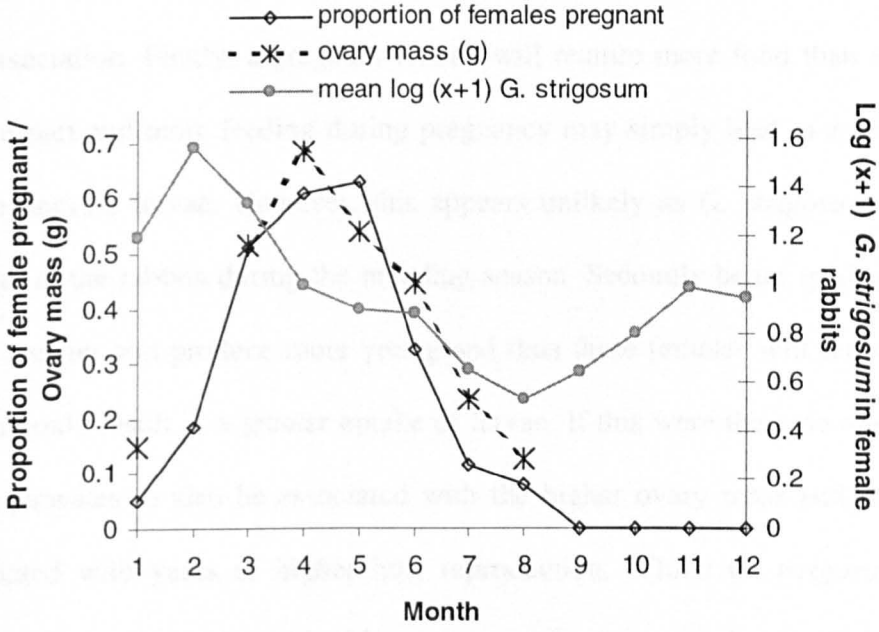


Figure 9. Proportion of female rabbits pregnant, ovary mass and Log (x+1) *Graphidium strigosum* values calculated from the raw data. Note that *G. strigosum* intensity peaks two months earlier than the peaks in pregnancy and in ovary mass

Unlike Australia and New Zealand, the peak period of abundance for *G. strigosum* in the UK is actually one to two months prior to the peak in rabbit breeding (figure 9). Even though *G. strigosum* intensity is actually declining during the peak period of breeding, the parasite is still associated with larger ovaries.

There was a positive association between the mean number of foetuses per female (March through June) in one year and both the mean log (x+1) intensity and prevalence of *G. strigosum* in juveniles later that year. This could simply indicate that a high reproductive year is associated with more susceptible young, but that hypothesis does not explain the positive association of *G. strigosum* with ovary mass during the breeding season. These factors may indicate that *G. strigosum* does positively affect ovary mass and, indirectly, fecundity. Conversely, there are three alternative hypotheses to explain the association. Firstly, a pregnant female will require more food than a non-pregnant counterpart and more feeding during pregnancy may simply lead to a greater exposure to the parasite larvae. However, this appears unlikely as *G. strigosum* intensity is in decline in the rabbits during the breeding season. Secondly better quality females tend to be heavier and produce more young and thus these females will require more food, which could result in a greater uptake of larvae. If this were the case one might expect other parasites to also be associated with the higher ovary mass and to be positively associated with years of higher host reproduction. Whilst *G. strigosum* is the only species of parasite associated with ovary mass, higher mean log (x+1) intensities of *T. retortaeformis* were found in the kitten cohort from high reproductive years. Nevertheless, the inclusion in the model of total host mass, should have controlled for the potential effect of larger rabbits simply having larger ovaries. Finally, whatever external conditions produce a good year for reproduction of the host population, may

also produce a good year for the survival / development of *G. strigosum* transmission stages. Experimental manipulation will be necessary to test between these hypotheses.

