

**The Ecological and Evolutionary Dynamics of
Multiple Natural Enemies**

Edward Owen Jones

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Department of Animal and Plant Sciences

University of Sheffield

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Summary

Natural organisms are infected by many different parasites, and how these parasites interact with each other has important ecological and evolutionary implications. This study is to understand how organisms with different transmission strategies effect the evolution of each other. Parasites that transmit vertically benefit from the host having a high reproductive rate whilst hosts that transmit horizontally are not affected by host reproduction. Some horizontal parasites may reduce host fecundity to benefit their own replication within the host. Therefore there will be clear conflict between vertically and horizontally transmitted parasites. The evolution of traits such as vertically transmitted 'protection' and 'sabotage' are likely to be costly in terms of other life history traits. In the presence of such 'trade-offs,' the parasite population will evolve towards the evolutionarily stable strategy (ESS) that balances the costs and benefits of these strategies in response to a horizontally transmitted parasite. The ecology of the interaction of vertically and horizontally transmitting parasites is also examined. Further to this, field data is used to construct a model of two horizontally transmitting parasites, a pathogen and a parasitoid that occur on the Orkney Isles. We demonstrate how each of them, and both in combination, may have a large effect on resultant dynamics of winter moth, *Operophtera brumata*, populations.

'A little nonsense now and then is relished by the wisest men'

Roald Dahl

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Declaration

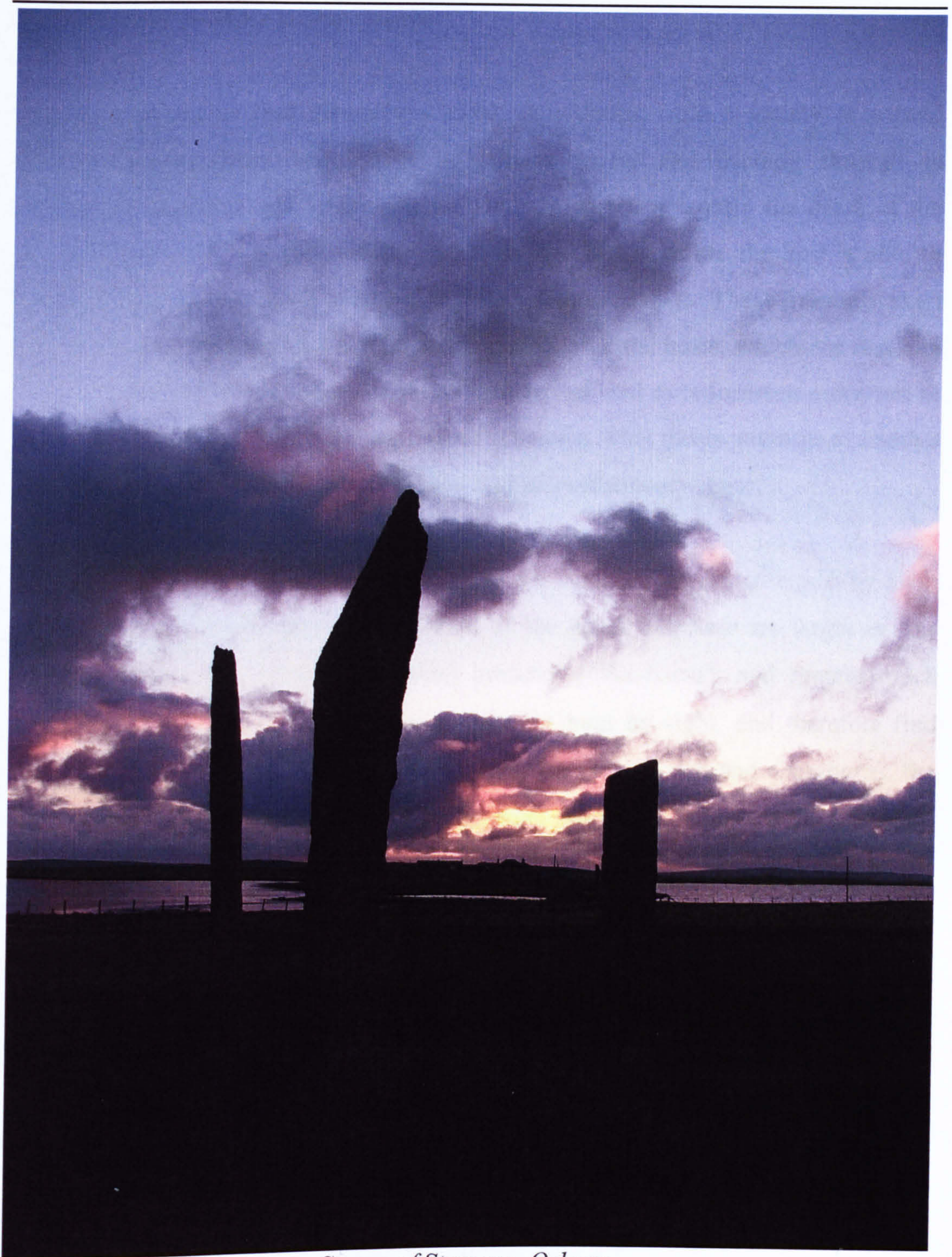
This dissertation summarizes the research I have conducted since October 2003, under the supervision of Dr. Michael Boots, Dr. Andrew White and Dr Rosie Hails MBE.

Chapter 2 forms the basis of a manuscript published in the *Journal of Theoretical Biology* 246 (2007), pp. 10-17.

Chapters 3 and 4 are currently being written up as manuscripts. Chapter 5 & 6 are to be written up in combination as a manuscript.

All photos on heading pages of chapters where produced by E. O. Jones, during fieldwork on Orkney for this thesis.

1. Introduction



Stones of Stenness, Orkney

1.1 Ecological Outline: Host and multiple enemy interactions

1.1.1 Ecology of multiple enemy systems

Most host organisms find themselves under exploitation from a variety of natural enemies, ranging from very small infectious viruses and bacteria through to multicellular parasites and larger predators. Many parasites require the death of the host to transmit, i.e. obligate killers, and those that do so before the host is able to reproduce can be seen as functional predators (Boots, 2004). These enemies exert many different ecological and evolutionary pressures on the hosts, which can result in a range of effects. Experiencing multiple enemies can lead to unforeseen outcomes in both host response and resultant population dynamics. This thesis attempts to address multi-enemy systems, both in an ecological and an evolutionary sense.

The host may respond differently to two different enemies when challenged by them in isolation. The zooplanktonic cladocerans of the genus *Daphnia* are larger in size when in the presence of a gape-limited predator (*Chaoborus*), and remain much smaller in the presence of planktivorous fish that hunt by sight, and therefore find larger prey easier to find. However, in the presence of both fish and *Chaoborus*, the *Daphnia* respond to counter the fish threat rather than the *Chaoborus* (Engelmayer, 2004; Weber & Declerck, 2004). Water spiders reduce mating duration in the presence of a predator (*Leponis cyanellus*) but increase the duration when exposed to a food competitor (*Pimephales promelas*). However, when both these enemies are in the same system, the host demonstrates no significant response (Sih & Krupa, 1996). This emphasises that often it is hard to predict the hosts' behavioural response in a multiple enemy system, even given the knowledge of its individual response with each predator/parasite.

The effects of different enemies can also lead to changes in population dynamics, either by direct removal or through changes in host behaviour. For example, changes in host population equilibrium and population cycles were seen in the red grouse

population interacting with the parasite, *Trichostrongylus tenuis*. The reduction of parasite burdens prevented cyclic fluctuations by averting population crashes, despite the grouses' constant exposure to predation (Hudson *et al.*, 1998). This demonstrates that the interaction between multiple enemies can specifically affect population dynamics. This emphasises that less obvious factors such as parasitism may actually determine the dynamics of a system rather than the more obvious ones such as predation. Indeed, it has been demonstrated that the removal of predation can lead to increased parasite control of hosts and can actually reduce host levels (Packer *et al.*, 2003). This finding may not hold in systems with a parasitoid, which act as functional predators (Boots, 2004), as they will be less able to superinfect the host due to earlier death by the viral infection.

While the above deals specifically with the effect of multiple enemy systems on the host's life history strategies and population dynamics, it is also interesting to consider the interactions between the natural enemies themselves. All the enemies are trying to exploit the host in their own selfish way. We see many different actions taken by natural enemies to 'own' their host and further their fitness. In parasitoid wasps there are strategies of multiple-parasitism (infecting an already infected host and possibly displacing a previous parasite), hyperparasitism (parasitising a parasitoid larvae) and avoidance (where the female wasp avoids a host already infected by other parasites). There is also a complex interaction between viral infection and parasitism by parasitoids. The braconid egg-larval parasitoid *Chelonus insularis* does not discriminate between virus-contaminated and uncontaminated *Spodoptera frugiperda* eggs, resulting in high mortality of parasitoid larvae due to the death of the host larvae before they can emerge. A superinfecting parasitoid, ichneumonid *Campoletis sonorensis* 'won' over 80% of multiparasitoided hosts and emerged to pupate when in competition with *C. insularis*. *C. sonorensis* was also able to survive its host being infected by the nucleopolyhedrosis virus, as long as the host was parasitized a full two days before viral infection. Conversely, *C. insularis* was unable to use hosts with the viral infection (Escribano *et al.*, 2000).

Multi-enemy interactions can have varying effects on the host, with the population level being dependent on the assemblage of enemies that the host encounters. The inclusion of an additional enemy can actually increase the host numbers because the enemies interfere with each other. For example, after the outbreak of the parasitoid *Erwinia sphaerosperma*, the diamond-back moth host, *Plutella xylostella*, actually experienced an increase in population size, as the second parasitoid reduces the numbers of other native parasitoids (Ullyett, 1947). An increase in host numbers can also be brought about by intraguild predation of controlling agents, as seen in the predators of the aphid *Aphis gossypii* (Rosenheim *et al.*, 1993). This is an example of non-additive, combinatorial effects of natural enemies lessening the host mortality. There are many examples of the non-additive effects in nature, where we assume there is some interference between natural enemies. These are often seen between predators and parasitoids, as predators consume both hosts that are infected by the parasitoid and those that are not, thus leading to a reduced mortality from the parasitoid. For example, ants predate the cabbage butterfly larvae, *Pieris rapae*, which are already parasitized by parasitoid wasps (Jones, 1987). Similarly, ladybird larvae, *Cycloneda sanguinea* L, predate the saltmarsh aphids *Dactynotus*, which are already parasitized by *Aphidius floridaensis* (Ferguson & Stiling, 1996).

1.1.2 Models of multiple enemy systems

There is much interest in understanding the mechanisms that allow coexistence of multiple enemies and the effect they have on the dynamics of the system. One strong impetus for such an understanding has been the increasing use of natural enemies in the biocontrol of economic and ecological pest species (Denoth *et al.*, 2002). Theoretical modelling has emerged as a useful tool to understand the mechanistic processes behind population dynamics of two species. The first models of Lotka (1925) and Volterra (1926) have been further elaborated to focus on host-species interactions with one pathogen or parasitoid (Bowers *et al.*, 1993; Hochberg, 1989; Rohani *et al.*, 1994) and in a seminal paper of invertebrate hosts and their pathogens (Anderson & May, 1981). These landmark papers, however, only dealt with the interactions and dynamics between a host and a single enemy. Of even greater interest

and more complexity are the interactions of hosts with multiple natural enemies, including how coexistence of multiple parasites is supported and their effect on the dynamics of the system. Models of multiple parasites have included competing parasites species or strains for the same host (Dobson, 1985; Hochberg & Holt, 1990), host-parasite-hyperparasite interactions (Beddington & Hammond, 1977; Holt & Hochberg, 1998), host-parasitoid-parasitoid systems (Briggs, 1993; May & Hassell, 1981), host-parasitoid-pathogen interactions (Hochberg, Hassell and Holt, 1990) and host-vertically transmitted disease-horizontally transmitted disease systems (Jones *et al.*, 2007).

Dobson (1985) describes the competition between natural enemies on two levels: 'exploitation' and 'interference'. In exploitation, two or more parasite species have a joint utilization of a host species, while in interference, one parasite species reduces the other's fecundity or survival by an antagonistic interaction or displacing its use of the host. Coexistence is found to be easiest when both parasite species are aggregated in their distribution. Another study which investigated two competing parasites on two different species of host (Hochberg & Holt, 1990) found that the two parasites would co-exist if there were trade-offs between transmission and competition in each species. Additionally, host mortality by the parasites without allowing reproduction leads to persistent oscillations in the host population. The models investigating hyperparasitism showed that the hyperparasite was able to affect host growth rate, and also rate of recovery from the primary parasite, and therefore could both stabilise and destabilise systems. It showed that the host growth rate largely determined the interaction, with low growth rate leading to exclusion of the hyperparasite and high growth rate leading to the hyperparasite persisting and destabilising the system (Holt & Hochberg, 1998).

There are few models that deal with the interaction of a pathogen with a parasitoid or predator. Anderson and May (1986) demonstrated that the invasion of a pathogen into a predator-prey interaction could depress host numbers to a level that no longer supports the predator and, therefore, leads to its exclusion. More recently, it has been shown that the addition of a generalist predator to a classical host-pathogen model can

create a stable, low-density equilibrium. The interactions between this equilibrium and limit cycles induced by the pathogen lead to stochastically induced complex dynamics, and thus high variability in the time between insect outbreaks (Dwyer *et al.*, 2004). A model has been developed that combines a parasitoid with a free-living pathogen to form a complex discrete time model with continuous pathogen infectious periods, solved by integration of the infectious time (Hochberg *et al* 1990). The bringing together of both free-living pathogen and parasitoid showed that both could either eliminate the other or result in some intermediate outcome. Both were able to coexist in constant, cyclic or chaotic populations. Four factors were found to determine the outcome: (1) The finite rate of increase in the host, (2) The clumping of parasitoid (and pathogen) attacks, (3) The relative extrinsic potentials of the competitors, (4) The relative intrinsic potentials of the competitors. This is one of the few studies that focuses on the ecological and biological differences of pathogens and parasitoids. It also incorporates an interference parameter, explicitly a within-host competition factor, as it is known that competitors can co-inhabit hosts (Beegle & Oatman, 1975; Powell *et al.*, 1986; Vail, 1981) and this of course affected the intrinsic potential of each competitor. While the model has parallels with other multiple interactions and provides similar outcomes to other multispecies models (Hochberg & Holt, 1990b; Holt & Pickering, 1985; May & Hassell, 1981), these four key points can also be applied across most multi-enemy systems, with the rate of increase of the host often determining the stability of the whole system. An intermediate level of clumping, or the heterogeneity of the enemy attack, promotes coexistence by increasing the range at which two species may compete evenly.

The intrinsic and extrinsic competition of each enemy species is important, with one natural enemy being possibly superior at one of the two types of competition and inferior in the other and enabling coexistence. Other facilitators of coexistence include spatial (Amarasekare, 2000) and temporal factors.

1.1.3 The Model system: The Winter Moth on Orkney

Our empirical system centres on the population dynamics of the winter moth, *Operophtera brumata*, and its natural enemies on a closed system on the Orkney Isles. We intend to survey the island populations for different natural enemies and their associated stage and density dependent infections. The winter moth itself is a polyphagous folivorous insect, feeding on many varieties of trees and shrubs. Populations live on oak (*Quercus* spp.), Sitka spruce (*Picea sitchensis*), heather (*Calluna vulgaris*) and bilberry (*Vaccinium myrtillus*) in moorland across Great Britain (Wint, 1983). They have been found to reach outbreak levels in heather (Kerslake *et al.*, 1996; Picozzi, 1981) and Sitka spruce in Scotland (Hunter *et al.*, 1991; Stoakley, 1985) and can defoliate large areas, leading to host plant death on heather moorland and the invasion of grasses into the area (Kerslake *et al.*, 1996). This is of importance as heather moorland is a sensitive habitat that supports a wide array of bird life. Outbreaks have also occurred on mature oak forest, causing large defoliation (Kerslake *et al.*, 1996). The model of the isolated system will help the understanding of winter moth outbreak events on nutritionally poor heather.

The winter moth is distributed across a large range of Europe, only being absent from the most northeastern and southwestern areas (Tenow 1972). The supercooling point for overwintering eggs is -33°C (Macphee, 1967), restricting the northern limit of the winter moth. The species was also introduced by accident to North America, where populations outbreaks have occurred too (Edland, 1971; Gillespie *et al.*, 1978; Kimberling *et al.*, 1986). The winter moth is a univoltine species, which emerges as an adult in November and December. The females of the species are brachypterous and therefore flightless, hampering dispersal, while the males are fully capable of flight (Varley *et al.*, 1973). Despite their ability to fly, males have also been found to have a low dispersal. This is possibly an adaptation to increase egg hatch synchronization with local bud burst (Van Dongen *et al.*, 1996). Genetic differentiation has been found to exist on a fine scale of habitat fragmentation, with distances between sites from only a few hundred metres up to 3 km apart (Van Dongen *et al.*, 1998). This shows

that the winter moth has very little dispersal and supports the assumption that the Orkney populations are isolated from the Scottish mainland.

Once the females have emerged from the pupae, they crawl up a tree or shrub close by (Graf *et al.*, 1995) and mate. The fertilised females then crawl further up the plant and lay eggs in crevices in the bark and the stem. On average, females produce 150 eggs, but can carry as many as 300. The size of the eggs is small, only 0.7-0.8 mm in length and 0.5 mm in width (Tenow, 1972). The eggs hatch the following spring and are often synchronized with budburst of the host plant, which enables the larvae to feed on the young shoots (Larsson & Ohmart, 1988). The phenology affected the fitness of larvae feeding on oak (Holliday, 1977; Varley & Gradwell, 1968) and on spruce (Watt & McFarlane, 1991). Conversely, larvae feeding on heather were unaffected by a delay of hatching of 30 days (Kerslake & Hartley, 1997). This was despite a decline in the nitrogen concentration in the foliage over this period. The larvae may have a nutritional compensatory mechanism that, at a certain level, buffers the effect of reduced nitrogen content in the host plant. The lack of effect of heather means temporal synchrony of hatch date and bud burst need not be modelled.

Neonate larvae of *O. brumata* disperse by becoming air buoyant, producing a long strand of silk to facilitate ballooning (Edland, 1971; Holliday, 1977). The distance of dispersal relies upon weather conditions and the height of the host plant they are in. Larvae in low lying heather on open moorland have different dispersal ability from that of larvae high up in an oak forest canopy. Edland (1971) found ballooning could disperse larvae by distances of up to several hundred metres. Dispersal is a behavioural adaptation to the quality of the host plant. Dispersion may occur due to chemical cues from the plant, when dispersion to another host plant will be more beneficial than remaining on the original (Tikkanen, 2000).

1.1.4 Natural Enemies: Parasitoids, Pathogens and Predators

O. brumata has a number of parasitoids that attack the larval form, both parasitic wasps and flies. Parasitoids from the Hymenoptera group are found to attack the

winter moth. Varley *et al* (1973) identified *Cratichneumon culex* to be an important parasitoid in oak woodland (Wytham Wood, Berks). The wasp directly injects an egg into the pupa of the winter moth. The adults emerge the following summer, after killing and ingesting the host larvae. Another is a koinobiotic parasitoid, *Phobocampe neglecta*; this attacks the winter moth larvae in the third and fourth instars. The wasp larvae consume the host larvae after pupation. *P. neglecta* is not present in high altitude moorland over 500m, but has been present in 27% of winter moth larvae in particular sites on Orkney (Kerslake *et al.*, 1996).

A lethal pathogen to the winter moth is a nucleopolyhedrovirus (ObNPV). This is a baculovirus, and there have been reported epidemics in many Lepidoptera outbreaks (Entwistle & Evans, 1985; Tanada & Fuxa, 1987), including in the winter moth (Embree, 1966; Stoakley, 1985). The baculoviruses are arthropod DNA-specific viruses. Their persistence outside the host is prolonged due to a proteinaceous occlusion body, which increases their transmission capabilities (Miller, 1997). The virus particles are ingested orally and infect through the gut. The host replicates the virus until the host tissues are broken down to such an extent that it dies. The cuticle then ruptures, releasing occlusion bodies that contaminate the surrounding soil (Raymond *et al.*, 2002a).

A parasitic fly, *Cyzenis albicans*, is a well known parasite of the winter moth. It lays eggs on the foliage upon which the moth larvae graze. The larvae consume the eggs of the fly during foliage ingestion. The fly egg hatches and breaks through the gut wall and lodges in the salivary gland of a larva. The fly larvae consumes the moth larvae during pupation, resulting in host death (Hassell, 1980). This process is stage dependent, as eggs consumed by larvae smaller than the third instar are normally ruptured and killed (Hassell, 1980). *C. albicans* has been introduced to control winter moth populations in British Columbia (Graham, 1958), where its presence appears to control the moth in oak forest but not on apple orchards, despite similar parasitism levels (Roland, 1986). Maintenance of low winter moth densities in British Columbia was caused primarily by pupal mortality from generalist predators, after an initial drop in densities caused by increased parasitism (Roland, 1994). Despite claims that pupal

mortality was not density dependent (Bonsall & Hassell, 1995), predation remained the major and persistent source of winter moth mortality in study sites in Nova Scotia (Pearsall & Walde, 1994; Roland, 1995). *C. albicans* is present in the U.K., but it was not found to be the major source of population control (Varley et al 1973). As yet, it is not present on the Orkney Isles.

The main source of predation on the pupae is from beetles (Coleoptera), which accounted for over half of the pupal predation in one study (Frank, 1967). Small mammals are important over the winter months in oak habitat (Varley *et al*, 1973), accounting for over half the pupal mortality during the winter in another study (Buckner, 1969). Therefore, it is clear that both are important in winter moth predation. It has been suggested that pupal predation is density dependent, due to generalist predators, such as shrews, only targeting pupae when they are in high abundance (Varley *et al*, 1973). The absence of pupal predation has been proposed as a potential cause of outbreaks in the moth, with carabid predator numbers being found to be 10-100 times lower in outbreak-prone heather and spruce habitats compared to in oak woodland (Raymond et al., 2002b).

Studying the multiple enemies of the winter moth on Orkney will further the understanding of multiple species interactions. With the empirical data outlined in Chapter 5, it is shown how the parasites respond to changes in host density, as well as the developmental stage. This is then used to develop more theoretical work in Chapter 6, describing the possible outcomes of population dynamics and species coexistence.

1.2 Evolutionary Outline: Host and Multiple Enemy Interactions

1.2.1 Evolution of Parasites

Parasites are known to adapt to their hosts in order to promote their persistence in a host population. For example, the myxomatosis virus was extremely virulent when first introduced to Australian rabbits in 1950, with nearly 100% mortality of infected rabbits. Virulence was then seen to decrease in magnitude over the following years (Fenner & Ratcliffe, 1965), clearly indicating a change in, and hence evolution of, the myxomatosis strain.

However, virulence by the parasite may often be an unavoidable consequence due to pathogen replication to increase transmission to new hosts (Anderson & May, 1982; Lipsitch & Moxon, 1997; Mackinnon & Read, 1999a, b; Messenger *et al.*, 1999) Often a host can have multiple infections and then selection of the parasite is not merely an interaction between it and the host, but also an interaction of the host and the other competing parasites. As natural system hosts are often challenged by multiple parasites (Chen *et al.*, 2004; Haine *et al.*, 2005; Haine *et al.*, 2004; Hodgson *et al.*, 2004). This can have important implications for the evolutionary dynamics of parasites. Multiple infections within a single host, in particular, are thought to select for more virulent parasites in a scramble competition for resources. In nematodes infecting fig wasps, increased horizontal transmission was correlated with higher virulence, and this was explained by within-host competition (Bull, 1994; Herre, 1993). The fact that the mode of transmission also influences virulence evolution is widely recognised. Pathogens transmitted via free-living infective stages (e.g. tuberculosis, smallpox) or through insect vectors (e.g. malaria) generally have much higher virulence compared to those requiring direct host-to-host contact (Ewald, 1993). Parasites, which compete for the same host, that have different transmission strategies consequently also have different selective pressures acting upon them. Therefore, a parasite that transmits vertically will be in direct conflict with a parasite that is an obligate killer, especially if the latter parasite kills the host before the vertically-transmitting parasite is able to reproduce.

1.2.2 Models of the Evolution of Parasites

Theoretical models often assume a trade-off between transmission and virulence in horizontally transmitting parasites. The dominant theory is that parasites achieve transmission via within-host replication, with virulence seen as an unavoidable consequence. If transmission is modelled as an increasing saturating function of virulence, this selects for an intermediate level of virulence (Frank, 1996; Restif & Koella, 2003; van Baalen & Sabelis, 1995). However, many pathogens transmit via free-living infective stages, capable of surviving for long periods of time outside the host (Anderson and May 1981). This partially separates transmission from the lifespan of infected hosts, and therefore can select for higher virulence (Day 2002). If free-living particles are released upon death of the infected host, as found in the nuclear polyhedrosis and granulosis viruses, then this potentially selects for even higher virulence (Day 2002). Obligately killing parasites, in particular, may be expected to evolve very high virulence (Ebert and Weisser 1997). However, there is also a trade-off between time to death and numbers of infectious particles produced, which is directly linked to future transmission of the disease. This relationship is seen in the insect baculovirus, with a positive correlation of time to death and virus yield (Hernandez-Crespo et al., 2001), and also in obligate killers in *Daphnia* (Ebert & Weisser, 1997). However, some free-living pathogens, such as the bacterium *Bacillus anthracis*, produce toxic substances that have no clear connection to within-host replication (Mock & Fouet, 2001; Prescott *et al.*, 1999). Recent theory has shown that such toxicity effects may actually confer a selective advantage, particularly in, although not limited to, obligate killers (Day, 2002).

1.2.3 Models of the Evolution of Multiple Parasites

Superinfection, where a more virulent strain displaces a less virulent strain in an individual host, selects for the more virulent and competitive parasites (Levin & Pimentel, 1981; Nowak & May, 1994). Where parasites coinfect, such that hosts harbour more than one infection at a time, both within-host and population level interactions are important (May & Nowak, 1995; Mosquera & Adler, 1998; van

Baalen & Sabelis, 1995). Models of coinfection by horizontally transmitted parasites (HTPs) indicate that parasites with greater virulence are favoured and that parasite coexistence is supported (Mosquera & Adler, 1998). Often in simultaneous infection, when both parasites are transmitted similarly, the evolution of virulence is mediated by the intensity of within-host competition, with the best within-host competitor often being the most virulent. However, when parasites with different transmission modes infect the same host, the selective pressures may be different.

There is a clear distinction between two key modes of parasite transmission. Horizontal transmission occurs between all individuals within a host population, for example through contact with either an infected individual or a free-living parasite. In contrast, vertical transmission is transgenerational, passing from parent to offspring. Although found across many taxa (Mims, 1981), purely vertically transmitting parasites (VTPs) are most commonly found in invertebrates and pass from mother to offspring (Hogg *et al.*, 2002; Kelly *et al.*, 2003; Stouthamer *et al.*, 1999; Werren, 1997).

Pure VTPs that cause virulence to their host will not persist without some other mechanism that compensates for the virulence (Fine, 1975; Lipsitch *et al.*, 1995). Parasites that transmit vertically may persist if they also transmit horizontally (Busenberg *et al.*, 1983; Regniere, 1984). This theory is supported in nature by many insect viruses (e.g. granulosis and baculoviruses) having both means of transmission (Burden *et al.*, 2002; Hackett *et al.*, 2000; Zhou *et al.*, 2005). The evolutionary dynamics of parasites that possess both vertical and horizontal transmission modes have been previously outlined (Lipsitch *et al.*, 1996). A key result was that, as parasite numbers increase, they select for greater vertical transmission, due to the higher potential for generation-to-generation infection and reduced encounters with susceptible individuals.

There is also evidence that some VTPs may reduce the effect of other natural enemies (Haine *et al.*, 2005; Oliver *et al.*, 2003). The dynamics of such interactions has become increasingly studied (Faeth *et al.*, 2007; Jones *et al.*, 2007; Lively *et al.*, 2005). All

these studies showed that a purely vertically transmitted parasite that causes some virulence to the host is able to persist by protecting the host from a virulent horizontally transmitted parasite. It is our intention to explore the evolutionary dynamics of this conflict between a vertically transmitting parasite and a horizontally transmitted parasite.

We will also address the situation where a parasite coevolves with another parasite within the same system. While there are many coevolution models between host resistance and parasites, there is very little theory or example of this occurring. This is mainly due to most parasites competing in similar ways and thus resulting in one winner for the host and, ultimately, only one parasite remaining in the system. When their life histories enable coexistence, it can be quite certain that these parasites will exercise selective pressures on each other to maximise their own fitness. We deal with this issue specifically in Chapters 3 and 4.

1.3 Theoretical Approach

1.3.1 Population Dynamics

Anderson and May (1981) developed models to describe the population dynamics of microparasites and their invertebrate hosts. This basic, yet influential, model assumes that transmission occurs through direct contact between an uninfected (susceptible) and an infected host. Once infected, individuals suffer an increased mortality rate (virulence) and recover at a constant rate. The model can be adapted to include morbidity, vertical transmission, density-dependent birth rates, and transmission via free-living infective stages. The inclusion of density-dependence in the birth rate provides a stabilizing mechanism that increases dynamic stability and reduces the likelihood of cycles (Bowers *et al.* 1993; Dwyer 1994; White *et al.* 1996). These models are constructed in continuous time and are very amenable to analysis of invasion boundaries and system stability. However, other models can be better suited when the host being modelled has distinct, discrete annual seasons. Thus, the Anderson and May model can be incorporated into a much more complex discrete-

continuous model that can better reflect the ecology (Hochberg *et al.*, 1990). In addition, changes in modelling are required in systems when stage structure proves to be the important factor in the dynamics and coexistence of multiple species (Briggs *et al.*, 2000; Gurney & Nisbet, 1985; Gurney *et al.*, 1983; Wearing *et al.*, 2004). The models often have a delay component in either the host and/or the parasite phase to model the development time of the host, or the period between infection and symptoms of the disease. The feature of the simpler models is the ability to analytically solve them, whilst the more complex models, although increasingly difficult to analyse, can provide mechanisms that will drive coexistence and also the dynamics of the system. However, these complex models often require numerical approximations to solve them as no explicit terms can be derived.

1.3.2 Winter moth dynamics

Due to the univoltine nature of the winter moth, models that have continuous generations may not be as suitable as they are to populations of invertebrates in tropical climes. Therefore, in a highly seasonal habitat, a combination of discrete and continuous modelling (Hochberg *et al.*, 1990) is used to better describe the jumps from larval season to larval season and the mortality effects over these periods. With the ability to parameterise more of the developmental stages of the larval moth, it is possible to add stage structure to the continuous part of the model. Most stage structured modelling use delay differential equations, with generations of host and larvae overlapping, as well as those of any parasites (Bonsall, 2004; Bonsall *et al.*, 2002; Briggs & Godfray, 1995; Briggs *et al.*, 1999; Murdoch *et al.*, 1986; Wearing *et al.*, 2004). The added complexity does inhibit some of the analytical convenience of the model, but this slight constriction is tempered by the fact that possible parameter space is reduced due to empirical knowledge gained from the field system. This will be expanded further in Chapter 6.

1.3.3 Evolutionarily Stable Strategies and Adaptive Dynamics

An evolutionarily stable strategy (ESS) is a strategy that, if adopted by a population, cannot be invaded by any competing alternative strategy. The theory of evolutionarily

stable strategies was formalised mathematically (Maynard Smith, 1982; Maynard Smith & Price, 1973) but has its origins in the survival of the fittest (Darwin, 1859), the workings of Fisher (1930), and the concept of the unbeatable strategy (Hamilton, 1967). The phenotypic approach is particularly useful as genetic considerations may be circumvented to some extent. However, the ESS-definition does not specify whether the singular strategy will actually become established in the first place (Eshel, 1983). Taylor (1989) produced two distinct conditions for evolutionary stability, the first being the local ESS stability, which he termed δ -stability, and *m*-stability, which refers to a condition for convergence to a trait value. This has led to an additional definition of evolutionary stability; a singular strategy is called 'convergence stable' (CS), if nearby residents may be invaded by those closer to the singular strategy (Christiansen, 1991). Only when the strategy is both a locally stable ESS and convergently stable is there a singular point of maximum phenotype fitness that is an evolutionary attractor. As such, it is these points that are the endpoint of evolution.

To further investigate an ESS of a particular organism, it is possible to use other mathematical theories. We use adaptive dynamics, a mathematical theory that explicitly links population dynamics to long-term evolution, driven by mutation and natural selection. This was developed largely in the studies by Metz et al. (1996) and Geritz et al. (1998). The main notions in adaptive dynamics are, firstly, that the resident population can be assumed to be in a dynamical equilibrium when new mutant phenotypes appear. Secondly, the eventual fate of such mutants can be inferred from their initial growth rate when rare in an environment consisting of the resident phenotypes. The dynamical feedback from the resulting interactions means that the fitness of a particular phenotype not only depends on its own life history parameters, but also on those of the resident phenotype it is invading. If the growth rate of the mutant that is invading, also known as the invasion exponent, is positive, then the mutant can be maintained and may go on to become the resident phenotype. It is then possible to choose specific traits to undergo evolutionary change. These are often linked by incorporating simple trade-off theory (Stearns, 1992). This can then produce expression of fitness and the position and nature of singular points of evolution. Mutation in adaptive dynamics does not have any large steps, but rather only occurs

locally to the resident phenotype strategy. It is also assumed to occur over such a time period that the mutation, if selected for, will become fixed, i.e. become the resident, before future mutations occur. Further detail of the methods in adaptive dynamics will be found in Chapters 3 and 4 that deal with evolution of traits in two parasites. However, it is important to outline the possible results than can be gained by different local ESS and CS properties.

There are four classifications of singular points. The first occurs when there is no evolutionary or convergence stability, creating a 'repellor' point and thus all mutations would be selected away from this singular point. The converse of this is the 'attractor', which occurs when the local ESS is stable, resisting invasion, and it is convergence stable, that is to say that mutations all proceed towards the ESS (Bowers *et al.*, 2005; Bowers & White, 2002). The third is where a 'branching point' in evolution occurs, where there is convergence stability but is not an ESS. This can lead to the coexistence of more than one strategy (Geritz *et al.* 1998, Nowak & Sigmund 1989). And finally is a singular point that has been termed 'the Garden of Eden' (Bowers *et al.*, 2005; Nowak & Sigmund, 1989), where the ESS is stable and cannot be invaded whilst there is no convergence and hence no way of getting there. There may also be more than one locally stable strategy, a phenomenon known as evolutionary bistability, where the outcome is dependent on the initial resident.

We use these approaches to examine the evolutionary implications of parasites with different transmission modes sharing the same host. Here the ecological feedbacks in the interaction are critical to the outcome and approaches of adaptive dynamics explicitly include these feedbacks.

1-4 Thesis outline

This dissertation provides an investigation into the evolution and population dynamics of host and multiple-parasite systems. This is done by utilising both theoretical approaches as well as field ecology. Chapter 2 explores the interaction of vertically and horizontally transmitting parasites. The vertically transmitting parasite is virulent

but provides the host it infects with protection against the horizontally transmitting parasite. The population dynamics and stability are investigated. Chapters 3 and 4 deal with the evolutionary dynamics of the interactions between vertically and horizontally transmitted parasites. Chapter 3 outlines what occurs between a vertical parasite that persist in the system through feminisation, whilst chapter 4 deals with vertical parasites that directly affect the horizontally transmitted parasite to aid in the vertical parasites persistence. Chapter 5 summarises the data collect from the field sites in Orkney outlining the prevalence of the natural enemies (pathogens and parasitoid) and the stage dependence affects in the system. Chapter 6 applies the data gained to construct and partly parameterise a stage dependent, discrete-continuous model of the winter moth system. This gives insight into the possible population dynamics of the system.

2. Interference and the Persistence of Vertically Transmitted Parasites



Detail of Emperor moth (*Pavonia pavonia*) wing, hatching from pupae at Stettiscarth, Orkney. 2005.

2.1 Introduction

Despite the fact that many parasites are transmitted vertically from mother to offspring (Goulson & Cory, 1995; Smits & Vlak, 1988), the persistence of virulent, purely vertically transmitted parasites in nature runs counter to simple ecological theory (Busenberg *et al.*, 1983; Lipsitch *et al.*, 1995; Regniere, 1984). This classical theory suggests that horizontal transmission is also required to enable persistence. Furthermore, Busenberg *et al.* (1983) showed that vertically transmitted parasites that increase the host's death rate have a threshold for persistence, which becomes increasingly harder to meet as the rate of horizontal transmission is reduced. A vertically transmitted parasite (VTP) was also shown to be unable to persist without some horizontal transmission in a model in which infection was assumed to reduce fecundity, rather than increase the death rate (Regniere 1984). Lipsitch *et al.* (1995) then showed that vertical transmission alone is not enough to maintain a parasite in a self-limited host population, if infection either lowers birth rate or increases death rate. So although vertical transmission in addition to horizontal transmission is well known to increase parasite persistence (Anderson & May, 1981) and affect the stability (Bonsall *et al.*, 2005; Boots *et al.*, 2003), theory suggests that purely VTPs will not persist in nature.

The classic theoretical results have raised the question of what other mechanisms may allow the persistence of purely VTPs. VTPs are commonly found in male-killing bacteria, of the genera *Rickettsia* and *Wolbachia* (Hurst *et al.*, 1999; Werren *et al.*, 1994). These distort the sex ratio of the host population by reducing the number of males. The death of the males can enhance the fitness of the surviving brood members due to factors such as reduced intrabrood competition and inbreeding. Models have shown that this fitness compensation allows the parasite to persist despite the death of infected males and female fitness costs (Hurst, 1991, 1993). Another possibility is host metapopulation structure. A model showed that vertically transmitted parasites that reduce host fitness may persist within a metapopulation, if infected offspring are able to invade new patches (Saikkonen *et al.*, 2002).

Here we examine another mechanism by which a completely VTP may be maintained in the population. We consider in detail, the situation where a vertical infection gives the host some form of interference protection against other natural enemies (Lipsitch *et al.*, 1996). This interference can arise in a number of ways. Firstly the infected host's behaviour may be modified so that encounters with other enemies are reduced. One obvious example is where the hosts feeding rates are reduced due to VTP infection (Bellonick, 1996) which results in a reduction in the ingestion of microparasitic infective stages. There are also many examples of hosts having their behaviour modified by parasitic infections, commonly to increase parasite dispersal and transmission via increased predation in intermittent hosts (Latham & Poulin, 2002; Levri & Fisher, 2000; McCurdy *et al.*, 1999). As VTPs are passed on from mother to offspring, behaviour that increased predation would reduce the ability of the VTPs to be passed on to the next generation. This conflict between horizontally and vertically transmitted diseases has been neatly discussed by Haine *et al.* (2005).

In addition to behavioural changes, the VTP may offer protection by restricting the uptake or development/replication of a secondary horizontally transmitted parasite (HTP). Despite the relative paucity of multi-enemy studies, interference has been shown to occur in a number of multi-parasite interactions. For example, it has been reported that vertically transmitted bacterial symbionts in aphid hosts interfere with the development of the larvae of parasitic wasps (Oliver *et al.*, 2003). Viruses have also been seen to have an effect on each other's intra-cell replication rate. In *Helicoverpa zea*, a slower killing virus (granulose virus) has been found to inhibit the replication rate of a more pathogenic NPV (nucleopolyhedrovirus) in the host, thereby increasing host fitness (Haskett *et al.*, 2000). Although this occurs in two HTPs, it demonstrates a mechanism of possible viral interference. Rohani *et al.* (2003) described the interaction of fatal diseases, where infection by one pathogen removed possible susceptibles from the population during a quarantine period, thus preventing infection from another. Modelled in this way, ecological interference was shown to have a profound effect on the dynamics of the system, making multi-annual outbreaks of different infections out of phase. Thus the dynamics can only be explained by the

interaction between the two infections and not simply looking at either one in isolation.

A related form of cross-immunity between one horizontally and one vertically transmitted parasite has previously been modelled by Allen *et al.* (2003). In this model, however, the VTP also had a component of horizontal transmission and as such the model did not examine the role of protection in the coexistence and persistence of a purely VTP. Here we present a simpler model that shows how ecological protection can lead to the persistence of an exclusively VTP in a host-parasite system. We assume that hosts infected with the VTP have an increased death rate and lower fecundity, but also gain protection from the HTP in the form of a reduced transmission rate. We consider both directly transmitted and free-living forms of HTPs in order to examine the effect of the persistence of VTPs on host-parasite dynamics. In addition we compare HTPs that castrate their hosts with true parasites that allow reproduction from infected individuals (see Boots, 2004).

2.2 Models and Analysis

The first model considers the density of the susceptible host population, X , the host population infected with the VTP, V , and the host population infected with the HTP, Y . The HTP is assumed to be directly transmitted. The following model represents the dynamics.

$$\frac{dX}{dt} = (r - q(X + V + Y))X + (1 - p)(af - q(X + V + Y))V - \beta XY, \quad (1a)$$

$$\frac{dV}{dt} = p(af - q(X + Y + V))V - (\alpha_v + b)V - (1 - \delta)\beta VY, \quad (1b)$$

$$\frac{dY}{dt} = (X + (1 - \delta)V)\beta Y - (\alpha_y + b)Y. \quad (1c)$$

Here, r (assumed > 0) is the intrinsic rate of growth of the host, equal to the birth rate a , minus the natural death rate b , with density dependence from the total host population acting on the birth rate via the crowding parameter, q . The birth rate of

hosts infected with the VTP is reduced by a proportion $(1-f)$, and a proportion, p , of their offspring are born infected with the vertical parasite (therefore $(1-p)$ are born into the susceptible class). Those infected with the HTP are assumed not to reproduce as the parasite acts in a ‘predatory’ manner (we will relax this assumption later; see below). Both susceptible and vertically infected hosts are susceptible to horizontal infection, at rates βY and $\beta\delta Y$ respectively. We assume a process of super-infection so that there is no co-infection with the two parasites. The parameter $\delta \in [0,1]$ acts to reduce horizontal transmission so that vertically infected hosts are afforded a level of protection from the HTP. Here, $\delta = 1$ equates to total protection while $\delta = 0$ equates to none. The parameters α_v and α_y are the additional death rates due to infection from the VTP and HTP respectively.

The model, equations (1a-c), has three biologically relevant equilibria at $(X, V, Y) = (X_1, 0, 0)$, $(X_2, 0, Y_2)$ and (X_3, V_3, Y_3) . (Note: the trivial equilibrium at the origin is always unstable and equilibria of the form $(0, V, 0)$, $(X, V, 0)$ and $(0, V, Y)$ are only attainable when $p = 1$ and always unstable. We therefore do not consider these equilibria. The disease-free equilibrium $(X_1, 0, 0)$ is given in equation (2).

$$(X_1, 0, 0) = \left(\frac{r}{q}, 0, 0 \right). \quad (2)$$

This equilibrium is stable provided the following inequality holds

$$\frac{r\beta}{q(\alpha_y + b)} < 1. \quad (3)$$

This is equivalent to $R_0 < 1$ where R_0 is the reproductive ratio of the HTP (Anderson & May, 1981). The equilibrium $(X_2, 0, Y_2)$, which includes the host and the HTP, is given by

$$(X_2, 0, Y_2) = \left(\frac{\alpha_v + b}{\beta}, 0, \frac{r\beta - q(\alpha_v + b)}{\beta(\beta + q)} \right). \quad (4)$$

A necessary condition for stability is that the inequality in equation (3) is reversed, as this allows the HTP to invade and coexist with the host. Stability also requires that the VTP is unable to invade (if inequality (5) below is reversed). The equilibrium (X_3, V_3, Y_3) is stable if the following inequality holds

$$p[af - q(X_2 + Y_2)] > (\alpha_v + b) + (1 - \delta)\beta Y_2. \quad (5)$$

Inequality (5) can be easily interpreted biologically. It represents the fact that, at the host-HTP equilibrium, net births to the VTP class must be greater than net losses (from natural death, death due to the VTP and from contracting the horizontally transmitted infection). If there is complete protection, $\delta = 0$, the inequality is

$$p[af - q(X_2 + Y_2)] > \alpha_v + b. \quad (6)$$

This is more easily satisfied than that inequality (5); however, it does not mean that V can always invade into the host-HTP equilibrium. Inequality (6) emphasises, rather intuitively, that VTPs with high vertical efficiency (high p) that do not significantly reduce host reproduction (high f) and have low virulence (low α_v) are most likely to coexist.

It is always easier for the VTP to coexist in species with low susceptibility to crowding, (low q) (Figure 2.1A). There is a threshold level of the birth rate, below which the VTP cannot coexist (Figure 2.1B). Above this threshold, as the birth rate increases, the VTP can persist for relatively lower levels of protection against the HTP. This effect saturates as the birth rate increases. Productive hosts with high carrying capacities are therefore more likely to support the VTP. The capacity for persistence of the VTP responds to changes in the HTP transmission parameter, β , in a similar way as to changes in birth rate (Figure 2.1C). Again there is a threshold level

of transmission, below which the VTP does not persist. As the transmission rate of the HTP increases, the VTP can persist at lower levels of protection. Similarly, if the proportion, p , born with the VTP infection is above a threshold, then further increases in p allows the persistence of VTPs that offer lower levels of protection (Figure 2.1 D).

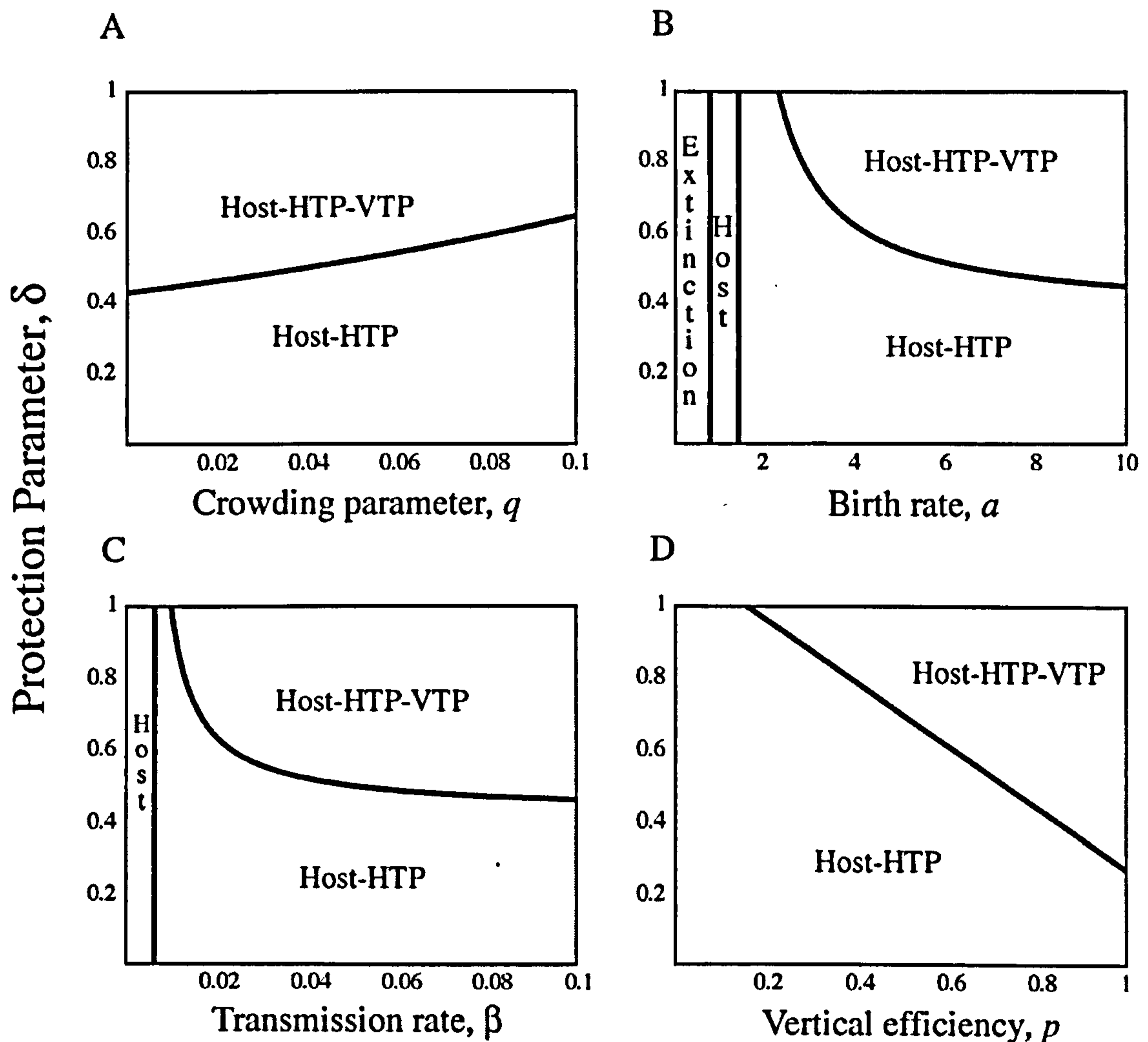


Figure 2.1: The role of protection (δ) vs susceptibility to crowding (q), birth rate (a), transmission rate (β) and vertical transmission efficiency (v) in coexistence of the natural enemies. Parameter space portraits indicating where the different population equilibria are stable for the model defined by equations (1a-c). Parameters used are $\beta = 0.2$; $f = 0.8$; $r = 10$; $q = 0.01$; $b = 1$; $p = 0.8$; $\alpha_x = 0.25$, $\alpha_y = 4$, unless varied in the plots.