The first of these hypotheses, i.e. that ovary mass of the host may be increased by a parasite is novel. Parasite induced decreases of host fecundity may result in increased host survival as resources, which would otherwise be used for reproduction, may be redirected. This may be an adaptive strategy by the parasite to increase its transmission potential, i.e. the longer the host lives the longer the parasite has to transmit (Feore, Bennett, Chantrey, *et al.*, 1997; Hurd, Warr & Polwart, 2001; Telfer, Bennett, Bown, *et al.*, 2002). However, in many non-rodent vertebrates, helminth parasites are shorter lived than their host and thus extending host lifespan may not always be useful transmission strategy in these species. An alternative strategy for parasite species that have shorter life spans than their hosts, would be to increase host fecundity rather than longevity, thereby ensuring fresh hosts for the parasite offspring to infect. Whilst it is perhaps more likely that one of the alternate hypotheses is true, the novelty of hypothesis one and its possible explanation as an alternate parasite transmission strategy, makes it worthy of further investigation.

The relationship between ovary mass and fecundity is not clear and it has yet to be proven that ovary mass leads directly to an increased number of offspring. However, ovary mass has been related in this species, to faster growing young. The prevalence of *G. strigosum* in juvenile rabbits is considerably higher than in kittens (9.5% and 38.5% respectively). It may be that kittens are not a very suitable host environment for *G. strigosum* and increasing the speed with which hosts mature could also be a useful strategy for this parasite.

Testes mass is correlated not only with reproductive success but also with dominance status (Stockley, Searle, Macdonald, *et al.*, 1996; Moreira, Macdonald & Clarke, 1997; Byrne, Roberts & Simmons, 2002; Coker, McKinney, Hays, *et al.*, 2002). The potential negative effect of *M. pectinata* and the positive effect of *C. denticulata* may therefore have implications for social interactions in addition to the possible effects upon reproduction. Negative effects of experimental infections with *Trichinella spiralis* upon social status of captive mice have been examined (Edwards, 1988; Barnard, Behnke & Sewell, 1993) and as such there is a precedent for this form of interaction. However, it is possible that the consequence of increasing testes size is to increase testosterone. Therefore, an alternative hypothesis is that testosterone positively effects *C. denticulata* and negatively effects *M. pectinata,* but as both species of cestode have very similar life history strategies, it is difficult to see how such divergent effects would come about. Even if the parasites are responding to the host and not the host to the parasite, the relationship between testosterone, dominance and the degree of parasitism may be important to population dynamics (dominant animals tending to harbour more parasites) and clearly the rabbit host would be a useful and relatively accessible system with which to work.

Rabbits are a major pest species in Europe and in the Antipodeans (Bull, 1964; Bell, Byrne & Watson, 1998; Croft, Fleming & van de Ven, 2002; Fleming, Croft & Nicol, 2002). Myxomatosis has been used, though rather unsuccessfully, as a biological control agent in rabbit populations for many years (Ross & Sanders, 1984a, 1984b; Barnes & Tapper, 1986; Ross & Tittensor, 1986; Flowerdew, Trout & Ross, 1992; Trout, Ross, Tittensor, *et al.*, 1992; Fenner & Ross, 1994) and rabbit haemorrhagic disease has also

been suggested as an alternative control agent (Fa, Sharples, Bell, *et al.*, 2001; Calvete, Estrada, Villafuerte, *et al.*, 2002; Reddiex, Hickling, Norbury, *et al.*, 2002). However, both of these pathogens are relatively new to their European hosts and their relationships are still rapidly evolving, making it difficult to anticipate the effect of using them as control agents. The relationship between the rabbit and its helminths are well established and are likely to be more consistent and predictable. If the rabbit helminths do have negative effects upon their host then they may have the potential to provide a more effective and predictable control strategy to keep the rabbit populations in check.

The correlative nature of the data means that a measure of caution should be applied to the interpretation of the analyses and experimental manipulation is required to conclusively determine the direction of the associations. Nevertheless, the evidence does support the hypothesis that rabbit parasites may have substantial effects upon both host condition and upon host fecundity.

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# GENERAL DISCUSSION

# General Discussion

Identifying successful and environmentally friendly treatments against parasites and pathogens that infect humans, domestic animals and free-ranging animals is one of the greatest challenges facing biologists today (Scott, 1988; Waller, 1993; Tembely & Hansen, 1996; Stromberg & Averbeck, 1999; Tisdell, Harrison & Ramsay, 1999; Wakelin, 2000). With parasite species becoming increasingly resistant to the drug therapies applied against infections, alternative strategies are urgently required (Waller, 1993; Smith, Grenfell, Isham, *et al.*, 1999; Bounias, 2000; Geerts & Gryseels, 2001; Leathwick, Pomroy & Heath, 2001; Atanasio, Boomaker & Sitoe, 2002). For such strategies to be found and effectively implemented, we need to obtain a more complete understanding of parasite biology, not only those factors which influence the population dynamics of individual parasite species but also how the factors and parasites interact to affect the abundance, structure, and dynamics of the whole parasite community.

## Parasite Aggregation

According to the theoretical models of Anderson and May (1978) and May and Anderson (1978), hereafter referred to as A & M, the distribution of parasites among hosts can have major influences on the stability of both host and parasite populations. These models predict that some degree of parasite aggregation is necessary if parasites are to stably regulate host populations, but if aggregation is too high, with large numbers of parasites concentrated in a few hosts, then too many parasites are lost when a heavily infected hosts dies and the host escapes the parasites regulatory role.

The few models that explore interspecific interactions between parasites suggest that aggregation is important and that it is necessary for parasite coexistence (Dobson, A. P. & Roberts, 1994b; Pugliese, 2000). However, these models describe aggregation using the mean and the negative binomial parameter k. Indeed aggregation may not be a static parameter. Some recent models have incorporated dynamic aggregation, with model outcomes considerably different from those where k was fixed (Pugliese, Rosa & Damaggio, 1998; Pugliese, 2000). These studies found that where aggregation was dynamic, it made less difference to the parasite population dynamics, whereas the fixed k used in the A & M models, made a marked difference to both the host and parasite dynamics.

Not surprisingly, the aggregated distribution of parasites has important implications for parasite control strategies as the majority of parasites will be found in relatively few hosts (Wilson, Bjørnstad, Dobson, *et al.*, 2001). Thus for a control strategy to be effective it must adequately target these highly infected hosts. It is therefore important to know if and how a parasite species' distribution varies. Another important consideration with respect to aggregation, is the evolution of anthelmintic resistance. Smith, Grenfell, Isham, *et al.* (1999) and Cornell, Isham & Grenfell (2000) examined the evolution of anthelmintic resistance using both deterministic and stochastic models. Both studies concluded that under circumstances where parasite transmission stages are aggregated, and there is a high degree of relatedness between the transmission stages in an infective patch, anthelmintic resistance can spread much more rapidly in a parasite population compared to parasites following a Poisson distribution. This phenomenon comes about because of the increased probability of heterozygotes, carrying recessive resistance genes, mating and producing homozygous resistant offspring. A large degree

of relatedness between worms is likely in any infective patch. However, these models also found that if unrelated strains were spatially separated and aggregated, that resistance would develop more slowly than in the case where the parasites exhibited a Poisson distribution.

The negative binomial distribution is not a good fit to these rabbit parasite data (Boag, Lello, Fenton, *et al.*, 2001). There are numerous alternative measures of aggregation: namely Poulin's D, the coefficient of variation (CV), the variance to mean ratio and Taylor's power law (Wilson, Bjørnstad, Dobson, *et al.*, 2001). Each measure considers aggregation in subtly different ways. The variance to mean ratio is considered to take more account of the tail of the distribution, whilst k provides more information about the spread of data about the mean. Additionally, there are limitations to the use of the different measures. For example, the most commonly used measure, i.e. the negative binomial distribution, cannot be used to compare between samples with different means (Boag, Lello, Fenton, *et al.*, 2001; Wilson, Bjørnstad, Dobson, *et al.*, 2001). As the data were not a good fit to the negative binomial distribution and because we needed to compare between samples with different means, we investigated the data using Taylor's power law. This is the linear relationship between the log variance and the log mean of counts described by the simple equation, log variance = a + $b$*log mean. The slope of the line, $b$, is the index of aggregation and in his classic study Taylor claimed that the $b$ is a fixed species characteristic (Taylor, 1979).