The level of protection offered by the VTP also alters the equilibrium values and therefore the proportions of the population in the different classes (Figure 2.2). Both types of infected hosts (VTP & HTP) increase as the protection increases up to a

threshold at which the protection becomes so large that there are very few individuals infected with the VTP becoming infected by the HTP (and therefore the HTP density decreases). The susceptible host density decreases with increasing protection until they are mostly produced due to the VTPs imperfect vertical transmission ($p < 1$). Due to protection the VTP experiences a lower transmission rate from the HTP and therefore supports a higher density VTP infected host. This in turn supports a higher density of HTP infected hosts that acts to depress the susceptible host density further. As protection tends towards 100% ($\delta \rightarrow 0$), the density of hosts infected with the HTP decreases. The decrease in HTP infected hosts decreases the infection pressure on susceptible hosts, thus, as well as the increased input from the imperfect efficiency of the VTPs, their density to increase.

The above results correspond to a HTP that castrates the host. If we modify the model (equations 1a-c) to allow reproduction from individuals infected with the HTP in a more traditional 'parasitic' sense, then equation (1a) becomes

$$\frac{dX}{dt} = (r - q(X + V + Y))(X + Y) + (1 - p)(af - q(X + V + Y))V - \beta XY. \quad (7)$$

This modification does not change the type of equilibria that can be obtained or the conditions on their stability (equations (3) and (5)). It does however change the population values at the equilibria $(X_2, 0, Y_2)$ and (X_3, V_3, Y_3) and, therefore, affects the parameter regions over which the VTP can persist (Figure 2.3). Here it is assumed the HTP is a super-infection. However, simulations have shown the same qualitative results occur if co-infection is allowed and the HTP can contribute to the VTP growth rate.

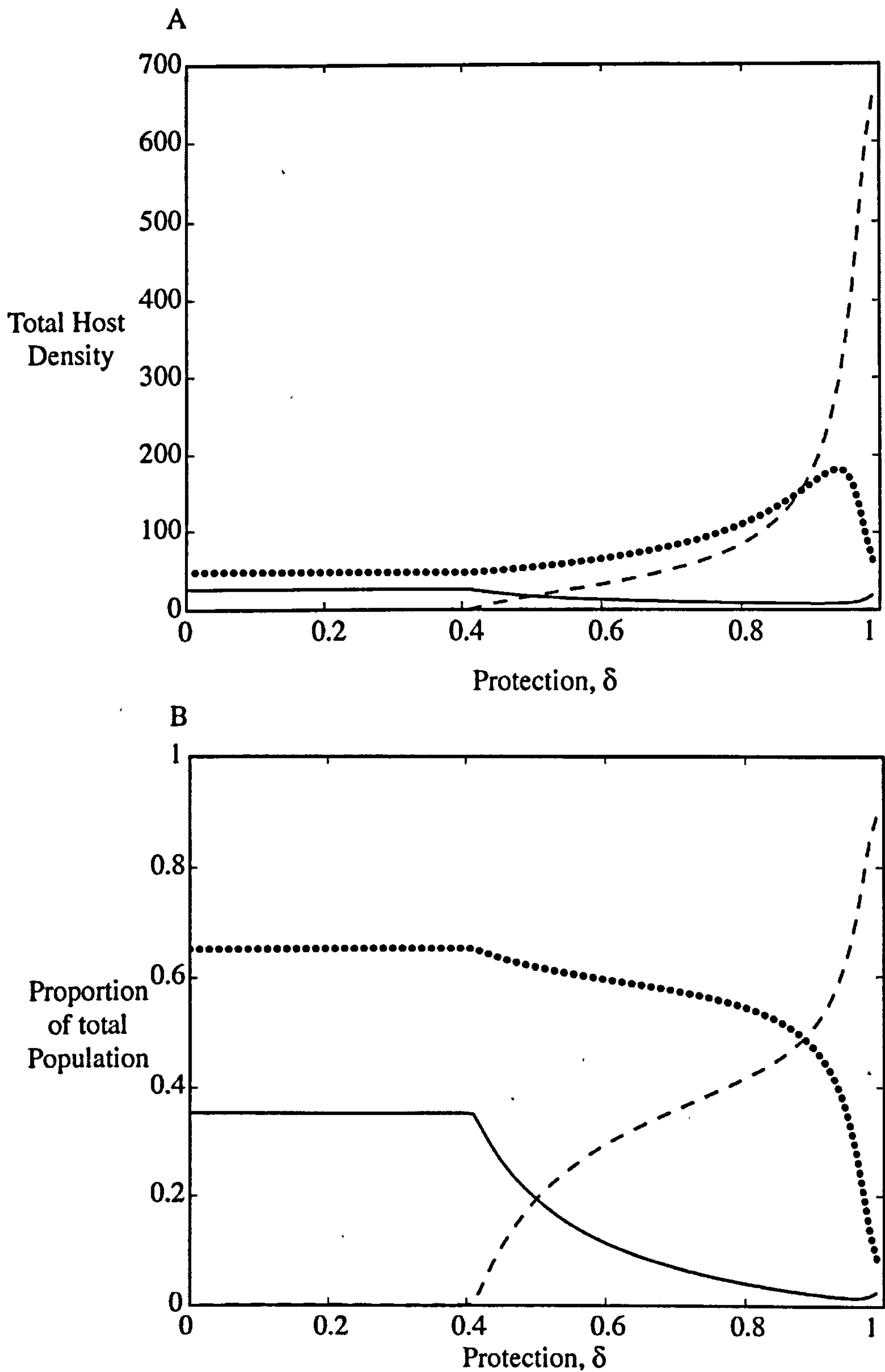


Figure 2.2; A. The equilibrium densities of susceptibles X (solid line), horizontally transmitted infecteds Y (dotted line), vertically transmitted infecteds V (dashed line) to increasing protection against the HTP for the model defined by equations (1a-c). B. The proportional densities are shown. Parameters used are $\beta=0.2$; $p=0.8$; $f=0.8$; $b=1$; $r=10$; $q=0.01$; $\alpha_v=0.25$; $\alpha_y=4$.

The distinction between infectious organisms that allow reproduction and those that castrate their host is important, since in the absence of recovery, it distinguishes ecologically predatory and parasitic life-histories (Godfray 1994; Boots 2004). Figure 2.3 shows the difference between a ‘parasitic’ and a ‘predatory’ HTP. In all the plots it is clear that the VTP is supported for a greater range of parameters if the HTP is predatory. For a predatory HTP, persistence of the VTP decreases as the crowding parameter increases. For the parasitic HTP, the VTP shows maximum persistence at intermediate values of crowding (Figure 2.3A). For a parasitic HTP the VTP can only persist at intermediate levels of the birth rate, whereas it shows an increasing saturating response to birth rate for a predatory HTP (Figure 2.3B). VTP persistence shows an increasing saturating response to HTP transmission for a predatory HTP and a similar response to a parasitic HTP, although here VTP persistence gradually declines as HTP transmission increases, rather than saturates (Figure 2.3C). Both predatory and parasitic HTP allow for similar persistence of the VTP for high levels of the death rate due to the HTP, α_Y . However the VTP cannot be supported at low levels of α_Y when the HTP is parasitic (Figure 2.3D).

The ability of the VTP to better persist in a ‘predatory’ rather than a ‘parasitic’ system is due to the uninfected hosts having no contribution to the growth rate from the HTP. This reduces the ability of the uninfected host to out-compete the VTP for resources and therefore means the VTP is required to provide less protection from the HTP to persist in the system. Simulations have shown that these effects are also apparent when the HTP reduces the fecundity of infected individuals rather than completely castrating them and therefore falls between our ‘predatory’ and ‘parasitic’ limiting cases.

The model (equations 1a-c) was also modified to consider the persistence of a VTP when the HTP takes the form of a free-living stage. The model is described as follows

$$\frac{dX}{dt} = (r - q(X + V + Y))X + (1 - p)(af - q(X + V + Y))V - \beta XW \quad (8a)$$

$$\frac{dV}{dt} = p(af - q(X + V + Y))V - (\alpha_v + b)V - (1 - \delta)\beta VW \quad (8b)$$

$$\frac{dY}{dt} = (X + (1 - \delta)V)\beta W - (\alpha_y + b)Y \quad (8c)$$

$$\frac{dW}{dt} = \lambda Y - \mu W \quad (8d)$$

The model is based on the free-living infective stage model G of Anderson & May (1981). The classes and parameters are the same as in equations (1a-c) except we additionally include a new class, W , which represents the density of the external free-living infective stage of the parasite. These external stages decay at a rate μ in the environment and are produced at a rate λ per individual, ($\lambda = \Lambda(\alpha_y + b)$) where Λ is the number of viral particles released upon death of an infected host. Transmission of the HTP therefore takes place when a susceptible host comes into contact with free-living infective stage, W . The uptake of free-living particles by the hosts has been shown to be negligible relative to typical biological rates of production and decay, and thus has little or no effect on the dynamics of host and parasite (Boots, 1999; Dwyer, 1994) and is thus passed over here to maintain a simple model. In the free-living model, the VTP is able to invade the host-HTP equilibrium if it is stable when inequality (9) is satisfied

$$p[af - q(X^* + Y^*)] > \delta\beta W^* + (\alpha_v + b). \quad (9)$$

Where the host-HTP equilibrium is given by

$$(X^*, 0, Y^*, W^*) = \left(\frac{\mu}{\Lambda\beta}, 0, \frac{\Lambda\beta\Psi - 2q\mu \pm \sqrt{\Lambda\beta\Psi^2 + 4\Lambda\beta\alpha_y q\mu}}{2q\Lambda\beta}, \frac{\Lambda(\alpha_y + b)}{\mu} Y^* \right) \quad (10)$$

Where $\Psi = a - b - \alpha_y$.

Equation (9) is analogous to equation (5). However, it is possible for the host-HTP populations to coexist in the form of population cycles (Anderson & May, 1981; White *et al.*, 1996). When this is the case, inequality (9) cannot be used to

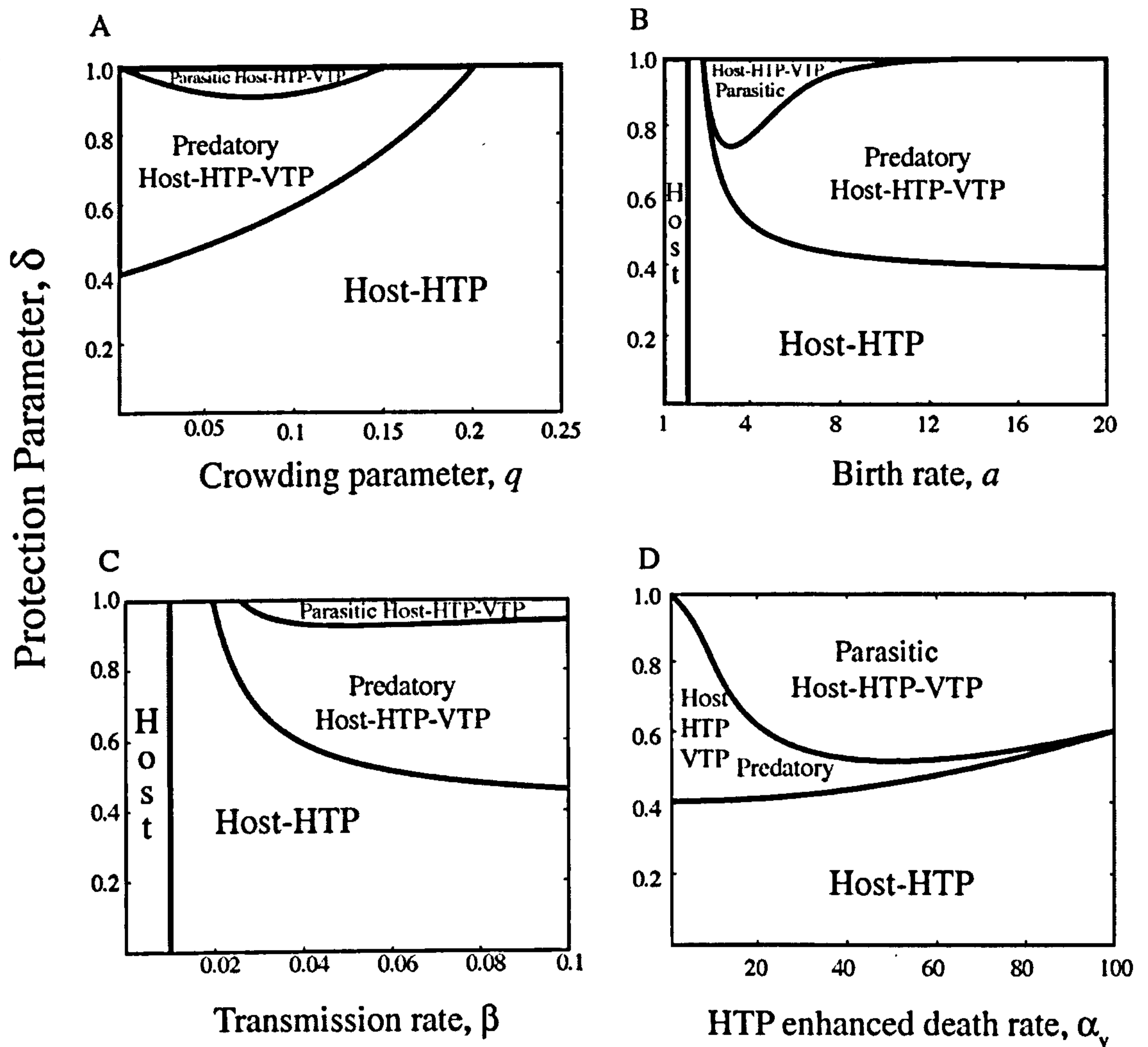


Figure 2.3. A comparison of coexistence of parasitic and predatory natural enemies. Parameter space portraits indicating where the different population equilibria are stable for the model defined by equations (1a-c) (predatory HTP) and the modified model where equation (1a) is replaced by equation (8), (parasitic HTP). Parameter are $r = 10$; $b = 1$; $\alpha_y = 0.25$; $q = 0.01$; $\beta = 0.1$; $f = 0.8$; $p = 0.8$, unless varied in the plots.

determine whether the VTP can invade. Instead the invasion success of the VTP is determined by numerical calculation of the Floquet multiplier (Ferriere & Gatto, 1993). This equates to calculating whether the growth rate of invading VTP population is positive or negative over time in a varying host-HTP population cycle. The analysis showed the invasion of the VTP stabilised the dynamics, either by decreasing the

amplitude of the systems oscillations or stabilising cycles to point equilibrium (Figure 2.4). Thus, persistence of VTPs may aid the stability of host-parasite systems. The presence of the VTP reduces the likelihood of cycles, by affording the host a level of protection from the HTP that reduces its overall transmission, which has been seen to cause cycles when high (Anderson and May, 1981).

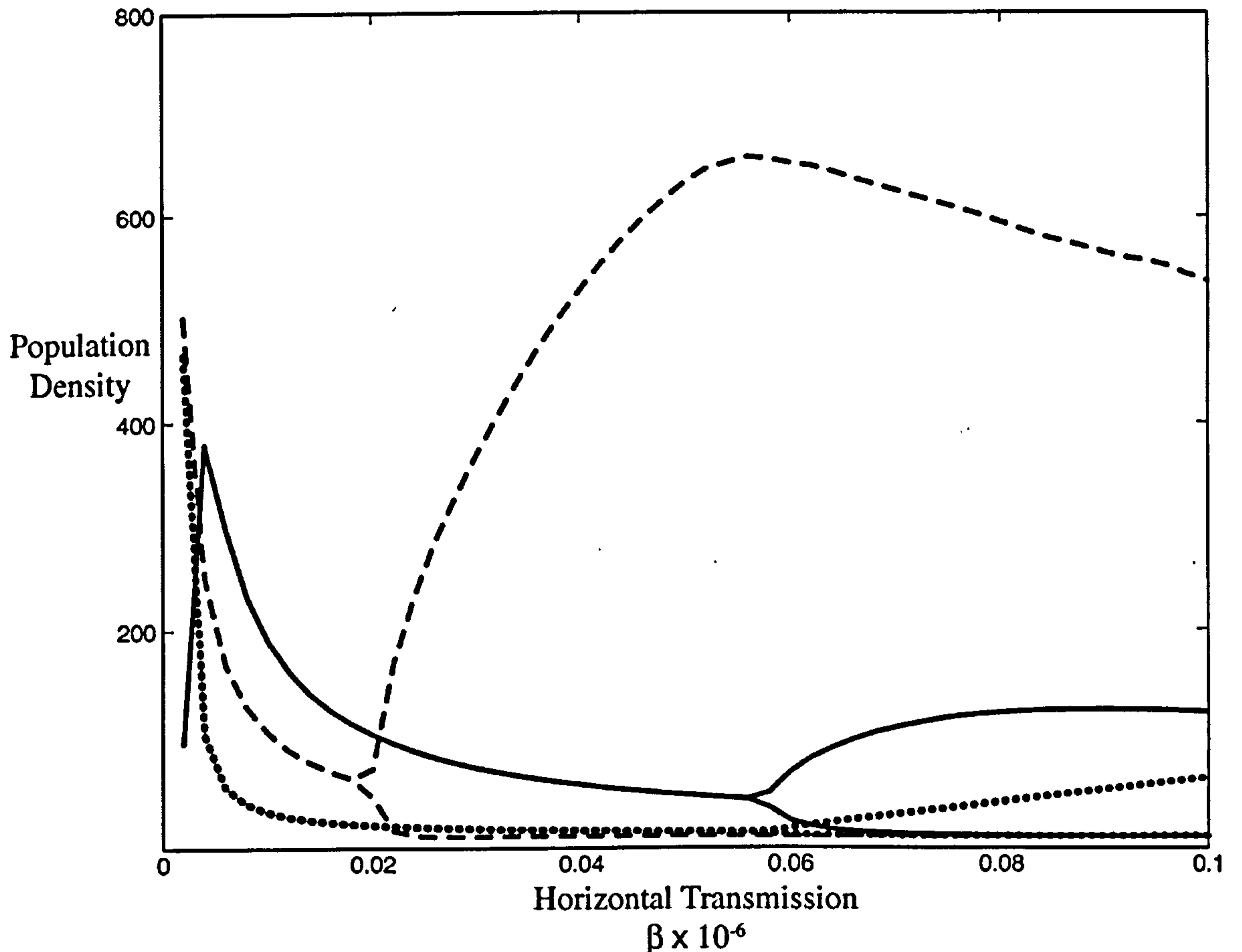


Figure 2.4. Bifurcation diagram that plots maximum and minimum values produced in the dynamics of host-HTP system and the host-HTP-VTP system with free-living parasites. The dashed line denotes the dynamics of the uninfected host in the presence of the HTP without a VTP present in the system (where the line splits it indicates that the dynamics change from point equilibrium to population cycles). The solid and dotted lines are the VTP and uninfected host dynamics, respectively. Parameters are $f = 0.8$; $r = 10$; $q = 0.01$; $\alpha_y = 4$; $b = 1$; $p = 0.8$; $\Lambda = 1 \times 10^6$; $\delta = 0.6$; $\alpha_v = 0.25$;

Figure 2.5 shows the invasion ability of the VTP into the coexisting host-free-living HTP population. Parameters in Figure 2.5 are chosen such that the HTP-host population exhibits oscillatory dynamics. The parameter region in which the VTP can

invade is similar for parasites with free-living stages as for directly transmitted ones (compare Figures 1 and 5). Again there is a threshold level in the vertical efficiency of the VTP, above which the VTP can persist. As vertical efficiency increases, persistence of the VTP can be achieved at lower levels of protection (Figure 2.5A). Persistence of the VTP shows an increasing saturating response for birth rate against protection (Figure 2.5B). Importantly, both figures show that for a large region of parameter space, VTP persistence acts to remove the oscillations and stabilise the population dynamics. This occurs most readily at high levels of protection (Figure 2.5). For the purpose of comparison the region of VTP invasion as determined from equation (9) is also plotted in Figure 2.5. If the HTP-host dynamics were in equilibrium then the VTP could invade below this line. The actual region of VTP invasion requires higher levels of protection, indicating that it is more difficult for the VTP to invade oscillatory populations.

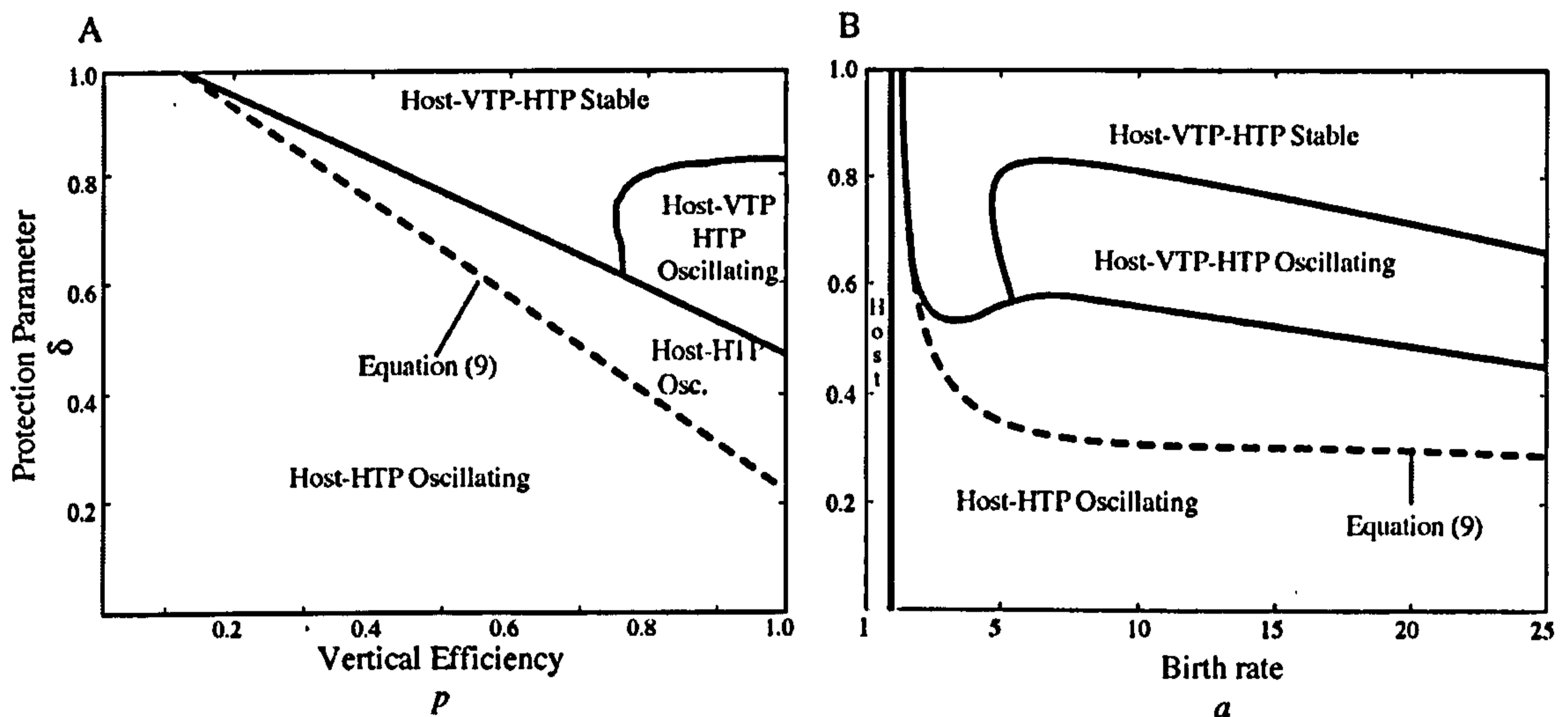


Figure 2.5. The role of protection (δ) vs. vertical efficiency and birth rate in coexistence and stability. The parameter space portraits indicate where the different population equilibria are stable for the model with free-living HTP defined by equations (8a-d). The dashed line represents the invasion boundary for the VTP determined by equation (9) and is plotted for comparison with the true invasion lines. Parameters are $f = 0.8$; $p = 0.8$; $r = 10$; $q = 0.01$; $\alpha_y = 4$; $\alpha_v = 0.25$; $b = 1$; $\Lambda = 1 \times 10^6$, unless varied in the plots.

2.3 Discussion

A vertically transmitted parasite's reduction of host fitness (whether by decreasing fecundity and/or increasing host mortality) is generally thought to lead its exclusion from a population (Lipsitch *et al.*, 1995; Regniere, 1984). Here we have shown that interference can allow a VTP, without any horizontal transmission, to be maintained in a host population. This occurs when the VTP confers protection against a secondary, horizontally transmitted, parasite in the system. Persistence depends critically on the pathogenicity of the HTP and the transmission efficiency of the VTP. In addition the mode of action of the HTP is important. If the HTP is a functional predator (Boots, 2004) it is much more likely to maintain the VTP in the population. In addition we have shown that the persistence of VTP through interference may stabilise host population dynamics. Interference may therefore be an important factor in the maintenance of complex multi-enemy systems in the wild, allowing persistence of VTPs and stabilization. It is, however, more difficult for the VTP to persist if the system it invades is oscillating rather than stable.

The vertical efficiency of the VTP, p , is crucial to persistence. If the efficiency decreases, then it not only reduces the VTP infected host's competitive ability but also mechanistically increases the uninfected host's birth rate and, therefore, its competitive ability. Vertically transmitted parasites benefit linearly from increased vertical transmission efficiency, with correspondingly less protection required for persistence. Therefore, providing that the parasite has a high vertical transmission, only a relatively small level of protection is required to allow persistence. The microsporidia *Dictyocoela* sp. (roeselum) possess a moderate vertical transmission efficiency (55%) (Haine *et al.*, 2004), which would suggest that a substantial reduction in virulence of a horizontally transmitted parasite is needed to enable its persistence. Vertical transmission efficiencies of over 70% have been seen in some granuloviruses and cytoplasmic polyhedral viruses (CPV) (Burden *et al.*, 2002; Sikorowski *et al.*, 1973). This high transmission efficiency leads to the requirement of much less interference with a HTP virus to enable persistence. CPVs are also known to be passed into the environment via larval faeces (Bong & Sikorowski, 1991; Sikorowski *et al.*,

1973). However there is little known of the scale at which this possible horizontal transmission occurs and therefore it may be less important in the persistence of these VTPs than an interference interaction with other parasites.

The host-HTP system displays a dramatic increase in overall population density when the VTP also persists (Figure 2). VTPs may therefore help to allow the persistence of host-parasite interactions in nature. Increasing the protection by the VTP also somewhat counter-intuitively may benefit the HTP since more HTP infected hosts can be supported. Therefore whilst the two parasites may be viewed as competing for the host in some sense, both the HTP and VTP may benefit from the interaction. The free-living model showed that the presence of a protecting VTP acts to reduce the amplitude of population oscillations (or replace them with a stable equilibrium). Therefore VTPs may be of value as biological controls in systems that have a tendency to exhibit large population oscillations since they reduce the tendency to cycles and hence limit population outbreaks.

It is increasingly recognised that there are important differences between parasites that act as functional predators (obligate killers from which the host cannot recover) compared with those that act as classical parasites (Boots 2004). Functional predators are common insect natural enemies, including the baculoviruses, and of course many, but not all parasitoids (Godfray, 1994). We have shown that such predatory HTPs always provide a greater opportunity for a VTP to persist compared to when the HTP acts in a parasitic manner. Furthermore, when the HTP has low virulence the VTP is only likely to persist with a predatory HTP, whereas it can persist with both predatory and parasitic HTPs that have high virulence. These differences reflect the contrasting effects of virulence on the prevalence of infection of the two types of parasite.

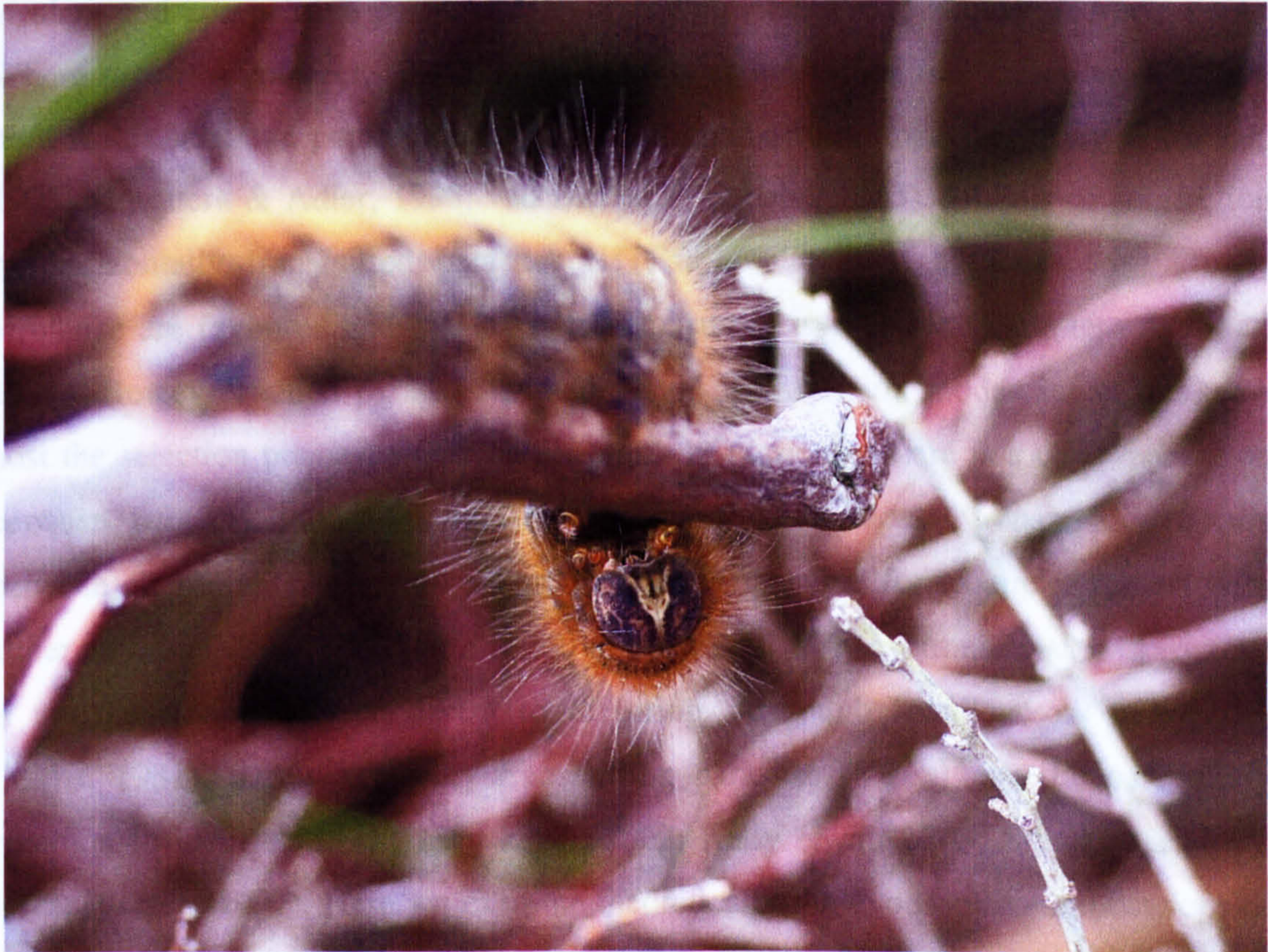
Productive hosts that have high carrying capacities through low susceptibility to crowding are more likely to support the VTP if the HTP is predatory. In contrast, when the HTP is parasitic the VTP is supported over a greatly reduced region of parameter space, which is maximised at an intermediate susceptibility to crowding. We may therefore expect that VTPs would be seen in r-selected species, such as pest

species that are also vulnerable to other HTPs, particularly if these are predatory. For example the winter moth, *Operophtera brumata*, is affected by viral predatory pathogens and parasitoids that infect the host's larval stage (Graham *et al.*, 2004; Kerslake & Hartley, 1997; Kerslake *et al.*, 1996). It is therefore a prime host for a VTP that provides some interference protection. Further investigation into the vertically transmitted cytoplasmic viruses that occur in winter moth populations (Graham *et al.*, 2006) should examine whether they protect their hosts from the HTPs.

Here we have assumed that the interaction is between a VTP that reduces the chance of parasitism from another HTP. This is closely related to the findings of Oliver *et al.* (2003) who showed that a vertically transmitted bacterial endosymbiont could persist if it provides a level of protection to an aphid host that is attacked by a *Braconidae* parasitic wasp. Hosts infected with the vertically transmitted bacteria exhibited a reduction in parasitism. This is analogous to the protection parameter that reduces the HTP transmission ability in our study. Oliver *et al.* (2003) do not consider the classical host parasitoid system with discrete generations. In their study the aphids can reproduce when parasitized and so it is equivalent to the parasitic HTP in our study. This is one example where protection may allow the persistence of a VTP. Our model suggests that persistence is even more likely with the predatory infections that are common in insect pathogens. Many vertically transmitted diseases seem to persist asymptotically or have very little pathogenic effect, but any deleterious effect on the host may be masked by the fact that they are protecting the host from a more pathogenic HTP in the environment.

Our models show it is important to determine if the VTPs commonly found in many different insects occur due to ecological interference with more pathogenic, horizontally transmitted parasites. We have shown here that there are important implications to the host population dynamics from the coexistence of these different parasites. Given the widespread presence of virulent vertically transmitted parasites in nature, understanding the mechanisms by which they persist remains a key theoretical and empirical challenge.

3. The Evolutionary implications of conflict between parasites with different transmission modes



Oak Eggar (*Lasiocampa quercus*) larvae in heather Orkney, 2005.

3.1 Introduction

Theory relating to host parasite interactions often considers the impact of a single parasite on the dynamics of its host. However, in natural systems hosts are often challenged by multiple parasites (Chen *et al.*, 2004; Haine *et al.*, 2005; Haine *et al.*, 2004; Hodgson *et al.*, 2004) and this can have important implications to the evolutionary dynamics of parasites. For example, superinfection, where a more virulent strain displaces less virulent strains in individual hosts, selects for the more virulent and competitive parasites (Levin & Pimentel, 1981; Nowak & May, 1994). Where parasites coinfect, such that hosts harbour more than one infection at a time, both within host and population level interactions become important (May & Nowak, 1995; Mosquera & Adler, 1998; van Baalen & Sabelis, 1995). Models of coinfection by horizontally transmitted parasites (HTPs) indicate that parasites with greater virulence are favoured and that parasite coexistence is possible (Mosquera & Adler, 1998). However, when parasites with different transmission modes infect the same host the selection pressures may be different. In particular there is a clear distinction between two key modes of parasite transmission. Horizontal transmission occurs between all individuals within a host population through, for example, contact with either an infected individual or a free-living parasite. Vertical transmission in contrast is transgenerational, from parent to offspring. Although found across many taxa (Mims, 1981), purely vertically transmitting parasites (VTPs) may be particularly common in invertebrates, (Hogg *et al.*, 2002; Kelly *et al.*, 2003; Stouthamer *et al.*, 1999; Werren, 1997). Here we examine theoretically the evolutionary implications of the conflict between parasites with different transmission modes.

VTPs that cause virulence to their host will not persist without some other mechanism that compensates for this virulence (Fine, 1975; Lipsitch *et al.*, 1995). Virulent parasites that transmit vertically may persist if they also transmit horizontally (Busenberg *et al.*, 1983; Regniere, 1984) and in nature many insect viruses (e.g. granulosis and baculoviruses) may indeed possess both means of transmission (Burden *et al.*, 2002; Hackett *et al.*, 2000; Zhou *et al.*, 2005). Another well understood mechanism by which a virulent VTP may persist, is the manipulation of the host's

reproductive output through altering the sex ratio of the host by either increasing the total number of females born, converting males to females (feminisation) or by male killing (Cordaux *et al.*, 2004; Kageyama *et al.*, 2002; Werren, 1997; Zeh & Zeh, 2006). Indeed Hurst (1993) showed theoretically that a sex-ratio distorting purely vertically transmitting parasite can be maintained in a population even when there is selection against infected hosts by increasing the number of females born in each cohort. There is also evidence that some VTPs may reduce the effect of other natural enemies (Haine *et al.*, 2005; Oliver *et al.*, 2003). Theory has shown that a protecting purely vertically transmitted parasite that causes some virulence to the host is able to persist when by protecting the host from a virulent horizontally transmitted parasite. Indeed the greater the virulence of the horizontally transmitting parasite the easier for the vertical host to persist (Faeth *et al.*, 2007; Jones *et al.*, 2007; Lively *et al.*, 2005).

The evolutionary dynamics of parasites that possess both vertical and horizontal transmission modes have been examined in detail by Lipsitch *et al.* (1996). A key result was that as parasite numbers increase, they select for greater vertical transmission, due to both the higher potential for generation-to-generation infection and reduced encounters with susceptible individuals. Vertically and horizontally transmitted parasites are both selected to minimise the additional mortality that infection causes. Reduced virulence benefits VTPs by extending the period of host reproduction from infected individuals and benefits HTPs by lengthening the infectious period. Whilst the birth rate of the infected host is vital for the persistence and spread of a VTP, the fitness of HTPs is not directly affected by a change in host birth rate. Indeed HTPs may be selected to castrate hosts if this leads to increased transmission (O'Keefe and Antonovics 2004). Here our aim is understand the evolutionary behaviour arising from conflict between two separate parasites that both infect the same host. We will consider a purely vertically transmitted parasite, maintained through either manipulation of host reproduction, and a horizontally transmitted parasite. We will construct a theoretical model to represent the host-HTP-VTP interaction and consider the evolution of the HTP and VTP characteristics separately in this system prior to considering the coevolution of both parasites.

3.2 The Model

We develop a general theoretical model based on Anderson and May (1981). This consists of a system of ordinary differential equations to represent the rate of change of the density of the susceptible host population, X , the population infected with the VTP alone, V , the population infected with the HTP alone, Y_x , and the population infected by both the VTP and HTP, Y_v . We assume that the HTP is directly transmitted. The dynamics are represented by the following system of equations..

$$\frac{dX}{dt} = (a - qH)(X + (1 - \kappa)Y_x) + (1 - p)(af - qH)(V + (1 - \kappa)Y_v) - (\beta Y + b)X \quad (1)$$

$$\frac{dV}{dt} = p(af - qH)(V + (1 - \kappa)Y_v) - \beta VY - (\alpha_v + b)V \quad (2)$$

$$\frac{dY_v}{dt} = \beta VY - (\alpha_v + \alpha_y + b)Y_v \quad (3)$$

$$\frac{dY_x}{dt} = \beta XY - (\alpha_y + b)Y_x \quad (4)$$

where $H = X + V + Y_x + Y_v$ and $Y = Y_x + Y_v$.

Hosts are born at rate a , and have a natural death rate b , with density dependence from the total host population acting on the birth rate via the crowding parameter, q . The birth rate of hosts infected with the VTP is increased by the feminisation parameter, f , where $1 \leq f \leq 2$. When $f = 1$ it represents a 50% female sex ratio and when $f = 2$ it equates to 100% females. A proportion, p , (where $0 \leq p < 1$) of the offspring are born infected with the vertical parasite (therefore $(1-p)$ are born into the susceptible class). Those infected with the HTP potentially reproduce at a reduced rate due to the level of castration, κ , by the HTP, (where $0 \leq \kappa \leq 1$). Susceptible and vertically infected hosts are equally susceptible to horizontal infection, at rate βY . The parameters α_v and α_y are the additional death rates due to infection from the VTP and HTP respectively and assuming a process of coinfection an individual infected with both the HTP and VTP experiences the sum of these mortality rates.

3.2.1 Evolution of the horizontally transmitted parasite

For the horizontally transmitted parasite we assume a trade-off between transmission rate and virulence (Anderson & May, 1982; Bremermann & Pickering, 1983; Restif & Koella, 2003; van Baalen & Sabelis, 1995). This is based on the assumption that the parasites' growth rate has a positive relationship on both the transmission and virulence of the HTP on the host (Ebert, 1998; Mackinnon & Read, 1999a, b). Thus an increase in transmission leads to an increase in virulence and we further assume that the costs to transmission in terms of virulence are accelerating. This relationship is likely for many parasites as transmission saturates more quickly with parasite growth rates than virulence (Ebert, 1998; Mackinnon & Read, 1999a, b) and leads to an evolutionarily stable transmission and virulence rate. Since our aim here is to examine the effect of co-infection on parasite life history, we need to assume there is an evolutionarily attracting and stable level of transmission and virulence (a Continuously Stable Strategy, CSS, (Eshel & Motro, 1981)). We restrict our analysis to a part of the parameter space where all three parasites coexist and denote this stable equilibrium as $(X_r, V_r, Y_{X_r}, Y_{V_r})$. The techniques of adaptive dynamics (Geritz et al 1998) are then used to examine the invasion of rare mutants. The invasion exponent, I_m , of a given mutant with parameters (β_m, α_{Y_m}) , attempting to invade a resident strain with parameters (β_r, α_{Y_r}) at equilibrium can be determined by considering the determinant of the resident-mutant Jacobian matrix at the resident equilibrium (see Miller et al 2005).

Assuming there is a trade-off between virulence and transmission such that $\beta_i = g(\alpha_{Y_i})$ the invasion exponent (or fitness) can be expressed as follows

$$I_m = g(\alpha_{Y_m})(V_r(\alpha_{Y_m} + b) + X_r(\alpha_{Y_m} + \alpha_{Y_r} + b)) - (\alpha_{Y_m} + \alpha_{Y_r} + b)(\alpha_{Y_m} + b). \quad (5)$$

When $I_m > 0$ the mutant can invade the resident strain. Evolutionary singularities occur when the fitness gradient is zero and this occurs when

$$\frac{dI_m}{d\alpha_{Ym}} = g'(\alpha_{Ym})(V_r(\alpha_{Ym} + b) + X_r(\alpha_{Ym} + \alpha_v + b)) + g(\alpha_{Ym})(X_r + V_r) - (2(\alpha_{Ym} + b) + \alpha_v) = 0 \quad (6)$$

Therefore the evolutionarily stable (ES) virulence (and transmission) occur when

$$g'(\alpha_Y^*) = \frac{(2(\alpha_Y^* + b) + \alpha_v) - g(\alpha_Y^*)(X_r + V_r)}{(V_r(\alpha_Y^* + b) + X_r(\alpha_Y^* + \alpha_v + b))}, \quad (7)$$

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where $\alpha_{Ym} = \alpha_{Yr} = \alpha_Y^*$.

We vary the characteristics of the vertically transmitted parasite to determine their selective pressures on the HTP. The important life-history characteristics of the vertically transmitted parasite are the rates of feminisation, f , vertical efficiency, p and virulence α_v . Numerical methods were used to verify that the evolutionary singularity is a CSS. These included the construction of pairwise-invasibility plots and simulations to determine values of the variables at equilibrium.

Above a threshold where the VTP is able to persist, the presence of a VTP selects the HTP for higher transmission and virulence (Figure 3.1 A & B). This effect tends to saturate as feminization increases to its maximum, although higher feminisation always selects for greater transmission and virulence in the HTP (Figure 3.1 A). This is because the overall intrinsic growth rate of the host is increased due to the increased proportion of females, which in turn leads to increases in susceptible numbers. This selects for horizontally transmitted parasites with higher transmission rates. Also VTPs with higher vertical efficiencies, p , select for higher HTP transmission and virulence due to the higher VTP prevalence that they have and hence again a higher overall host birth rate. It is VTPs with intermediate rates of virulence that lead to the evolution of the highest transmission and virulence in the HTP (Figure 3.1 B). This reflects the fact that the greatest number of susceptibles in the system also occurs when the VTP has intermediate values virulence (Figure 3.2 A). This arises as when

VTP virulence is small the majority of the total population susceptible to the HTP are infected by the VTP. This supports a relatively high population infected with the HTP. As VTP virulence becomes large the total population susceptible to the HTP becomes dominated by uninfected hosts which have a lower birth rate (than hosts infected with the VTP) and therefore supports fewer hosts infected by the HTP. At intermediate VTP virulence the total population susceptible to the HTP is maximised as it is composed of host infected with the VTP, thereby having the benefit of the higher birth rate and uninfected hosts, thereby having the benefit of supporting fewer hosts infected by the HTP.