Previously it was only possible to use Taylor's power law if data were available from more than one sample, e.g. from different sites (Wilson, Bjørnstad, Dobson, *et al.*, 2001). Our development of a bootstrapping method to produce the regression means

that this aggregation measure may now be used even on single data sets although care should still be taken to obtain adequately sized samples which give a true picture of the aggregation in the population.

In chapter 1 we revealed that Taylor's *b* did not remain stable for different subsets of the data. Taylor's *b* was observed to vary with month, year, host sex, host age class, and myxomatosis status of the host. As is the case with k, it appears that environmental conditions, aspects of host biology and the presence of other parasite species can all influence the degree of aggregation of the helminths.

Whilst we did not find a stable value of *b* for the parasites, the rabbit nematodes were consistently more aggregated than the cestode parasites in this study. Parasite populations that are less aggregated tend to destabilise host populations and reduce the host equilibrium values if they negatively affect host condition and /or fecundity. However, whilst the cestodes are less aggregated they also tend to have lower prevalences than the nematodes. The 'ideal' parasite to use as a biological control agent for the host population would therefore be one that strikes a balance between its aggregation level, its prevalence and also in the degree to which it affects host fecundity and survival. Of course, prevalence and virulence both play a role in the distribution of parasites, and so the three parameters are linked in a complex fashion and cannot be considered as independent entities.

Since the financial costs of sampling wild rabbits and maintaining captive rabbits are relatively low compared with the costs of maintaining sheep or cattle, the rabbit is an excellent model system in which to consider issues relevant to effective parasite control.

For example in the rabbit system, male hosts showed higher levels of parasite aggregation than females, for four of the five helminths (*T. retortaeformis*, *G. strigosum*, *M. pectinata* and *C. denticulata*) (Boag, Lello, Fenton, *et al.*, 2001). If we intended to control these four parasite species we might, therefore, decide upon different treatment strategies for male and female hosts. We could then assess the effects of such differential control in this relatively low cost system and extrapolate findings to control in domestic grazers.

This study indicates that aggregation is dynamic and is an important consideration in control strategies. However, we must be careful to consider parasite aggregation in association with other measures of parasite population dynamics, since aggregation is a product of both the prevalence and the intensity of a species. For example, a consequence of myxoma infection was to considerably lower the levels of aggregation in all rabbit age classes for all rabbit helminths (with the exception of *C. denticulata* in adult rabbits). However, this low aggregation may result from one or a combination of two factors. Firstly this reduction could be due to a shortening of the tail of the distribution i.e. high parasite counts may be missing from the data because rabbits infected with heavy burdens as well as with myxomatosis may die and be lost from the data. Alternatively the suppression of the immune system by myxomatosis would be likely to result in more rabbits harbouring heavier helminth parasite burdens, thus increasing the mean of the distribution. Furthermore, these effects may vary between different species of parasite and even for different host age classes. Considering the parasite distribution alongside intensity and prevalence measures may therefore give us a clearer insight into the processes that form the distribution.

The temporal variation we see in the parasite data will also have important implications for the control of parasites. For example, the best time of year to treat the host population with anthelmintics might be prior to the peak in parasite fecundity since fewer transmission stages are released onto pasture. Knowing the degree of aggregation at that time point could be useful as it could minimise treatment costs and reduce the infection of newly born offspring. If the host group in which the parasites are concentrated can be identified, only those, relatively few, animals would require treatment.

**Interspecific Interactions Between Parasites**

In the majority of studies, evidence for interspecific interactions between parasites have been sought through an examination of the community structure, most frequently through an analysis of pair-wise associations (Tokeshi, 1993; Kelt, Taper & Meserve, 1995; Poulin, 1996; Haukisalmi & Henttonen, 1998; Poulin & Guegan, 2000; Alves & Luque, 2001; Behnke, Barnard, Bajer, *et al.*, 2001; Poulin, 2001). Such analyses do not control for other factors such as host biology, or external environment except by subdividing the data set prior to analysis, which would result in a loss of explanatory power. In this thesis, I utilised generalised linear models (GLM) and restricted maximum likelihood (REML) linear mixed models, to consider the association between species. The use of these models enabled us to include a wide variety of explanatory variables, which may otherwise have confounded the elucidation of the parasite associations.

While the findings suggest the direction of the relationship it is still necessary to test the interactions predicted in chapter 2 via experimental infections under controlled

conditions. However, the biology and life histories of the parasites and the inclusion in the models of many environmental and host attributes, suggests that the interactions are genuine. We could find no other study where there has been consistent evidence of interactions between parasites within wild mammalian hosts. The implications are wide reaching and have consequences for current and future disease treatment programs.

The parasites in this system are not only predicted to affect each other's dynamics, but those effects are predicted to be substantial. Only the influence of month was more important to the intensities of the parasites, when we considered the whole helminth community. This is important to note, as season will have profound effects upon all aspects of the parasite life cycle e.g. changes in weather conditions over the seasons will differentially affect the transmission stages of the parasites (Boag & Thomas, 1985; Stromberg, 1997; Saunders, Tompkins & Hudson, 2002), and season may impact indirectly upon the adult parasites by influencing host biology directly through food quality and indirectly through day length and hormone secretion. It is therefore particularly significant that interspecific parasite interactions are the second most important influence on the helminth intensities.

Failure to take account of interactions may result in reduced efficacy of control strategies or in concomitant infections becoming more severe. Conversely, knowledge of interactions could be used to the advantage of those working towards more effective control. For example, relatively benign parasite species could be used to guard against more deleterious species. Such a strategy would also have the advantage that both species of parasite would be evolving and that resistance to the effects of the control

agent might never occur, or if it did is likely to take longer than the one sided evolution of parasites against antibiotic (e.g. anthelmintic) agents.

In chapter 2 we examined the effects of parasites on the individual population dynamics of other species. In chapter 3 we considered the parasite interactions at the community level using a mathematical model. We adapted a simple constant uptake model (Woolhouse, 1992; Chan, Srividya, Norman, *et al.*, 1998; Norman, Chan, Srividya, *et al.*, 2000) and assumed that all the helminths in the rabbit system were interacting through acquired immunity. Since chapter 2 had revealed the extreme importance of season to the parasite dynamics we also incorporated this factor into the model using a simple sine function. We found that this seasonal function did not prevent interactions between the parasites but that when parasites cycled out of phase with one another, the degree of influence the parasites exerted upon another's dynamics, was affected. This was further complicated by an interaction with the immune decay rate. Lower rates produced the unexpected emergent property of shifting the immune response out of phase with the parasite species against which it was produced. This immune shift means that a parasite species can potentially have a greater effect on a second species if the second species cycles in phase with the immune response to the first parasite rather than with the parasite itself. This phenomenon may be specific to this model but it is certainly worthy of further investigation since it could have major implications for parasite control.

Using this model we also considered the relative effects of two treatment strategies, an anticestodal and a vaccine against one of the cestodes, *C. denticulata*. The vaccine had the most substantial impact upon the modelled community because of the cascade of

immune effects between parasites. However, it should be noted that the vaccination strategy had very little effect against the other cestodes species. Therefore care should be taken to clearly decide upon the goals of any strategy before implementation.

The production of anthelmintic vaccines has been problematic (Dalton & Mulcahy, 2001). Successful trial vaccines have been raised for some species of parasites whilst attempts to produce them for other species have failed. In sheep, successful steps have been made in the production of vaccine for *Haemonchus contortus* and yet the attempts to produce a vaccine for *Teladorsagia circumcincta* have been fraught with difficulty. In fact, attempts to produce a *T. circumcincta* vaccine, in one study, resulted in a product that, ironically, is partially active against *H. contortus* whilst having no effect upon *T. circumcincta* itself (Adams, Anderson & Windon, 1989). Similarly there are numerous research programmes seeking vaccines for a wide range of human helminth infections. Again, these programmes have met with mixed success, whilst the production of a vaccine against schistosomiasis has progressed well (Pearce, 2003). The search for a vaccine against oncherciasis has had little success to date, although the search continues (Cook, Steel & Ottesen, 2001).