Evolution of the horizontally transmitted parasite

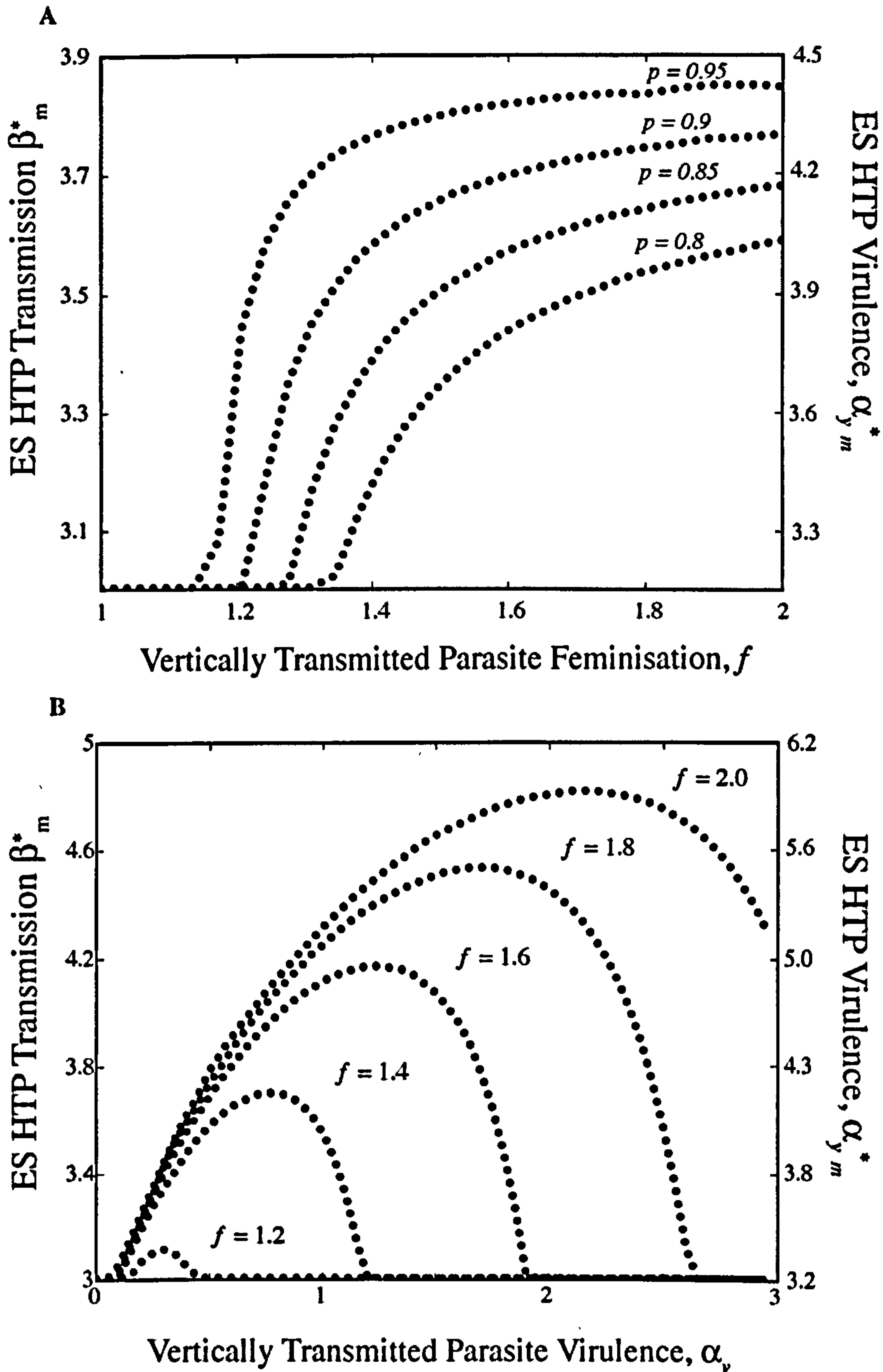


Figure 3.1A. The evolutionarily stable (ES) HTP transmission rate when co-infecting with VTPs with different feminization rates, and different rates of vertical transmission. B. The ES HTP transmission rate at varying values of VTP virulence and with varying amounts of feminisation. The parameters are $a = 4$, $b = 1$, $\kappa = 0$, $q = 1$ and unless varied in the figure $\alpha_v = 0.5$ $p = 0.9$. The trade-off relationship is, $\alpha_y = 15 - 4.5(8 - \beta)^{0.6}$.

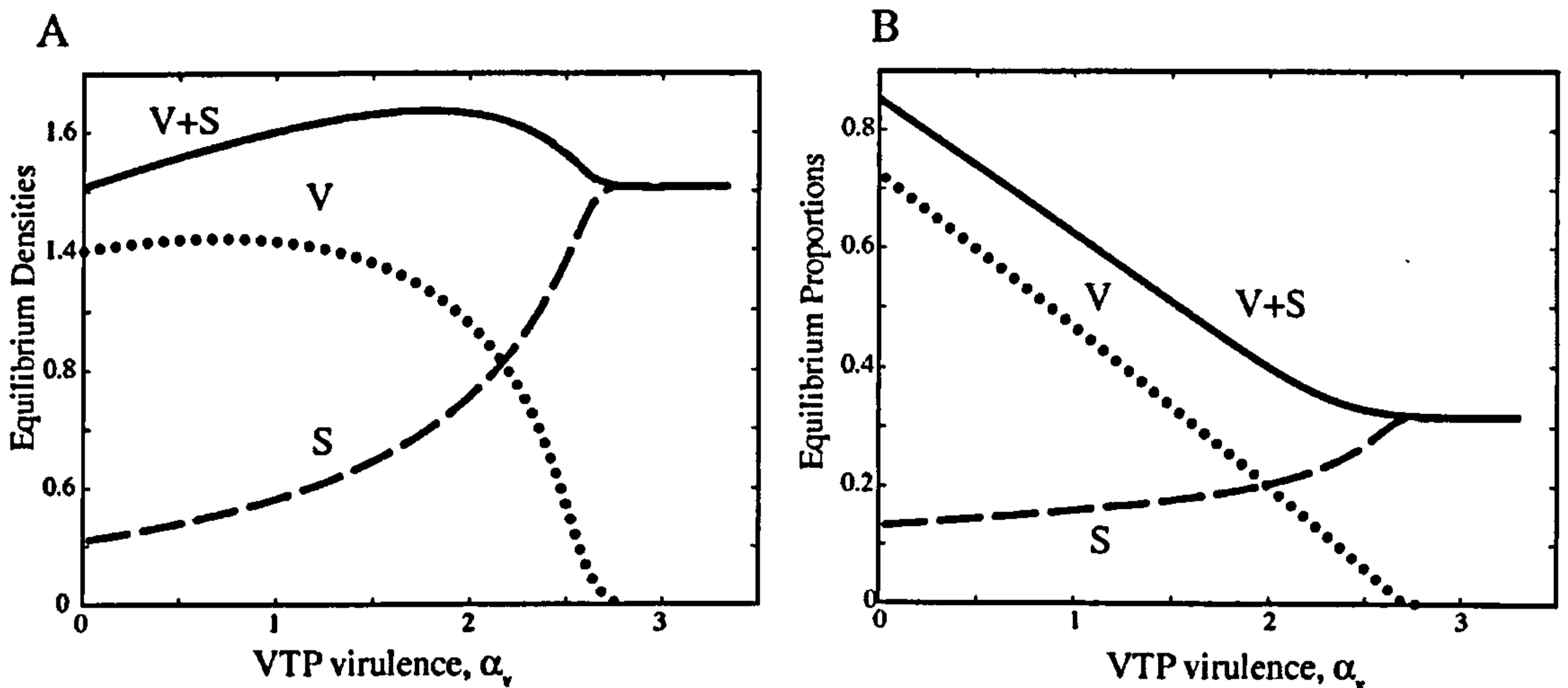


Figure 3.2:A) Equilibrium densities of susceptible host, S , (dashed-line), vertically infected hosts, V , (dotted line) and total susceptible hosts, $S + V$, (solid line). B) Equilibrium densities of horizontally infected susceptible hosts, Y_x , (dashed-line), horizontally and vertically infected hosts, Y_v , (dotted line) and total horizontally infected hosts, $Y_x + Y_v$, (solid line). The parameters are $a=4$, $b=1$, $\kappa=0$, $q=1$, $f=1.8$, $p=0.9$, $\beta=4$, and $\alpha_v = 15 - 4.5(8 - \beta)^{0.6}$.

3.2.2 Evolution of the vertically transmitted parasite

We now examine the evolution of the VTP when faced with a horizontally transmitted parasite. Our aim is to examine how different characteristics in the HTP alter the optimal strategy of the VTP. Trade-offs in vertically transmitting parasites are less well established than the transmission virulence trade-off in horizontally transmitted parasites, although there is now data to suggest that the high replication rates needed for vertical transmission (Mouton et al., 2004; Power, 1992) and feminisation (Min & Benzer, 1997) are detrimental to the hosts. We therefore assume these two different potential trade-offs and analyse them separately. The first is between the vertical parasites feminisation ability and its virulence and the second between the vertical parasites vertical efficiency and its virulence. As before the trade-offs are assumed to have accelerating costs. Both the trade-offs incorporate the increase of virulence alongside some increase in vertical parasite birth rate. The subtle difference between the two is that by increasing the feminisation ability, the overall input of new susceptibles into the system also increases, where as the vertical efficiency only

directly effects the proportion of susceptibles inputted into either uninfected or vertically infected hosts.

For the VTP feminisation-virulence trade-off the invasion exponent of the rare mutant strain (f_m, α_{vm}) invading a resident strain (f_r, α_{vr}) at equilibrium is given by the following expression

$$I_m = \left(p(af_m - qH) - (\beta Y + \alpha_{vm} + b) \right) + \frac{(1 - \kappa)p\beta Y(af_m - qH)}{(\alpha_y + \alpha_{vm} + b)} \quad (8a)$$

Similarly for the VTP efficiency-virulence trade-off with mutant (p_m, α_{vm}) resident (p_r, α_{vr}) the invasion exponent is

$$I_m = \left(p_m(af - qH) - (\beta Y + \alpha_{vm} + b) \right) + \frac{(1 - \kappa)p_m\beta Y(af - qH)}{(\alpha_y + \alpha_{vm} + b)} \quad (8b)$$

In biological terms, invasion occurs when the input of new vertically transmitted parasites from the singly infected hosts plus the reproductive output from the co-infected class are greater than the loss from horizontally transmitted parasite infection and the increased vertical and horizontal virulence. As castration tends to completeness the invasion component is simplified such that

$$I_m = p(af_m - qH) - (\alpha_{vm} + b + \beta Y) > 0 \quad (9a)$$

$$I_m = p_m(af - qH) - (\alpha_{vm} + b + \beta Y) > 0 \quad (9b)$$

The invasion of the VTP is then not dependent on the input of individuals from the co-infected class and is determined by the balance between the increase in hosts with the VTP and the loss due to mortality and acquiring infection from the HTP.

Feminisation and Virulence Trade-Off

The ES level of VTP feminisation and virulence is determined from the invasion exponent (equation 8a) in a similar manner as for the evolution of the HTP (equations 5-7). At full and zero castration the ES level of VTP feminisation and virulence do not vary substantially with changes in HTP transmission or virulence (Figure 3.3). At full castration, there is little change in selection as the HTP infected individuals do not reproduce and as such both uninfected host and vertically infected hosts are equally susceptible to HTP infection. At zero castration there is no cost to fecundity by being infected with the HTP infection. At intermediate vales of castration lower feminisation and virulence are selected (Figure 3.3). This occurs because of contrasting selection occurring on hosts infected by the HTP and hosts not infected by the HTP. At low castration the hosts infected by the HTP are in great abundance and prevalence and thus are the major factor in the selection of the ES feminisation. By increasing HTP castration the birth rate of HTP infected individuals decreases while their death rates remain constant. This means even though the hosts infected by the VTP, Y_v , have a larger birth rate, due to feminisation, than VTP free hosts, Y_x , the effect of increasing castration tends to reduce their competitive advantage. This is due to the coinfecting class having the shortest lifespan. This therefore leads initially to the decrease in feminisation and virulence in the VTP from low to intermediate levels of castration. As castration continues to increase the HTP infected hosts become less important compared to the HTP uninfected hosts (X & V) and higher feminisation and virulence is selected for as the advantage of the high feminisation returns.

Evolution of the vertically transmitting parasite: Feminisation-virulence trade-off

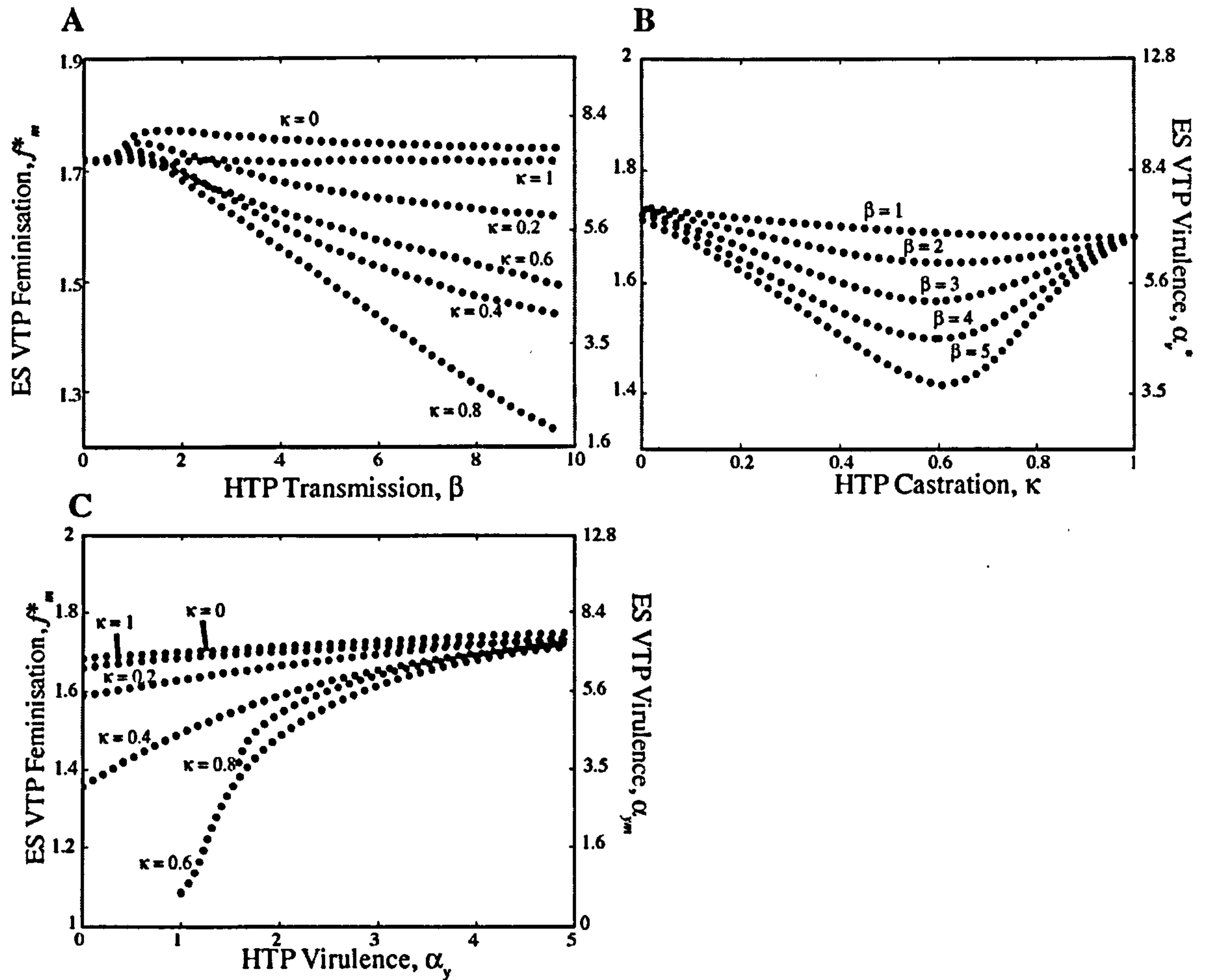


Figure 3.3: A) ES vertical parasite feminisation against horizontal castration at varying horizontal transmission values B) ES vertical parasite feminisation against horizontal castration at varying horizontal transmission values, C) ES vertical parasite feminisation against horizontal virulence (on death rate) at varying horizontal castration values. Parameter values unless otherwise stated: $a=15; b=1; r=a-b; p=0.95; \beta=2; \alpha_y=4; \alpha_v=20.8-20(2.1-f)^{0.4}$.

Vertical Transmission Efficiency and Virulence Trade-Off

Figure 3.4 give the results for a trade-off between VTP transmission efficiency and virulence. If both the horizontal transmission and virulence of the HTP is increased, a more virulent VTP with higher vertically transmitting efficiency is selected. This occurs since high horizontal parasite transmission leads to an increase in the prevalence of horizontally infected hosts and therefore greater prevalence of the coinfecting class. As a consequence the level of parasite induced death is increased across the whole population and in general the average lifespan is reduced across the whole population. This selects for vertical parasites with greater vertical transmission as this benefits the overall birth rate of the host. An increase in the HTP virulence reduces the relative effect of the VTP virulence in the coinfecting class. In turn, this is balanced by an increase in VTP virulence with the benefit of increasing VTP transmission. When there is complete castration, this no longer happens (Figure 4 A) because the coinfecting class does not reproduce and therefore it is not the major influence on selection.

The ES vertical transmission level and virulence increases as the horizontal parasites castration level increases. In contrast to the feminisation trade-off, a minimum level of ES virulence for the VTP is not observed. This result must be due to the subtle difference in trade-off form whereby changes in the level of feminisation affect the overall input of new susceptibles where as changes in vertical efficiency only affects the partitioning of hosts susceptible to the HTP between uninfected or vertically infected hosts.

Evolution of the vertically transmitting parasite: Vertical efficiency-virulence trade-off

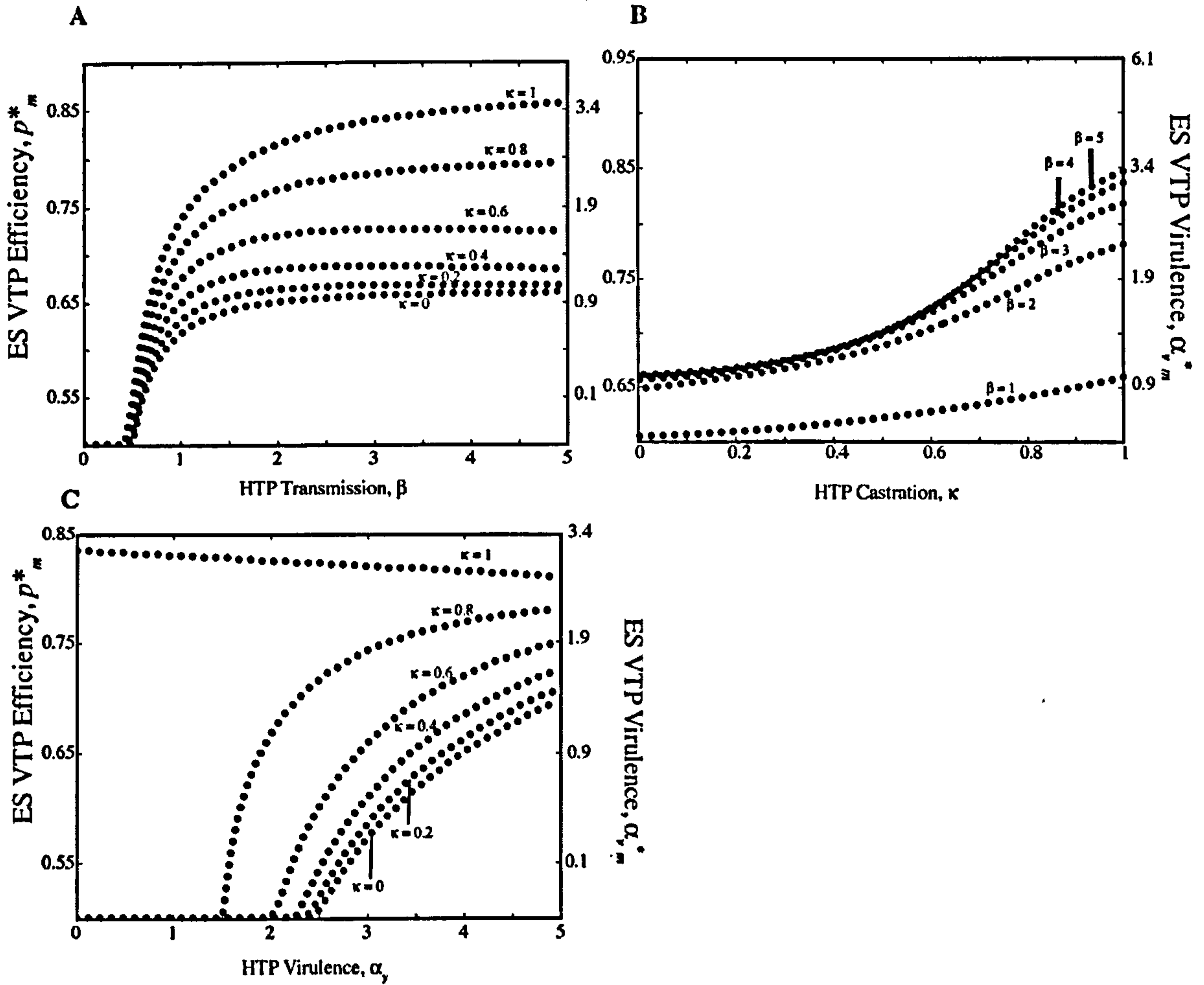


Figure 3.4: A) ES vertical parasite transmission against horizontal castration at varying horizontal transmission values B) ES vertical parasite transmission against horizontal castration at varying horizontal transmission values, C) ES vertical parasite transmission against horizontal virulence (on death rate) at varying horizontal castration values $a=15; b=1; r=a-b; \beta=2; \alpha_v=0; \alpha_v=32.5-35(1.1-p)^{0.1}; f=1.5;$

3.2.4 Coevolution of both the horizontally and vertically transmitted parasites

We now consider the situation where both the HTP and VTP can co-evolve. We use the invasion conditions determined for the evolution of the parasites in isolation (equation 5 and 8a or 8b) to graphically plot the position of the CSS of each parasite. The position of the coevolutionary stable strategy (CoESS) is determined as the intersection of the CSSs for the HTP and VTP. Numerical tests were undertaken to ensure that the CoESS is convergent stable.

VTP feminisation and virulence trade-off

We first examined how the CoESS between the horizontal and vertical parasite varies in hosts with different life spans under the feminisation-virulence trade-off in the VTP (Figure 3.5). A decrease in lifespan (an increase in the death rate) leads to an increase in virulence in both the horizontal and the vertical parasite and its consequential increase in horizontal transmission and feminisation. With high natural death rates the host is more likely die before contracting either infection. The virulence of the two parasites are a lower proportion of the overall death rate and so increasing the virulence of either parasite has a lower relative cost but the associated benefit of increasing VTP feminisation and HTP transmission.

Figure 3.5 (B) shows how the CoESS of the parasites is affected by the castration rate of the HTP. Both the HTP and VTP exhibit minimum virulence at intermediate levels of castration and maximum virulence when there is no castration. The response of VTP virulence and feminisation is similar to the response when the VTP evolves in isolation (Figure 3.3B). The HTP tracks the trend of the VTP since a reduction in VTP feminisation leads to reduced HTP transmission (Figure 3.2).

Coevolution of both parasites: Feminisation-virulence trade-off

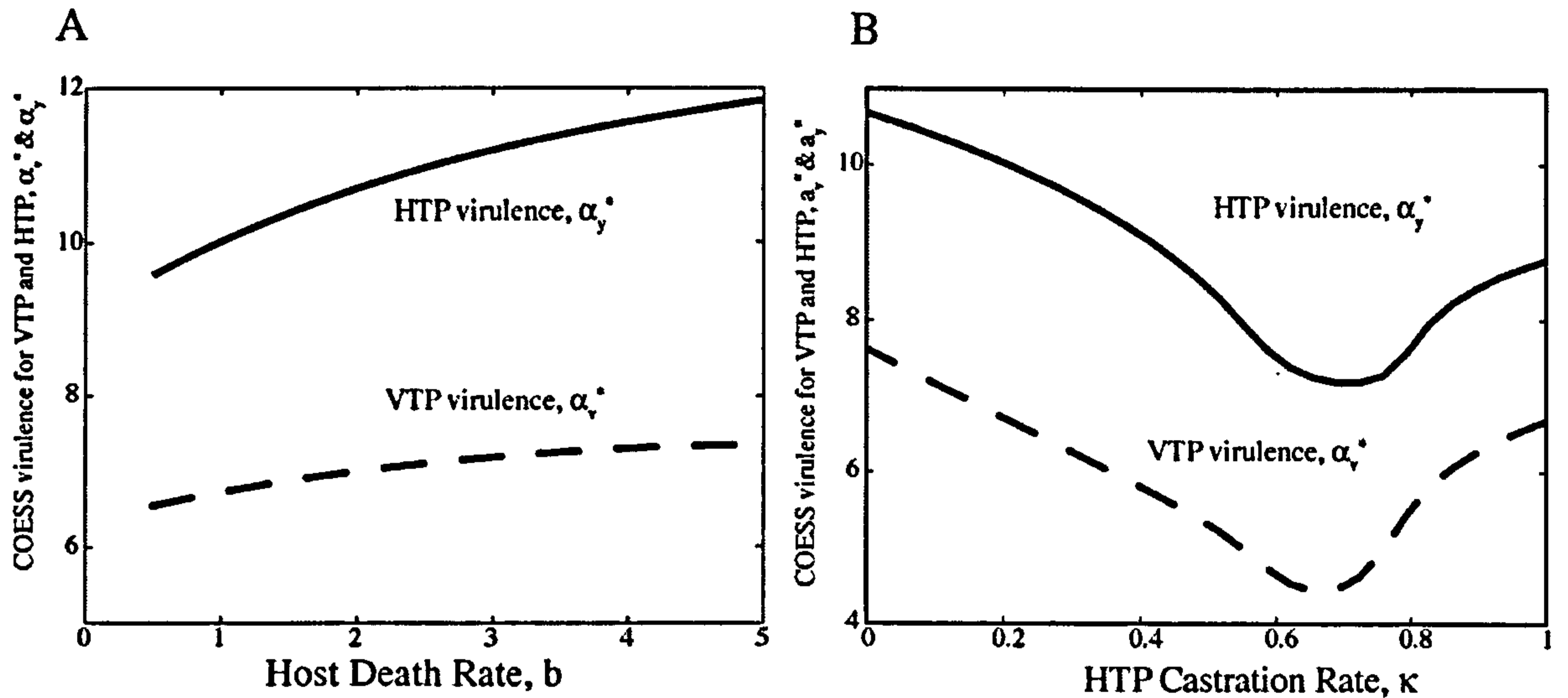


Figure 3.5. A) CoESS of horizontal parasite virulence (solid line, α_h^*) and vertical parasite virulence (dashed line, α_v^*) against natural death rate. B) CoESS (solid line, α_h^* , dashed line, α_v^*) plotted against HTP castration levels. $a=15$; $b=1$; $p=0.9$; $\alpha_h=15-4.5(8-\beta)^{0.6}$; $\alpha_v=20.8-20(2.1-f)^{0.4}$; $\kappa=0.2$;

VTP vertical efficiency and virulence trade-off

Figure 3.6 shows the effect of the change in host death rate and HTP castration on the CoESS strategy of the HTP and a VTP with a trade-off between vertical transmission efficiency and virulence. In a similar manner to the virulence-feminisation trade-off (above) the increase in host longevity selects for an increase in the virulence and vertical efficiency of the VTP and an increase in virulence and transmission for the HTP (Figure 3.6 A). The coevolutionary effect of changes in castration for the VTP (Figure 3.6 B) are similar to those exhibited when the VTP evolves in isolation. The HTP shows an initial decrease in virulence in transmission to a minimum at intermediate castration and an increasing trend for high castration levels. The initial decrease arises since increased castration levels reduces the overall susceptible population and the vertical transmission efficiency of the VTP and therefore select for reduced HTP virulence. The increase in HTP virulence is as a result of the increase in the VTP characteristics at high castration levels.

Coevolution of both parasites: Vertical efficiency-virulence trade-off

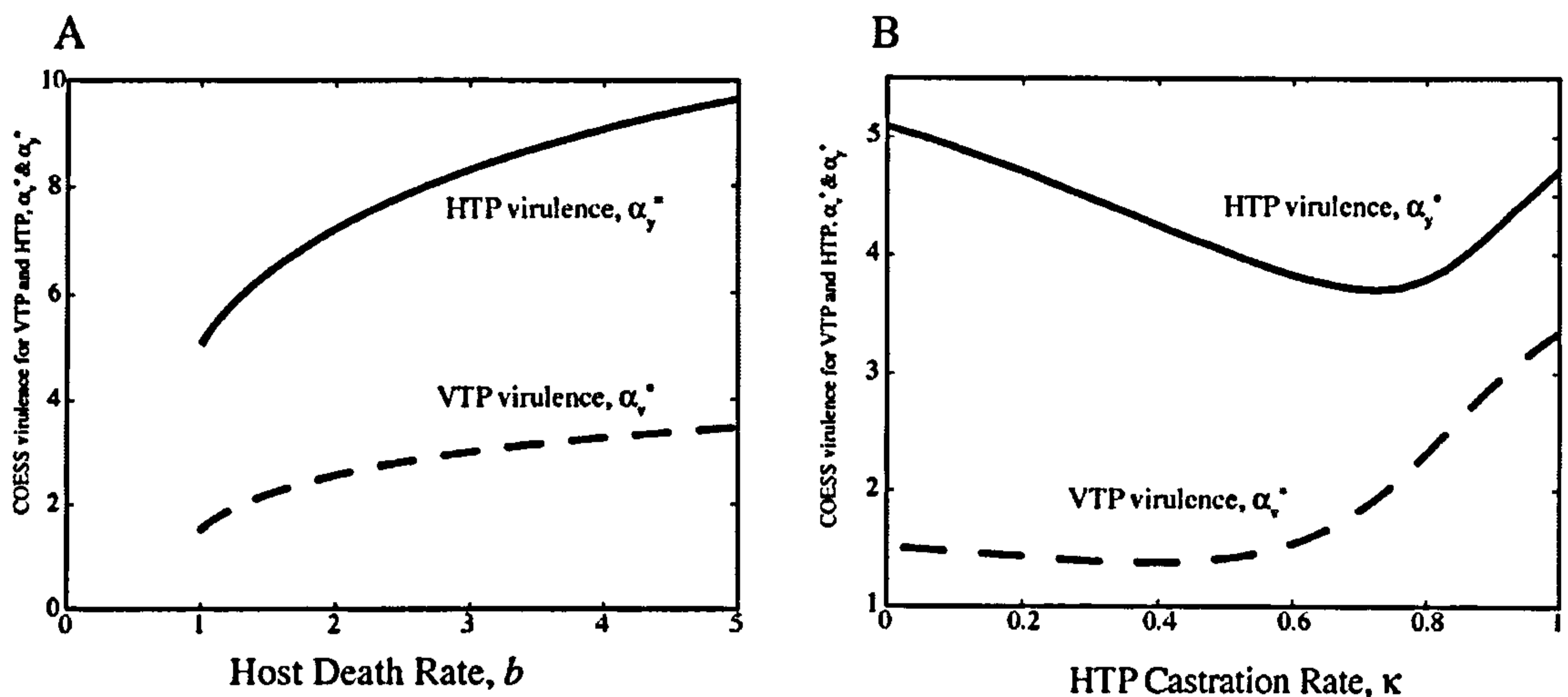


Figure 3.6. CoESS plots of virulence of VTP with vertical efficiency and virulence trade-off (solid line, α_y^*) and a HTP with a transmission virulence trade-off (dashed line, α_v^*). A) Change in CoESS with increasing host death rate. B) Change in CoESS with change in horizontally transmitted parasite castration ability. $a=15$; $b=1$; $f=1.5$; $\alpha_v=32.5-35(1.1-p)^{0.1}$; $\alpha_y=15-4.5*(8-\beta)^{0.6}$; $\kappa=0$.

3.3 Discussion

The main goal of this study was to gain insight into how two parasites with different transmission modes influence each other's evolution when they compete for the same host. We have shown that parasites with different transmission strategies have important selective pressures on each other when competing for the same host. Unconstrained, a horizontally transmitted parasite will clearly be selected to increase transmission whilst trying to minimise virulence (Levin & Pimental, 1981), which leads to the assumption that there is a trade-off between transmission and virulence (Anderson & May, 1991; Antia *et al.*, 1994; Levin & Pimental, 1981; Mosquera & Adler, 1998). It is also clear that an unconstrained optimal vertically transmitting parasite would have high feminisation, low virulence and high vertical efficiency. We have shown that such Darwinian demonic (Law, 1979) vertically transmitting parasites would select for fast transmitting, highly virulent horizontally transmitting parasites if they shared their hosts. Indeed the presence of a vertically transmitting parasite in the system is enough to increase the virulence and transmission of a HTP. In one sense, the VTP can simply be considered as a form of the host with greater reproductive and

death rates, which leads to parallels with the results of Gandon et al (2002) who showed that host reproductive effort also increases parasite virulence.

It is clear, however, that the evolution of vertically transmitted parasites is likely to be constrained due to trade-offs between the key life-history characteristics. We found that when feminisation is traded-off against virulence, high transmission rates and low virulence in the HTP, select for low feminisation rates and virulence in the VTP. In contrast when the VTP has a trade-off between transmission efficiency and virulence we observe that both low transmission and virulence in the HTP select for low transmission efficiency and virulence in the VTP. When there is a trade-off in the HTP (between transmission and virulence) we would tend to expect high virulence to be associated with high transmission and therefore this may select for intermediate VTP characteristics under the feminisation and virulence trade-off but high characteristics for the vertical efficiency and virulence trade-off. The selective pressure of a HTP on a VTP therefore crucially depends on the nature of the trade-off in the VTP. In particular VTPs are selected for intermediate feminization and virulence in the presence of highly infective, highly virulent HTPs.

Several theoretical studies have shown that the competition created by multiple infections of horizontally transmitted parasites can select for increased virulence (May & Nowak, 1995; Nowak & May, 1994; van Baalen & Sabelis, 1995), with the selection dependent on the force of infection (van Baalen & Sabelis, 1995). These studies are between strains that infect using the same transmission modes and not between parasites with different transmission modes. Other studies have shown that when sub-lethal effects have been incorporated in simple multiple infection models, evolution of lower virulence can be found (Schjorring & Koella, 2003). Here we show that virulence of the HTP would tend to be increased by a feminising parasite. However the effect of increasing HTP transmission and virulence, respectively, has a different effect on the VTP feminisation and virulence. It is unclear, therefore, how the virulence of vertically transmitted parasites will be selected by multiple infection with HTPs. The outcome will depend on the trade-off relationships of the life-history characteristics of each parasite.

There is a clear conflict between a vertically transmitting parasite and a horizontally transmitting one that reduces the fecundity of its host. High rates of castration significantly reduce VTP transmission, but may have no effect on the HTP fitness (O'Keefe & Antonovics, 2002). The degree of castration can therefore have profound effects on the evolution of the VTP. When virulence is traded off against feminization, the minimum level occurs at intermediate levels of castration, whereas when it is traded-off against vertical efficiency the minimum occurs at low castration levels. When we examine the coevolutionary dynamics we find a similar pattern such that for a HTP and VTP with a feminisation/virulence trade-off, virulence is minimised at intermediate levels of castration. With a vertical efficiency/virulence trade-off, coevolution also leads to minimum virulence for the HTP at intermediate levels of castration, but minimum VTP virulence at low castration.

In this model where the virulence of the HTP was placed on the death rate, castration is selectively neutral to the horizontally transmitting parasite and can only act through a change in the VTP evolution and retrospectively select the HTP. However it is clear from nature that there is often a level of induced castration/reduced fecundity from many HTP diseases. Indeed many studies suggest that castration should be maximised if the hosts energy is then used to further the parasites transmission (Jaenike, 1996; Jokela *et al.*, 1993; O'Keefe & Antonovics, 2002). However castration does not always reach 100% (Young & Yearian, 1982) and is under other selection pressures such as possible reallocation of host resources, from fecundity, to resistance against the horizontal parasite (Bonds, 2006; Gandon *et al.*, 2001; Rothman & Myers, 1996). Here I have assumed that the host does not react evolutionarily to the change in each of the parasites. But it is clear that the level to which a HTP infected hosts is castrated does have a large impact on how the virulence is selected for in both parasites when they are allowed to coevolve. Therefore to achieve lower virulence in a system with both HTP and VTP, a host could facilitate this by reducing HTP infected host reproduction to intermediate levels. The implications of castration and shared defence to host resistance should be considered in more detail

The coevolution of HTP and VTP characteristics is affected by the life-span of the host. Figures 5A and 6B show that as hosts become shorter-lived, this selects for more virulent strains of both parasites and therefore selects for an increase in either feminisation or vertical efficiency, in the VTP and transmission of the HTP. This follows classical life-history theory, where parasites invest more in host exploitation and hence virulence as the cost of virulence is low because of low survival likelihood in the infected host. However it has been argued that with increasing host death rate a reduction in the force of infection would occur that may lead to a lower parasite virulence (Ebert and Mangin, 1997). A theoretical study by Gandon *et al* (2001) showed a reduction in virulence occurred in systems where superinfection was high as host death rate and/or host recovery increased. They made the point that as superinfection became more prevalent selection occurs not only through the force of infection but also by the competition within the host. Our study assumes coinfection between parasites with different transmission processes and showed that the coevolutionarily stable virulence increased with reduced lifespan, for both parasites. Restif & Koella (2003) showed conversely when a host and parasite coevolved that a decrease in virulence occurred in response to host lifespan decrease. This selection was due to both the host and parasite having some control over the replication rate of the parasite which contrasts to this study where we assume that the host has no active affect on the parasite replication rate of either the two parasites.

The evolution of a VTP which shares its host with a HTP depends on the nature of the trade-offs found in the VTP (Figures 3 and 4). The two VTP trade-offs considered in this study are similar in the fact they both affect the reproductive rate of the VTP. However the feminisation trade-off has the capacity to alter the total reproductive rate of the whole system, while the vertical efficiency trade-off merely partitions the vertically infected hosts reproduction into uninfected and VTP infected offspring. This subtlety leads to contrasting results when the VTP is exposed to a HTP in a system. With an increase in HTP transmission, a greater proportion of the hosts in the system are infected with the HTP, and therefore the total numbers of hosts supported in the system is reduced as more are dying. The two types of VTP react to this in different ways. The VTP with the vertical efficiency trade-off is able to increase its

competitiveness by increasing vertical efficiency and virulence as the HTP prevalence rises. This means on average the host has a shorter lifespan so that the VTP virulence is less of a proportion in the overall host death rate and thus can be increased with the added benefit of increasing vertical efficiency. This effect also occurs with increased castration, as getting infected with the HTP becomes even more costly, although the virulence remains constant the reproductive output decreases. The VTP with the feminisation trade-off however, at complete castration, selects for maximum feminisation and virulence, whatever the HTP transmission. This is because at complete castration, there is no reproduction from the HTP infected class and thus infection by the HTP is just an added death rate that acts equally on both host and VTP. At zero castration, infection by the HTP does not affect the reproductive ability of the infected host and so being infected by the HTP is only costly in the fact that you suffer from greater virulence. This increased virulence due to greater HTP infection allows for greater feminisation and virulence of the VTP as an increase in virulence is less costly as the overall death rate has increased. We see decreases in feminisation and virulence in the VTP at intermediate castration because, as mentioned before, the decrease in birth rate from the HTP infected hosts effects the feminising VTP more than the host and reduces its competitive superiority. Thus the VTP is selected to reduce its virulence to maximise its competitiveness.

Our model has assumed that both parasites evolve at a similar rate, and that host resistance itself is not relevant on an evolutionary time scale. It is of future interest, however, to understand the evolution of host resistance to multiple infections, especially when the infections act quite differently on the host. The evolution of a resistance strategy would undoubtedly depend on the interactions between the infections and how they impact on the fitness of the host. Systems in the wild often have multiple infections and it is vital understand how these influence the coevolution of the host and its shared parasites.

4. The evolution of protection and sabotage: direct conflict between parasites



Old Man of Hoy, Orkney. 2004

4.1 Introduction

There is considerable interest in understanding how virulent vertically transmitted parasites persist in nature (Jones *et al.*, 2007). Persistence can occur due to an increase in infected reproductive potential through feminization or male killing (Hurst & Jiggins, 2000; Hurst *et al.*, 1994; Hurst, 1991; Terry *et al.*, 1998). Another mechanism that can allow persistence is when infection by the vertically transmitted parasite provides protection from a horizontally transmitted one (Jones *et al.*, 2007, Oliver *et al.*, 2003). This protection may be direct through restricting the uptake or development/replication of a secondary horizontally transmitted parasite. For example, it has been reported that vertically transmitted bacterial parasites in aphid hosts interfere with the development of the larvae of parasitic wasps (Oliver *et al.*, 2003). The bacteria are not found across the whole host population therefore it is assumed some cost must be incurred by the infected host preventing them being ubiquitous throughout the population. Indeed costs to fecundity have been seen with superinfections of these symbionts (Oliver *et al.*, 2006). Viruses have also been seen to have both additive and interfering effects on each others virulence and transmission when coinfecting the same host (Bird, 1959; Tanada, 1959; Tanada, 1985).

As well as these direct interactions, there is also evidence of behavioural modifications by vertical transmitted parasites that lead to effective protection for the host. A particularly well studied example of this is the Acanthocephala, *Polymorphus minutus*, that infects a species of freshwater shrimp, *Gammarus roselii* by horizontal transmission of eggs in the water column that are then consumed by the amphipod host. The Acanthocephala uses the shrimp as an intermediate host, before transmitting to its definitive bird host (Bauer *et al.*, 2005; Hynes & Nicholas, 1963). Transmission is facilitated by the manipulation of the host geotaxis behaviour increasing its predation rate (Bauer *et al.*, 2005), a behaviour that is common with acanthocephalan infections (Bethel & Holmes, 1977). The intermediate host, *Gammarus roselii* is also infected by a number of vertically transmitted microsporidia, in which there is no evidence of horizontal transmission (Haine *et al.*, 2004). There is therefore a clear conflict between the vertical transmission of the microsporidia and the increased predation through

infection by the acanthocephalan, which of course reduces life span and therefore reproduction. Haine *et al.*, (2005) showed that there was a significant reduction in the geotaxis behaviour, induced by the horizontally transmitted acanthocephalan, when the host contained a vertically transmitted microsporidia. This effect is assumed to be an evolved response by the vertical transmitted parasites and has been described as sabotage (Thomas *et al.*, 2002).

There is now increasing recognition of the role of mixed infections to the ecology and evolution of host-parasite interactions (Read & Taylor, 2001). Parasites may use the host in differing ways to promote their transmission to the next generation (Thomas *et al.*, 2002) and this can produce conflict between parasites that co-occur in the same host but often require different life history outcomes for the host to maximise their life cycle (Oliver *et al.*, 2003; Thomas *et al.*, 2002). For example parasites that infect using the same transmission mode but requiring different secondary hosts have a clear conflict trying to cause the host to succumb to the correct secondary host (Cezilly *et al.*, 2000; Lafferty, 1999). An even more obvious conflict occurs between vertically and horizontally transmitted parasites (Rigaud & Haine, 2005) and recent theoretical models have examined the evolutionary consequences to parasite life histories when there is coinfection by parasites with different transmission modes (Jones: Chapter 3).

Our aim here is to examine theoretically the conditions under which protective interactions between parasites may evolve and the evolutionary implications of protection itself. We first present a model that examines the evolution of protection based on the ecological dynamics described by Jones *et al.*, (2007). We then extend this theoretical framework to examine the more specific example of the evolution of sabotage represented by the acanthocephala system. We model the evolutionary dynamics of sabotage and the implications to the evolution of the horizontally transmitted parasite. The model examines the conditions under which selection of sabotage occurs and how the optimal level is affected by the horizontal parasites' life history.

4.2 The Evolutionary Dynamics of Protection

Consider the density of the susceptible host population, X , the host population infected with a vertically transmitted parasite (VTP), V , the host population infected with a horizontally transmitted parasite (HTP), Y_x , and the vertically infected host, V , that is also infected by the horizontally transmitted parasite, Y_v .

$$\frac{dX}{dt} = (a - qH)(X + (1 - \kappa)Y_x) + (1 - p)(af - qH)(V + (1 - \kappa)Y_v) - (\beta Y + b)X \quad (1)$$

$$\frac{dV}{dt} = p(af - qH)(V + (1 - \kappa)Y_v) - (1 - \delta)\beta VY - (\alpha_v + b)V \quad (2)$$

$$\frac{dY_v}{dt} = (1 - \delta)\beta VY - (\alpha_y + \alpha_v + b)Y_v \quad (3)$$

$$\frac{dY_x}{dt} = \beta XY - (\alpha_y + b)Y_x \quad (4)$$

where $H = X + V + Y_x + Y_v$, $Y = Y_x + Y_v$ and all parameters are positive.

Hosts are born at rate a , and have a natural death rate b , with density dependence from the total host population acting on the birth rate via the crowding parameter, q . The birth rate of hosts infected with the VTP is increased by the feminisation parameter, $f \in [1, 2]$, with 1 representing 50% female sex ratio and 2 equating to 100% females. A proportion, p , of the offspring are born infected with the vertical parasite (therefore $(1 - p)$ are born into the susceptible class). Those infected with the HTP have fecundity reproduced by a proportion κ . The HTP has transmission coefficient β with the vertically infected hosts gaining protection against the HTP through a reduction in transmission by a proportion $\delta \in [0, 1]$. We assume a process of co-infection and α_v and α_y are the additional death rates due to infection from the VTP and HTP respectively.

4.2.1 The evolution of protection in vertically transmitted parasites

We first look at how much protection, δ , will evolve in response to horizontally transmitted parasites with different characteristics. We assume that there is a saturating trade-off between protection rates (δ) and the virulence that the vertically transmitted parasite causes (α_v). Therefore strains of the VTP that give higher protection against the HTP cause more damage to the host. There are costs to certain secondary facultative symbionts (Chen *et al.*, 2000; Oliver *et al.*, 2006; Russell & Moran, 2006) although direct links between protection and increased virulence in the vertically transmitted parasite have not been measured in detail. We assume that Host-HTP and a resident strain of the VTP (δ_r, α_{vr}) are at a stable equilibrium. The invasion criteria of a mutant strain (δ_m, α_{vm}) attempting to invade this equilibrium is

$$I_m = \left(p(af - qH) - ((1 - \delta_m)\beta Y + \alpha_{vm} + b) \right) + \frac{(1 - \delta_m)(1 - \kappa)p\beta Y(af - qH)}{(\alpha_y + \alpha_{vm} + b)} \quad (5)$$

If I_m is positive the mutant strain can invade. To examine how the VTP will evolve we construct pairwise invasibility plots (Geritz *et al.*, 1998) which indicate when the mutant invasion criteria is positive or negative. If castration due to the HTP is low then no protection will evolve (Figure 4.1A). As castration increases a repeller and an attracting ESS are generated as a result of a saddle node bifurcation (Figure 4.1B). At high levels of castration only the attracting ESS remains and selects for high protection (Figure 4.1C and D). Costly protection will therefore only evolve in systems where there is significant castration by the horizontally transmitted parasite. If the vertically transmitted parasite also displays feminisation we may find an evolutionary repeller at lower protection levels than the convergent stable CSS point (Figure 4.1 B). Thus bi-stability can be found in the system (Figure 4.1), where the vertically transmitted parasite will either minimise the protection and virulence or have very high protection (Figure 4.1 B). This again suggests that significant levels of protection are much more likely when there is relatively high castration. It should be noted that the evolutionary repeller only occurs if the vertical parasite itself feminises

the host. This allows the parasite to persist in the system without any form of protection.

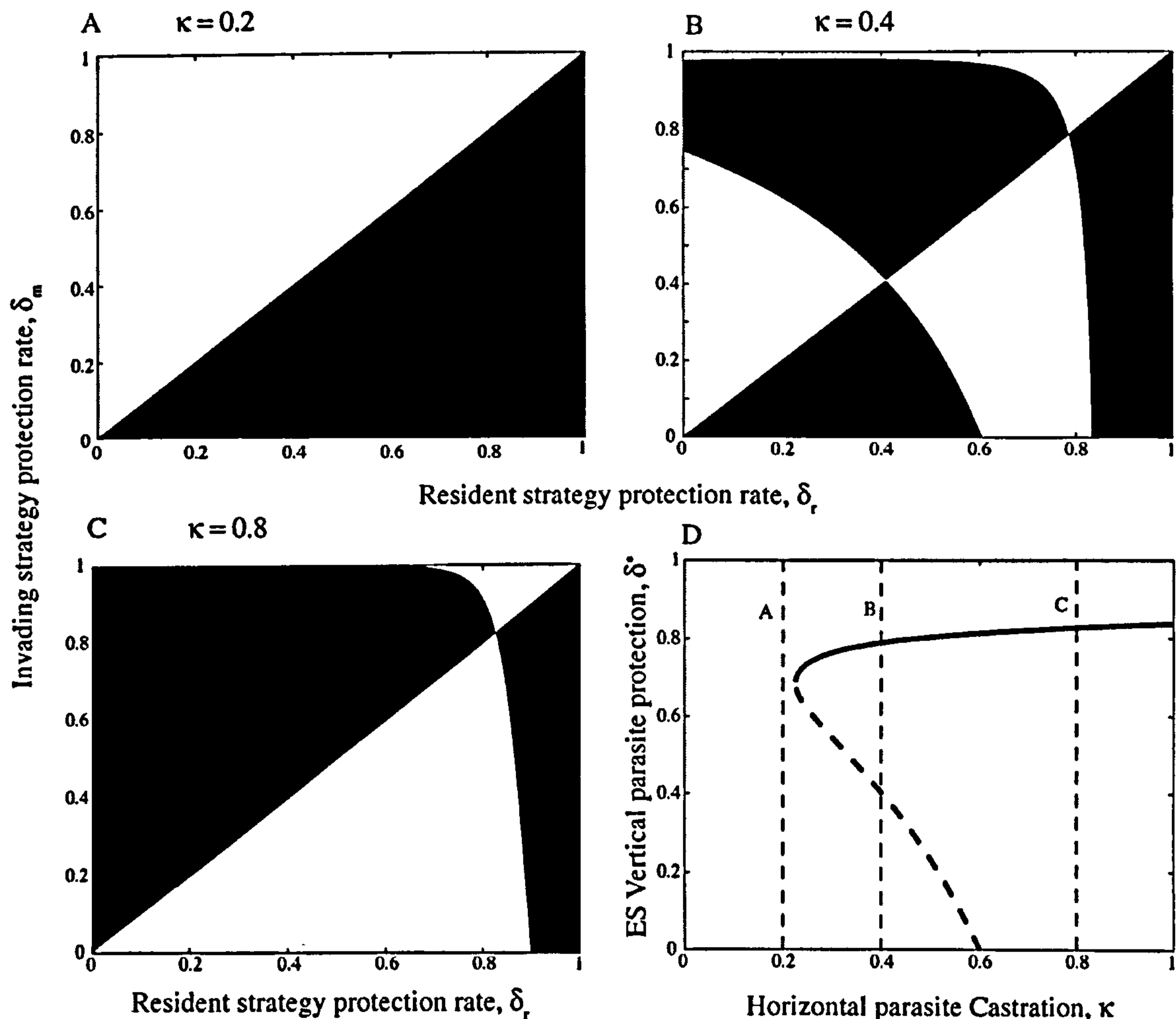


Figure 4.1: A-C, Pairwise invadability plots (PIP) of protection rates of invading mutants (*y-axis*) against residents (*x-axis*). The marginal growth rate of the mutant strain is positive in the shaded region and negative in the unshaded regions. The plots show how convergent and repelling points alter when castration is increased (A. $\kappa = 0.2$, B. $\kappa = 0.4$, C $\kappa = 0.8$), in a feminising host. Plot D Shows the change in the local ESS with increasing castration (solid line) and also the position of the evolutionary repeller (dashed line). $a=15$; $b=1$; $p=0.95$; $\beta=4$; $\alpha_y=2.5$; $f=1.2$; $\alpha_v = 10.5 - 10(1 - \delta)^{0.1}$

Generally HTPs with higher transmission rates select for VTPs to invest more in costly protection (Figure 4.2A). However there is a threshold in the transmission rate above which an evolutionary bistability occurs and therefore for a wide range of transmission rates, there tends to be the evolution of high protection or zero protection (Figure 4.2A). Bistabilities due to repellers also occur below a threshold in virulence

of the horizontally transmitted parasite (Figure 4.2B). Above this threshold protection will be selected and therefore protection is more likely against more virulent pathogens. When protection is selected it shows a slight decrease as the virulence of the HTP increases (Figure 4.2B). The decrease occurs as the directly transmitting HTP has a shorter lifespan as virulence increases. This reduces the number of secondary infections of the HTP and therefore reduces the level of protection that is required.

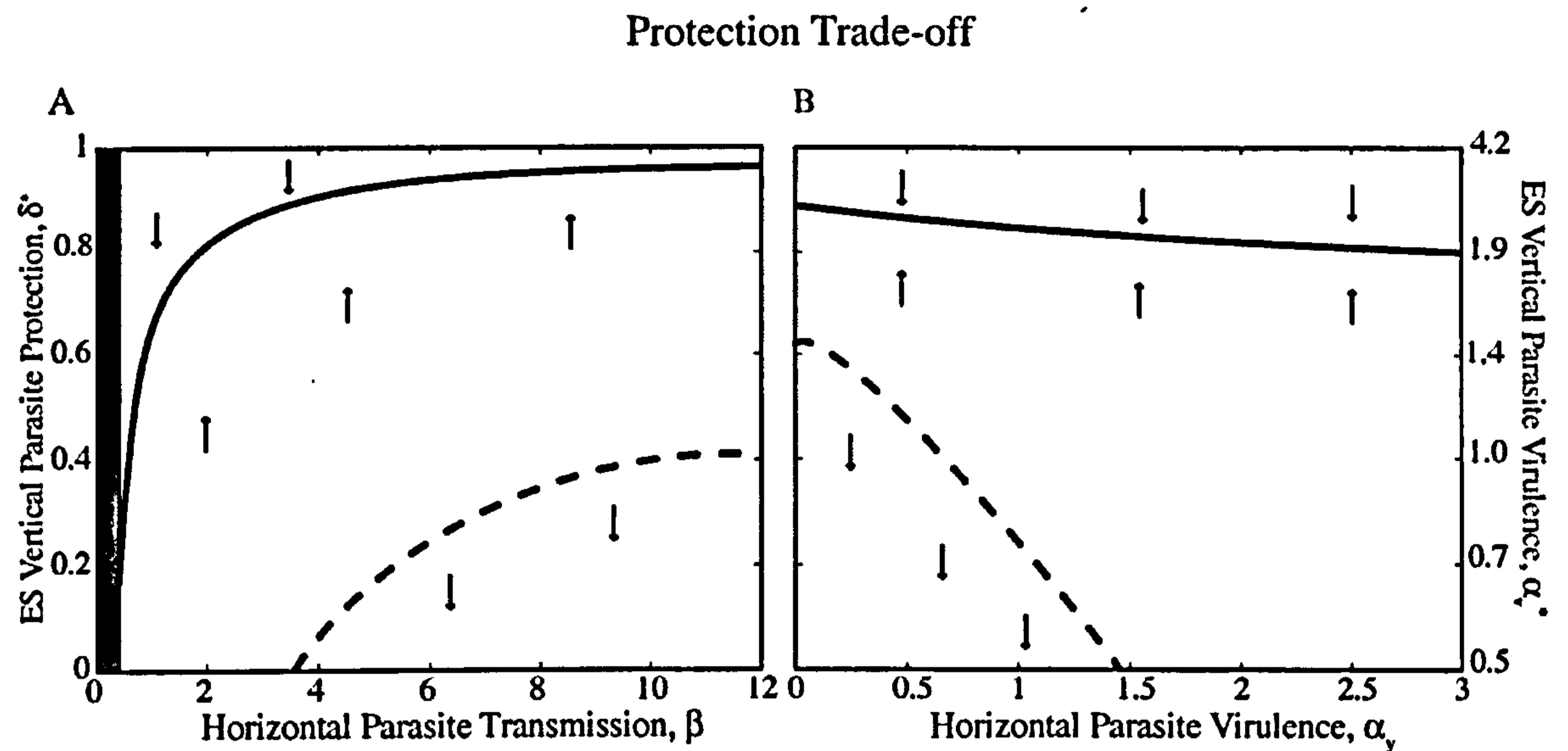


Figure 4.2. A & B The solid line is a convergent stable ESS (CSS) and the dashed line is an evolutionary repellor, from which protection either minimises or fixes at the CSS. Arrows denote selection gradient direction. A) ES protection of the vertically transmitted parasite at different transmission values of the horizontally transmitted parasite, grey area denotes where horizontally transmitted parasite was unable to persist in the system. B) ES protection of the vertically transmitted parasite at different virulence values of the horizontally transmitted parasite. $a=15; b=1; p=0.95; B=2; \kappa=0.7; \alpha_y=2; f=1.2; \alpha_v=0.5$; The trade-off is $\alpha_v = 10.5 - 10(1 - \delta)^{0.1}$.

4.2.2 Evolution of a horizontally transmitted parasite in the presence of a protecting vertically transmitted parasite

We have examined elsewhere the evolutionary implication to the HTP of a VTP for a range of feminisation levels, VTP virulence and vertical transmission efficiency (Jones: Chapter 3). This reported that increasing feminisation and vertical efficiency select for a more virulent and highly transmitting HTP. While maximum HTP

pathogenicities are selected at intermediate values of VTP virulence due to the increase in susceptibles that occur as a result. Here we look for the first time at how HTP selection depends on the level of protection in the vertically transmitted parasite. We assume a trade-off with saturating costs between transmission and virulence in the horizontally transmitted parasite (Anderson and May 1982). This assumption rests on the idea that increased within host growth rates of the parasite cause damage and therefore increased virulence but also results in increased transmission (Ebert, 1998; Mackinnon & Read, 1999a, b). The invasion exponent of a given mutant (β_m, α_{ym}) , attempting to invade a host-VTP and HTP with resident strain (β_r, α_{yr}) at equilibrium is

$$I_m = (\alpha_{ym} + \alpha_v + b)(\alpha_{ym} + b) - \beta_m \left((1 - \delta)V_r(\alpha_{ym} + b) + X_r(\alpha_{ym} + \alpha_v + b) \right) \quad (6)$$

Investigations using pairwise invadability plots indicate that an attracting ESS level of HTP transmission (and therefore virulence) will evolve. The evolutionarily stable level of transmission and virulence increases as the level of protection offered by the vertically transmitted parasites increases and results in a peak at a high (but not maximum) level of protection (Figure 4.3). This suggests there is a threshold, above which an increase in protection by the vertically transmitting parasite changes the horizontally transmitting parasite strategy. When protection is high there are considerably less available susceptibles for the horizontally transmitted parasite to infect since the population is dominated by protected individuals. The occurrence of the maximum peak is due to the change in the availability of susceptibles to the horizontally transmitted parasite. The increasing protection reduces the proportion of vertically infected hosts that are available for horizontal infection, but increases their abundance in the system. The increase in vertically infected numbers also boosts susceptible uninfected host, X , numbers due to a birth rate contribution through the vertical transmission efficiency falling below unity. Thus numbers susceptible to HTP infection increases with increasing protection until the protection level is high.

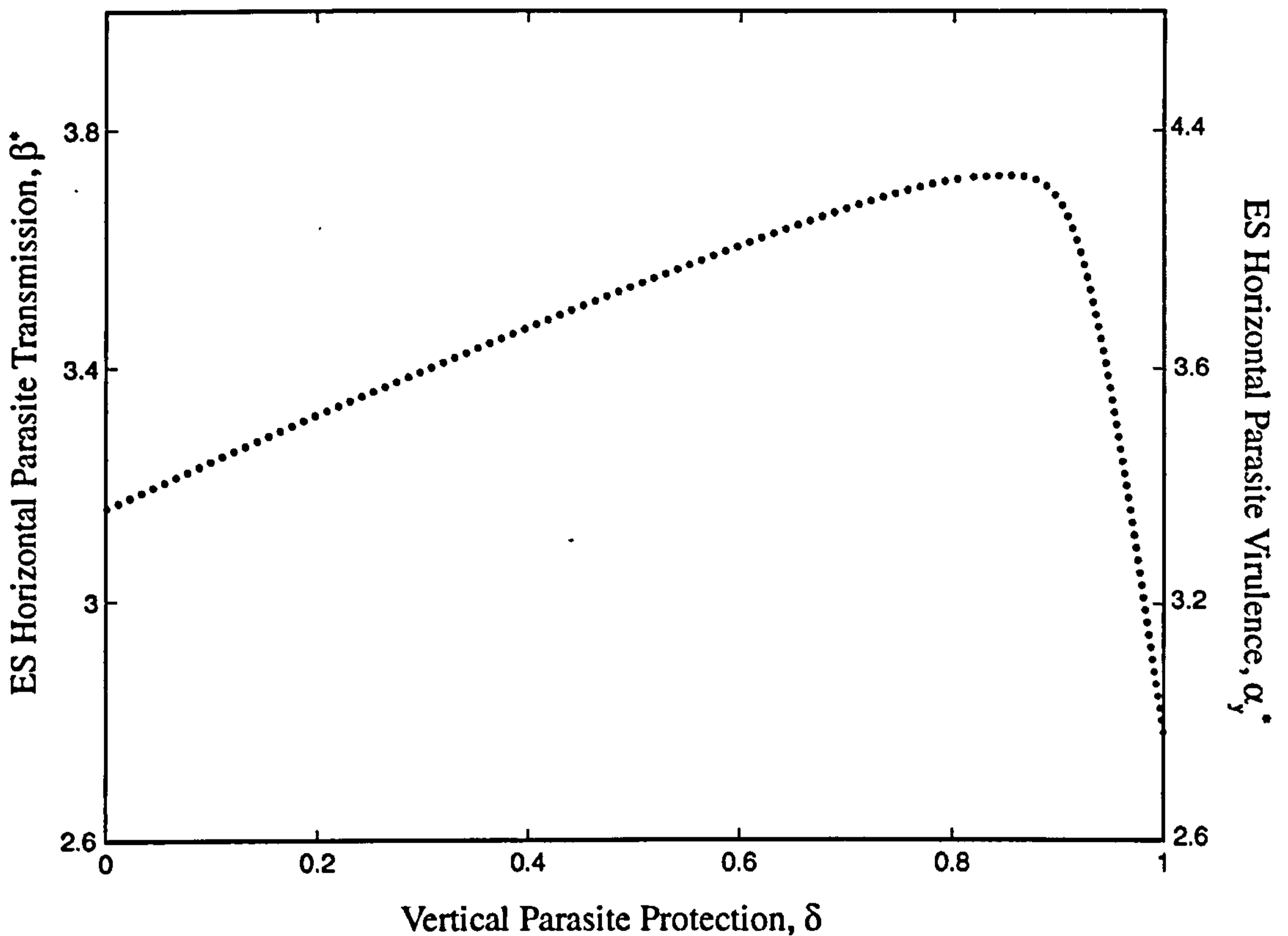


Figure 4.3. The plot shows how ES transmission, β^* of the horizontally transmitted parasite at different protection levels of the vertically transmitted parasite. The parameters used are $a=15; b=1; p=0.9; \kappa=0.3; \alpha_y=2; f=1.1; \alpha_v=0.5$; The trade-off is $\alpha_y = 15 - 4.5(8 - \beta)^{0.6}$

4.2.3 Coevolution of a protecting vertically transmitted parasite and horizontally transmitted parasite

We now let the two parasites co-evolve. Here we have assumed that the vertically transmitted parasite is neutral on the hosts' birth rate ($f=1$) and therefore the VTP only exhibits an attracting ESS. The position of the coevolutionarily stable strategy (CoESS) occurs when the invasion exponents of the VTP and HTP (equation 5 and 6) simultaneously equal zero (which can be determined numerically).

Increases in the level of castration of the HTP leads to selection of increasing virulence in the horizontally transmitted parasite and decreasing virulence vertically

transmitted parasite (Figure 4.4 A). The increase in horizontally transmitted parasite castration means that it is more costly for the vertically transmitted parasite and the host if they are infected by the horizontally transmitted parasite. Therefore it would be expected that the vertically transmitted parasite would select for greater protection the greater castration is, as is seen when the vertically transmitted parasite is allowed to evolve on its own (Figure 4.1 D). However we see protection decreasing with an increase in castration, and also an increase in horizontally transmitted parasite transmission. The decrease in protection maybe brought about therefore by the increase in virulence in the horizontally transmitted parasite, which decreases selection for protection (Figure 4.2 B). A change in castration has little effect on the HTP when it evolves on its own. Therefore, the increase in horizontal virulence and transmission with increasing castration could be due to the decrease in the vertical transmitted parasites level of protection and virulence. Therefore it maybe concluded that the virulence of the HTP is more important in the selection of the VTP as the selection of the VTP appears counter to the other two traits.

We also see how a possible CoESS point is affected by host lifespan. Protection and virulence decrease in the vertically transmitted parasite in shorter-lived hosts whereas transmission and virulence in the horizontally transmitted parasite both increased (Figure 4.4 B). As host lifespan decreases, it is less costly for the horizontally transmitted parasite to select for greater transmission and virulence. However the same increase in death rate leads to the vertically transmitted parasite selecting for lower virulence and less protection despite the corresponding increase in the horizontally transmitted parasite transmission (and counter to when the VTP evolves alone (Figure 4.2). The decrease in VTP levels is due to a reduction in the overall force of infection from the HTP. Higher natural death rates and higher HTP virulence reduce the HTP infected host lifespan which reduces the overall force of infection.

Coevolution of parasites: HTP and protecting VTP

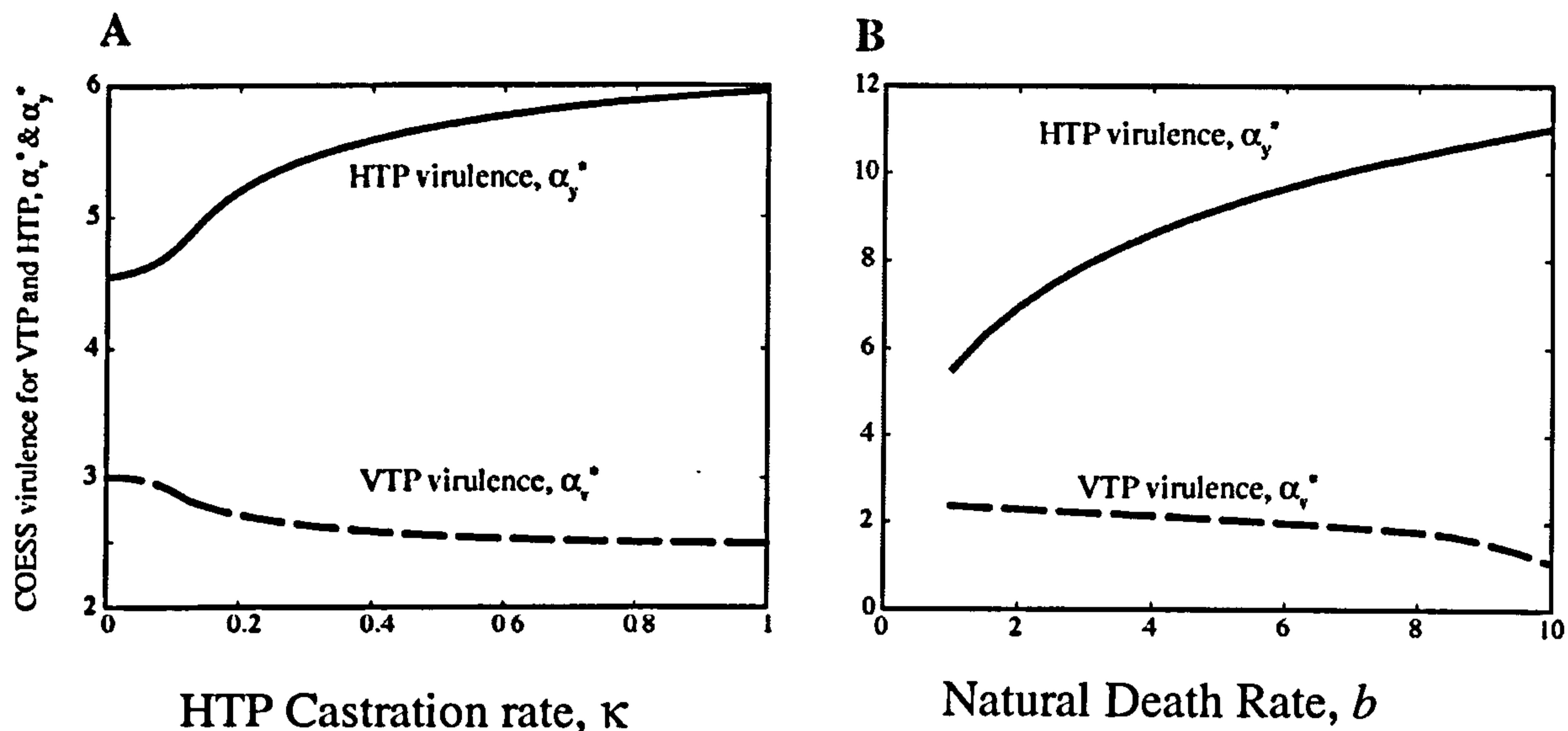


Figure 4.4. A. The effect of on the CoESS of the virulence of the horizontally transmitted parasite (solid line) and vertically transmitted parasite (dashed line). B. The effect of an increase in natural death rate in the host on the CoESS of the virulences of the horizontally transmitted parasite (solid line) and vertically transmitted parasite (dashed line). The virulences of the horizontal parasite and vertical parasite are linked to horizontal transmission and protection respectively. Parameter values are $a=15$; $b=1.5$; $f=1$; $\alpha_h = 15 - 4.5(8 - \beta)^{0.6}$; $\alpha_v = 10.5 - 10(1 - \delta)^{0.1}$; $\kappa=0.5$; $q=1$;

4.3 The evolutionary dynamics of ‘Sabotage’

Our second model introduces two subtleties, the first is that the HTP transmits to susceptible hosts only on the death of an infected host and hence by increasing the death rate directly increases the transmission ability. This implicitly represents the effect of the acanthocephalan worm that infects a *Gammarus* host and modifies its behaviour to increase its predation risk and therefore induces death. The induced death rate due to the HTP, Γ , is analogous to virulence and is necessary for transmission as if it is zero the HTP can not transmit (and is therefore an obligate killer). Therefore overall transmission is dependent on the induced death rate of the HTP and its ability to transmit on death $\beta\Gamma$. The second subtlety is to describe a ‘sabotage’ effect in the VTP that acts to reduce the induced death rate, and hence the total transmission ability, of the HTP. This acts to reduce the induced death rate, Γ , by a proportion $(1-\Delta)$, where $\Delta \in [0,1]$. The sabotage effect is found in hosts infected by the vertically transmitted microsporidia and reduces the tendency of the HTP infection to lead to greater predation (Haine *et al.*, 2005). There is also no other protection factor in this model and hence host and VTP infected host are equally susceptible to HTP infection. The following model represents the dynamics.

$$X' = (a - qH)((X + (1 - \kappa)Y_x) + (1 - p)(V + (1 - \kappa)Y_v)) - \beta\Gamma X(Y_x + (1 - \Delta)Y_v) - bX \quad (7)$$

$$V' = p(af - qH)(V + (1 - \kappa)Y_v) - \beta\Gamma V(Y_x + (1 - \Delta)Y_v) - (\alpha_v + b)V \quad (8)$$

$$Y_x' = \beta\Gamma(Y_x + (1 - \Delta)Y_v)X - (\Gamma + b)Y_x \quad (9)$$

$$Y_v' = \beta\Gamma(Y_x + (1 - \Delta)Y_v)V - ((1 - \Delta)\Gamma + b + \alpha_v)Y_v \quad (10)$$

where $H = X + V + Y_x + Y_v$.

The parameters have the same notation as in the previous model apart from the changes detailed above.

4.3.1 Evolution of the horizontally transmitting parasite in the presence of a sabotaging vertically transmitting parasite

In this system in contrast to the general parasite we examined above, the transmission of the parasite is linked to the induced death rate and hence there is a different shape to the trade-off curve (than in the above investigation of equations (1)-(4)). The induced death rate is not the same as conventional virulence, as without it the parasite is unable to transmit. We therefore assume a saturating trade-off between the obligate killing, Γ , and its ability to transmit once it has killed the host, β . The assumption is that to gain a high transmission, the horizontally transmitted parasites need to replicate in the host for longer, i.e. a small Γ . This relationship is seen in insect baculovirus with a positive correlation of time to death and virus yield (Hernandez-Crespo *et al.*, 2001) and also in obligate killers in daphnia (Ebert & Weisser, 1997). The invasion exponent is derived as

$$I_m = (\Gamma_m \beta_m X_r - \Gamma_m - b)(\Gamma_m \beta_m V_r \Delta - \Gamma_m - b - \alpha_v) - \Gamma_m^2 \beta_m^2 X_r V_r \Delta, \quad (11)$$

and assuming there is a trade off between transmission and induced death rate, $\beta_i = g(\Gamma_i)$, this can be expressed as follows

$$I_m = (\Gamma_m g(\Gamma_m) X_r - \Gamma_m - b)(\Gamma_m g(\Gamma_m) V_r \Delta - \Gamma_m - b - \alpha_v) - \Gamma_m^2 g(\Gamma_m)^2 X_r V_r \Delta \quad (12)$$

Which can be read, as number of secondary infections must occur at a rate faster than the death rate of the HTP infected hosts.

We now examine how varying the critical life-history parameters in the vertical parasite affect the optimal horizontal parasites transmission and induced death rate. Here we are mainly concerned with the effect of sabotage on the evolutionarily stable transmission rate and corresponding virulence of the horizontally transmitted parasite. Figure 4.5 (A) shows that increasing sabotage selects for a horizontally transmitted parasite with lower transmission and higher induced death rate. Figure 4.5 (B) shows the importance of sabotage in conjunction with other parameters. As sabotage increases, the magnitude of the effect of feminisation is seen to also increase. Raising feminisation rates reduces horizontal transmission whilst increasing the induced death rate of the horizontally

transmitted parasite. The decrease of transmission and increase in induced death rate occurs because the sabotage rate directly reduces the infection rate of horizontally transmitted parasites. The high sabotage rate leads to a greater proportion of co-infected hosts dying due to natural mortality. Therefore to counter this, the induced death rate is increased at the cost of losing some transmission. Feminisation works similarly to sabotage in reducing horizontal transmission. However, it does not act as directly. As feminisation increases, the proportion of susceptibles that are infected with the vertical parasite increases in the system. As the vertically parasitized hosts have equal susceptibility as the completely uninfected hosts, the proportion of horizontally transmitted parasite infected hosts with the vertical parasite also increases. Therefore the horizontally transmitted parasite infection is experiencing a greater proportion of sabotage and hence combats it by increasing the induced death rate.

Evolution of the Horizontally Transmitted Parasite

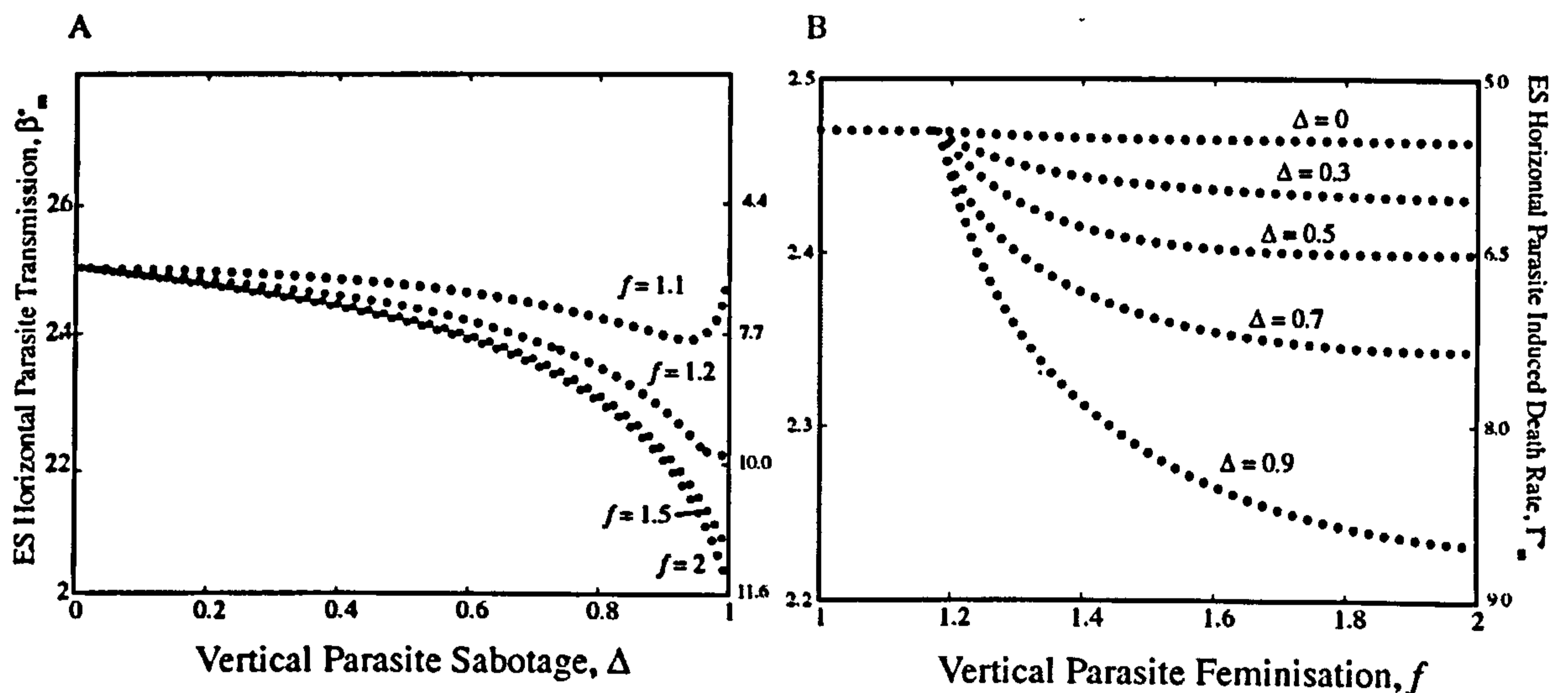


Figure 4.5: A. ES horizontal transmission against different levels of sabotage with varying feminisation B. ES horizontal transmission against varying vertical feminisation values at different sabotage rates. $a=4; b=1; p=0.9; \beta=10-9(15-\Gamma)^{-0.08}; q=1; av=0.1; \kappa=0; .$

4.3.2 Evolution of sabotage in a vertically transmitted parasite in the presence of a castrating horizontally transmitted parasite

We now assume that the horizontally transmitted parasite does not evolve and examine selection of the VTP traits. It is known that feminising microsporidia (our vertically transmitted parasite) do reduce development and clutch size (Haine *et al.*, 2005). Therefore when we examine the sabotage strategy we assume a trade off with saturating costs where an increase in the sabotaging capability of the microsporidia, Δ , leads to a reduction in feminisation ability, f . Thus one may expect the microsporidia to favour feminisation in a system with a weakly pathogenic horizontally transmitted parasite and a highly sabotaging vertically transmitted parasite in a system where the horizontally transmitted parasite infection is very costly to the host.

Castration by the horizontal hosts maximises selection of sabotage at intermediate values and increases in induced death rate and transmission both increase the selection for greater sabotage (Figure 4.6). Increasing the castration initially leads to an increase in sabotage, due to the advantage of vertical parasite reducing the induced death rate of the co-infected class, Y_v . Reducing the induced death rate enhances the input of the vertical parasite into the next generation relative to the hosts via births from the horizontally infected hosts (Y_x and Y_v). By increasing the sabotage rate, the number of the co-infected class that go on to infect horizontally is reduced. This benefits both the host and the vertical parasite. However, with increasing castration, the effectiveness of sabotaging the horizontally transmitted parasite is gradually reduced as fewer of the co-infected class are able to reproduce and hence find it more profitable to enhance the fecundity of the singly vertically infected host, V . The increased horizontal transmission leads to a greater proportion of the population being infected by the horizontal parasite. It is therefore beneficial to increase sabotage as it gives the co-infected hosts better relative fitness compared to the host infected only with the horizontal parasite. The increase in induced death rate also increases the proportion of horizontal infection since it is directly linked to the total level of HTP transmission.

Evolution of the Sabotaging VTP

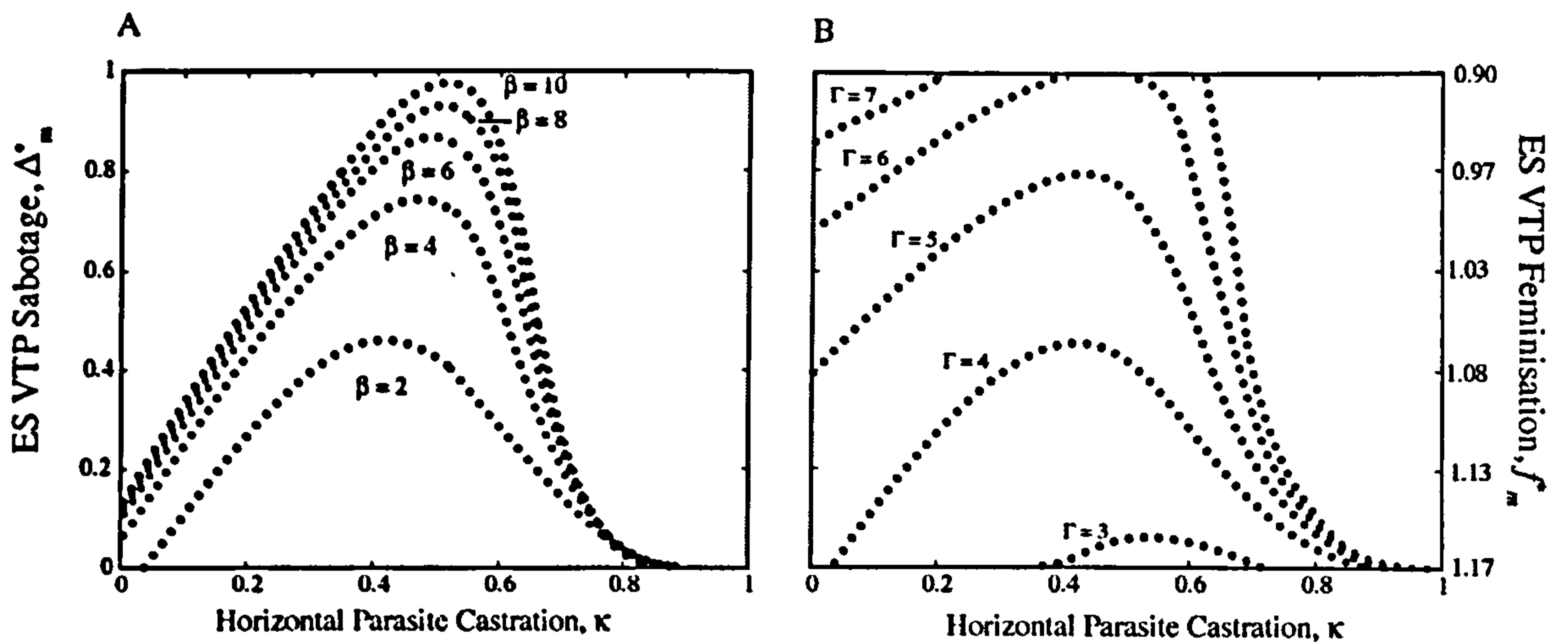


Figure 4.6: A. ES vertical parasite sabotage level against castration by the horizontal parasite at different transmission values. B. ES vertical parasite sabotage level against castration by the horizontal parasite at different induced death values. $a=20$; $b=7$; $p=0.9$; $T=4$; $\beta=2$; $\alpha_v=0.5$; $f=(1.71-\Delta)^{0.3}$; $q=1$

4.3.3 How do the horizontally transmitted parasite and the vertically transmitted parasite coevolve?

In the system described in this study the parasites share the same hosts. Therefore it could be assumed that coevolution may occur between these parasites with similar generation times. Both the vertical parasite and horizontal parasite are assumed to have the same respective trade offs as described above. The fitness function for both was solved graphically such that the change in fitness for each was equal to zero. A coevolutionarily stable strategy (CoESS) occurs when both parasites are at an ESS for corresponding parameter values. Figure 4.7 shows how the castrating effect of the horizontally transmitted parasite affects the CoESS. Maximal sabotage levels are found at intermediate levels of castration, whereas the horizontally transmitted parasite induced death rate decreases as castration increases. The effect on horizontally transmitted parasite induced death rate by castration is very small. Castration in itself would not directly effect selection of the of horizontally transmitted

Coevolution of parasites in response to HTP castration

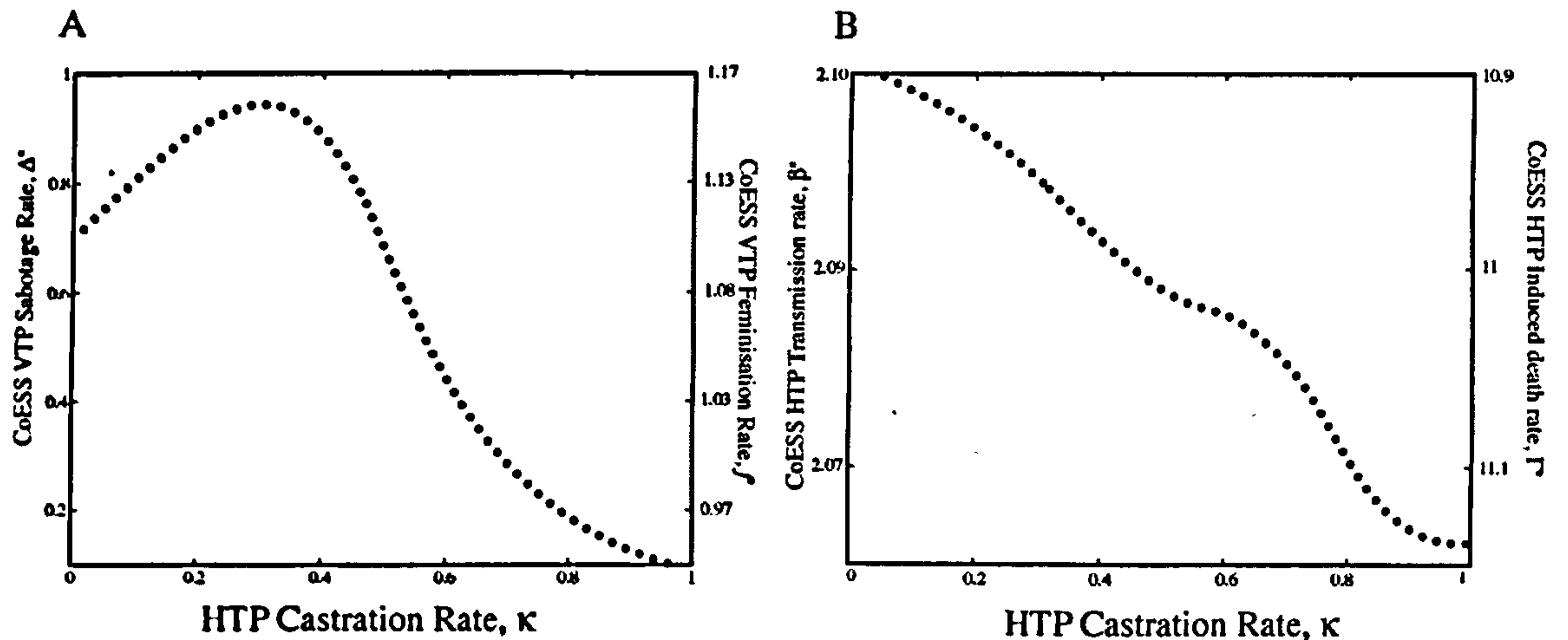


Figure 4.7. Plots showing CoESS values for A. The vertical parasite sabotage level Δ^* & B. The horizontal parasites induced death rate, Γ^* , both for a range of horizontally transmitted parasite castration. $\alpha=40$; $b=20$; $p=0.9$; $f = (1.71 - \Delta)^{0.3}$; $\beta = 10 - 9(16 - \Gamma)^{-0.08}$; $\alpha_v = 0$;

parasite transmission, however, selection may occur through the effect of castration on traits of the vertically transmitted parasite, sabotage and feminisation. These then may feed back to alter the traits in the horizontally transmitted parasite. Figure 4.8 shows how changes in the host death rate affect the CoESS. The increase in host death rate was used as a proxy to understand a change in environmental quality and also lifespan of the host. As natural host mortality increases, the vertical parasite selects for less sabotage and more for fecundity. Long lived hosts would expect to have a vertically transmitted parasite that is selected to have more sabotaging strategy. The horizontally transmitted parasite shows decreases its transmission and increases its induced death rate when faced with worsening habitat quality or shorter-lived hosts. The decrease in sabotage is brought about by the increased natural death rate of the host reducing the transmission of the horizontally transmitted parasite, as a greater proportion die naturally rather than by the induced route of the horizontally transmitted parasite. Therefore the vertical parasite can invest less in sabotage and concentrate on maximising fecundity. The increase in host death rate requires the horizontally transmitted parasite to increase its induced death rate as a higher proportion of those infected by the horizontally transmitted parasite die without transmitting the disease.

Coevolution of parasites in response to host death rate

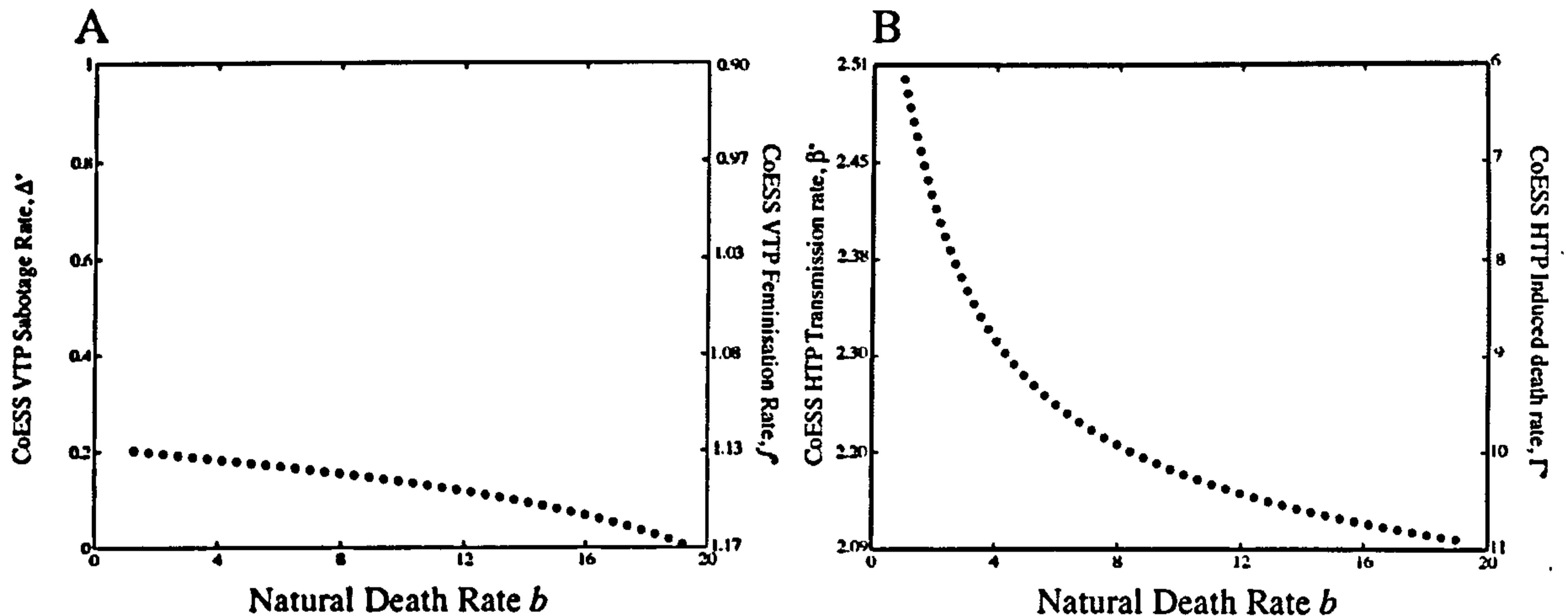


Figure 4.8. Plot showing CoESS values for A. The vertical parasite sabotage level Δ^* & B. The horizontal parasites induced death rate, Γ^* . This shows how the COESS varies with change in natural host mortality rate. $\alpha=40$; $p=0.9$; $f=(1.71-\Delta)^{0.3}$; $\beta=10-9(16-\Gamma)^{-0.08}$; $\alpha_v=0$;

4.4 Discussion

We have shown in both scenarios that a vertically transmitted parasite that directly interacts with a horizontally transmitted parasite to promote its persistence will alter the horizontally transmitted parasite transmission and virulence across a range of host parameters. The protection model (equations (1)-(4)) shows that high and low protection from the vertical parasite leads to a lower virulence in the horizontally transmitted parasite. It is intermediate protection that selects for higher virulence and transmission in the horizontally transmitted parasite as this provides the greatest number of available susceptibles. In the sabotage model the interaction between the vertically transmitted parasite and horizontally transmitted parasite is slightly different due to the different mode of infection of the horizontally transmitted parasite and the resultant strategy of the vertically transmitted parasite. Here an increase in sabotage leads to a decrease in the transmission ability of the horizontally transmitted parasite but an increase in the induced death rate. In the sabotage model an increase in feminisation leads to a decrease in transmission in the horizontally transmitted parasite

There were a number of interesting evolutionary outcomes with the introduction of the protection-virulence trade-off when the VTP can feminise the host. It was possible to get bi-stability in the system with either the vertically transmitted parasite minimising virulence and protection or selecting for a convergent stable point with relatively high protection and vertically transmitted parasite virulence (Figure 4.1). This means that the selection of both a very low virulent vertically transmitted parasite offering little protection and a vertically transmitted parasite strain offering high protection as well as high virulence could evolve in the same system.

We also showed that when both parasites co-evolve, there was a differential selection of virulence in the vertically transmitted as opposed to the horizontally transmitted parasite. When the parasites coevolve, the horizontally transmitted parasite virulence increases with a reduced lifespan whereas vertically transmitted parasite virulence was decreased. The increase in virulence and transmission of the HTP has been highlighted in theoretical work when the a parasite evolves in isolation (Gandon *et al.*, 2001; Restif & Koella, 2003; van Baalen & Sabelis, 1995). This framework allows a reduction of virulence with increasing host death rate, this interaction has been shown to be due to a reduction in host resistance rather than due to increase in host mortality (Restif & Koella, 2003). Here it should be noted that it is the vertically transmitted parasite rather than the horizontally transmitted parasite that shows the decrease in virulence. This is in response to the reduced need for the protection offered by the vertically transmitted parasite as the force of horizontally transmitted parasite infection decreases.

We have also shown that the effect of a VTP that sabotages the response of a HTP has important evolutionary implications to each other whilst competing for the same host. As the vertically transmitted parasite increases its level of sabotage, it selects for a horizontal parasite that kills the host quickly. If the horizontal transmission and induced death rate is increased by a horizontally transmitted parasite, the horizontal parasite selects for a highly sabotaging vertical parasite. Intermediate levels of castration maximise sabotage. So in the Haine *et al* (2005) system, the horizontal

parasite, the acanthocephalan, would evolve in response to a sabotaging vertical parasite, the microsporidia, by increasing its virulence, leading to an increase in the manipulating geotaxis behaviour. The extreme geotaxis seen in this system may even be a result of the sabotaging vertical parasite. In the *Gammarus* system the castration rate of the acanthocephalan was measured as 73.2% (Haine *et al.* 2005). This is a slightly greater rate of castration at which maximum selection of sabotage was seen in the model, but still in an intermediate zone. With this level of castration the ES sabotage rate is affected greatly by variation in transmission and induced death rates. Therefore it is expected that the selection of sabotage in the system maybe very variable and dependent on the prevalence and possible absence of a horizontally transmitted parasite.

Sabotage can effect the selection of the horizontally transmitted parasite even at total castration. However, at total castration the vertically transmitted parasite is selected to have minimal sabotage. For some reason, in nature, the acanthocephala does not completely castrate the host, showing that there must be costs for the horizontally transmitted parasite or resistance from the host. By not completely castrating, the horizontal parasite suffers from the sabotage effect from the vertically transmitted parasite. It would be beneficial for the horizontally transmitted parasite to increase its castration rate to suffer less sabotage. There must be therefore a selective pressure on castration itself, such as limited host resource usage. If the horizontally transmitted parasite does lower transmission or induced death rate, this tends to lower not only the vertically transmitted parasite sabotage rates, but also their prevalence in the population. If the horizontally transmitted parasite evolves its characteristics to reduce sabotage, then the reduction in sabotage would conversely lead to an increase in transmission and reduction in induced death rate.