Our model predicts that where negative interactions between parasite species exist, a vaccine raised against one species may be used to control (at least partially) the interacting species. This may be a way around the difficulties involved in producing some of the vaccines. However, on a note of caution the model also predicted the counterintuitive effect that immunising against *C. denticulata* produced a massive gain in the numbers of *T. retortaeformis*. This was because, in the model, the latter parasite positively affected the former specifically via the host immune system. Thus stimulating

the immune system (by vaccination) against *C. denticulata* positively affected *T. retortaeformis*. This was only a prediction from a model and may seem unlikely but it is known that some parasite species are affected by $Th_1$ (T-helper cells) immune response whilst for others immune action is via the $Th_2$ response (Behnke, Bajer, Sinski, *et al.*, 2001; Cox, 2001). The two cell responses are in opposition such that if the host produces more $Th_1$ then it will produce less $Th_2$ (Behnke, Bajer, Sinski, *et al.*, 2001; Cox, 2001). As a number of parasite species are capable of pushing the immune response away from the Th cell response that is active against themselves, it is conceivable that *C. denticulata* creates an immune response, which is in opposition to the response produced against *T. retortaeformis*. Under these circumstances the second species would benefit from the vaccination against the first.

There are limitations to the model in its current form. One of the most important is that the model can only consider the average parasite population in the average host. In the A & M models discussed earlier, parasite aggregation is very important to the dynamics of both parasite and host populations. The majority of the host parasite models are based around the A & M formulation, and therefore utilise this measure of aggregation. The current models, incorporating the negative binomial distribution, only allow coexistence of multiple species in a parasite host population when levels of aggregation are high, effectively separating the parasites into different hosts (Dobson, A. & Roberts, 1994a; Pugliese, 2000). Whilst the A & M models, have allowed an enormous theoretical step forward in the understanding of parasite population dynamics, the rabbit parasite system (along with many others, Christensen, Nansen, Fagbemi, *et al.*, 1987; Petney & Andrews, 1998; Cox, 2001; Drake & Bundy, 2001) involves concomitant infections, with a number of different species commonly found together in any one host. Therefore,

the A & M models are unsuitable in their current form for the consideration of parasite interactions via the host immune system. As aggregation is likely to be important, the essential next step for the model used in this study will be to incorporate a suitable aggregation parameter in order to assess its affect on the interactions. An assessment of whether the incorporation of a dynamic aggregation parameter would change the outcome in this type of model structure would also be an interesting consideration. Unfortunately, such adaptations were beyond the scope of the current study.

**Parasite Influences on Host Health and Fecundity**

The majority of pathogens, which have been considered to have a major impact upon their host population, are organisms that cause epidemics and population crashes, (Scott, 1988). This has been a natural first step, as these systems tend to have relatively high levels of variation within them, which thereby allow the elucidation of the parasites' action on the host population. However, a number of empirical and theoretical studies have highlighted the potential for endemic parasites, either directly through their effects upon host fecundity and survival or through interactions between host health and extrinsic factors (such as predation) to regulate their host population (Dunsmore, 1980; Dobson, A. P. & Hudson, 1992; Hudson, Dobson & Newborn, 1992; Hudson, Newborn & Dobson, 1992; Holmes, 1995; Ives & Murray, 1997; Murray, Cary & Keith, 1997; Hudson, Dobson & Newborn, 1998; Irvine, Stien, Dallas, *et al.*, 2001; Albon, Stien, Irvine, *et al.*, 2002). In the final chapter of this thesis I considered the effects of the rabbit helminths, and of myxomatosis and *Eimeria stiedae* on aspects of host health and fecundity.