Although there are few empirical descriptions of co-evolution in parasites, it is reasonable to expect that two parasites such as the ones describe by Haine *et al.*, (2005), will effect each other's selection. This is because they coexist and there is a documented effect of the microsporidia on the acanthocephalan. We showed that in hosts with long life spans, high sabotage rates are expected. As lifespan decreases the

ESS level of sabotage decreases, while, the ES induced death rate increases. Horizontal parasites with intermediate values of castration were seen to select most strongly for a highly sabotaging vertical parasite and a greater rate of death inducing behaviour.

As suggested in Jones: Chapter 3, the castration effect of the horizontal infection can be due to the parasite using host resources (Bonds, 2006) or also looked at as the host using resources to resist the infection (Boots & Bowers, 1999, 2004; Miller *et al.*, 2005; Restif & Koella, 2003). The resistance to the parasite can be seen to reduce infected host fecundity or to reduce other effects on host fitness by the infection. Either way, a level of reduced fecundity or incomplete castration will lead to sabotage.

The models have two extremes of parasite transmission, either totally vertical or totally horizontal. It is known that different parasites often have both modes, with some utilising one more than the other. How a parasite with both modes of transmission may affect either the vertical or horizontal or both parasites is again something of interest. It is without doubt that many hosts suffer from both specific and more general parasites that display a range of different transmission strategies. How they select for each other in competition for the same host resource must be critical in understanding parasite virulences and transmission strategies. With the advance of molecular techniques in describing the plethora of vertical parasites hitherto unnoticed (Terry *et al.*, 2004), it is of great interest to understand how these parasites persist and transmit. More interestingly, as we have shown here, we can observe how their interactions may affect the evolution of other more overt parasites.

5. The winter moth and its natural enemies on Orkney



Fifth instar larvae feeding on heather tip, Trumalnd, Orkney. June 2005

5.1 Introduction

Natural enemy systems are often complex, with each host or prey species being under attack from a number of predators, pathogens and/or parasitoids (Crawley, 1992; Hawkins *et al.*, 1997). The effects of multiple enemies in a system tend not to be the simple summation of their effects on the host, but are often non-additive in effect (Ferguson & Stiling, 1996; Rossi, 2004). Interference has also been demonstrated between natural enemies; for example, parasitoids acting as vectors for viral pathogens in *Plodia interpunctella* (Sait *et al.*, 1996), thus reducing their own transmission effectiveness. Other disrupting interactions occur in generalist predators and specialist parasitoids in the pea aphid (*Acyrtosiphon pisum*), as the carabid beetle reduces the effectiveness of the specialist parasitoid by preying on parasitized aphids (Snyder & Ives, 2001). Dynamics in the field therefore are often complicated because of these interactions between natural enemies even before seasonal environmental and stochastic mechanisms are taken into account. It is of great interest to understand how the interactions result in host population changes, and indeed there are many theoretical models dealing with natural enemy competition and coexistence. The mechanism that is often suggested for coexistence between competing natural enemies is resource partitioning. This can occur both temporally (Briggs, 1993; Godfray & Waage, 1991) and spatially (Comins *et al.*, 1992; Hassell *et al.*, 1994), as well as resource partitioning due to life history traits in competing parasitoids (Bonsall *et al.*, 2002) or foraging behaviour such as in gastropods grazing of microalgae (Wilson *et al.*, 1999). These mechanisms coexistence and competition attempt to explain the driving factors in multiple enemy system dynamics and community assemblages.

A practical understanding of competition and coexistence is important in biocontrol. Biocontrol agents include species specific viruses (Tinsley, 1977) and parasitoids (Embree, 1966; Itioka *et al.*, 1997). Biocontrol has been well described in theoretical models (May & Hassell, 1988). However, there may be a complex interplay when more than one species is expressing infection/predation pressure on the host, with unforeseen pairwise interactions. Combinations of specific models and data have been

constructed to aid control strategies for specific pest control issues, such as the Asian gypsy moth, *Lymantria dispar*, invading New Zealand using NPVs (Barlow *et al.*, 2000).

Here we have examined a moth, *Operophtera brumata*, and its natural enemy assemblage that has been undergoing pest level outbreaks on the Orkney Isles (Stoakley, 1985). The winter moth is a univoltine species with brachipterous females with very low dispersal (Embree; 1966, East, 1974; Graf *et al.*, 1995). Adults emerge from pupation during the months of November and December and reproduce laying up to approximately 300 eggs per female. These eggs remain dormant until April on the mainland of Britain but hatch during mid-May on Orkney, where they remain on the heather, *Calluna vulgaris*, until mid to late June before the 5th instar larvae burrow into the soil and pupate (Varley *et al.*, 1973). In Orkney it co-occurs with its specific baculovirus (OpbuNPV) and a generalist parasitoid, *Phobocampe tempestiva*. These natural enemies both attack the larval stage of the winter moth.



Frame 5.1. Fourth instar winter moth larvae consumed by *Phobocampe tempestiva* larvae, on heather, at Wideford site. 2004.

Periodic population out-breaks are a feature of many winter moth populations studied in Scotland (Hunter *et al.*, 1991; Kerlake *et al.*, 1996; Stoakley, 1985), with larval densities varying as much as 500-1400 m² (Kerlake *et al.*, 1996). Also, the winter

moth has been seen as a pest on many orchards in North America, reaching economically damaging densities and requiring biocontrol through the parasitoid fly, *Cyzenis albicans* (Tachinidae) (Embree, 1966; Graf *et al.*, 1995). Populations of winter moth have had their dynamics described using life-table analysis in Wytham wood in Oxfordshire (Varley & Gradwell, 1968; Varley *et al.*, 1973). The main source of predation there occurs on the pupa by beetles (Coleoptera) (Frank, 1967) and small mammals (Buckner, 1969) over the winter months in oak habitat (Varley *et al.*, 1973). It is clear that both are important in winter moth predation. Pupal predation is suggested to be density dependent due to generalist predators, such as shrews, as they will only target pupae when they are in high abundance (Varley *et al.*, 1973). However, the fauna on heather moorland in Scotland is somewhat different from that found elsewhere in heavily studied winter moth systems. The absence of pupal predation is a potential cause of outbreaks in the moth, as carabid predator numbers are found to be 10-100 times lower in outbreak-prone heather and spruce habitats compared to oak woodland (Raymond *et al.*, 2002b).

There are a number of theoretical studies of invertebrate hosts and multiple enemies such as pathogens and generalist predators (Dwyer *et al.*, 2004) and pathogens and parasitoids (Hochberg *et al.*, 1990). These studies showed the importance in understanding the interaction between the natural enemies in understanding the system they occur in. Descriptive data is utilised in order to understand how the natural enemies attack and infect the hosts. This study will expand the field of multi-enemy systems in the wild. Theoretical models also show the stage dependency can provide conditions for coexistence (Briggs, 1993) and hence we examine viral infection of the winter moth and levels of parasitoid prevalence in different populations across Orkney. The focus is on hosts' stage dependent susceptibilities and development time. A comprehensive amalgam of the data collected is presented and the patterns within them are described. The different ways the natural enemies may be affecting and exerting an influence on the densities of *Operophtera brumata* larvae are alluded to.

5.2 Methods

5.2.1 Larval Collection

The survey of larval populations on Orkney was undertaken at 15 sites from 2003 till 2005 in the summer months of May and June when the larvae are present on the heather. The 15 sites were selected for study in 2003 on the criteria of available habitat, *Calluna vulgaris*, and their geographical separation from each other, (Graham *et al.*, 2004). The sites sampled each year are shown and grid refs provided in Figure 4.1. Larval densities were estimated using transect and quadrat methodologies. A 10m transect was measured out across the chosen site. At each 1m interval, a quadrat area of 25 cm² of foliage from the heather was collected and removed. In total 10 quadrats were taken on the 10 m transect. The heather was thoroughly examined to ensure capture of every larva from each quadrat. In addition, at each site a general collection of larvae was taken so as to increase sample size and hence aid in subsequent analysis. Here the aim was to try to collect 200 individuals. From these data collections, densities were estimated alongside the NPV prevalence at each site. All suspected NPV deaths were tested and confirmed by using standard Giemsa staining and phase contrast microscopy at the Centre of Ecology and Hydrology (CEH Oxford). The staining is necessary as the deaths of NPV in *O. brumata* do not always display the classic baculovirus symptoms (Wigley, 1976), such as liquefaction of the insect.

5.2.2 Stage Dependency

In May and June 2004, four of the study sites were chosen to examine the effect of stage structure in parasitism. These were Wideford (grid ref. 408 114); Settiscarth (356 184); Linnadale (322 062) and Hundland (310 264). All these sites were characterised by a large covering of heather, *Calluna vulgaris*.



Figure 5.1. Map showing location of *Opeorhthera brumata* L. populations sampled on the Orkney Isles. Shaded area indicates the coverage of *C. vulgaris* heather moorland.

Site 1: Trumland ⁱ ,	(grid ref. 427 281)	Site 9: Wideford hill ,	(408 114);
Site 2: Hundland ,	(310 264);	Site 10: Ireland,	(313 093);
Site 3: Midhill,	(334 250);	Site 11: Swartabeck (a),	(374 075);
Site 4: Graemshall,	(384 222);	Site 12: Swartabeck (b),	(376 076);
Site 5: Burn o' Rusht,	(341 207);	Site 13: Linnadale ,	(322 062);
Site 6: Settiscarth ,	(356 184);	Site 14: Mull Head,	(585 090);
Site 7: Syradale,	(351 158);	Site 15: Heldale ⁱⁱ ,	(277 918).
Site 8: Scarra,	(230 140);		

(i), site on the island of Rousay, (ii) site on the island of Hoy, all others on the mainland. Data on stage-structure was obtained for those in bold.

The sites were previously selected in 2003 and thus known to have different larval densities and different levels of parasitism from the NPV and parasitoid, *Phobocampe tempestiva*. Larval densities were again estimated using transects and quadrat methodologies. Each site was revisited every three days so as to regularly check the development of the larvae and parasitism levels. The winter moth has five active instars before it pupates. We collected from the 10th May 2004 till the 16th June, so as to sample from the earliest 1st instars to the 5th instars. The larvae were then reared on sterile artificial diet and sent to the Centre of Ecology and Hydrology (CEH Oxford), where all suspected viral deaths were tested. Confirmation of NPV infection was made using standard techniques (Giemsa staining and phase contrast microscopy). At the same time the number of parasitoids emerging was determined. In the second year, 2005, this was repeated on only two of the four sites, Wideford and Settiscarth, due to a much-reduced abundance at Hundland and Linnadale.

5.2.3 Bioassay

LD₅₀ values of second, third and fourth instars were calculated by inoculating larvae with a range of virus doses, and assessing virus-induced mortality. For each instar five different dose levels were given in addition to a control dose. Thirty-two larvae were used for each dose. The larvae were collected from the field from a location with high host density and low NPV prevalence, (Wideford 2004 1.56%). Since the larvae were collected in the wild each instar was available at different times and thus were inoculated at differing times. Each larva was starved for a period of 12 hours and then fed on a small tip of heather (*Calluna vulgaris*). Each tip of heather had been inoculated with 1 µL OpbuNPV occlusion bodies (OBs). The virus solution was diluted from a single batch of OpbuNPV(Sal1) to add the different number of OBs to the heather tip at different doses. The controls had the heather tip inoculated with 1µL of distilled water. After a period of 12 hours, and the full consumption of the inoculated heather tip, the larvae were each transferred to separate rearing pots and were fed on an excess of fresh heather. The heather was replaced every day and mortality was recorded every 12 hours. The heather used was cut from areas of

moorland where there was an absence of *O. brumata* and therefore it was unlikely there were OpbuNPV occlusion bodies.

5.2.4 Statistical analysis

Proportional infection rates of natural enemies and larval density were subjected to logit analysis; with model behaviour checked by examination of residual deviance and fitted values, testing for overdispersion in the model. For the bioassay, dose-mortality data was subjected to logit analysis with binomial errors, using the statistical package R (Crawley, 1993). To calculate time to death after infection, a Weibull distribution was fitted to the data; a method often used to allow the hazard function to vary with time (Goulson *et al.*, 1995; Murillo *et al.*, 2006). A general linear model was used to investigate if there were any patterns in the infectivity in the different instars and the development data. Other analyses used are outlined explicitly in the following results section.

5.3 Results

5.3.1 Field collection

Over the three years of study, a general decline in larval densities was observed with only three sites maintaining or increasing larval densities, (Wideford, Trumland and Linnadale) (Figure 5.2.1). Only Wideford, however, showed a year upon year increase in densities; 286, 388.8 and 540 m⁻². All other sites showed a decline in larval densities in each successive year. The change in NPV prevalence showed a general decline also over the three-year study window (Figure 5.2.2). Again, along with the density at Wideford, there was an increase in the prevalence of NPV, increasing from 1.56% in 2003 to 17.28% in 2005. Large decreases in NPV prevalences were also seen in sites that had also undergone reduction in host densities, e.g. Hundland 61.08% in 2003 to 4.08% in 2005. However, as seen at Burn O'Rusht, an increase in NPV prevalence coincided with a decrease in larval density. Figure 5.2.3 shows the decline in the prevalence of the parasitoid across the sites with it the parasitoid occurring at fewer

sites. However its prevalence did increase as sites such as Hundland, Midhill and Settiscarth.

Figure 5.3 demonstrates clearly the differences between sites and years. Only one site provided a positive increase in density over the study (Wideford). Between 2003 and 2004 there were four sites with a positive density growth rate, (Wideford, Settiscarth, Trumland and Linnadale), whilst between 2004 and 2005 that was reduced to two (Wideford and Syradale), with the rest decreasing in density. Also only one site, Syradale, demonstrated a recovery from a decrease in density between 2003-2004 to an increase between 2004-2005. The rest showed a negative trend for population densities. The prevalence of the NPV also showed variation between the years and sites. There was largely a decrease in prevalence between 2003 & 2004 with the exception of Graemshall (Figure 5.3.2). There is a trend of increasing prevalence in the year 2004 & 2005 with Wideford, Burn O'Rusht and Linnadale all increasing. Again it is Graemshall that goes largely against this trend by showing a sharp decline in prevalence.

Figure 5.3.3 shows the parasitoid prevalence increasing in only two sites between 2003-2004 (Hundland and Settiscarth). These two sites also showed an increase the following year (2004-2005) along with Trumland, Graemshall and Midhill. Midhill and Graemshall showed a marked decrease from 2003-2004 and a large increase in the following season (2004-2005). This was with both populations decreasing in density in consecutive years. Parasitoids seemed to be absent in 2005 from two sites that appeared to have a winter moth population that could sustain them, Swartabeck (b) and Linnadale. Also in 2005, Wideford had a density of over 500m^{-2} but still a very low prevalence of parasitoids (0.5%).

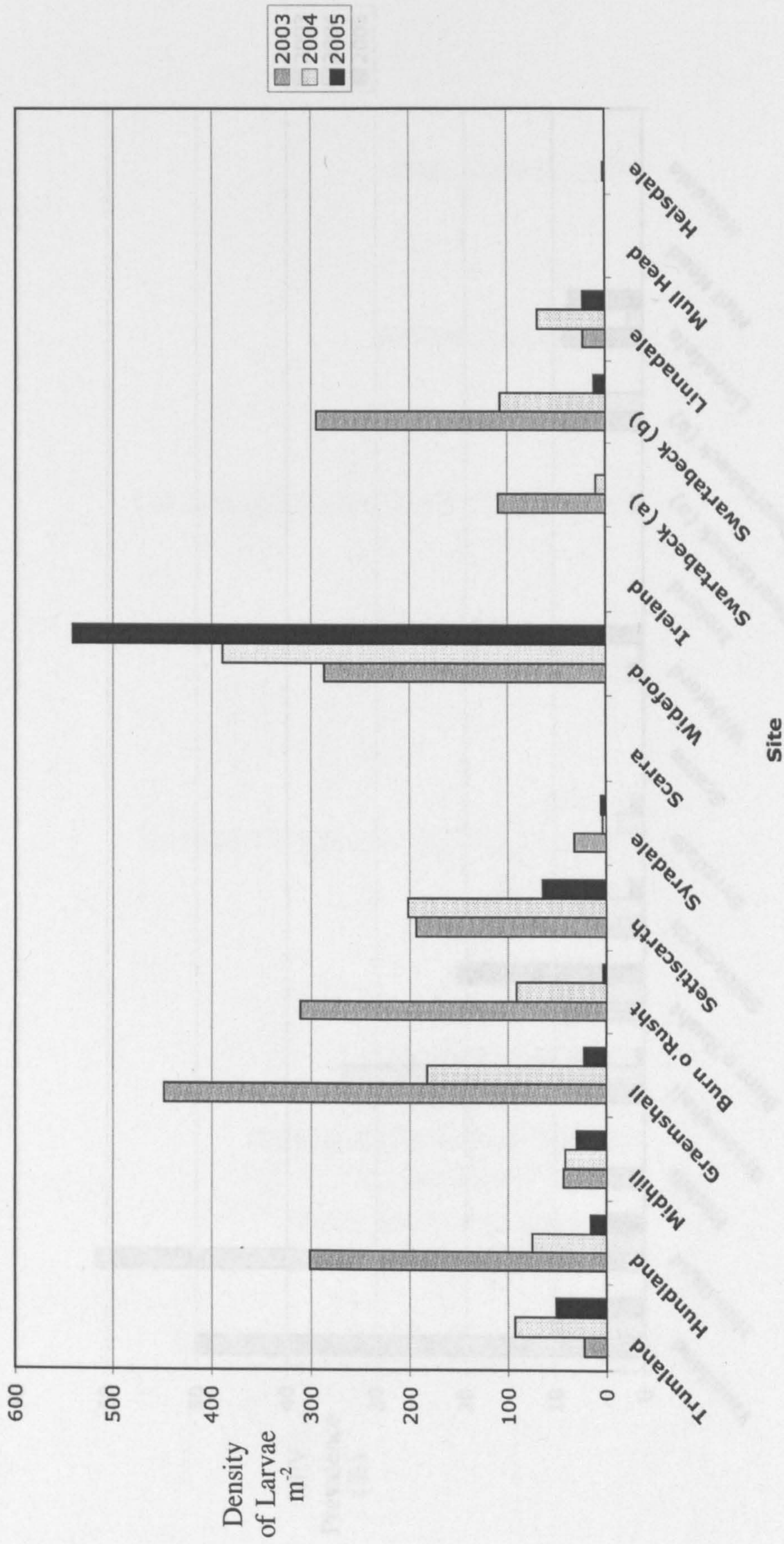


Figure 5.2.1 Figure shows densities in the sites over the years 2003-2005.

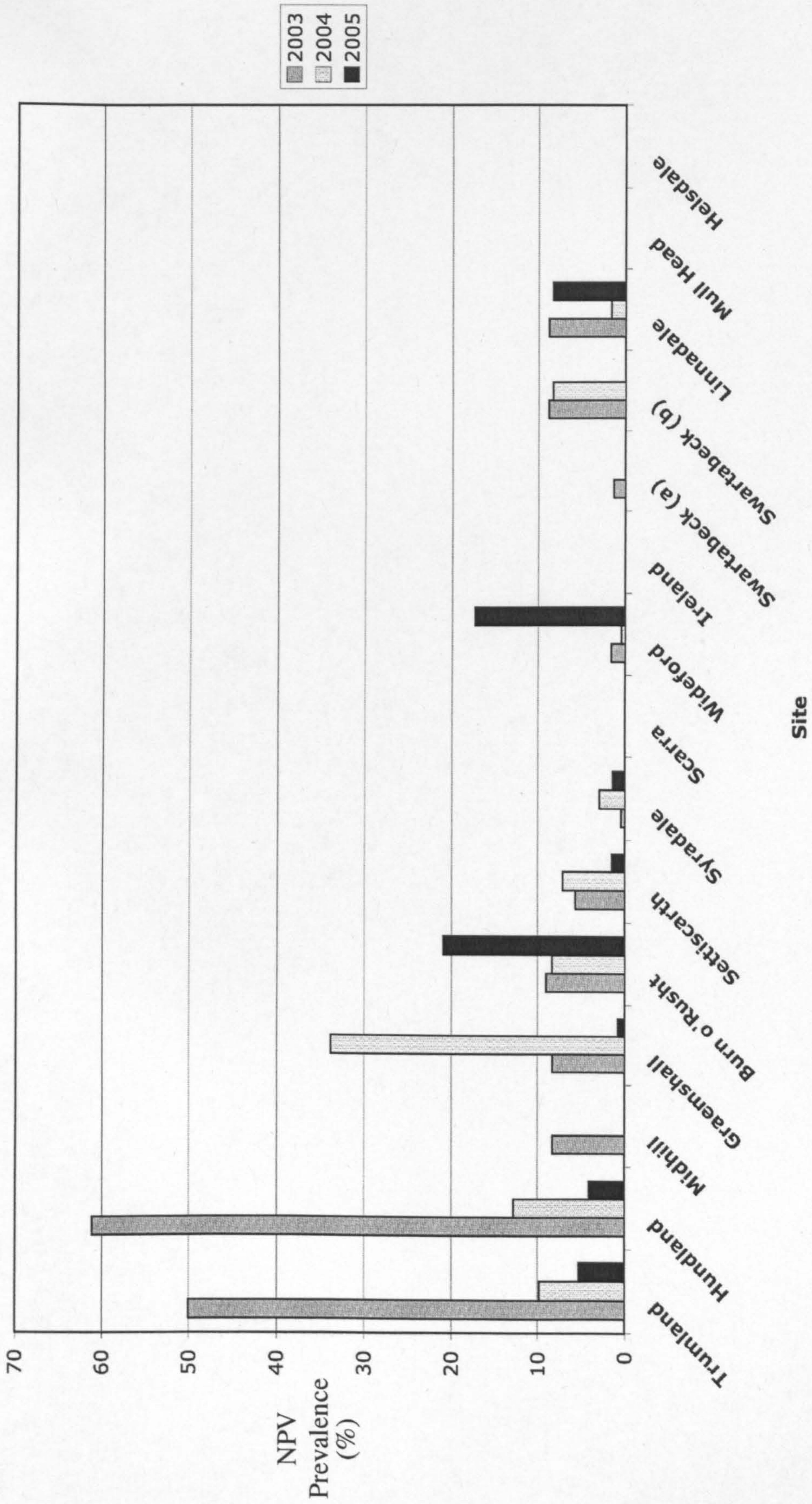


Figure 5.2.2 Figure shows NPV prevalence in the sites over the years 2003-2005.

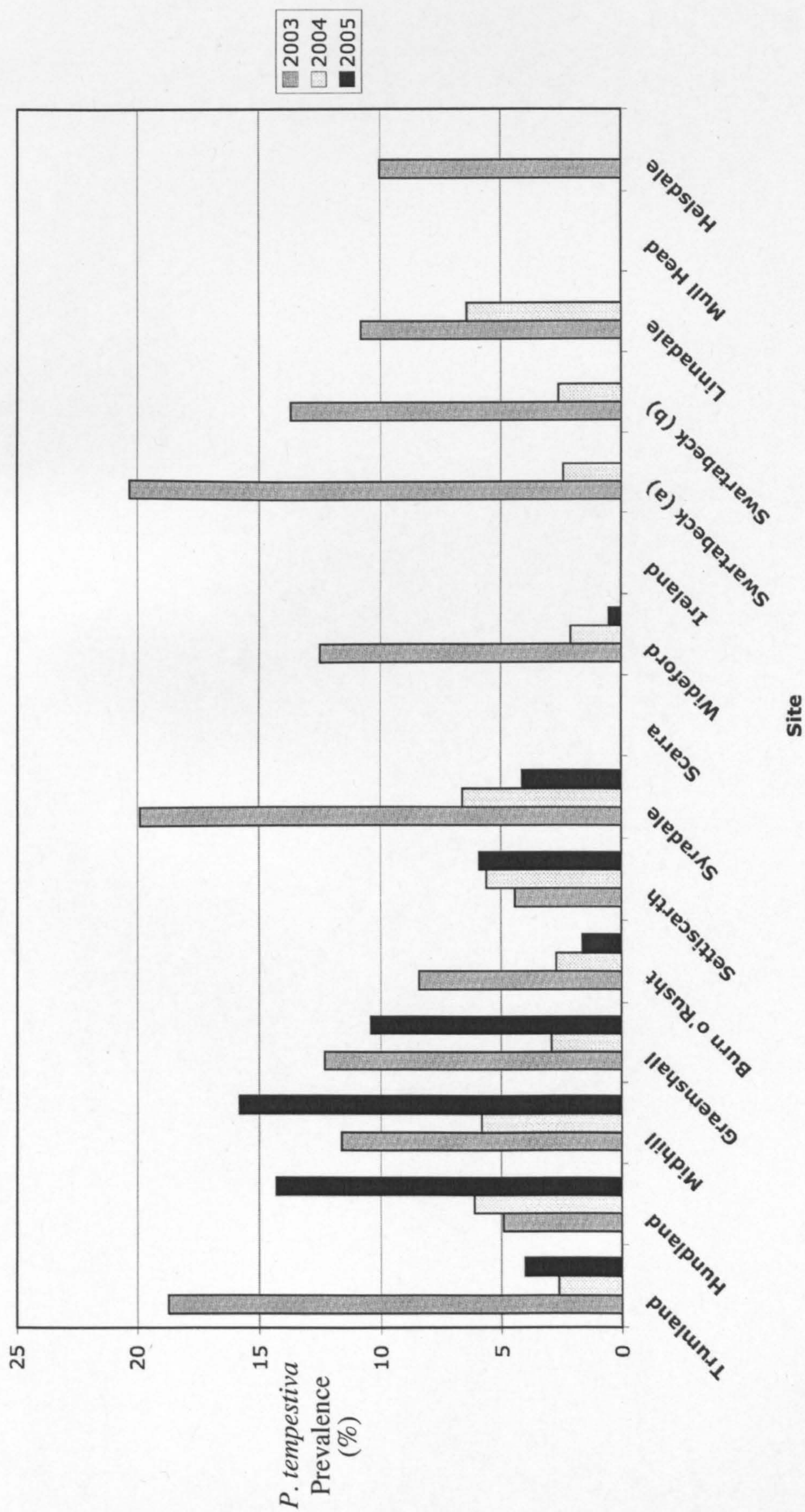


Figure 5.2.3 Figure shows parasitoid prevalence in the sites over the years 2003-2005.

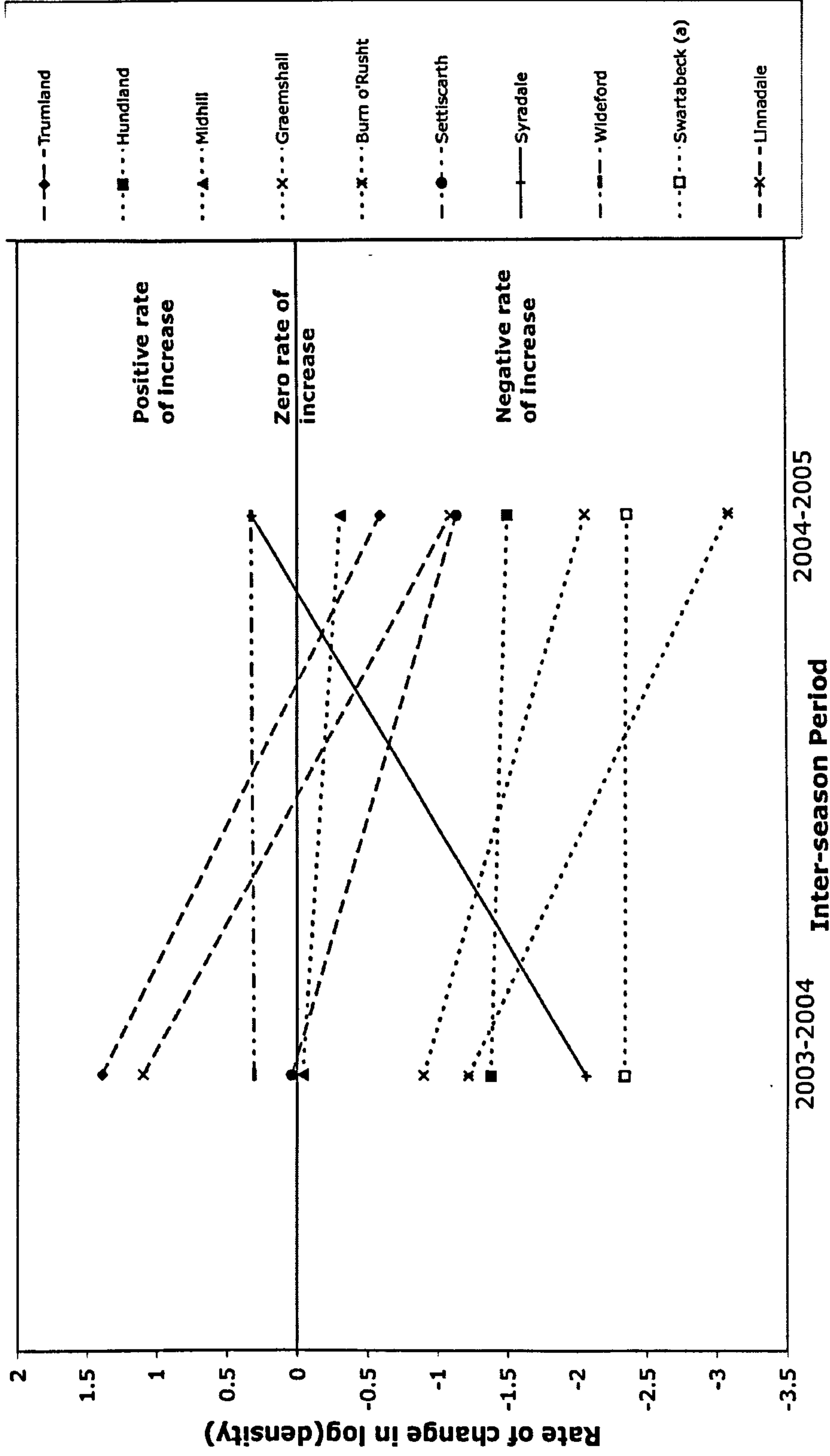


Figure 5.3.2 Graphs showing rate of change in percentage host density, between the years 2003-2004 and 2004-2005. Solid lines denote decrease in prevalence 2003-2004 and increase 2004-2005; dashed lines denote an increase in prevalence in 2003-2004 and a decrease in 2004-2005, dotted lines denote a negative change both inter year periods, dotted-dashed lines indicate positive growth in prevalence each period. Legend denotes study sites.

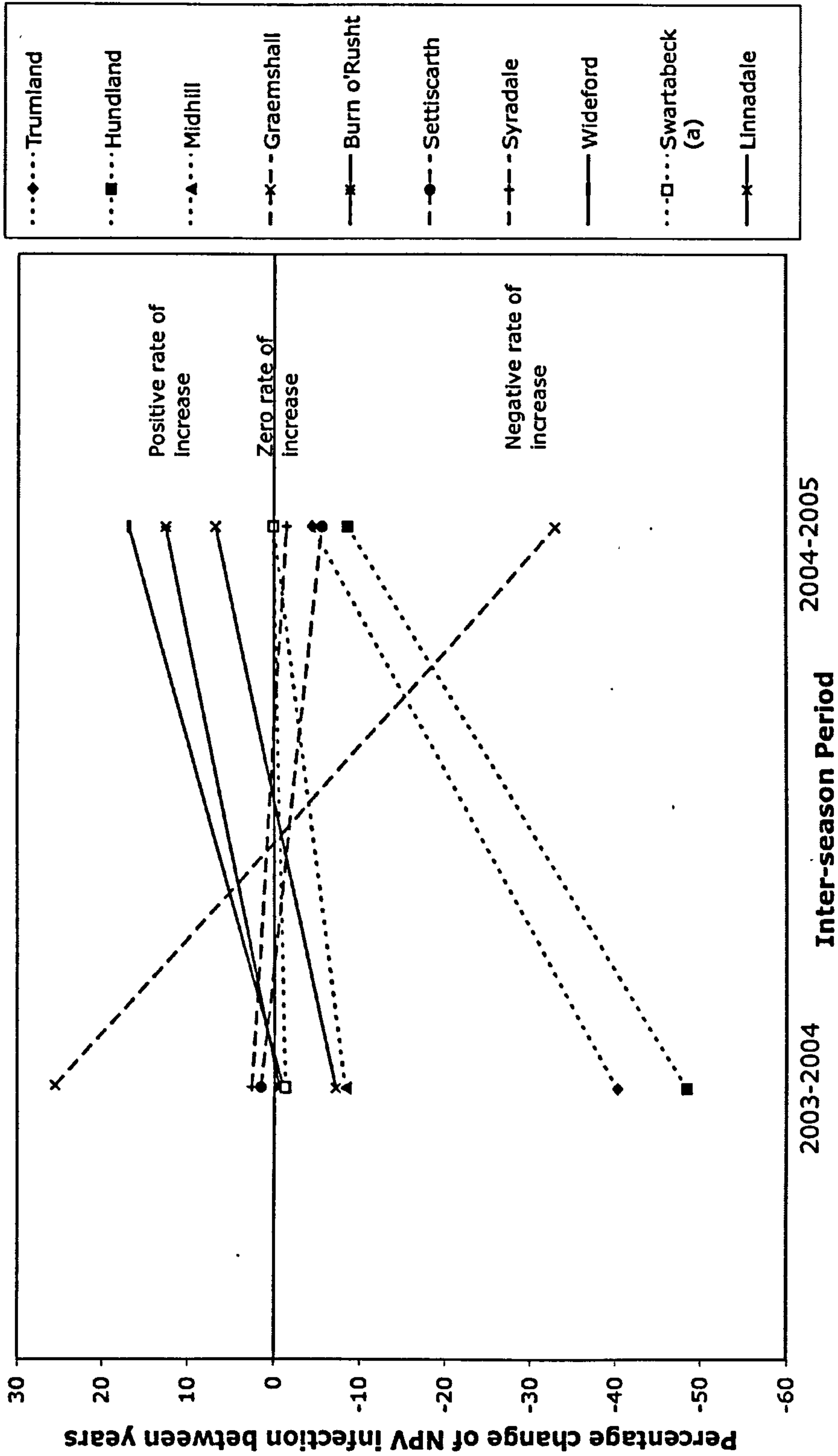


Figure 5.3.2 Graphs showing rate of change in percentage NPV prevalence, between the years 2003-2004 and 2004-2005. Solid lines denote decrease in prevalence 2003-2004 and increase 2004-2005; dashed lines denote an increase in prevalence in 2003-2004 and a decrease in 2004-2005, dotted lines denote a negative change both inter year periods, dotted-dashed lines indicate positive growth in prevalence each period. Legend denotes study sites.

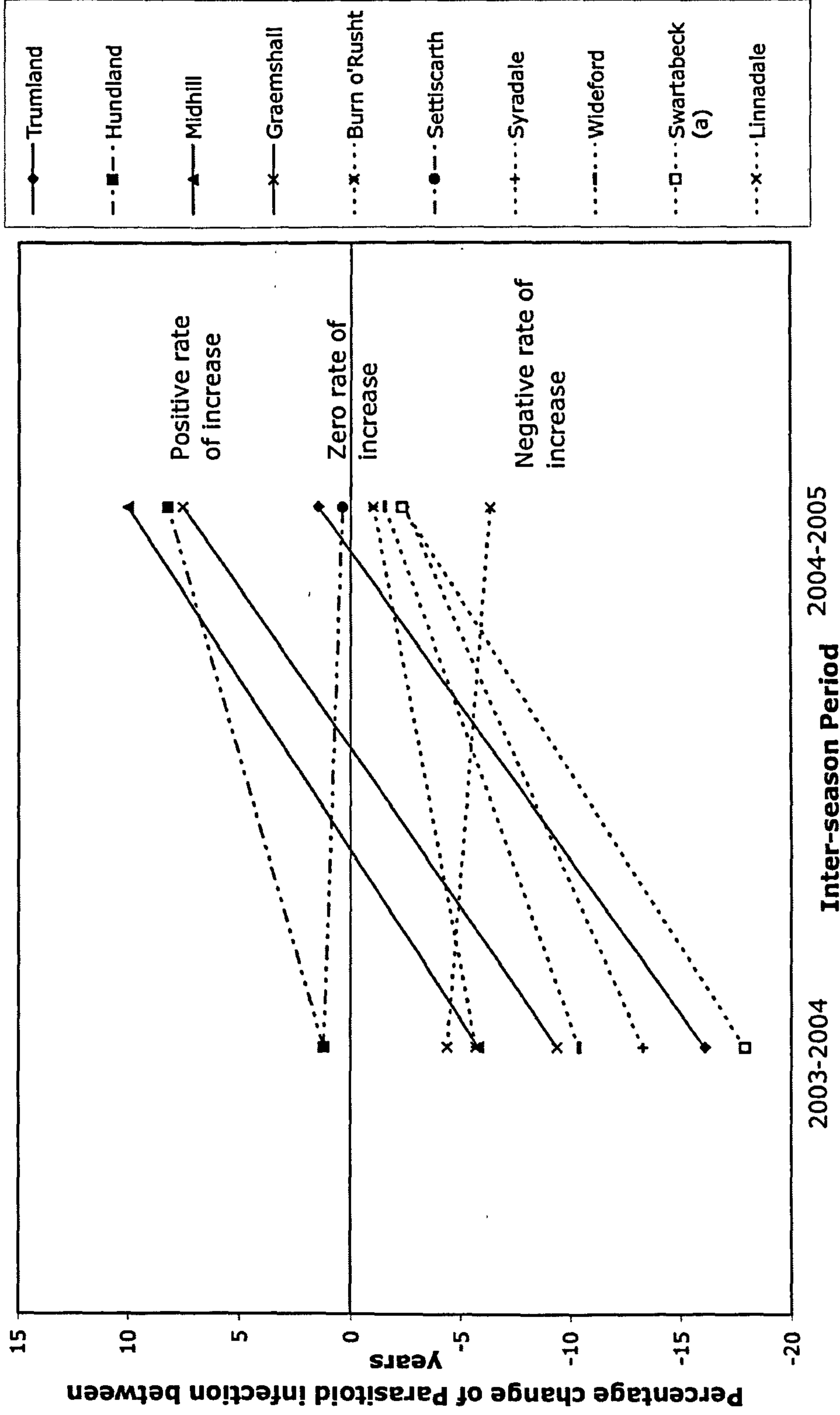


Figure 5.3.3 Graphs showing rate of change in percentage parasitoid prevalence, between the years 2003-2004 and 2004-2005. Solid lines denote decrease in prevalence 2003-2004 and increase 2004-2005; dashed lines denote an increase in prevalence in 2003-2004 and a decrease in 2004-2005, dotted lines denote a negative change both inter year periods, dotted-dashed lines indicate positive growth in prevalence each period. Legend denotes study sites.

If the average density of moth larvae is taken across sites where *Operophtera brumata* are found each of the three years, we see a decrease across the years along with the decrease in OpbuNPV. The decrease in density is significant if Wideford is omitted from the analysis ($F_{2, 27} = 6.4$, $p=0.03$). This seems prudent as it is clearly increasing whilst the other sites are declining. There is, however, no significant change in the average prevalence of viral infection ($F_{2, 30} = 0.72$, $p = 0.49$). The prevalence decreases over the three years, but not significantly due to the high variance in the prevalence across the sites. The parasitoid, *Phobocampe tempestiva*, prevalence however was seen to significantly vary between the years ($F_{2,30}=13.34$, $p<0.001$), With high parasitoid prevalence in 2003 and low prevalence in 2004 and 2005. A summary of densities and OpbuNPV and *Phobocampe tempestiva* prevalences recorded across the sites and sample years is found in Table 4.1.

Table 4.1. Densities (m^{-2}) of winter moth larvae at the study sites over the 3 years with corresponding OpbuNPV and *Phobocampe tempestiva* prevalence.

Site	Density per metre sq.			NPV %			Parasitoid %		
	2003	2004	2005	2003	2004	2005	2003	2004	2005
Trumland	22.4	92.8	51.2	50	9.79	5.25	18.7	2.6	4
Hundland	300.8	75.2	16	61.08	12.74	4.08	4.9	6.1	14.3
Midhill	43.2	41.6	30.4	8.3	0	0	11.6	5.8	15.8
Graemshall	448	182.4	22.4	8.3	33.81	0.8	12.3	2.9	10.4
Burn o'Rusht	310.4	91.2	3.2	9.02	8.33	20.9	8.4	2.7	1.6
Settiscarth	193.6	201.6	64	5.65	7.14	1.48	4.4	5.6	5.9
Syradale	32	3.2	4.8	0.42	2.92	1.35	19.9	6.6	4.1
Scarra	0	0	0	0	0	0	0	0	0
Wideford	286.4	388.8	540.8	1.56	0.41	17.28	12.5	2.1	0.5
Ireland	0	0	0	0	0	0	0	0	0
Swartabeck (a)	108.8	9.6	0	1.31	0	0	20.3	2.4	0
Swartabeck (b)	294.4	107.2	11.2	8.87	8.33	0	13.7	2.6	0
Linnadale	22.4	68.8	22.4	8.82	1.61	8.33	10.8	6.4	0
Mull Head	0	0	0	0	0	0	0	0	0
Helsdale	1.6	0	0	0	0	0	10	0	0
Mean	172.4	114.8	76.64	14.7	8.47	8.4	11.1	3.73	3.72
Standard Error	41.3	59.8	61.3	4.12	4.29	4.74	1.25	1	1.1

5.3.2 Stage structure

Development Rates

We examined both the development rate and the susceptibility to natural enemies at different larval instar stages. The percentage of each particular instar found in each collection is plotted against the day of sampling to ascertain the rate at which they change from one instar to another and indeed the time spent in that larval instar. Figure 5.4 shows this for the sites Wideford, Linnadale, Hundland and Settiscarth for the year

2004 and was also recorded in the year 2005 at only Wideford and Settiscarth. Figure 5.4 shows that the development of larvae occurs at different rates and at slightly different dates at different sites. We also can see that there is definitely a difference between the years, with all the sites monitored in 2004 having later instars earlier than in 2005. This is more obvious in Figure 5.5, where the maximum prevalence of instars 2-4 is plotted against time. We only examined the second to fourth instars, as we were not able to sample precisely the start and end of the season at all the sites, and hence cannot accurately determine where the maximum infestation of each instar occurs in time. This demonstrates that the sites of Hundland and Linnadale showed the quickest development times in 2004, while Wideford and Settiscarth took the longest. A large difference is observed between the fastest developing sites, Hundland and Linnadale, and the slower developing sites, Settiscarth and Wideford, in the date of greatest prevalence of the third instar, in 2004. However, there is little difference found in the second and fourth instar maximum prevalence dates. We also see a much later season in the year 2005 with the sites at Wideford and Settiscarth. The maximum densities at Wideford were found to lag by 8, 9 and 14 days in the 2nd, 3rd and 4th instars, respectively. At Settiscarth they lagged 14, 7 and 13 days for the instar peaks from the previous year. The larvae at Wideford took 18 days in 2004 from 2nd instar maximum prevalence to 4th instar maximum prevalence; in 2005 this took 22 days. At Settiscarth, despite the late start to the larval season, they developed in 18 days in 2005 and slower in 2004 taking, 19 days.

As we require an estimate of the development rate for a future stage-structured model, the period of each instar was recorded. This was carried out by using the average instar level as an indicator of the rate at which the larval population develops from 1st instars to 5th instars. We ran a simple regression to find a rate of change of instars against time (Figure 5.6). By using the most complete data set from each site in the year 2004 the average instar level was regressed against the day of sample, which gave a linear time scale for approximate instar stage development. The regression equation was; $\text{Instar} = -0.0018 \cdot \text{Day}^2 + 0.17 \cdot \text{Day} + 1.25$. ($R^2 = 0.92$, $F_{2, 49} = 319.4$, $P < 0.001$). From this equation approximate times for development in each stage could be estimated. These data are used in the parameterisation of the stage-structured model in Chapter 6

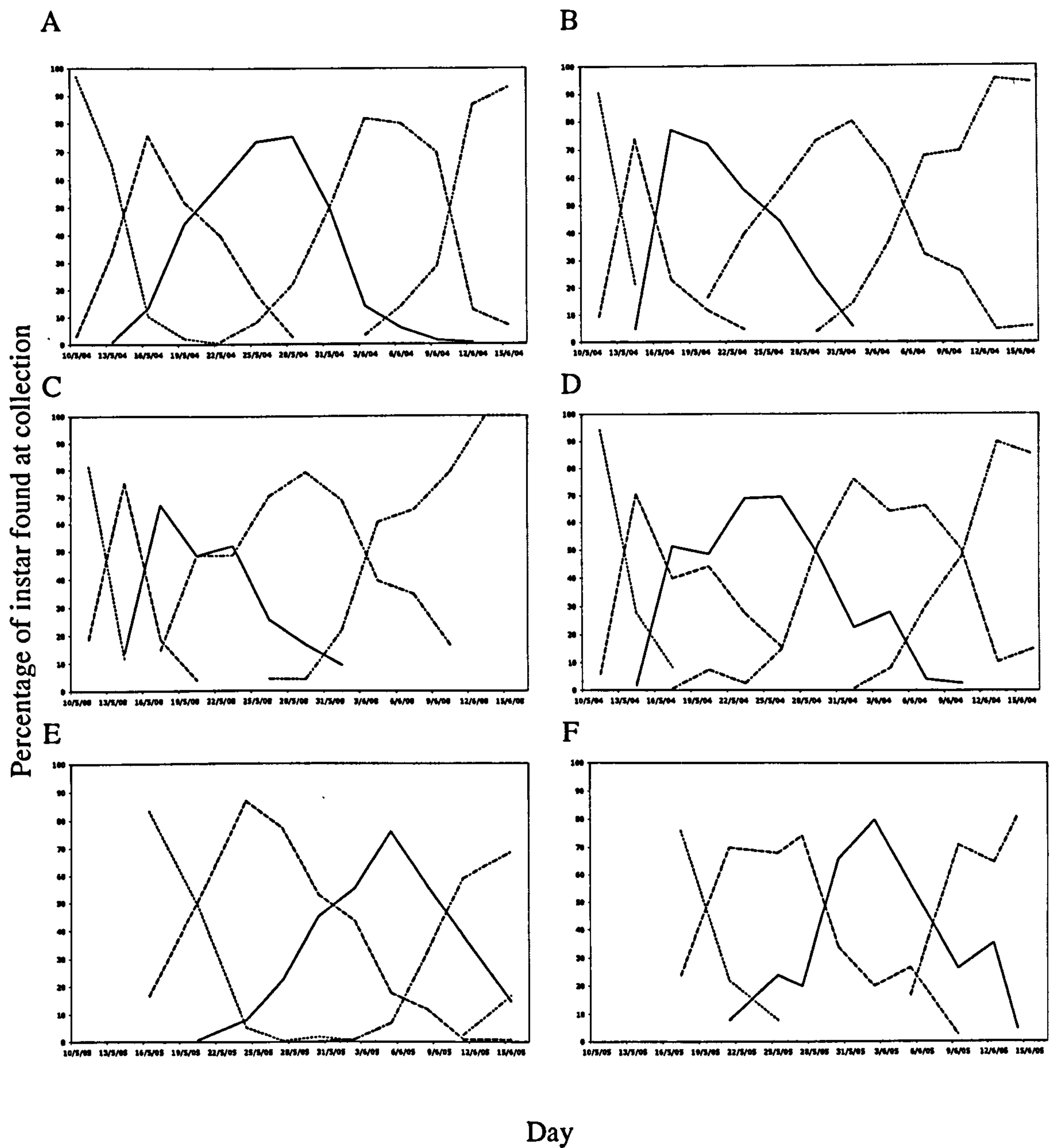


Figure 5.4. The percentages of each instar in collection over the sampling period at 1st instar (dotted line), 2nd instar (dashed line), 3rd instar (solid line), 4th instar (dash-dotted line) and 5th instar (dash-dot-dot line). The sites were sampled in May–June 2004, A) Wideford, B) Linnadale, C) Hundland & D) Settiscarth and May–June 2005, E) Wideford and F) Settiscarth.

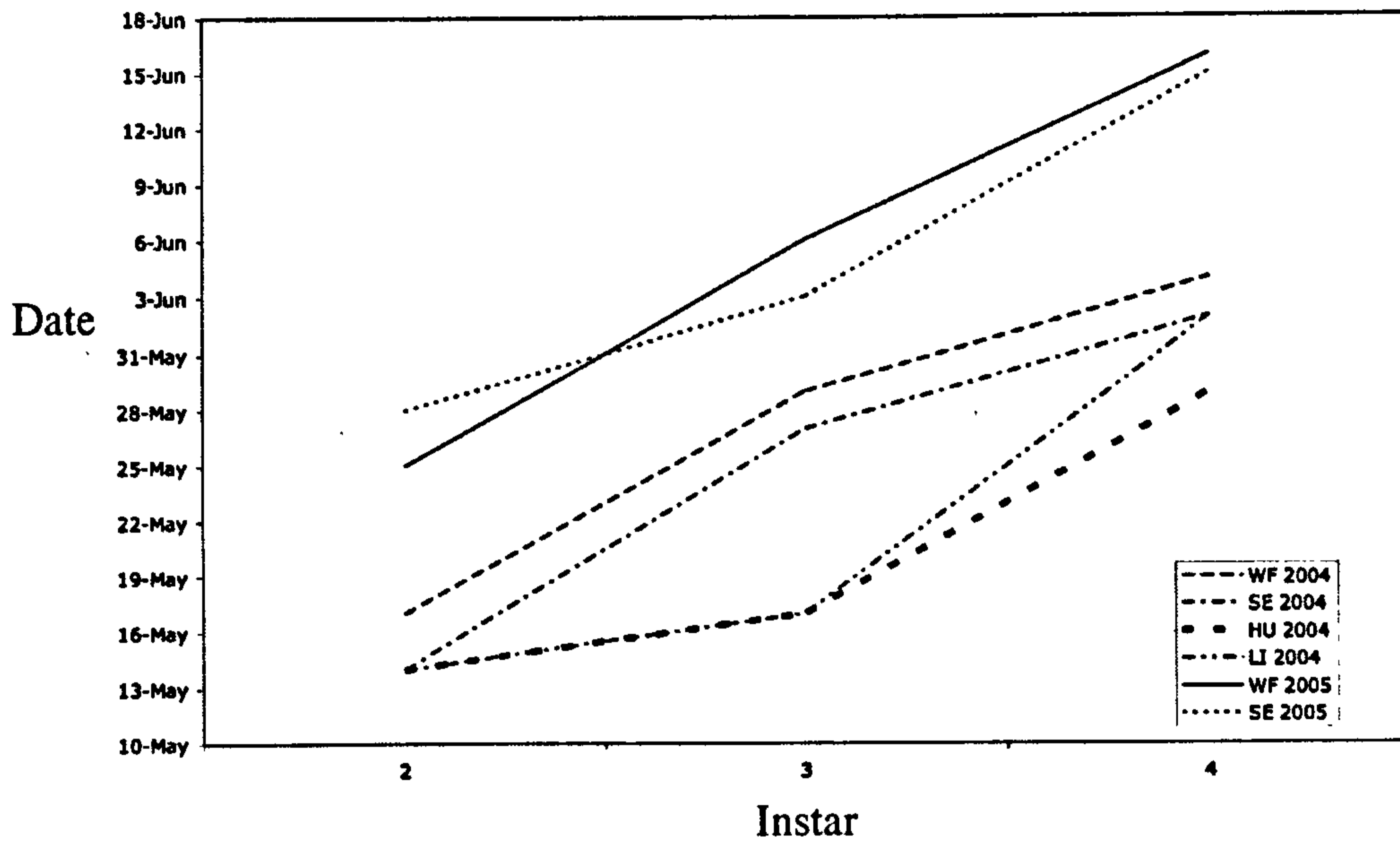


Figure 5.5. The date of maximum instar prevalence of the 2nd, 3rd & 4th instar in the stage structure sample site over the two sampling years.

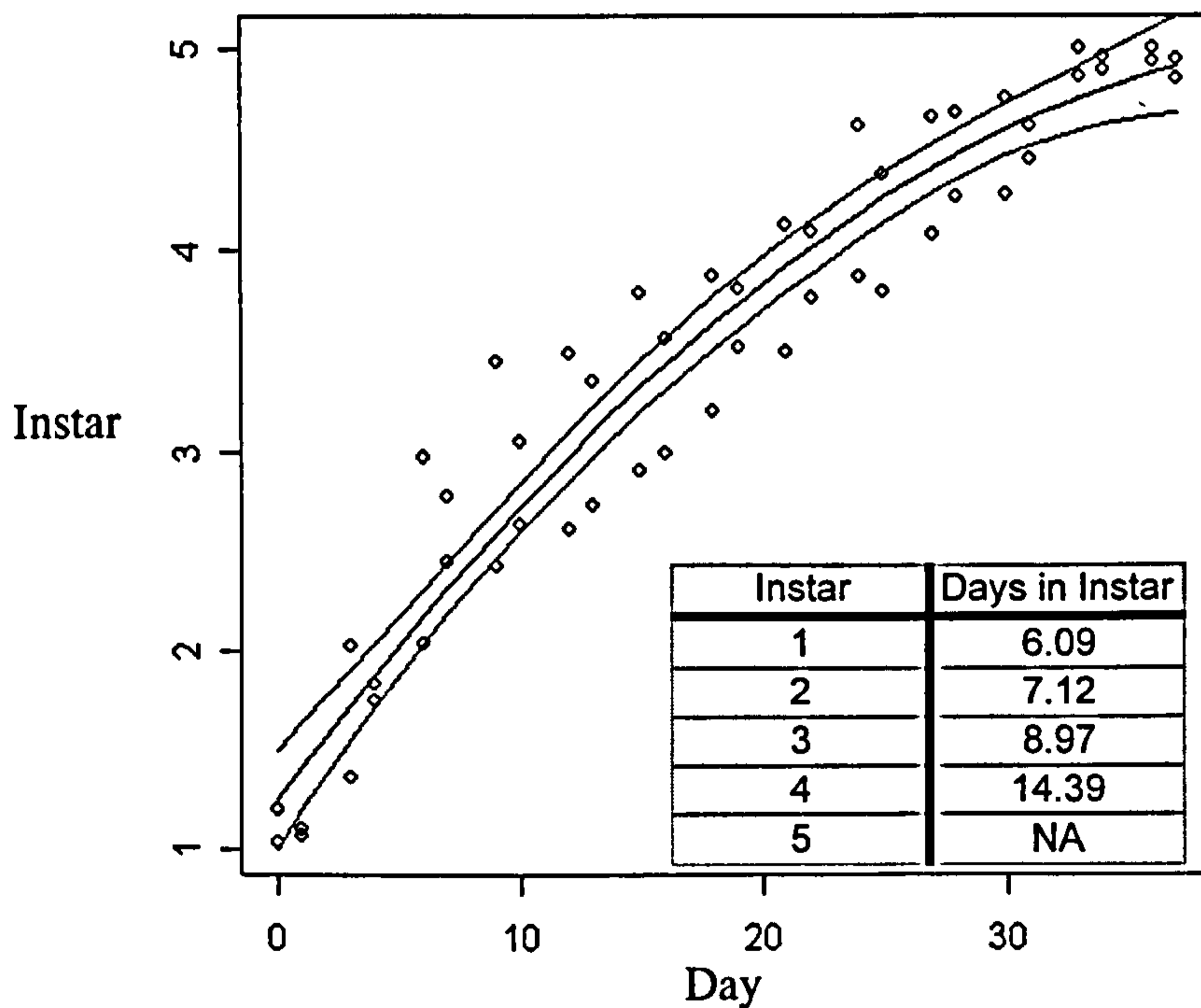
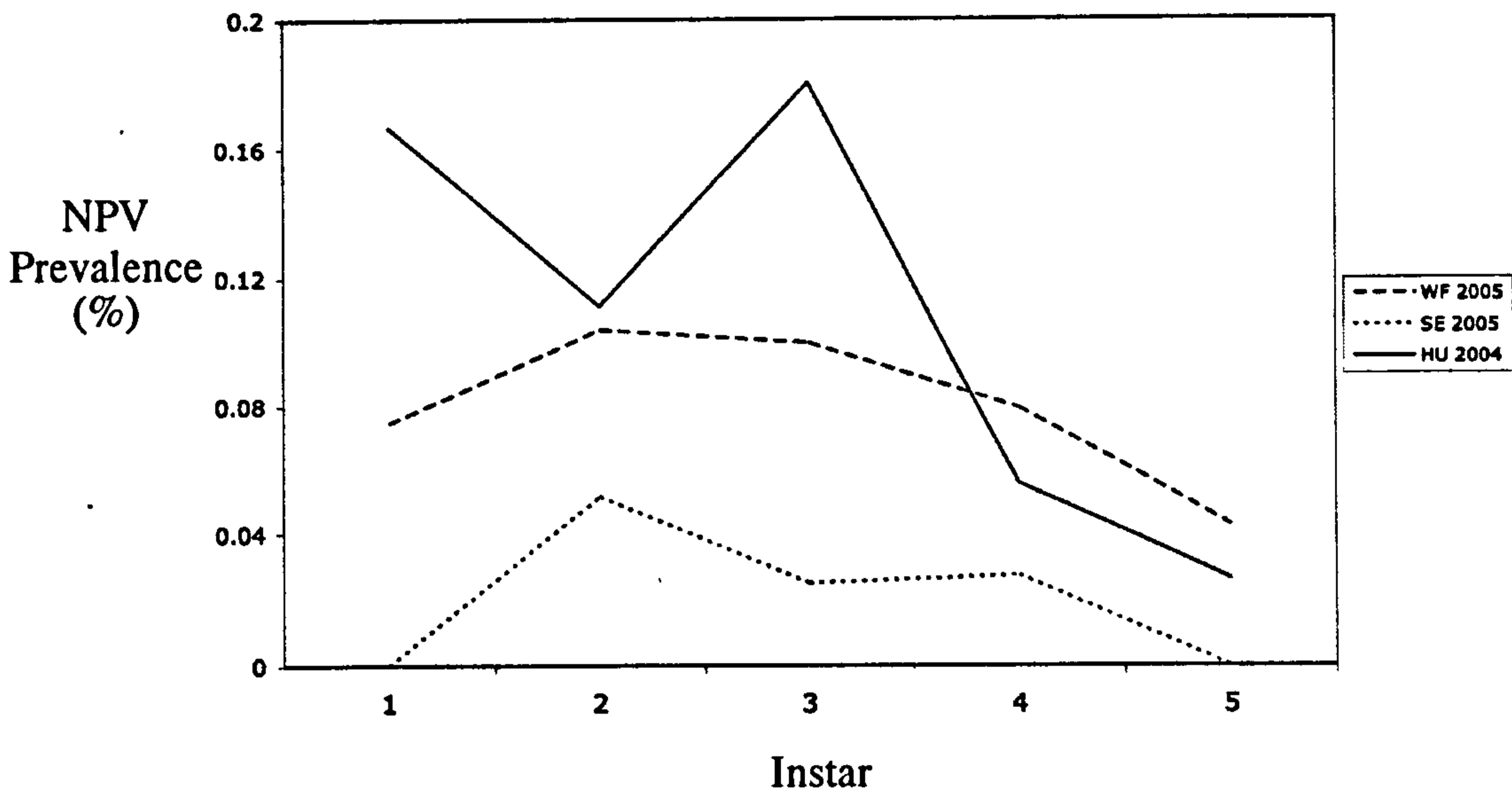


Figure 5.6. The development rate through different instar stages over the larval period in the summer of 2004, with 95% C.I. The regression equation was $\text{Instar} = -0.0018 \cdot \text{Day}^2 + 0.17 \cdot \text{Day} + 1.25$. $R^2 = 0.92$, $F_{2, 49} = 319.4$, $P < 0.001$.

Natural Enemies

NPV instar dependent prevalence: There was a difference in prevalence of OpbuNPV in the different years of study and in the different study sites ($F= 20.96$, $df = 1$, $P<0.05$, & $F=12.32$, $df = 3$, $P<0.05$, respectively).

A



B

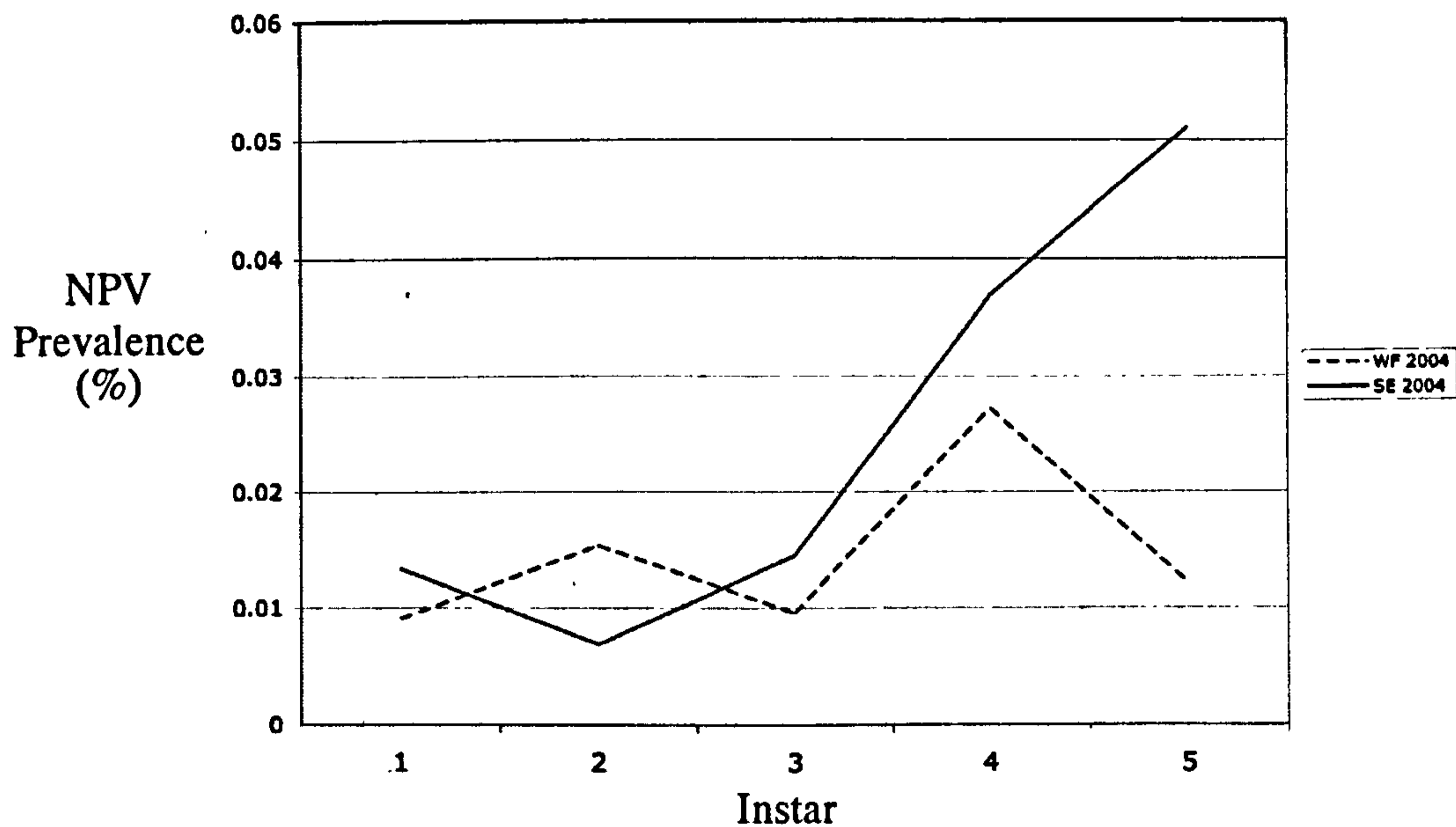


Figure 5.7 OpbuNPV prevalence in different *Operophtera brumata* instars in three sites, Wideford, Settiscarth and Hundland in the years 2004 and 2005. A. Prevalence decreasing with increasing instar and B. Sites where NPV prevalence increases with increasing instar.

However, no significant difference in the prevalence level of NPV infections in different instar stages was found ($F=0.807$, $df = 4$, $P>0.05$). We can see from Figure 5.7 the differences found between sites in how different instars are affected. Hundland shows high prevalence in the early instars (1-3) and lower prevalence in the later, larger instars (4-5). This was also seen to some extent in 2005 at Wideford and at Settiscarth, although there were no OpbuNPV deaths observed in the 1st and 5th instar stages in Settiscarth in that year. However, in 2004, both Wideford and Settiscarth provided a converse pattern where the later instars were seen to have a greater prevalence of the virus. Hence we see that there is little difference in the prevalence of OpbuNPV across instars (Figure 5.8 (a)) when they are pooled together. However with each site and year we see certain trends in instar and prevalence.

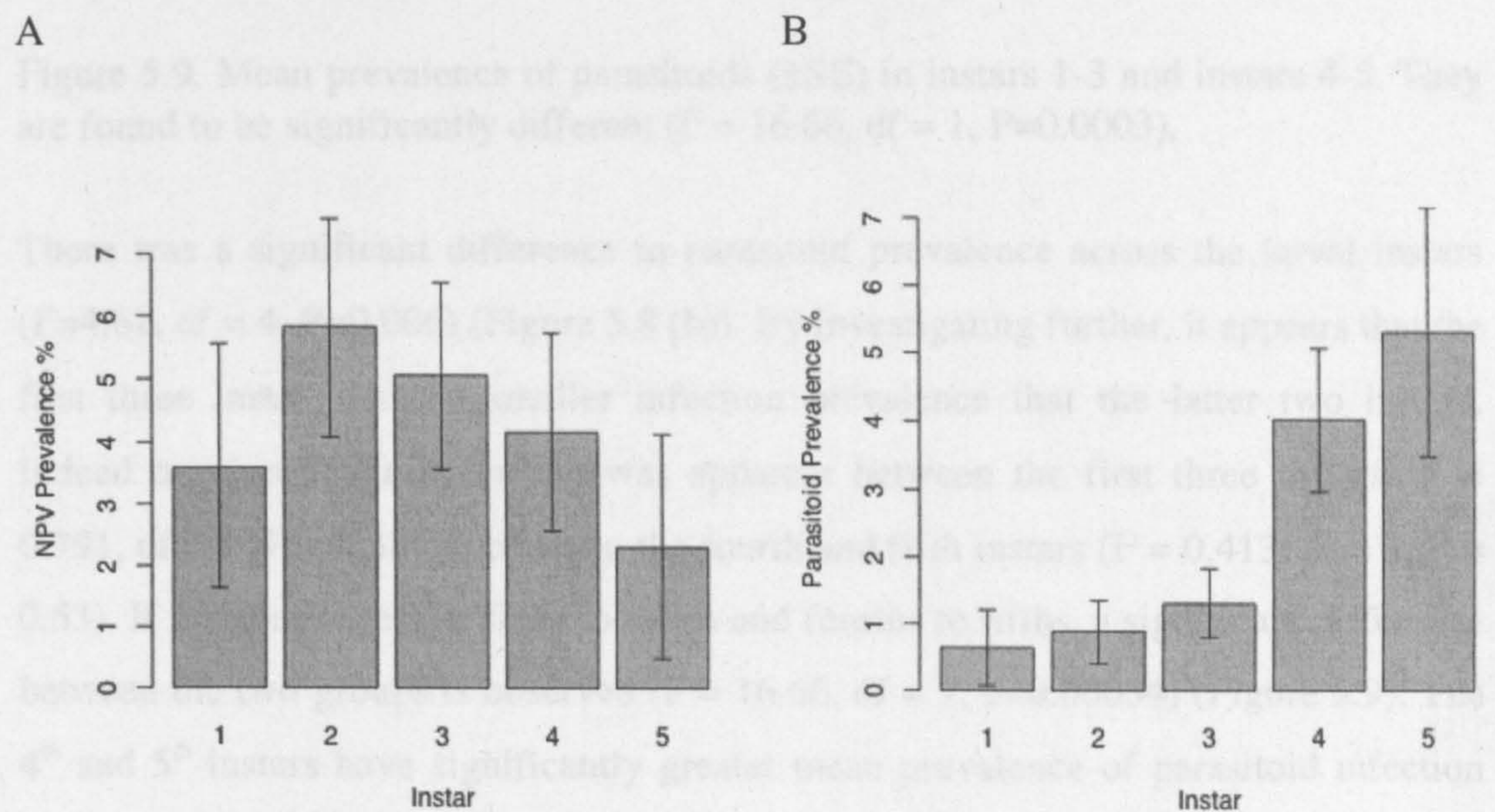


Figure 5.8 A. Mean NPV prevalence ($\pm 1SE$). B. Mean parasitoid, *Phobocampe tempestiva*, prevalence ($\pm 1SE$) in the 5 larval instars of the winter moth, *Operophtera brumata*

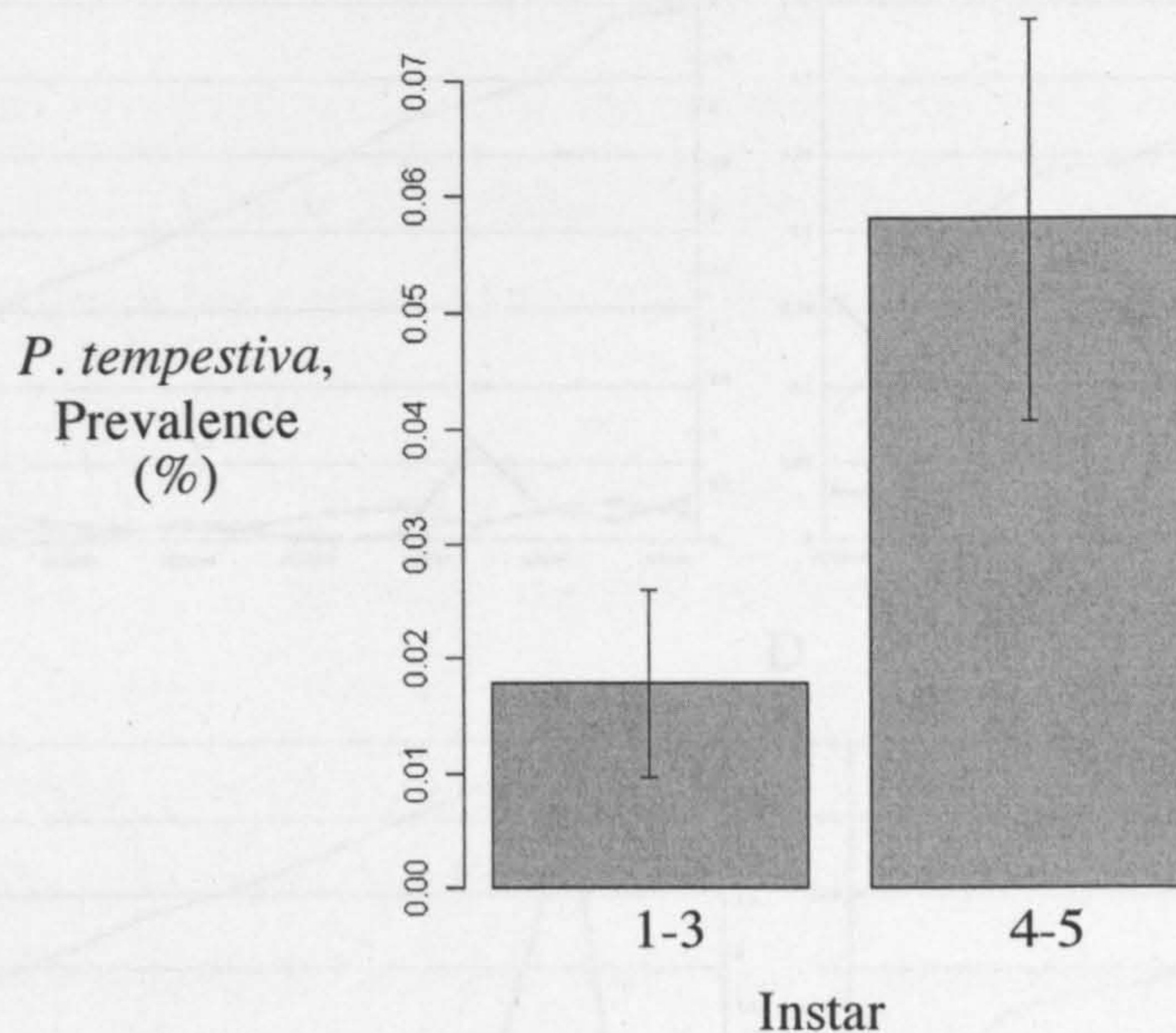


Figure 5.9. Mean prevalence of parasitoids (\pm SE) in instars 1-3 and instars 4-5. They are found to be significantly different ($F = 16.66$, $df = 1$, $P=0.0003$).

There was a significant difference in parasitoid prevalence across the larval instars ($F=4.61$, $df = 4$, $P=0.006$) (Figure 5.8 (b)). By investigating further, it appears that the first three instars have a smaller infection prevalence than the latter two instars. Indeed no significant difference was apparent between the first three instars ($F = 0.791$, $df = 2$, $P = 0.38$) or between the fourth and fifth instars ($F = 0.413$, $df = 1$, $P = 0.53$). If grouped together, firsts to thirds and fourths to fifths, a significant difference between the two groups is observed ($F = 16.66$, $df = 1$, $p=0.00034$) (Figure 5.9). The 4th and 5th instars have significantly greater mean prevalence of parasitoid infection ($5.8 \pm 1.7 \%$) than the smaller 1st, 2nd and 3rd instars ($1.8 \pm 0.8\%$).

The prevalence of natural enemy caused death is described in Figure 5.10. This shows similar levels of infection of the OpbuNPV and the parasitoid wasp over the time of the season within each site. Wideford in 2004 was a population that had very little death caused by these two natural enemies. A year later the parasitoid wasp remained at a similar level to the previous year. However the OpbuNPV was at a much higher prevalence.

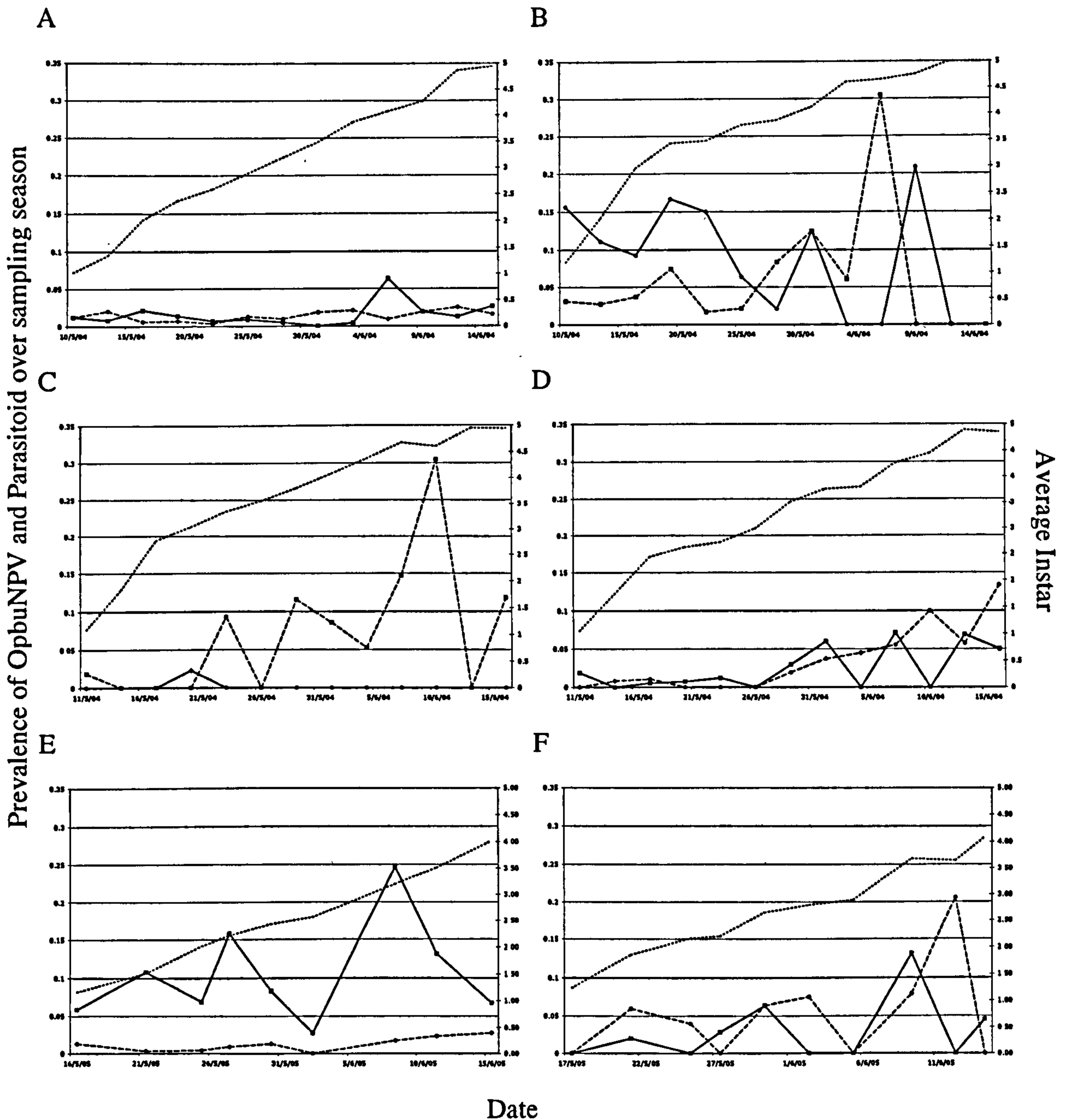


Figure 5.10. Prevalence of OpbuNPV (solid line) and the parasitoid *Phobocampe tempestiva*, (dashed-line) in 2004 in sites A) Wideford, B) Hundland, C) Linnadale & D) Settiscarth and in 2005 E) Wideford & F) Settiscarth. All plotted on the same y-axis scale. The dotted line denotes the average instar level, to show the level of development in the population.

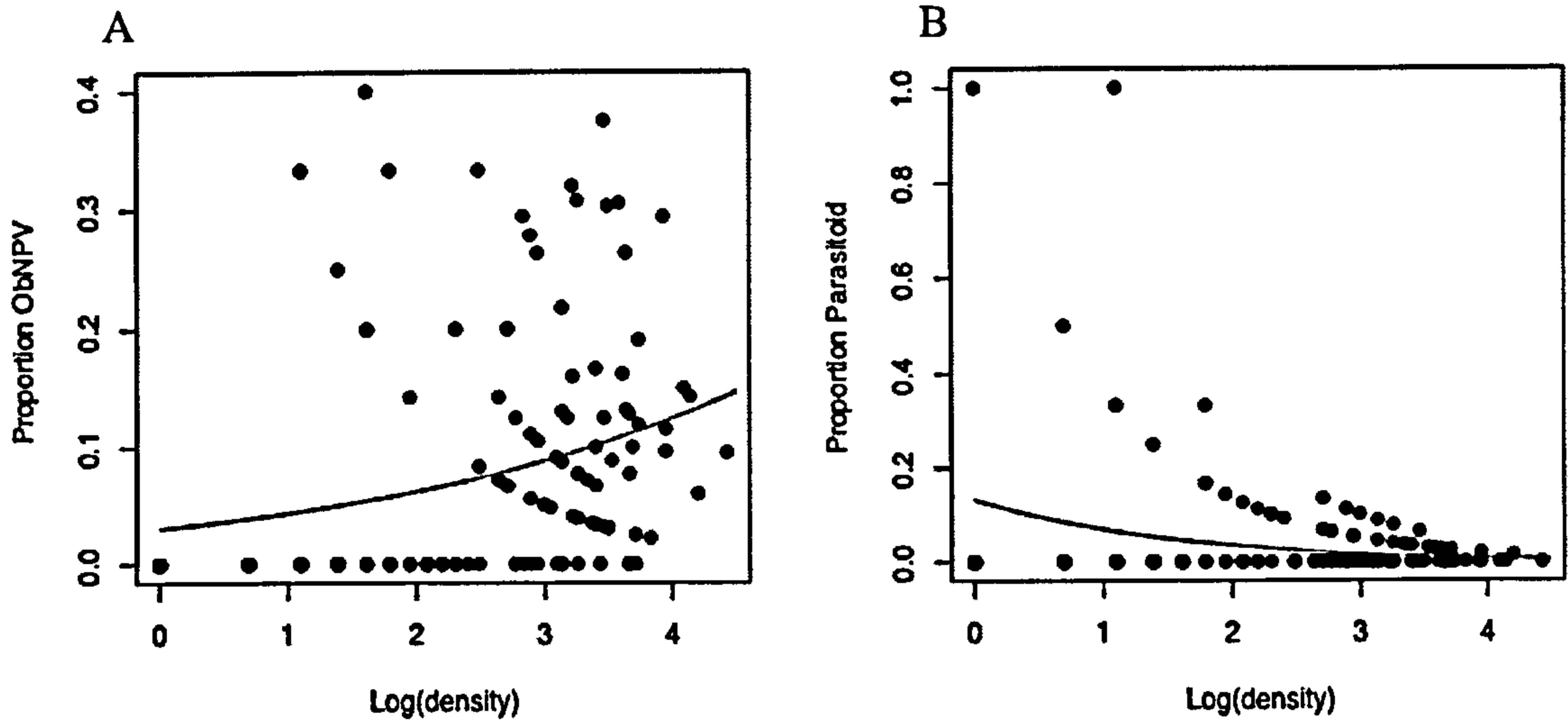
It appears there are two peaks in prevalence, with a trough in mid-season. A similar pattern is to be found in Hundland in 2004 where high prevalence was found at the start of the season (~20/5/04), decreasing mid season, before again increasing towards the end of the season (9/6/04). Indeed in Settiscarth in both years highest prevalences

were found towards the end of the seasons. Linnadale proved very low in OpbuNPV prevalence, and was found absent from larvae collected from the mid to end of the 2004 season. However, it did show the increase in the prevalence of parasitoid in the population as the season progressed. This is also clear in the sites of Hundland in 2004 and Wideford to a small extent in 2005 and at Settiscarth in both 2004 & 2005.

Over the three years (2003-2005), NPV prevalence varied widely across sites and between years. In a previous analysis of some of the data, the prevalence of OpbuNPV correlates with an increase in density, (Graham *et al.*, 2005). The statistical model used an arcsin transformation of the prevalence data against the log of the density, and a significant correlation was found using a simple linear regression ($F_{1,31} = 5.80$, $p < 0.05$ $R^2 = 0.13$). Here that updated data was reanalysed with a weighted regression that takes into account the numbers sampled. We have measured larval density, NPV prevalence and parasitoid prevalence data from each quadrat in each transect for each day sampled in 2005 for Wideford and Settiscarth. Within this fine scale data of natural enemies we see a positive significant relationship between proportion of larvae infected and the density of larvae ($F_{1,171} = 10.25$, $p = 0.0016$, Figure 5.11 (a)). Conversely we see a negative relationship in the density of the larvae and proportion infected by parasitoids ($\chi^2_{1,171} = 24.18$ $p < 0.001$, Figure 5.11 (b)). The model of NPV and host density dependence displayed a significant overdispersion in the data. Although there is a significant effect of larval density and proportion NPV infection, the model used does not show a good fit to the data, due to its dispersed nature. The logistic model used for the parasitoid is a better descriptor since it demonstrated lower variances as density increased, which means that quasibinomial errors were not required; not least because the variation occurred mainly at data points with low weighting effects. We also showed that over a large spatial scale, density of larvae has a significant positive correlation with NPV prevalence ($F_{1,31} = 4.82$, $p = 0.04$, Figure 5.11 (c)). Over the same spatial scale, the prevalence of *P. tempèstiva*, showed a significant negative relationship with density of the larvae after different yearly effects were incorporated into the model ($F_{1,30} = 7.69$, $p < 0.01$, Figure 5.11 (d)). A negative correlation was seen in both the year 2003

and in the grouped years 2004 and 2005, these two years were not significantly different when testing models, both when grouped and separate ($F_{30,31} = 0$, $p = 0.99$).

Density Dependence of Natural Enemies in Fine Scale Repeated Transects



Density Dependence of Natural Enemies on Large Scale Transects

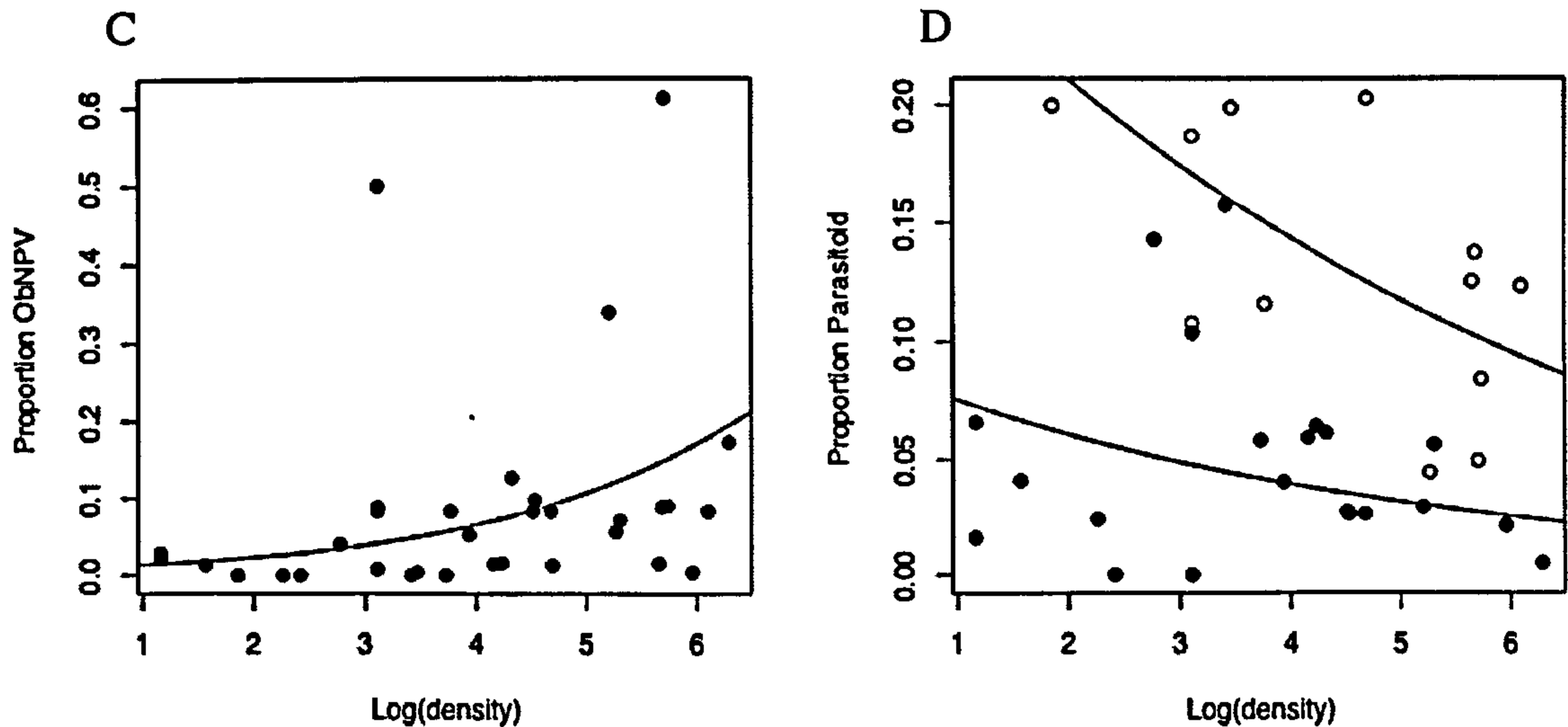


Figure 5.11. The relationship between log(larval density) and of A. NPV prevalence, $OpbuNPV = -3.8 + 0.38 \log(\text{density})$, $F_{1,171} = 10.25$, $p = 0.0016$, (quasibinomial errors) and B. parasitoid prevalence, with back-transformed fitted lines in each, logit regression using binomial errors, $Parasitoid = -1.8 - 0.73 \log(\text{density})$, $F_{1,171} = 24.18$, $p < 0.001$, C. NPV prevalence, $OpbuNPV = -4.8 + 0.54 \log(\text{density})$, $F_{1,31} = 4.82$, $p = 0.04$, (quasibinomial errors). D. parasitoid prevalence $F_{1,31} = 7.69$, $P < 0.01$, *P. tempestiva* prevalence in year 2003 (open-circles) was significantly greater than in 2004 & 2005 (closed circles), $F_{1,30} = 42.6$, $P < 0.001$ (quasibinomial errors). Years 2004 & 2005 showed no significant difference in parasitoid prevalence.

5.3.3 Bioassay

The bioassay provides LD_{50} curves for the three instars. Third and fourth instar larvae showed a significant relationship between $\log(\text{dose})$ and the proportion of the assay that died from NPV infection (Logit regression fits can be viewed in Figure 5.12). The curve for the second instar was not significant due to a probable anomalous result for the 50 OBs dose that showed a particularly low death rate. The LD_{50} for the instars are shown in Table 4.2. In all three treatments, none of the controls died from overt NPV infections. The background death rate for reasons other than NPV infections occurred highest in the second instars ($n=178$, 15.2%) then in the third instars ($n=192$, 9.37%) and finally the fourth instars ($n=192$, 2.6%). The high mortality in the earliest instars is understandable and the reduction in their numbers by these other means of mortality may have hampered the calculation for the LD_{50} .

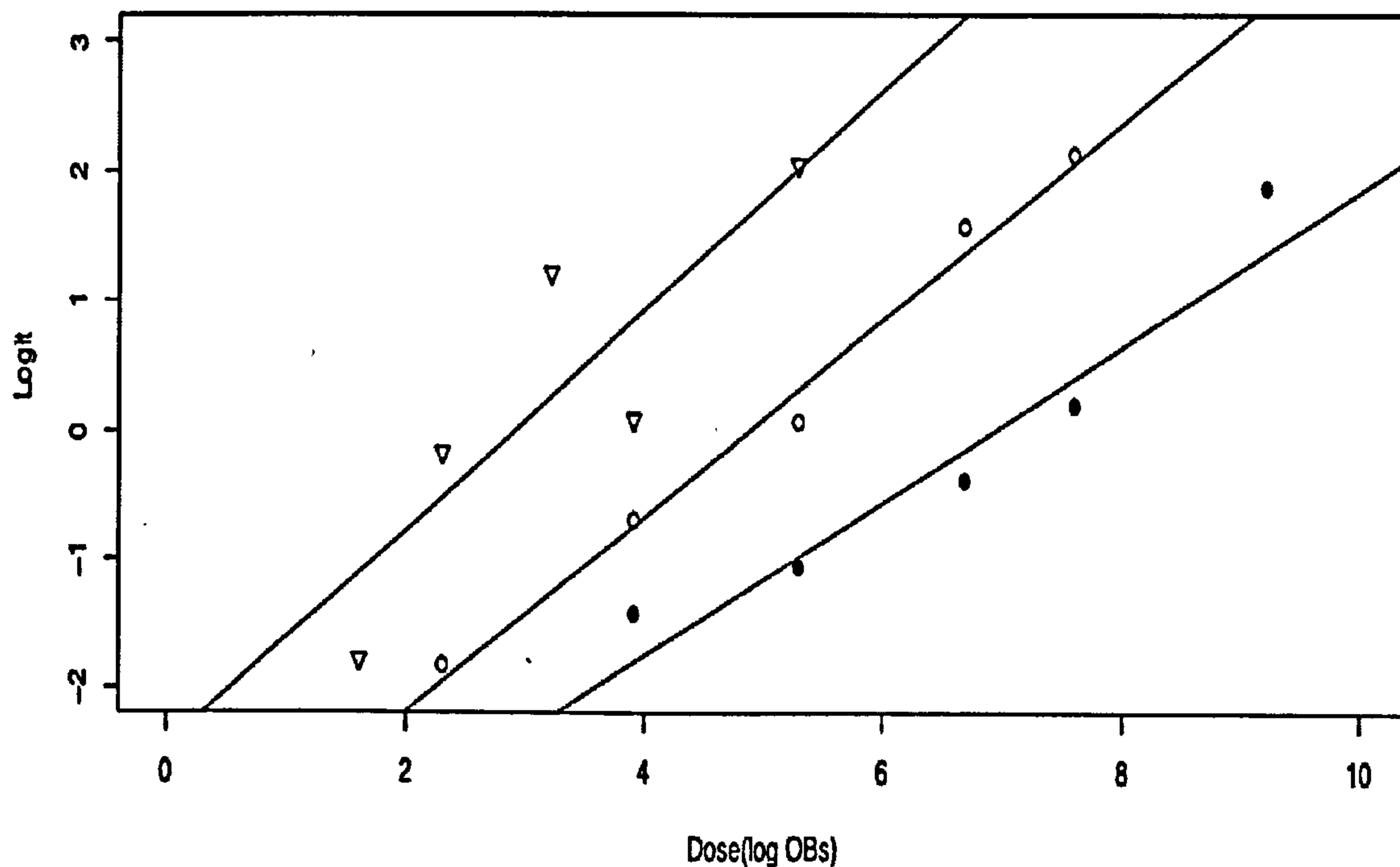


Figure 5.12. Log regression of dying *O. brumata* larvae against \log_e (virus dose) given in terms of \log_e odds ratio: $\log_e (p/q) = a + bx$. Second instars (open triangles) \log_e odds ratio = $0.84 \text{ Log}(\text{OBs}) - 2.46$, Third instars (open circles) \log_e odds ratio = $0.76 \text{ Log}(\text{OBs}) - 3.71$, Fourth instars (closed circles) \log_e odds ratio = $0.59 \text{ Log}(\text{OBs}) - 4.16$.

Table 4.2. Logit regression analysis of virus induced mortality in 2nd, 3rd and 4th instar larvae.

Instar	LD50(OBs/larvae)	Intercept \pm SE	<i>P</i>
2	18.45	-2.46 \pm 1.09	0.06
3	133.2	-3.71 \pm 0.67	<0.001
4	1030.76	-4.16 \pm 0.78	<0.001

The LD₅₀ for the second instar is estimated as 18.45 OBs, the third instar as 133.2 OBs and 1030.76 OBs for the fourth instar. That is 7.4 times from second to third instar, and an increase 7.7 times from third to fourth instar. The LD₅₀ dose for the fourth instars is therefore over 55 times than that of the second instar.

Time to death

The larvae were checked every twelve hours and larval deaths by OpbuNPV were recorded. The survival curve for each dose and instar was fitted with a Weibull distribution model (Goulson *et al.*, 1995; Murillo *et al.*, 2006), which allows a variation in the hazard function and provides a better description of the data than other distributions.

Table 4.3. Weibull model estimates of mean time of death (days) of infected larvae, τ , (excluding non-virus mortality) for second, third and fourth instars from each sampling dose.