As with chapter 2 the correlated nature of the data used in our study precludes us from stating that the predicted model effects are certain. We cannot be completely confident that what appears to be an effect of a parasite species upon the host is not in fact an effect of the host upon the parasite. However, the evidence (detailed in chapter 4) suggests that the effects reflect the actions of parasites upon the host condition although more caution must be applied to the data on fecundity. What cannot be determined from these data is whether the parasite effects are density dependent. If the helminth parasites act in a density dependent manner, even in combination with extrinsic interactions such as predation, then they may still be able to regulate a rabbit population and potentially provide an effective rabbit control strategy.

Another interesting effect predicted in this study, was that of the influence of helminth diversity on host condition. To our knowledge few studies have considered the effect of parasite diversity upon the host (Morand & Poulin, 2000). It may be that as a host becomes infected with more species, the immune system becomes overloaded as it tries to produce an effective response against different species. Returning to our earlier example, the host will struggle to respond to two species when they produce different groups of T-helper cell or other divergent immune responses. Alternatively, higher diversity may also equate to higher intensities of some species and so host health may suffer from shear weight of numbers.

Some surprising evidence from this portion of the study was the predicted positive influence of parasite infections on ovary mass by *Graphidium strigosum*. However, as ovary mass is not a direct measure of fecundity, it is not clear what effects such an interaction would have upon the host's actual breeding production. There is evidence

that larger ovaries are associated with larger follicles (Boag, pers comm.) and previous work with rabbits has revealed that larger follicles are associated with faster growth of the young (Fox, 1974). Therefore, if the apparent effect of *G. strigosum* is genuine, the induced increase in ovary mass would lead to greater production of susceptible young rabbits. One may speculate that this could be an adaptive strategy by which the parasite ensures the rapid development of suitable susceptible hosts and therefore increase its own fitness, particularly if mothers tend to infect their own offspring with these parasites. However, there are alternative explanations for the apparent association, and these are discussed in detail in chapter 4, but this was not the expected result and so is certainly worthy of further investigation.

**Final Thoughts**

Parasites are one of the most abundant groups of organisms on the planet (Anderson & May, 1995; Grenfell & Dobson 1995). They cause misery to millions and loss of income to millions more. In contrast, we are increasingly becoming aware of the potential to use parasites as biological control agents (Waller, 1993; Arras & Arru, 1997; Fenner & Fantini, 1999; Hood, Chesson & Pech, 2000; Collier & Hunter, 2001; Chandrawathani, Waller, Adnan, *et al.*, 2003). Without a full understanding of their dynamics and their interactions with their environment and other organisms we will continually fail to find effective methods with which to control them; equally we will fail in manipulating them for our benefit.

This thesis, as with other research, has probably produced many more questions than it has answered. Do the rabbit parasites interact as they appear to? Can the helminths be used successfully as pest control agents? Could a parasite positively affect mammalian

fecundity? What will be the effects of incorporating aggregation into models of interspecific parasite interaction, where that interaction is immune mediated? All these questions and more will be essential before a complete understanding of this system is gained. Nevertheless, we have examined some interesting issues and drawn some tentative conclusions and believe that in so doing we have improved our insight into this host parasite system but also that our conclusions may shed light on other parasite systems as well.

In conclusion, the bottom up approach, of examining individual parasite population dynamics and then combining them into a community model, has much to recommend it. It is a simple and yet powerful method with which to explore the emergent properties of the community. The model used in this thesis is simplistic and its use as a predictive tool will need to be compared with more complex models, which include aggregation and host population dynamics, before it is practically applied. However, it has been a very useful first step in examining this system and has pointed to the importance of taking a holistic view of parasite communities. Future work will need to confirm the parasite and host-parasite interactions experimentally and extend the use of the analyses in this thesis to other systems, to establish how widespread interspecific interactions are. It is likely that the application of these techniques will reveal that interactions between parasites and between parasites and their host are widespread. If this study has achieved anything, then I hope it will be as a further encouragement, to parasite ecologists and epidemiologists, to treat parasite communities as we would any other community, considering the impacts upon the whole system when we perturb any element within it.

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