Instar 2			Instar 3			Instar 4		
Dose	τ	SE	Dose	τ	SE	Dose	τ	SE
5	10.1	0.3	10	15.7	1.2	50	11.6	1.5
10	11.5	0.3	50	14.8	0.8	200	11.3	1.4
25	10.8	0.3	200	13.5	0.5	800	12.5	1.0
50	10.9	0.7	800	13.1	0.5	2000	11.3	0.9
200	11.7	0.4	2000	11.4	0.3	10000	12.0	0.7
Combined	11.1	0.2	Combined	13.0	0.3	Combined	11.8	0.4

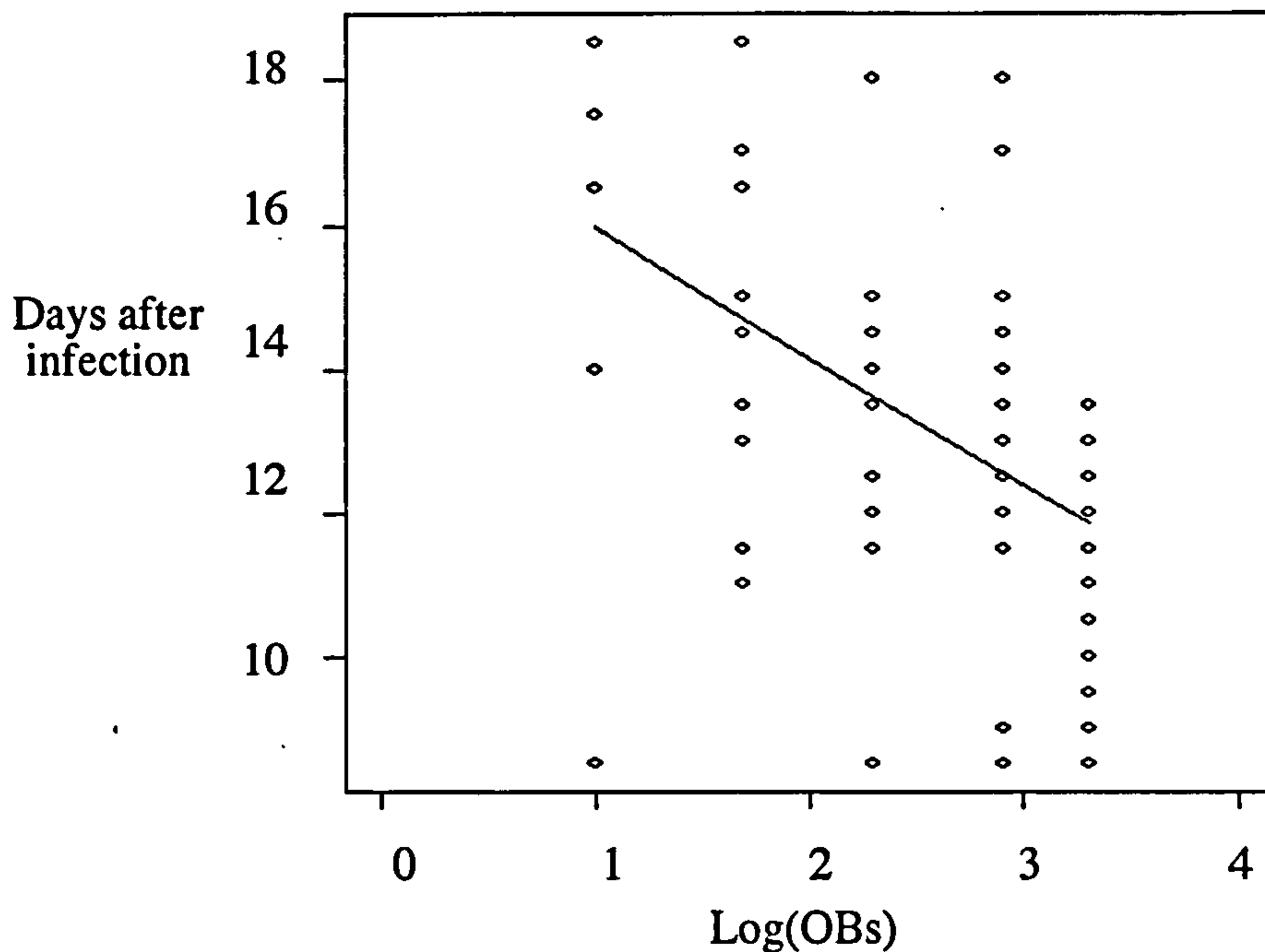


Figure 5.13. The relationship between the time to death after infection and dose of OpBuNPV occlusion bodies for third instars. $y = 17 - 1.72x$, where y equals days after infection and x refers to the log dose of NPV occlusion bodies. $F_{1,82} = 27.61$, $R^2 = 0.24$, $P < 0.05$.

Table 4.3 summarises the estimated time to death at different doses on different instar stages. Time to death was instar dependent. The third instars significantly took longer to die (13 ± 0.3 days (mean \pm SE)) than the other instars ($F_{1,222} = 16.3$, $p < 0.05$). There was no significant difference between the mean time to death for both the second and fourth instars, 11.1 ± 0.2 days and 11.8 ± 0.4 days, respectively ($F_{1,138} = 1.22$, $p = 0.27$). The time to death was not dose dependent in both the 2nd and 4th instars ($F_{1,62} = 1.93$, $p = 0.16$ and $F_{1,67} = 0.0749$, $p = 0.78$, respectively), but in the 3rd instars a significant negative relationship between dose and time to death was seen, Figure 5.13 ($F_{1,82} = 27.61$, $R^2 = 0.24$, $p < 0.05$).

5.4 Discussion

Our study of the developmental stages of the winter moth, *Operophtera brumata*, and the different mortality pressures from a species specific nucleopolyhedrovirus, OpbuNPV, and a more generalist parasitoid, *Phobocampe tempestiva*, showed considerable variation between sites, years and stages. The OpbuNPV virus was positively density dependent with the host, while the parasitoid *P. tempestiva* showed an inversely density dependent relationship with larval density. This was demonstrated using transects from sites across the whole Orkney Isles, and also on a finer scale using small quadrats from repeated transects in the sites of Wideford and Settiscarth in 2005.

Density dependence in larval viruses, such as the NPV that infects *Lymantria dispar* (Liebhold *et al.*, 2000; Woods *et al.*, 1991), the NPV that affects the pasture pest, *Wiseana cervinata* (Fleming *et al.*, 1986) and also the granulosis virus in *Plodia interpunctella* (Knell *et al.*, 1998), is well established. This is often most evident at high densities and weaker at low density populations (Liebhold *et al.*, 2000). Knell *et al.* (1998), showed that the transmission coefficient increased in a non-linear way with increasing density. However, an inverse relationship in density dependence has also been found in the gypsy moth (D'Amico *et al.*, 1996). Non-linearity in transmission rates has been modeled in the past and shown to affect stability of host-parasite interactions (Hochberg, 1991b). Most regulation of the host occurred when transmission efficiency increased with host density. There was a slight negative response in transmission efficiency with increasing parasite density. Instability occurred more frequently when transmission efficiency showed a decelerating response with both host and parasite density.

In this study, we also show non-linearity between *O. brumata* and prevalence of OpbuNPV, with the resultant model fits having exponents less than one. This indicates that the proportion infected increases more slowly as density increases. This deceleration in the increase of infection due to density can occur for a number of reasons, such as when increasing host density results in the obstruction of the

transmission of the disease (Hochberg, 1991). One explanation is that the larvae must consume more than a single viron to become infected and the infectious particle is relatively short lived; thus the infective particles become limiting. This could be the case in the winter moth system if the infectious particles are short lived on the heather, due to environmental factors such as an effect on inactivation of the NPV viruses by UV radiation (Shapiro & Domek, 2002). Simple patchiness in distribution of viral particles can also bring this about, as the viral occlusion bodies would be clustered most densely around the recently liquefied cadavers.

The saturating nature of the density dependence pathogen infection can also be explained due to the risk of exposure to the pathogen increasing with increasing host density, and hence larvae invest more in resistance. This concept is known as density-dependent prophylaxis (Wilson & Reeson, 1998). It is certainly a possibility in the winter moth system on Orkney. However, physiological resistance was not measured and therefore this must remain speculation. Work in sub-arctic populations of winter moth has shown distinct density dependent melanism (Hagen *et al.*, 2003), which is used as an indicator of investment in an immune response, having been found to positively correlate with phenoloxidase activity in *Spodoptera exempta* (Wilson *et al.*, 2001). Melanism has been found to have a positive relationship with parasitoid attack rate in *O. brumata*, where the moth is largely regulated by the parasitoid (Hagen *et al.*, 2006). The relationship between melanin and viral resistance has not been studied in the winter moth. The data set from which we draw our conclusions showed high dispersion and thus the significant relationship showed by the logistic regression also showed that there is much variance that is not explained by the increase in density. Density dependence may therefore vary in its effect on infection rates at different larval densities (Liebhold *et al.*, 2000).

The parasitoid, *Phobocampe tempestiva*, showed inverse density dependence to host larvae on both spatial scales. Density dependence has been found in parasitoids of Lepidoptera (Liljestrom & Bernstein, 1990) and indeed with the parasitoid *Cyzenis albicans* (Diptera: Tachinidae) in the winter moth in North America (Embree, 1966). However, there are also many examples of inverse density dependence (Hassell *et al.*,

1985; Kuhlmann, 1996; Sugiura & Osawa, 2002). This is explained by Walde and Murdoch (1988) as parasitoid egg limitation and handling time and also hosts being inaccessible to the parasitoid (Darrouzet-Nardi *et al.*, 2006). A review of the literature by Stiling (1987) showed from 171 examples in previous studies both inverse (23 %, $n = 39$) and directly density dependent (25%, $n = 43$) to be common, as well as density independent interactions (52%, $n = 89$) between hosts and parasitoids. The inverse density dependence seen in this system is not then unprecedented, even though optimal foraging behaviour would predict direct density dependency, as the parasitoids would be expected to preferentially target high-density sites to reduce search time. However, this is in a parasitoid without limiting attack rates through egg limitation and handling time of each oviposition. Also, when describing density dependence, it is important to determine what spatial scale the data is on (Heads & Lawton, 1983; Veldtman & McGeoch, 2004). In this study the scale ranges from, in one case, a single 10 metre transect covering the whole of the Orkney Isles and in the second, a scale within the same 10 metre transect of two sites, Wideford and Settiscarth. These both show inverse density dependence, and also over different years.

Inverse density dependence in parasitoids, displaying a Type II functional response (Holling, 1959a), can be destabilising if it allows the host to escape from parasite control and lead to possible outbreaks. The occurrence in the winter moth system of these inversely density dependent parasitoids could suggest a mode by which they are able to escape control from their parasitoids and reach large densities. Another possible cause of the pattern of density dependence is interference with NPV. When the larvae die of virus before the parasitoid is able to develop and pupate, then the parasitoid also dies. This interference has been shown in *Spodoptera frugiperda* larvae that, once infected with a lethal dose of a NPV, were unable to support the parasitoid, *Campoplex sonorensis*. However, there was a temporal effect with the parasitoid, *Chelonus insularis* that was able to develop normally as long as the viral infection occurred two days after the parasitoid oviposition (Escribano *et al.*, 2000). This interference maybe a factor in the inverse density dependence seen, but this is dependent on the parasitoid not being able to discriminate between infected and

uninfected larvae. A reduction in laying and discrimination against hosts infected with fungus (Fransen & van Lenteren, 2006) and granulosis virus (Hegazi & Abo Abd-Allah, 2004) have been seen in parasitoids. However, it is also true that some parasitoids show no such ability (Escribano *et al.*, 2000). The parasitoid in the winter moth system, *Phobocampe tempestiva*, is yet to be studied in detail regarding its interaction with the OpbuNPV. This is partly due to the difficulty of rearing both in the laboratory, with the *O. brumata* having a very long generation time for a laboratory study species, and the necessity for the *P. tempestiva* to find other hosts during the time the winter moth is not present. This generalist behaviour further unlinks parasitoid densities with that of the winter moth host. It is thought that, although the parasitoid, *P. tempestiva*, is a generalist, this occurs on a temporal continuum. Thus, while the winter moth is present, it acts very much as a specialist as in the heather there are very few other possible hosts to be found.

Infection from the NPV was not instar dependent in the natural population, although clearly there was an effect of instar on susceptibility to infection in the bioassay data. This showed LD₅₀ of OpbuNPV occlusion bodies of 19.84, 132.24 and 990.59 for 2nd, 3rd and 4th instars, respectively. However, *P. tempestiva* showed some instar preference with 4th and 5th instar larvae suffering significantly greater parasitoid prevalence. This would be expected by the method these data were collected, as the levels of parasitism in each instar are cumulative over time. There is, however, an unmistakable increase between the 3rd and the 4th instar. Many age-related susceptibility studies have been described (Boucias *et al.*, 1980; Dwyer, 1991; Evans, 1981, 1983; Goulson *et al.*, 1995). Goulson *et al.*, (1995) showed in field experiments since the leaf area consumed increased at a greater rate that despite the LD₅₀ increasing with instar, they predicted mortality to be greater in the 4th instar than in the 3rd and 2nd instars. They showed the LD₅₀ of the 2nd instar to be five times smaller than the LD₅₀ in the 4th instar of the *Mamestra brassicae*. However, in the *O. brumata* in this study here, the LD₅₀ of the 2nd instar appeared to be approximately twenty times smaller than that of the 4th instar, and hence the 4th instar would be required to consume a considerably greater amount of heather to suffer equal infection, assuming the density of occlusion bodies (OBs) remains the same. We have no quantitative

measures of instar dependent consumption or dispersal of the instars in the winter moth, although it can be observed that the later instars utilize more of the host plant, consuming a larger area in the wild. How this changes relative to instar is unknown, but from the results seen here it suggests that the increase in consumption may be comparable to the decrease in susceptibility to account for there being no difference in percentage infection through the instar stages. This may seem to contradict with the two infection peaks seen in the 2005 data; however, these were seen in temporal data and thus when fitted into instar data the peaks are lost. We also do not know how the density of OBs on the foliage varies over the season. The two peaks must presumably arise from an infectious pulse of those infected early in the season, releasing a high number of OBs into the environment. Thus, as they die, less are found to be infected and prevalence decreases, only to increase again as the newly released OBs start to infect new susceptible hosts. We therefore can predict that there is variation in occlusion body density over the season.

Virus yields are known to vary with instar size in viral infections of Lepidoptera (Biji, 2006; Hochberg, 1991a). Thus as size of larvae increases so too does the viral yield from an infected larva. How this alters the density of the OBs on the foliage as well as spatial spread and their accessibility to consumption by uninfected larvae is not known. Possibly, as has been suggested by Hails *et al.*, (2002), it is the number of cadavers that remain in the foliage rather than virus yield from each infected larva that has the greater influence on the transmission of the virus. This would require quite extensive specific field studies to gain accurate measures of transmission values of the virus in the winter moth, such as those that have been estimated in *Mamestra brassicae* (Goulson *et al.*, 1995), *Orgyia pseudotsugata* (Dwyer, 1991) and *Autographa californic* (Hails *et al.*, 2002).

We also showed that in the second year of the stage structured study; the developmental season of the larvae was behind that of the first year. It lagged just over a week at Wideford, two weeks and continually approximately two weeks behind at Settiscarth. Differences in development rate were seen between the sites. In the first year, Hundland showed the quickest development, followed by Linnadale,

with Settiscarth the third fastest and Wideford starting and finishing latest. The phenology of the winter moth with that of new shoot growth in *Calluna vulgaris*, and indeed other host plant species such as oak, *Quercus robur* (Visser & Holleman, 2001), has been found to be important in the stability of the larvae and plant interaction recognized through effects on *O. brumata* fitness. It is seen on certain host plants such as oak (Feeney, 1970), apple (Holliday, 1977) and a conifer, *Picea sitchensis*, (Watt & McFarlane, 1991) that survival rapidly declines after budburst as the foliage ages. Thus it is surprising that in *C. vulgaris*, which has recognizably a lower nitrogen concentration than other plants (Vanbergen *et al.*, 2003; Wint, 1979), synchrony between early shoot growth and egg hatching was important in neither survival nor development (Kerslake & Hartley, 1997). This suggests the larvae may be able to use compensatory feeding to balance the low nutritional food by simply eating more of it (Slansky & Feeney, 1977). Also, the effect of synchrony is less than that found on species of oak and spruce, as in heather the concentration of nitrogen although lower, does not degrade over time as in other species (Vanbergen *et al.*, 2003). This strategy of flexible feeding is quite beneficial to the *O. brumata* populations in the Scottish moorlands, due to the unpredictable climate and altitudes of different areas of habitat, as well as probable highly unpredictable wind dispersal of both early first instar larvae and adult males (Edland, 1971). The increased feeding rate on the *C. vulgaris* does however raise the possibility of increased exposure to orally infectious agents such as OpbuNPV.

The different development times could suggest different exposure to natural enemies and possibly greater parasitism by certain parasitoids, as predicted by the slow-growth-high-mortality hypothesis. Differing results have been seen regarding this hypothesis, with parasitism actually being greater in quicker developing larvae of *Orgyia leucostigma* (Medina *et al.*, 2005). Of course, in this study, the developmental differences were, only a matter of days, with Wideford being the slowest; therefore, whether or not that this would lead to heightened parasitism is not clear. The environmental conditions that elongate the development may also negatively affect parasitoid attack rate. What is certain is that low parasitism and high density were

seen in Wideford itself and therefore does not suggest that the slow-growth-high-mortality hypothesis applies here.

This field study of *O. brumata* on Orkney demonstrates there are many different factors that can affect the changing densities of the winter moth. More field studies in successive years to produce a long-term data set, such as those on the winter moth in other habitats (Embree, 1966; Kowolski, 1977; Varley & Gradwell, 1960; Varley *et al.*, 1973), are necessary to really expand our understanding of how these natural enemies are interacting. We have evidence for density dependence of the NPV and parasitoids with the host. However, other factors such as pupal mortality (East, 1974; Frank, 1967), climate conditions and winter disappearance may also have a large impact on populations. However, we have looked in detail at the largest factors in larval mortality and these can now be used to build a model that incorporates these details to see how our new knowledge of the system may lead to different population dynamics.

6. The winter moth and its natural enemies on Orkney: A Model



Winter moth, fifth instar, in silken nest in heather tip, Wideford, Orkney. 2005

6.1 Introduction

In this chapter we utilise the field data from Chapter 5 to build and parameterise a model of the winter moth system on Orkney in order to gain an insight into the role of multiple enemies in the ecological dynamics of the system. In addition our aim is to develop a model that can be used in the future to develop further work on the winter moth system. We focus on the larval stage of the winter moth, which undergoes infection by the viruses that we studied as well as attack from parasitoids.

Ecological models have mostly been two-species population type models (Bowers *et al.*, 1993; Hochberg, 1989; Lotka, 1925; Rohani *et al.*, 1994; Volterra, 1926) but here our aim is to examine the role of multiple enemies. Although multi-species models are less numerous, they include single parasitoid and multiple prey in apparent competition models (Bonsall & Hassell, 1999), specialist and generalist natural enemies affecting the same species (Hassell & May, 1986), competing parasites species or strains for the same host (Dobson, 1985; Hochberg & Holt, 1990), host-parasite-hyperparasite interactions (Beddington & Hammond, 1977) and host-parasitoid-pathogen interactions (Hochberg *et al.*, 1990). Within these there have been a number of models that deal with the interaction of a pathogen with either a parasitoid or a predator. For example, Anderson and May (1986) demonstrated that the invasion of a pathogen into a predator-prey interaction could depress host numbers to a level that no longer supports the predator and therefore leads to its exclusion. Multiple enemy interactions clearly therefore have the potential to influence the dynamics of natural systems.

Parasites are now well recognised as a major force in driving their host dynamics (Tompkins & Begon, 1999). One of the best of examples of this is the experimental manipulation of the red grouse, *Trichostrongylus tenuis* system that demonstrated that removal of the parasite lead to the loss of host cycles (Hudson *et al.*, 1998). Within this system however, theoretical work (Hudson *et al.*, 1992) has also suggested an important role of an interaction between parasitism and predation since infected individuals are preferentially taken. Another theoretical example of a parasite and

predator interaction showed that removal of predation can increase parasite control of hosts and can actually reduce host levels (Packer *et al.*, 2003) when the parasite is highly virulent and aggregating. This particular result may not hold in our system since the parasitoid, acts like a functional predator (Boots, 2004), not be able to superinfect the host very well due to the death via viral infection. That said, there is a clear need to understand in detail the role that interactions between different natural enemies may play in natural systems.

Hochberg *et al.* (1990) constructed a model that incorporated a host parasitoid and pathogen, where the parasitoid demonstrated variable attack behaviour from clumped to random. The pathogen was transmitted by external stages that spanned periods of low or even no host abundance as it moved out of a reservoir and the model included continuous, within-season dynamics as well as discrete between-season changes. In this way, the model represents a host with discrete generations with larval mortality caused by natural enemies. The continuous within season dynamics were solved in the model by integration over the pathogen-infectious time period, which gave proportions of the host to survive and changes in pathogen and parasitoid densities. The model showed how both natural enemies were able to exclude the other, sometimes dependent on initial starting densities, and also demonstrates how they can coexist with constant, cyclic or chaotic population dynamics. This scenario captures some of the features of our system but the model did not include stage structure with the larval stage of the host. Given that we have stage-structured data and that the interaction is inherently stage structured, we need to include these details in order to get at the dynamics of the actual system.

A number of models have included stage and age structure in insect hosts, often in the context of larval competition (Briggs *et al.*, 2000; Gurney & Nisbet, 1985; Gurney *et al.*, 1983; Wearing *et al.*, 2004) although the effect of pathogens (Bonsall, 2004; Briggs & Godfray, 1995) and single (Briggs *et al.*, 1999; Murdoch *et al.*, 1986; Wearing *et al.*, 2004) or multiple parasitoid assemblages (Bonsall *et al.*, 2002) have also been considered. The model proposed here is slightly different from most delay differential models since the species, *Operophtera brumata*, to be modelled is

univoltine, rather than multivoltine. We use a similar approach to Hochberg *et al* (1990) by modelling the within-season infectious period continuously and the between-season period discretely. However, in our model, unlike the one in Hochberg *et al.*, (1990), we use delay differential models in the within season dynamics to fully explain the stage structure. We consider a number of models that incorporate the different variables of the system to gain an insight into their possible effects. Firstly we look at the effect of the purely horizontally infecting virus, based on the NPV in the system. The role of the parasitoid in the system will also be studied. Winter moth undergoes parasitism from the parasitoid wasp, *Phobocampe tempestiva*, a generalist parasitoid that is thought to parasitise other Lepidoptera larvae. The model takes the form of delay-differential continuous equations within the larval season, with discrete jumps between seasons.

The field study of the developmental stages of the winter moth, *Operophtera brumata*, and the different mortality pressures from a species specific nucleopolyhedrovirus, OpbuNPV, and a more generalist parasitoid, *Phobocampe tempestiva*, showed considerable variation between sites, years and stages. The OpbuNPV virus was positively density dependent with the host, while the parasitoid *P. tempestiva* showed an inversely density dependent relationship with larval density. Using our data on instar dependent development times and susceptibility to viral infection, we use the model to gain an insight into the cause of the different dynamics and see if the interactions in the model can produce inherent oscillations or are the variations due to seasonality effects and climate.

6.2 Host Model

First we use knowledge from our field data to model host development both within and between seasons. The field data (i.e. viral bioassay and parasitoid prevalence) shows that in this system we have distinct stage susceptibility to both virus infection and parasitoid attack. Therefore, the host is modelled within a season by four age classes: neonates, early instar, late instar, and pupae, (E , L_1 , L_2 and P , respectively). It is the early instars and late instars that undergo the majority of infection from the natural enemies. Each season starts with a number of neonates $E_{(t+1)}$ that is dependent on the number of pupae at the end of the previous season $P_{(t)}$. Neonates hatch at the beginning of the season and mature into early larval stages at rate b , and suffer a density dependent removal rate $deE_{(t)}^2$, where $E_{(t)}$ represents the density of the host larval class with constant de that indicates the strength of competition for the heather resource. This is termed removal rate, as the density-dependent effect will cause both emigration as well as death due to resource competition. There would be high mortality here as the neonates utilise the ends of the heather to spin their nests and this is a finite resource. The density dependence acting here limits the host to a stable equilibrium. Hosts remain in early larval stage for a time Tl_1 where they undergo a death rate dl_1 . The rate at which early instars mature is $ML_1(t)$, the amount that mature is also dependent on the number that die over the time period Tl_1 and thus a survival function is required, equation (5),

$$\frac{dE}{dt} = -bE(t) - deE^2(t) \quad (1)$$

$$\frac{dL_1}{dt} = bE(t) - ML_1(t) - dl_1L_1(t) \quad (2)$$

$$\frac{dL_2}{dt} = ML_1(t) - ML_2(t) - dl_2L_2(t) \quad (3)$$

$$\frac{dP}{dt} = ML_2(t) \quad (4)$$

$S_{1(t)}$ = Probability that an individual born at time t survives at least to age Tl_1

Here the survival function is a constant, as survival is purely a linear function of host density. This is the same for both survival functions,

$$S_{1(t)} = \exp(-Tl_1 dl_1) \quad (5)$$

$$S_{2(t)} = \exp(-Tl_2 dl_2) \quad (6)$$

Thus $S_{1(t)}$ and $S_{2(t)}$ in this instance can be written more properly as constants $\sigma_{11} = \exp(-dl_1 Tl_1)$ & $\sigma_{12} = \exp(-dl_2 Tl_2)$ respectively, where dl_2 is the death rate during the late instar phase in which the host spends on average a time Tl_2 . The maturation rate from early instar to late instar is the rate of entry of the neonates into the early instar stage at time TL_1 days ago multiplied by the probability of surviving density-independent mortality during the late early stage

$$ML_1(t) = bE(t - TL_1)\sigma_{11} \quad (7)$$

and the late instar to pupae is similarly the maturation rate of individuals from the early larvae to the late instar Tl_2 days ago, multiplied by the probability of surviving density-independent mortality during the late instar stage

$$ML_2(t) = ML_1(t - Tl_2)\sigma_{12} \quad (8)$$

At the end of a season E , L_1 , L_2 all are equal to zero, with P containing the individuals that survived the developmental season. The change from season to season is modelled by a discrete stage, a stage which is analogous to the pupal stage, adult emergence and egg laying by the winter moth over the autumn and winter months. The number of neonates at the start of the next season is modelled as a function of those that become pupae in the previous season.

$$E_{\tau+1} = r\delta P_{\tau} \quad (9)$$

Where birth rate per individual is represented by r , the number of pupae surviving to reproduce is δ . I can find an expression for the steady state of the host without any natural enemies. This is done by assuming that the number of eggs produce in year τ is equal to those produced in year $\tau+1$, i.e. $E_{(\tau+1)} = E_{(\tau)}$, it is then assumed that the

survivorship of the pupae and subsequent reproduction between the seasons is matched by the loss of individuals in the larvae within seasons. I therefore can express the rate of change of pupae in terms of the neonates surviving the larval stages

$$\frac{dP}{dt} = bE_{(t-TL_1-TL_2)} e^{-(d_1TL_1+d_2TL_2)} \quad (10)$$

To solve this we require the solution for $E_{(t)}$. This is found by integration by separation of the variables and partial fractions of equation (1), for the derivation see the appendix. The equilibrium value is therefore

$$E_o = \frac{r\delta b \exp^{-(d_1TL_1+d_2TL_2)}}{de} \left[\ln \left(\frac{b + deE_o}{b} \right) \right], \quad (11)$$

However functions where $x = c \ln(x)$ are notoriously hard to solve analytically and in this instance, an accurate solution can best be found by employing the Newton-Raphson method of iteration.

$$x_{n+1} = x_n - \frac{f(x_n)}{f'(x_n)} \quad f(E_o) = \frac{be^{-(d_1TL_1+d_2TL_2)}}{de} \left[\ln \left(\frac{b + deE_o}{b} \right) \right] - \frac{E_o}{r\delta} \quad (12)$$

Further additions of variables such as a free living viral stage and parasitoid lead the equilibrium expression becoming too complex to express explicitly and thus all further equilibriums are estimation through simulation using the delay differential solver dde23, in Matlab.

6.3 Host and NPV model

The model system is represented schematically in Figure 6.1. The OpbuNPV has external infectious occlusion bodies; these are modelled as a transmissible free-living infectious stage, W , which are assumed to be distributed randomly in the environment. The infectious particles are lost from this environment at a constant rate μ , and by being passively taken up by the feeding larvae L_1 and L_2 at a rate of ω_1 and ω_2 respectively. They infect the two different larval stages, L_1 and L_2 , at a rate β_1 and β_2 respectively. From the data in the field studies it is noticed that the relationship is positive between infection and host density however, this is a decelerating saturating relationship.

There are many empirical studies highlighting a deviation away from the mass action assumption of transmission (D'Amico *et al.*, 1996; Dwyer, 1991; Knell *et al.*, 1998; Knell *et al.*, 1996), some in particular identify that an increase in infectious agents is associated with a decrease in transmission efficiency in bacteria (Knell *et al.*, 1996) a virus (D'Amico *et al.*, 1996; Knell *et al.*, 1998). As suggested by Hochberg (1991) the departure from the mass action assumption can be modelled by altering the function of βIS to $\beta(S^p I^q)IS$, where p and q are the responses of the susceptible, S , and infected, I , respectively and both are restricted by $-1 < p \text{ or } q < 0$.

This is somewhat analogous to the Type II functional responses with transmission efficiency decreasing with increasing host densities if $p < 0$. Here the mass action function is altered from $\beta L_{1,2}W$ to $\beta L_{1,2}W^n$ where $0 < n < 1$. Thus as infectious particles increase, the transmission efficiency decreases, which could be possible with a higher consumption of infectious particles than required for infection due to greater density on the leaf surfaces, thus lowering the average transmission efficiency of each infectious particle.

The infected larvae, Y_1 and Y_2 , produce infectious particles at a rate λ_1 and λ_2 respectively. It is assumed that once a host is infected, it remains in the infectious stage either Y_1 or Y_2 for a time period of Ty_1 or Ty_2 respectively.

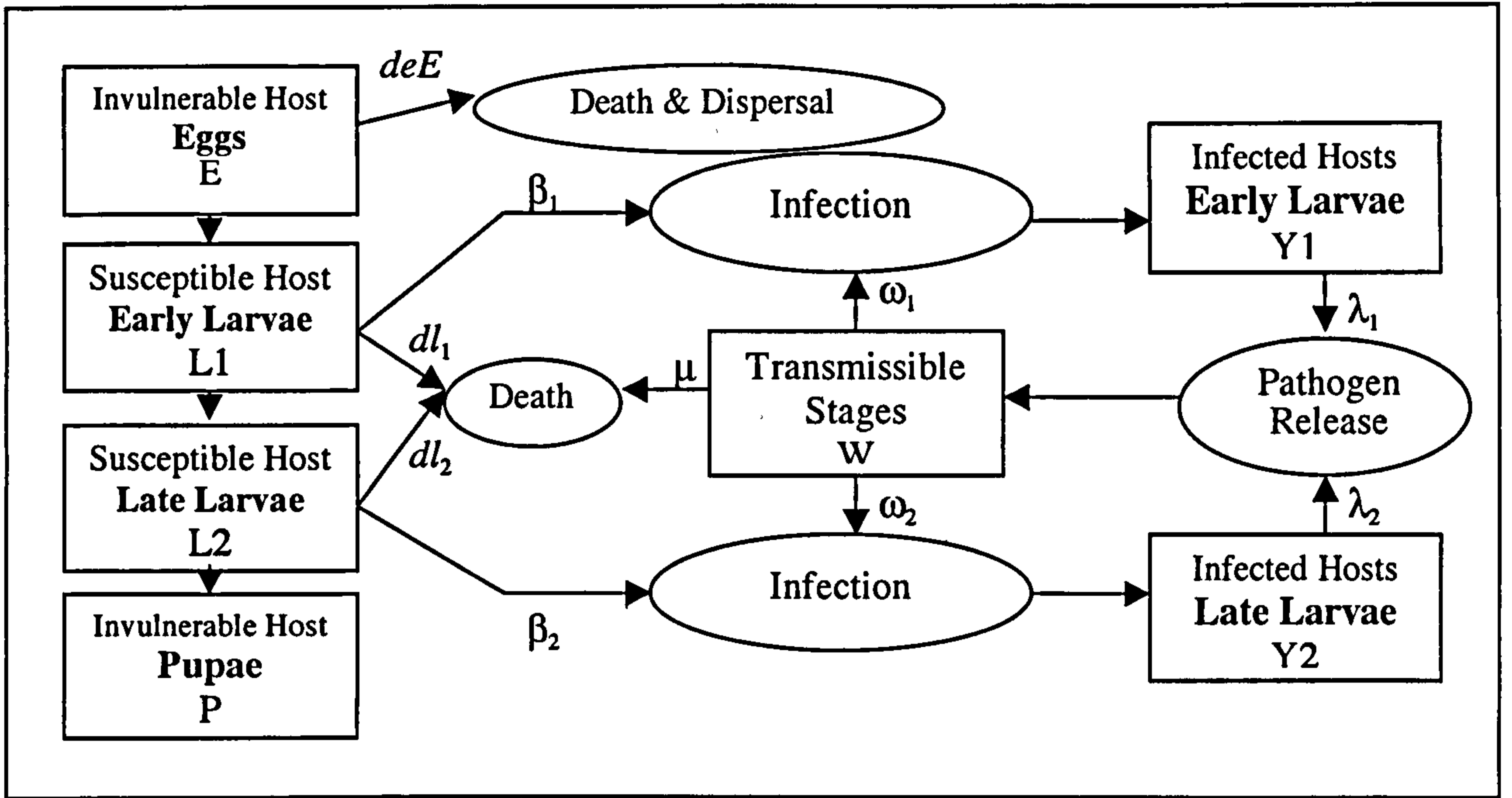


Figure 6.1. Diagrammatic flow chart of the host-pathogen model for the successive stages of the host, E , L_1 , L_2 , & P , infected stages Y_1 & Y_2 , transmissible stages of the pathogen, W . *Ovals*: Processes that occur during the infectious season.

The infected larvae Y_1 & Y_2 mature at a rate MY_1 & MY_2 , which is the number of surviving early instar larvae, L_1 & L_2 , that were infected TY_1 & TY_2 days ago, respectively. The probability of Y_1 & Y_2 surviving to the end of their respective stage is $\sigma_{Y_1} = \exp(-dl_1TY_1)$ & $\sigma_{Y_2} = \exp(-dl_2TY_2)$

The full dynamics of the system are described below

$$\frac{dE}{dt} = -bE(t) - deE^2(t) \quad (13)$$

$$\frac{dL_1}{dt} = bE(t) - ML_1(t) - dl_1L_1(t) - \beta_1W^n(t)L_1(t) \quad (14)$$

$$\frac{dL_2}{dt} = ML_1(t) - ML_2(t) - dl_2L_2(t) - \beta_2W^n(t)L_2(t) \quad (15)$$

$$\frac{dP}{dt} = ML_2(t) \quad (16)$$

$$\frac{dY_1}{dt} = \beta_1W^n(t)L_1(t) - MY_1(t - TY_1) - dl_1Y_1(t) \quad (17)$$

$$\frac{dY_2}{dt} = \beta_2W^n(t)L_2(t) - MY_2(t - TY_2) - dl_2Y_2(t) \quad (18)$$

$$\frac{dW}{dt} = \lambda_1MY_1(t - TY_1) + \lambda_2MY_2(t - TY_2) - (\omega_1\beta_1L_1(t) + \omega_2\beta_2L_2(t) + \mu)W^n(t) \quad (19)$$

The maturation rates are seen below.

$$ML_1(t) = bE(t - Tl_1) \exp\left(-\int_{t-Tl_1}^t \beta_1 W^n(x) + dl_1 dx\right) \quad (20)$$

$$ML_2(t) = ML_1(t - Tl_2) \exp\left(-\int_{t-Tl_2}^t \beta_2 W^n(x) + dl_2 dx\right) \quad (21)$$

$$MY_1(t) = Y_1(t - TY_1) \sigma_{Y_1} \quad (22)$$

$$MY_2(t) = Y_2(t - TY_2) \sigma_{Y_2} \quad (23)$$

The infection dependent survival functions of the hosts maturation in equations (24) and (25) can be described more conveniently in differential equation form,

$$\frac{dS_{Y_1}}{dt} = S_{Y_1}(t) \beta_1 [W^n(t - Tl_1) - W^n(t)] \quad (24)$$

$$\frac{dS_{Y_2}}{dt} = S_{Y_2}(t) \beta_2 [W^n(t - Tl_2) - W^n(t)] \quad (25)$$

which simplifies equations (24) and (25) when combined with the density independent probability of survival to

$$ML_1(t) = bE(t - Tl_1) S_{Y_1}(t) \sigma_{L_1} \quad (26)$$

$$ML_2(t) = bE(t - Tl_2) S_{Y_2}(t) \sigma_{L_2} \quad (27)$$

Between seasons, the NPV remains in the environment as occlusion bodies that somewhat protect the infectious viral particles. The bodies are often washed into the soil and off the foliage, aiding their persistence further by removing them from a surface that has greater exposure to the damaging U/V rays (Raymond *et al.*, 2005). During the between-season period, it is assumed that there is a reduction in both the number of surviving infectious particles and also in the numbers that are accessible to the host due to their redistribution by abiotic factors, the proportion surviving to be available to the host in the following season is, h .

$$W_{(\tau+1)} = hW_{(\tau)} \quad (28)$$

It is assumed that all infected larvae are converted into the transmissible stages at the end of the season. At equilibrium with the host, the pathogen must infect enough larvae during the infectious season so that it is balanced with those that are lost between the infectious seasons.

6.3.1 Host-pathogen dynamics

Model and field predictions

The model constructed is compared with the field data to see if it can describe the patterns found there. Time-series data that were collected over five weeks were used to see how the prevalence of the virus in the host population changed over time. We use the data from 2004 and 2005. The shape of the prevalence curves is quite distinctively different between the years 2004 and 2005; the model is able to recreate the qualitative shapes of both of these curves (Figure 6.2 A and B). By altering transmission levels and inter-season viral survival we can find stable equilibria that describe qualitatively those curves. Hundland in 2004 had high prevalence early on in the season but relatively low towards the end of the season. The model produces similar patterns if the pathogen has low transmission but high inter-seasonal survival. Conversely if it has higher transmission and a lower inter-seasonal survival then prevalences with two distinct peaks result as seen in Settiscarth and Wideford in 2005.

It could be argued that pathogen transmission rates at each site or in each year would be expected to be similar. However, the inter-season survival of the virus may vary considerably from year to year, dependent on climatic factors. Figure 6.3 A shows how the inter-season survival has little effect on the stability of the system but Figure 6.3 B shows the inter-season survival does have quite a major effect on the shape of the prevalence curve. My model therefore suggests that inter-season survivorship of

the virus may vary from year to year and that this may explain the pattern of within year prevalences. However this variation in virus survival is unlikely to have a major effect on the host parasite population dynamics year to year. The qualitative fits in Figure 6.2 are made at stable interactions and thus the prevalence shapes would be expected to remain the same year after year if the parameters remained the same. Different shaped within season prevalence curves from year to year may therefore indicate external changes in climatic and seasonal effects. However, from our data it is clear that the prevalence of the NPV along with host density has changed at many sites. This instability suggests that there is variation in the winter moth system, either induced intrinsically by host interaction with natural enemies or possibly driven by climatic factors. If the pathogen does intrinsically induce oscillations in the system it is interesting to see what the effect on the within season patterns in prevalence is.

Therefore the prevalence curves were mapped over an induced population cycle to understand if the prevalence curves alter from year to year in an oscillating system. Figure 6.4 A describes the densities of host and pathogen found over a one cycle of host and pathogen and the corresponding within season prevalences (Figure 6.4 B). We see that qualitatively similar changes in the shapes of curve are found over this five-year period in the model to those that are found from the data in Figure 6.2. Figure 6.4 B shows both high prevalence at the start and low prevalence at the end of a season (Year 4), and also two even peaks in prevalence for a number of years (Year 2, 3, 5). In year 4 we see the prevalence starting high and dropping throughout the season, as in the previous year there is high pathogen production leading to large number of pathogen particles at the start of the next year and only low numbers of host. This combination brings about a similar prevalence curve to that seen in Figure 6.2 A. This is a possible explanation for what occurred 2004, as we saw a density of winter moth in 2003 of over 300m^{-2} and high pathogen prevalence (61%), thus this would correspond to year 3 in Figure 6.4 A. Therefore this model would suggest that the shapes of prevalence that are seen in the field data could be brought about by the simple host-pathogen interaction when this interaction produces oscillations.

Therefore, although the changes in the prevalences within a season may be due to changes in the over winter survival of the NPV, they may also be due to intrinsic host parasite oscillatory dynamics in the system.

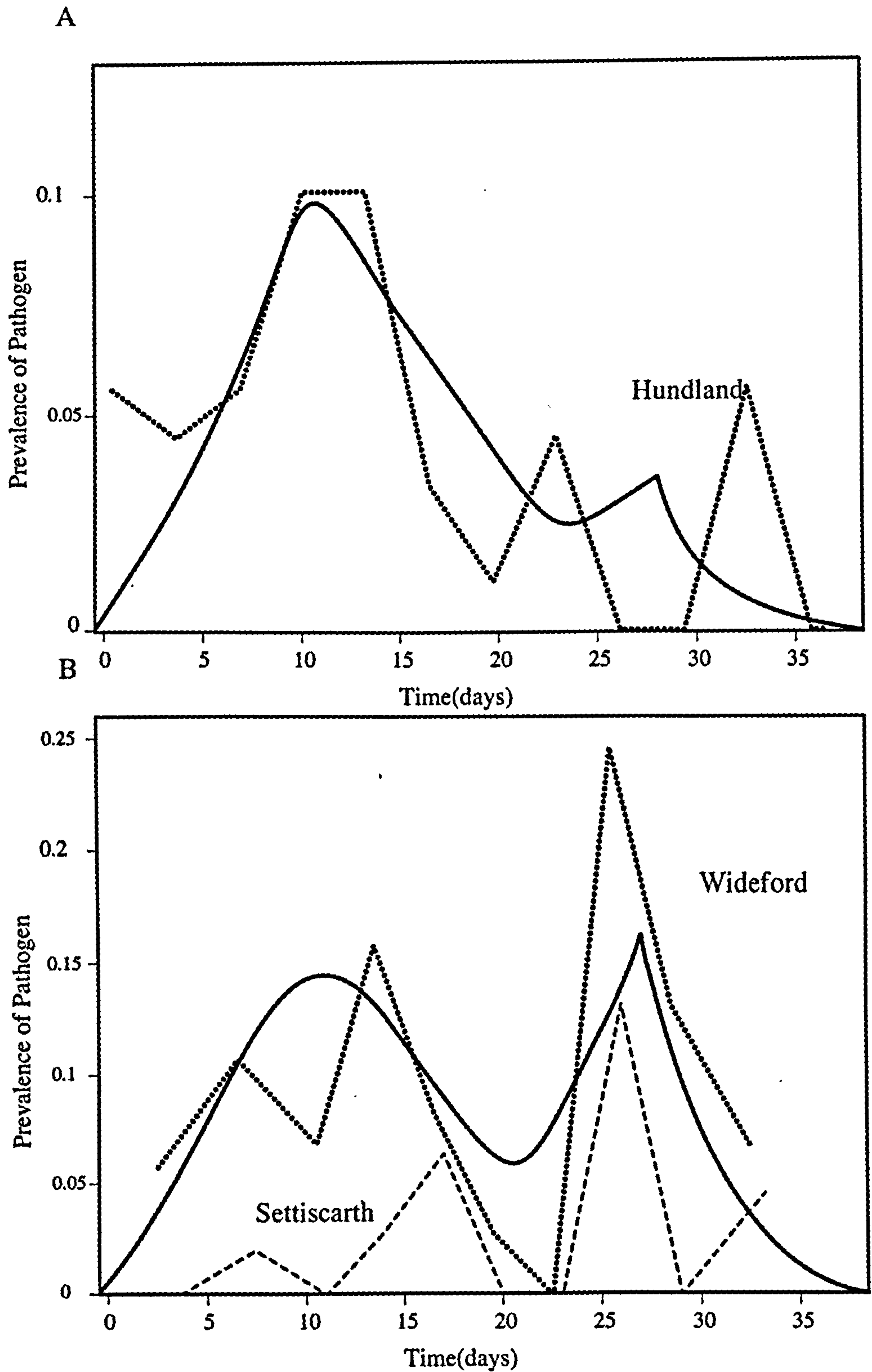
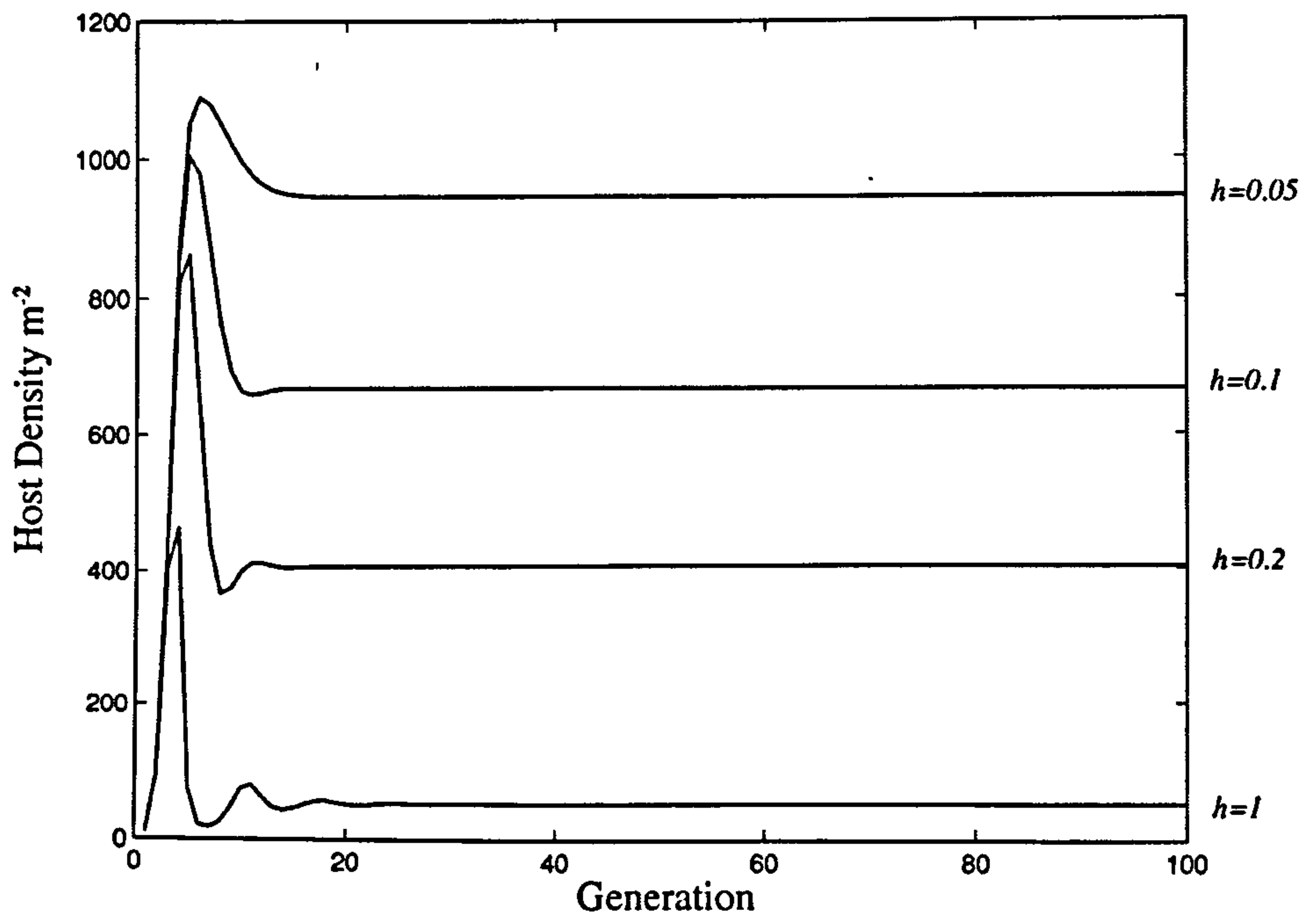


Figure 6.2. Proportion of winter moth infected with the baculovirus, ObNPV, in 2004 (A) and 2005 (B). Actual data from sites are labeled on each figure (solid-lines) and model predictions (dotted lines). A: $\beta=4.5 \times 10^{-8}$; $h=0.1$ B: $\beta=1.5 \times 10^{-7}$; $h=0.01$; $\beta_1=\beta/5$; $n=0.85$; $r=150$; $\delta=0.15$; $b=0.5$; $de=dl_1=dl_2=1 \times 10^{-3}$; Unless otherwise stated

A



B

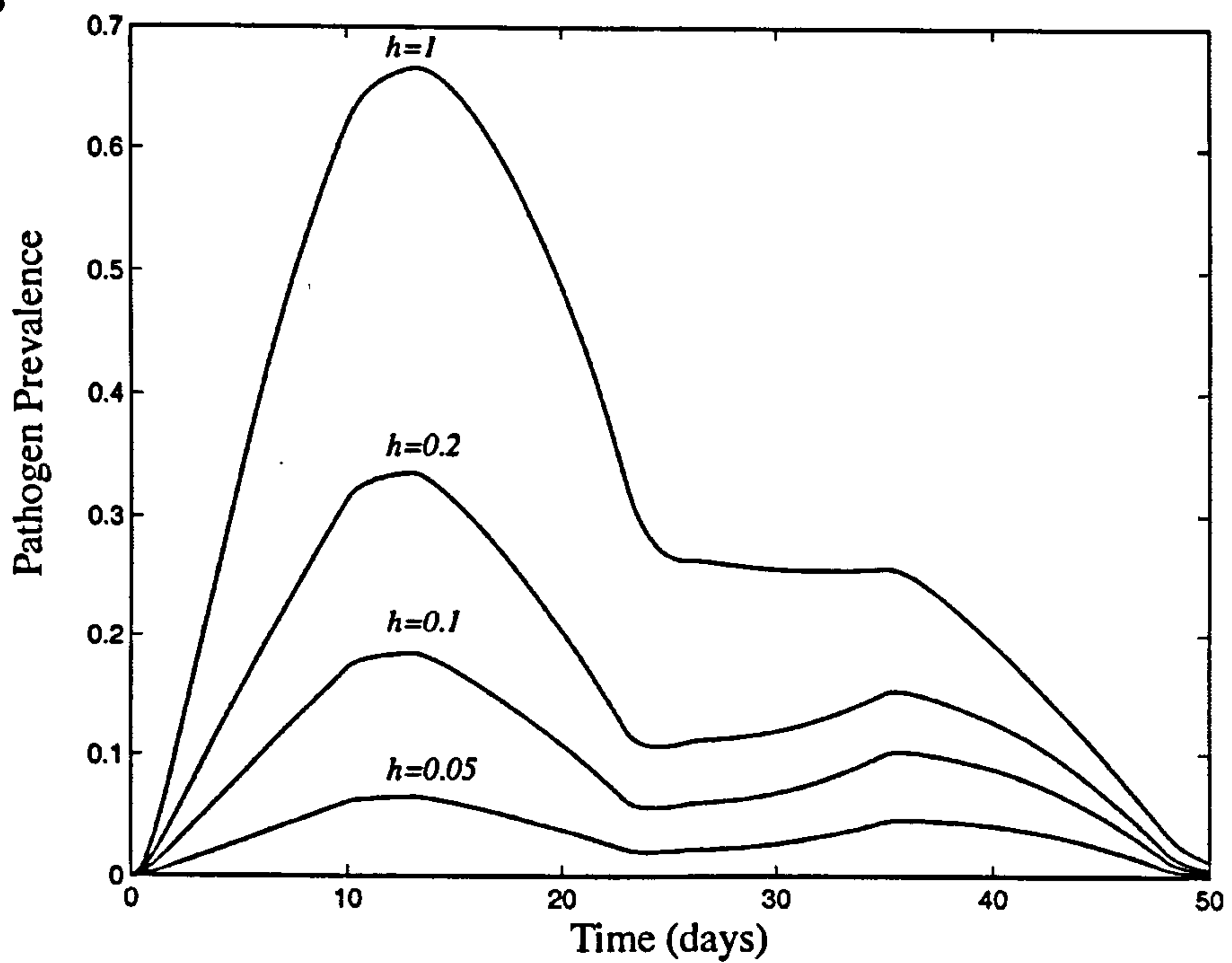


Figure 6.3. A) Effect on dynamics by the inter-season survival of the pathogen. B) The change in prevalence of infection within a season, showing the trace of the infection over time is dependent on the proportion of infectious particles that are available from the previous season at the start of the next season. . $\beta=4 \times 10^{-8}$; $\beta_1=\beta/5$; $n=0.85$; $h=0.01$; $r=150$; $\delta=0.15$; $b=0.5$; $d_e=d_{11}=d_{12}=1 \times 10^{-3}$;

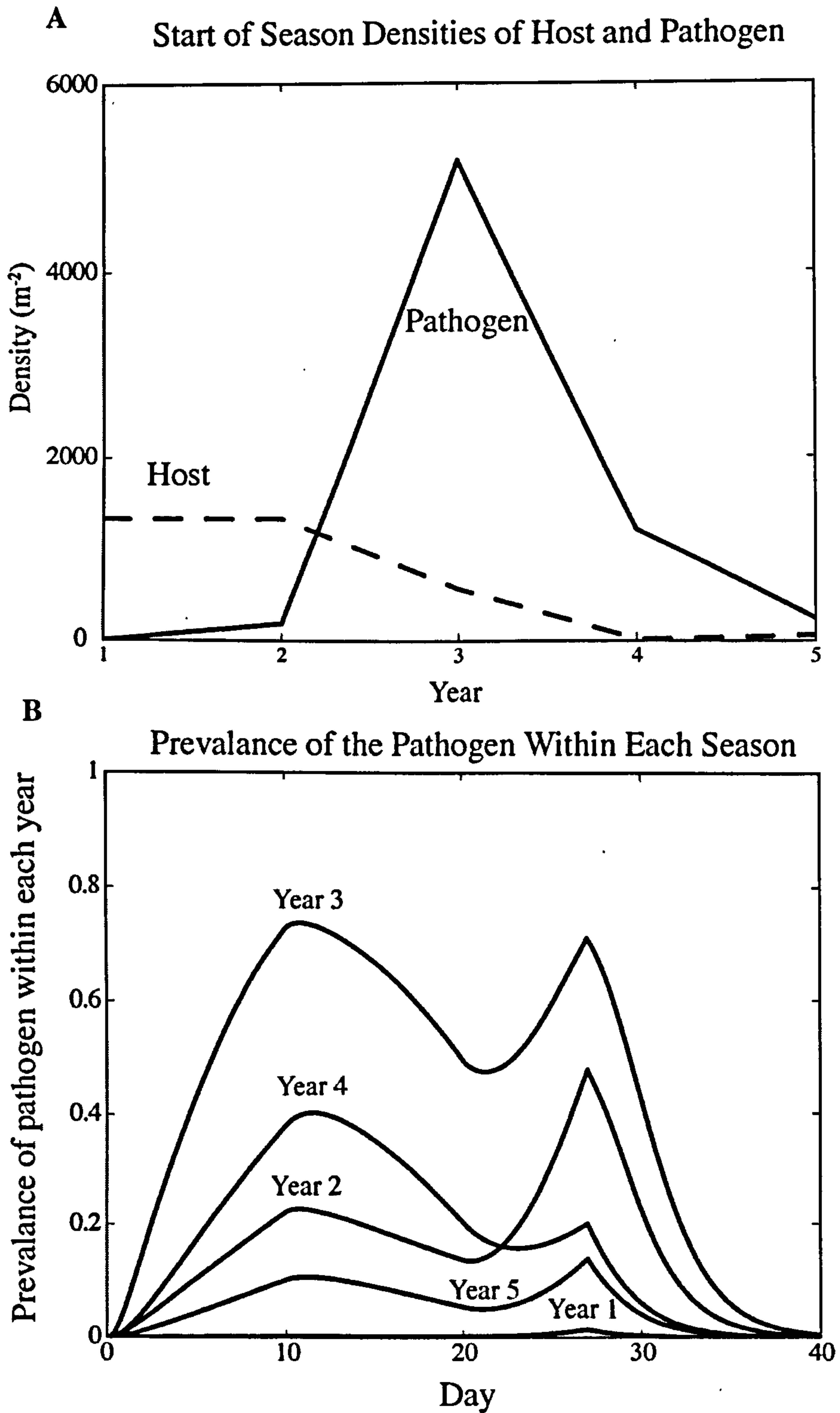


Figure 6.4. A) Densities of host and pathogen found over a one host and pathogen cycle over five generations (Pathogen density is scaled at 0.001). B) Within-season prevalence of the pathogen, in an oscillating interaction. $\beta=1 \times 10^{-7}$ $h=0.01$; $n=0.95$; $r=150$; $\delta=0.15$; $b=0.5$; $de=dl_1=dl_2=1 \times 10^{-3}$;

The effect of transmission rates and non-linearity on population dynamics

As we can see that the shape of prevalence curves can be generated in an oscillating host-pathogen system. The factors that may cause instability in the host-pathogen interaction are now investigated. Firstly, the dynamics are sensitive to the shape of the transmission function. As from the field data it is clear that the pathogen becomes less efficient at high densities of free-living particles, the host numbers increase and the dynamics become more stable. When pathogen transmission efficiency is increasingly density-independent ($n \rightarrow 1$), the interaction between the host and pathogen becomes less stable. When transmission efficiency is completely unaffected by the change in host and pathogen density populations, cycles can result. The cycles have varying amplitude and periodicity (Figure 6.5 A). The stability of the dynamics decreases at higher transmission rates (Figure 6.5 B). The increase in transmission rate suppresses the host density the greater it is. The inter-peak period increases with the increases in the pathogen transmission. To see this, simulations were run long enough to negate any effect of transient dynamics and then fast Fourier transforms were used to find the period of the oscillation. We see from Figure 6.5 B that an increase in the pathogen transmission efficiency leads firstly to host-pathogen oscillations and secondly to an increase in the inter-peak period.

The effect of pathogen decay rates on population dynamics

The decay rate of the particles between years was shown to have little effect on the stability with stable equilibria being found. However, at high survival rates of the free-living particles, the equilibrium was reached more slowly via damped oscillations, hinting at slower recovery of the host (Figure 6.5 C). An interesting result of high survival was the increased level of infection at the start of the larval season meaning a higher proportion of the early instars are infected due to the high numbers of infectious particles still present (Figure 6.5 D).

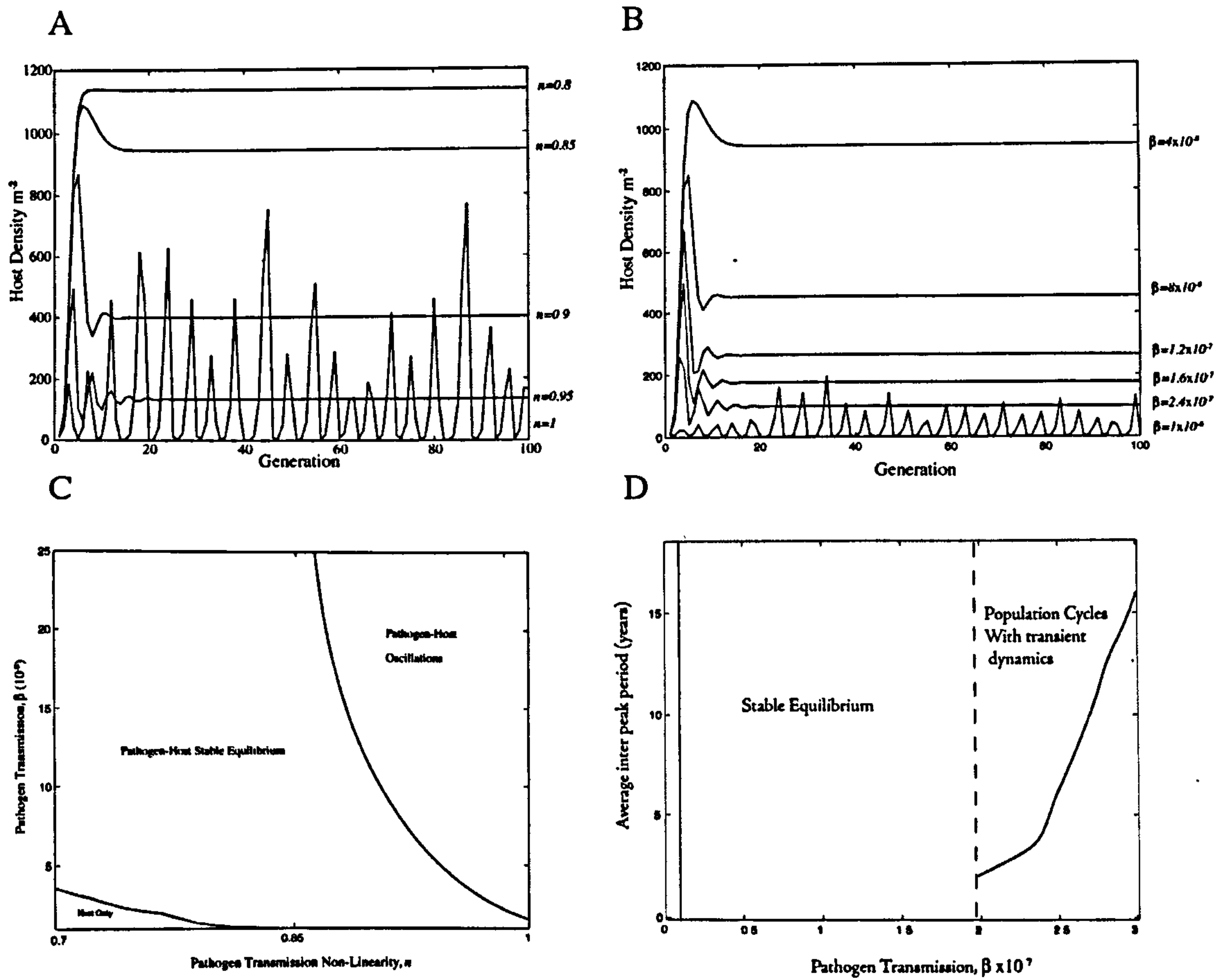


Figure 6.5. Population dynamics of the host density in a system containing a pathogen. These plots display changes, final dynamics, and host population numbers dependent on different parameters in the pathogen (A-C). The change in infection through a season is described in D, showing the trace of the infection over time dependent on the number of infectious particles that are available from the previous season at the start of the next season. $\beta=4 \times 10^8$; $\beta_1=\beta/5$; $n=0.85$; $h=0.01$; $r=150$; $\delta=0.15$; $b=0.5$; $d_e=d_{II}=d_{I2}=1 \times 10^3$;

6.4 Parasitoid and Host Model

The model was further extended by the addition of the parasitoid into the system. Since parasitism occurs throughout all larval instars, it is assumed that the parasitoids are present over the whole developmental period. Parasitoid emergence occurs in the fifth instar and the hatching of the adult parasitoid adults occurs after pupation of the winter moth larvae, it is assumed that numbers of parasitoids remain constant through the host larval period. It is then assumed that the numbers parasitised by the parasitoids by the end of the season directly influence the number of parasitoids at the start of the next winter moth larval season. However, in between the seasons, *Phobocampe tempestiva* is presumed to parasitize other Lepidopteron larvae present on the heather. Thus numbers, although dependent on the winter moth, are also dependent on other hosts at different times of the year. I then assume that the number of parasitoids at the start of the year is some function of those that remain in the host to pupation.

$$Y_{P(\tau+1)} = f(Y_{P(\tau)}) \quad (29a)$$

$$Y_{P(\tau+1)} = \Omega Y_{P(\tau)} \quad (29b)$$

The numbers of parasitoids at the end of one season are scaled by a growth term, $\Omega > 0$, that determines how many parasitoids start the next season, if Ω is greater than 1 then there is an increase in parasitoid numbers brought about by parasitism of other Lepidoptera, and if below 0 then the parasitoid must be competing less well for the rest of the year. The within season dynamics are of course altered by the introduction of the parasitoid into the system. From the data collected in the field we saw a clear increase in the prevalence of parasitoids in the last two instars of development and therefore it is assumed that there is a stage-dependent attack rate by the parasitoid and that it attacks the earlier instars at a lower rate than the later instars, A_1 and A_2 respectively. From the field data we saw a negative density dependent effect on parasitism by the parasitoid on host density, therefore it is assumed that the attack rate follows a Hollings type II response (Holling, 1959b) when faced with altering

prey densities. Thus the parasitoid can be saturated with prey due to the handling time of processing each prey item. Therefore at very high host densities, the parasitoid is limited to a maximum attack rate, and the half prey saturation density is represented by the parameter Lh . This parameter shapes the functional curve and its value denotes the density at which the parasitoid is at half its maximum attack rate. I also assume that the hosts that are successfully attacked by a parasitoid are still vulnerable to infection by the pathogen. The dynamical equations are shown below, with the parasitoid presumed to die at an equal rate to uninfected hosts, during the season. Thus the dynamics of the parasitoid are

$$\frac{dJ_1}{dt} = \frac{AJ_a L(t)}{L(t) + Lh} - MJ_1(t) - dl_1 J_1(t) - \beta_1 W^n(t) J_1(t), \quad (30)$$

$$\frac{dJ_2}{dt} = \frac{AJ_a L(t)}{L(t) + Lh} - MJ_2(t) - dl_2 J_2(t) - \beta_2 W^n(t) J_2(t), \quad (31)$$

$$L(t) = L_1(t) + L_2(t). \quad (32)$$

The functional response was dependent on the density of hosts as a whole, and thus when parasitizing the early host, the parasitoid attack rate is still affected by the density of late larvae. The pathogen-infected larvae are not included in the response, due to possible behavioural avoidance responses by the parasitoid.

The maturation rate of early parasitoid-infested instars to late instars can be written as the proportion of uninfected hosts that have survived pathogen infection and natural death at time Tl_1 , multiplied by the one minus the survival probability of infected by a parasitoid (S_{J_1}). Hence

$$MJ_1(t) = bE(t - Tl_1)S_{Y_1}(t)\sigma_{L_1}(1 - S_{J_1}(t)), \quad (33)$$

The maturation from late parasitoid-infested instars to infected pupae is equal to those infected that initially matured from the early to late instar that also survive pathogen infection and natural death rates till time Tl_2 , plus the hosts that initially remained

uninfected but subsequently were attacked by the parasitoid in the late instars.

$$MJ_2(t) = MJ_1(t - Tl_2)S_{Y_2}(t)\sigma_{L_2} + ML_1(t - Tl_2)S_{Y_2}(t)\sigma_{L_2}(1 - S_{J_2}(t)) \quad (34)$$

The survival functions of each stage are described below as dynamical equations.

$$\frac{dS_{J_1}}{dt} = S_{J_1}(t)A_1J_a \left[\frac{1}{L(t - Tl_2) + Lh} - \frac{1}{L(t) + Lh} \right] \quad (35)$$

$$\frac{dS_{J_2}}{dt} = S_{J_2}(t)A_2J_a \left[\frac{1}{L(t - Tl_2) + Lh} - \frac{1}{L(t) + Lh} \right] \quad (36)$$

I assume throughout the within season dynamics that the parasitoid level remains a constant, dependent on the previous seasons' parasitoid prevalence levels.

6.4.1 Host-parasitoid dynamics

Model and field predictions

From the field data it is clear that as time progresses through a season there is a cumulative increase in prevalence of the parasitoid in the host population. We run the host-parasitoid system to parameterize the model by examining how the deterministic model fits against the prevalence found in the field data (Figure 6.6). We can see that for parameters that place the parasitoid prevalence at a similar level to the field data, we have a relatively small attack rate and high half saturation density, and thus we would expect that the host-parasitoid interaction at these prevalences would be stable. In addition we can see that the model predicts the similar qualitative increase in prevalence of the parasitoid as the season progresses.

However, again there is the possibility that with oscillating dynamics, differently shaped prevalence curves would be found. The population cycles are induced by the

parasitoid (Figure 6.7 A), but there is no qualitative change in the prevalence curve as occurred with the pathogen, and only a change in the parasitoid infection prevalence (Figure 6.7 B). The prevalence level found however reach very high levels of 80% or above which are much greater than those found in the field, where the highest prevalence was found to be 20% (Swartabeck A). Therefore although the prevalence data is only from a three-year period it seems unlikely that the parasitoid will reach high prevalence of 80% or above in the field and thus we may conclude that attack rates of the parasitoid are relatively low.

Therefore we can conclude that the qualitative fit of the model to data seen does seem robust. However, the quantitative findings are somewhat high at times, although this may need prolonged field data to verify that parasitoid levels do not reach very high prevalences. The densities where the very high prevalences occur are characterized by very low densities In both host and parasitoid, thus may not be recorded in the field due to sampling errors such as not collecting enough larvae to avoid stochastic affects in sampling.

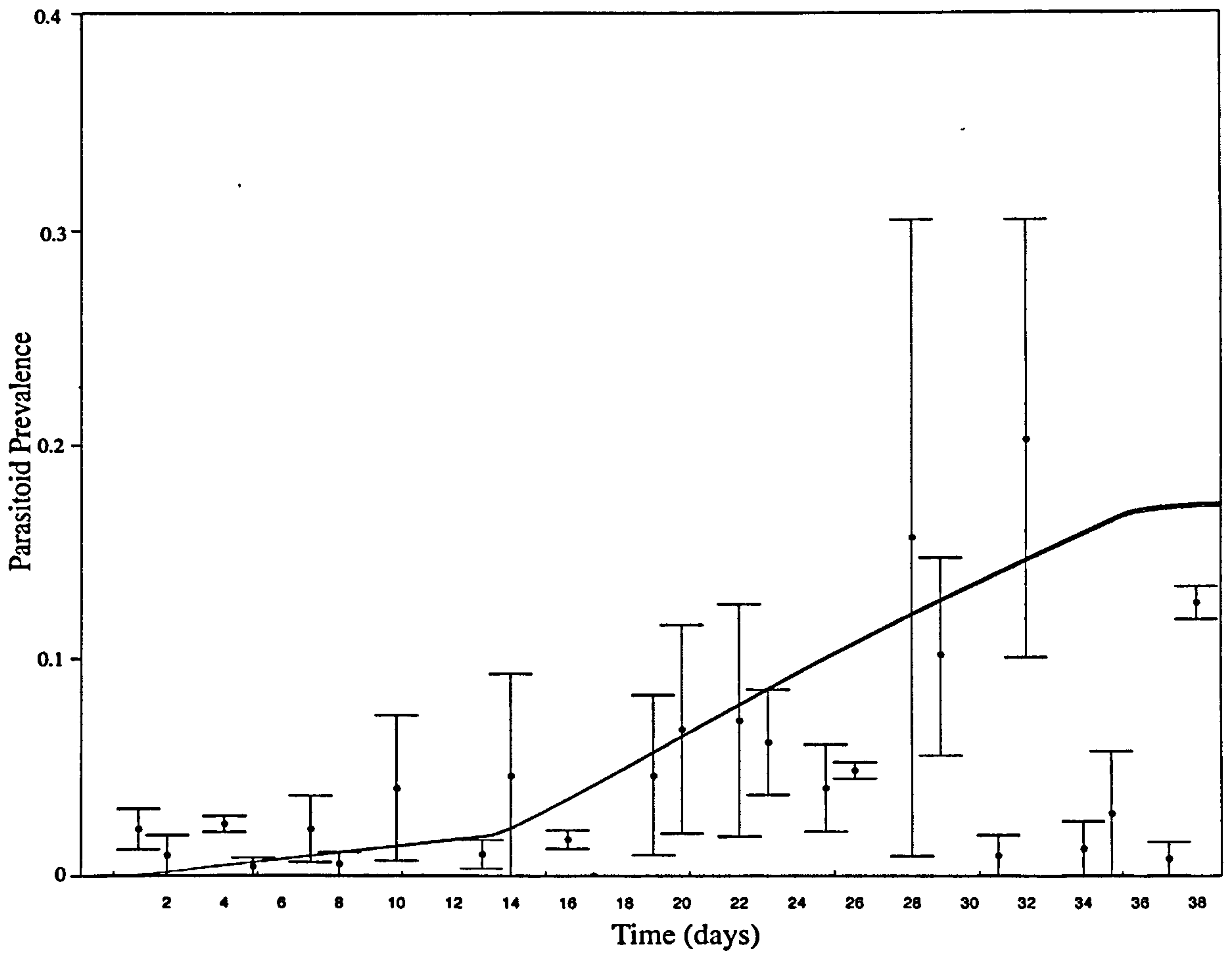


Figure 6.6. Change in parasitoid prevalence in the population of winter moth at different sampling days. The data is pooled from all sites over both 2003 and 2004. The solid-line denotes the deterministic prediction from the model. Parameters $L=5$; $A=0.47$; $Lh=200$; Host parameters $r=150$; $\delta=0.15$; $b=0.5$; $de=dl_1=dl_2=1 \times 10^{-3}$;

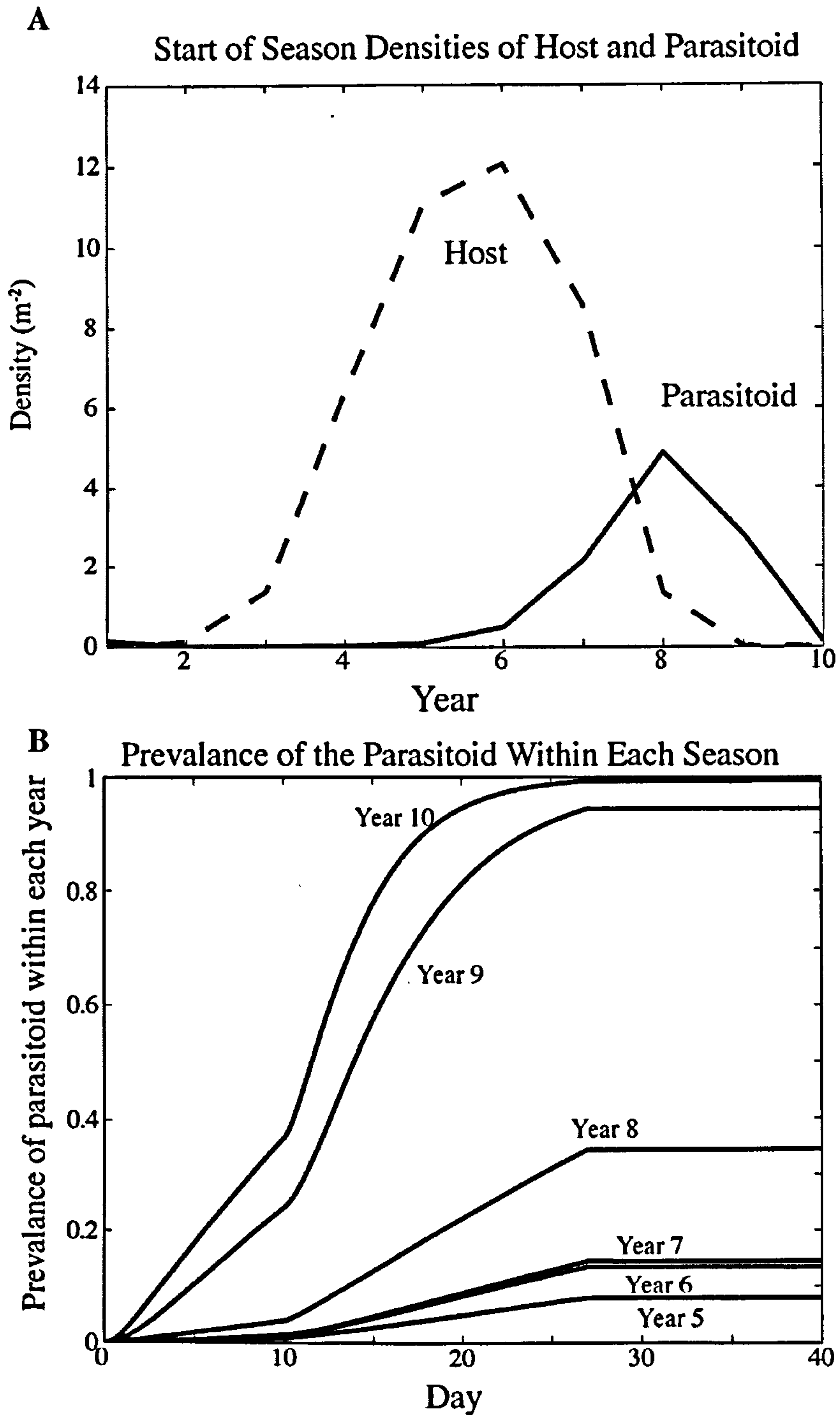


Figure 6.7. A) Densities of host and parasitoid found over a one host and pathogen cycle over five generations (host density is scaled by 0.1). B) Within-season prevalence of the parasitoid from season 5 to season 10, in an oscillating interaction. $A=2.5$; $Lh=200$; $r=150$; $\delta=0.15$; $b=0.5$; $de=dl_1=dl_2=1 \times 10^{-3}$;

Parasitoid Functional Response

With the model a good qualitative fit to the field data it is now important to understand further the effect of the parasitoid on the system. The functional response of the parasitoid is then investigated to understand how it can control the host and affect the stability of system. The ability of the parasitoid to control the host was increased by increasing the maximum attack rate, A_1 . This depressed host numbers as it is increased, however as it becomes larger in magnitude it tends to destabilize the host-parasitoid equilibrium and sets up oscillations (Figure 6.8 A). The slope of the Type II functional response also plays a role in stability, by adjusting the half food saturation value, Lh , we see that by decreasing it, so that parasitoid becomes saturated by prey at a lower host density, we see oscillations begin where as a high value lead to a stable interaction (Figure 6.8 B).

By performing fast Fourier transforms on the cyclic data we can also see that the increase in either maximum attack rate or also inter-season growth rate we see the increase in inter-peak distance (Figure 6.9 A). We see a decrease in the inter-peak distance as the half saturation prey density increases. This corresponds with the half saturation increasing the stability of the system (Figure 6.9 B). As the parasitoids are generalists and also attack other Lepidopteron larvae over the course of the year the inter season density is likely to change. Surprisingly, we also see, at very high attack rates the parasitoid depresses the host numbers to such a low level the parasitoid itself becomes extinct, and is unable to reinvade after the host recovers back to its equilibrium density when uncontrolled by a natural enemy (Figure 6.10). This of course is in a system where we presume no immigration occurs from other populations; therefore we may not see this taking place in the natural system.

Change in parasitoid density after each season

We see if parasitoid numbers at the start of the season are less than the numbers at the end of the previous season that this actually leads to an increase in stability

whereas if parasitoid numbers increase in that period this leads to a more unstable interaction and the onset of population cycles (Figure 6.11).

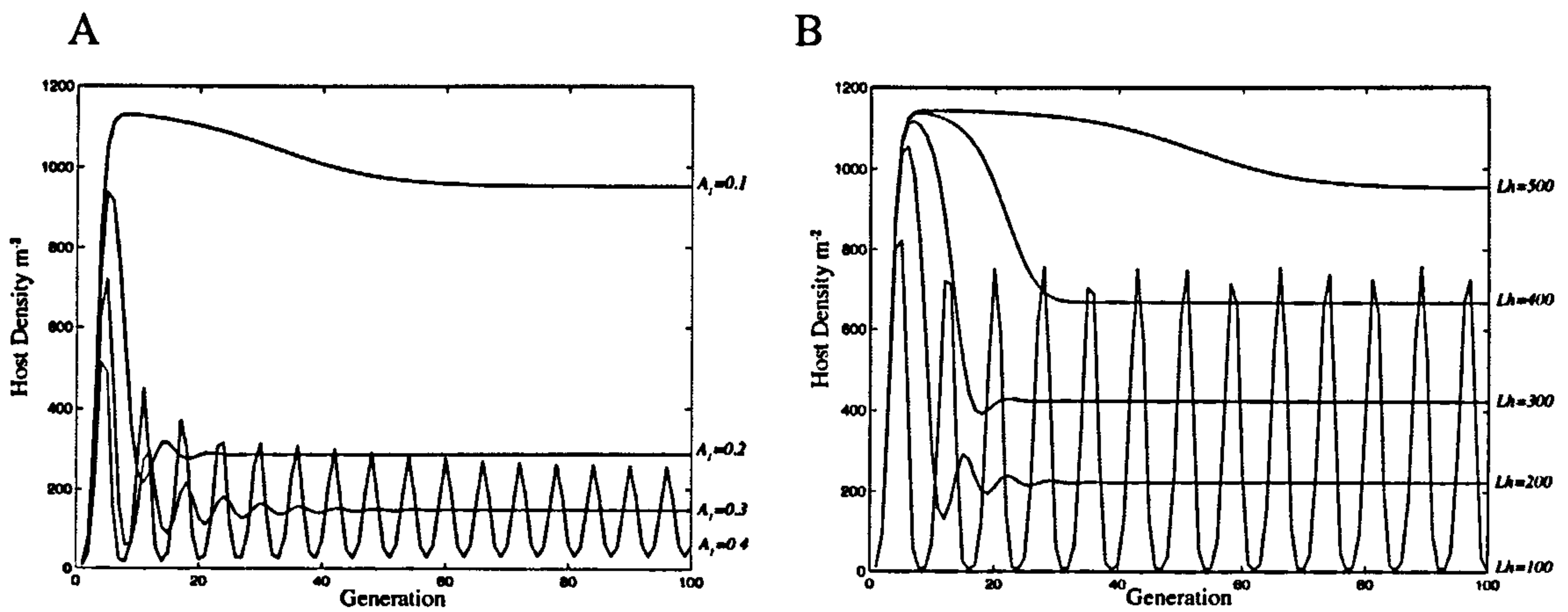


Figure 6.8. Population dynamics of the host density in a system containing a parasitoid. These plots display changes, final dynamics, and host population numbers dependent on different parameters in the parasitoid, change in saturation point of parasitoid, A and change in maximum attach rate of parasitoid, B. A $L_h=500$; and B $A=0.1$ other parameters are the same for both $L=5$; $r=150$; $\delta=0.15$; $b=0.5$; $\Omega=1$; $de=dl_1=dl_2=1 \times 10^{-3}$;

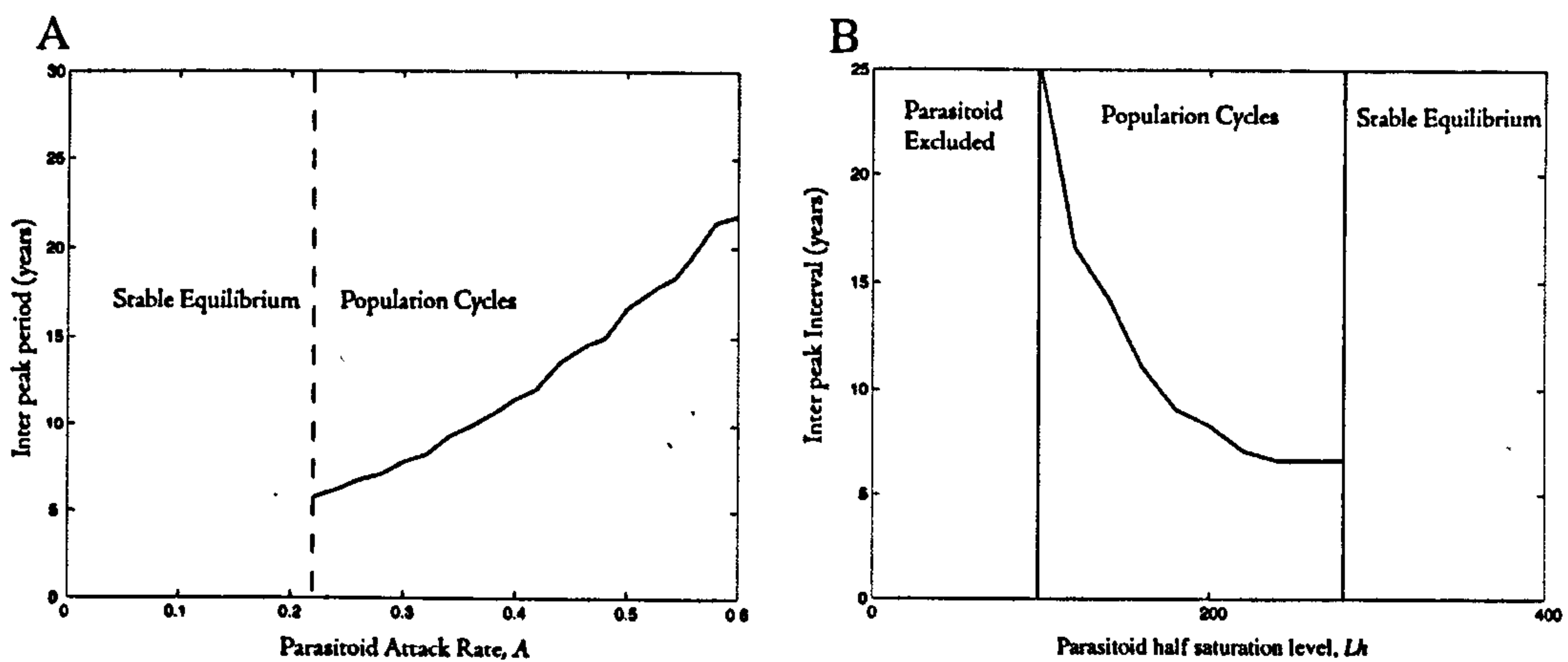


Figure 6.9. Plots describing the changing dynamics and also change in period between host outbreak peaks. When firstly altering parasitoid attack rate, A and also the level of food saturation, following Hollings Type II response equation, B. A $L_h=300$; and B $A=0.2$ other parameters are the same for both $L=5$; $r=150$; $\delta=0.15$; $b=0.5$; $\Omega=1$; $de=dl_1=dl_2=1 \times 10^{-3}$;

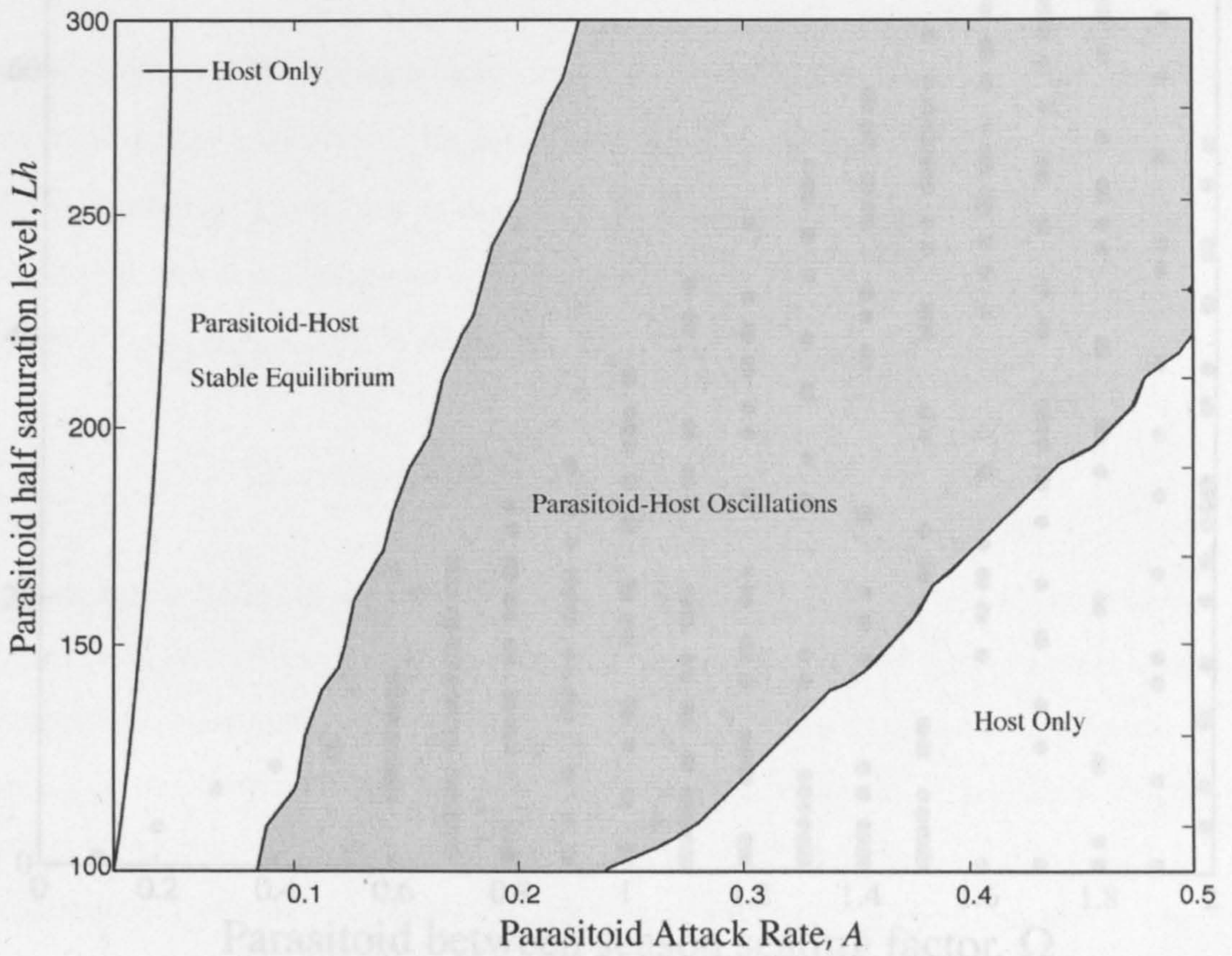


Figure 6.10. Plot of the boundaries of invasion and dynamics of the host parasitoid interaction with varying the strength of pathogen maximum attack rate and changing shape in the type II response. $L=5$; Host parameters $r=150$; $\delta=0.15$; $b=0.5$; $\Omega=1$; $d_e=d_{l_1}=d_{l_2}=1 \times 10^{-3}$;

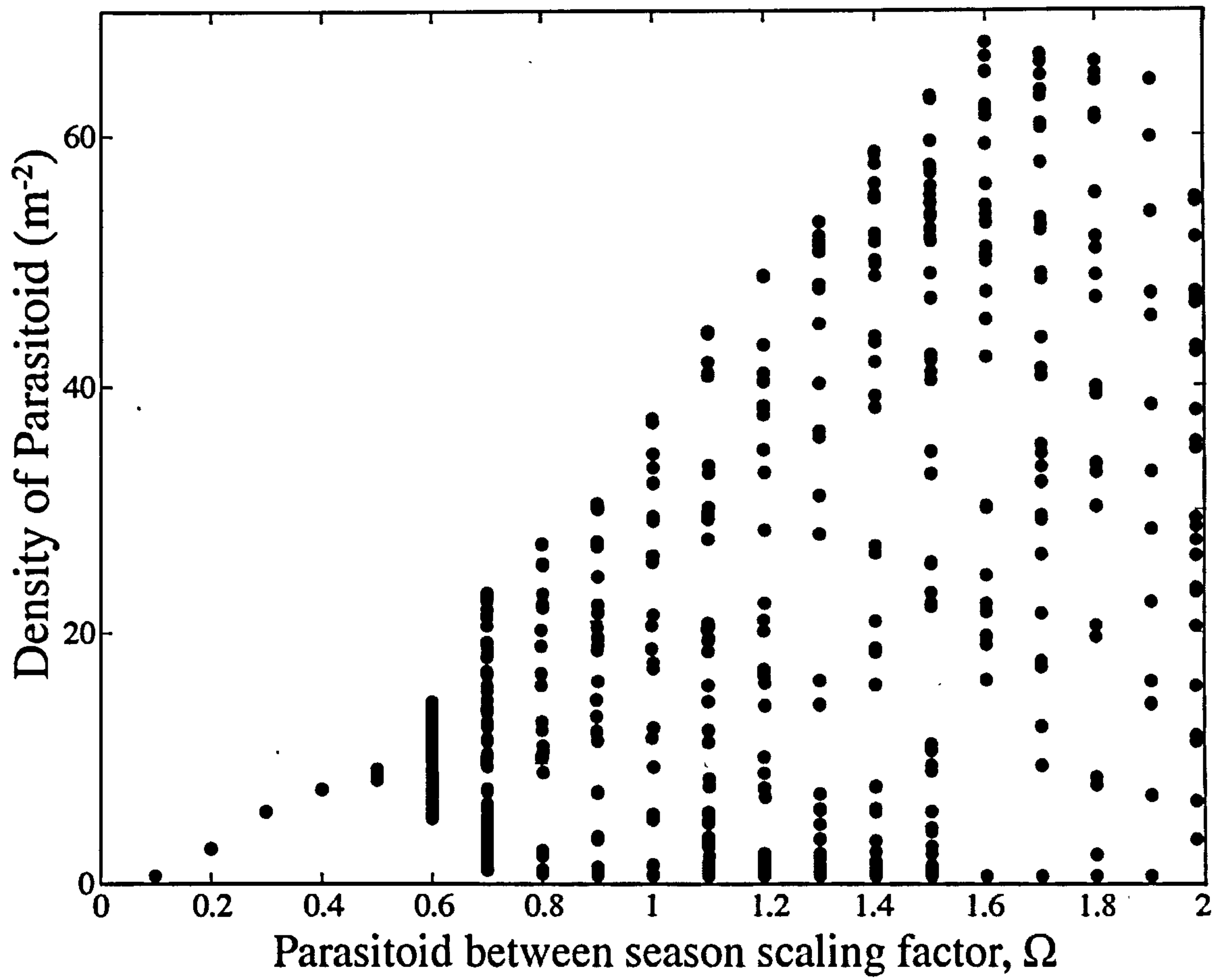


Figure 6.11. Displaying the effect of the between season scaling factor on the dynamics of the host-parasitoid system. As the scaling increases we see cycling occurring with larger amplitudes. $L=5; r=150; \delta=0.15; b=0.5; de=dl_1=dl_2=1 \times 10^3; A=0.5; Lh=400;$

6.5 Host-Pathogen-Parasitoid Model

The two different models are then combined. This explicitly defines the interaction of the pathogen on the parasitoid. The pathogen is able to infect both uninfected hosts and those infected by parasitoids at an equal rate. However, the parasitoid is assumed to be unable to develop inside a pathogen infected host. The affect of the interaction of the two is again explored using simulations.

6.5.1 Host-pathogen-parasitoid dynamics

When both natural enemies are present in the same system, we can piece together how their interaction will affect the host dynamics. Simulations are used to understand how the competitive interaction of the parasites affects not only the dynamics, but also their persistence in the system. The invasion of the parasitoid is able to destabilise a stable host-pathogen interaction and produce oscillations of all three species (Figure 6.12 A). In oscillating host-parasitoid systems the pathogen is able to invade and can reduce the oscillating dynamics and stabilise the interaction between the host and parasitoid (Figure 6.12 B). The pathogen competitively excludes the parasitoid, whilst still being susceptible to aperiodic reinvasion and extinctions of the parasitoid (Figure 6.12 C). In this case no three species equilibrium exists and the dynamics seem driven mainly by the frequent loss and reinvasion of the parasitoid. There is also the capability in the system for the population cycles to become uncoupled from each other. We see the parasitoid and host oscillate together, whilst the pathogen cycles once for approximately every three cycles of the host parasitoid oscillating interaction (Figure 6.12 D).

Intuitively, increasing the transmission in both pathogen and parasitoid that the respective natural enemy is better able to invade and persist. We also see that increasing transmission leads to instability in the system and a multitude of dynamical outcomes (Figure 6.13). If the parameter approximations from the earlier interactions are used then the pathogen competitively excludes the parasitoid. However over the three years we see a large variation in pathogen and parasitoid prevalence, which

means it would be unwise to restrict our parameter estimates to those which gives similar prevalence over the within season time period. It is also true that if the populations that were sampled are undergoing cycles then the prevalence of each natural enemy would be dependent on which stage they were in the cycle.

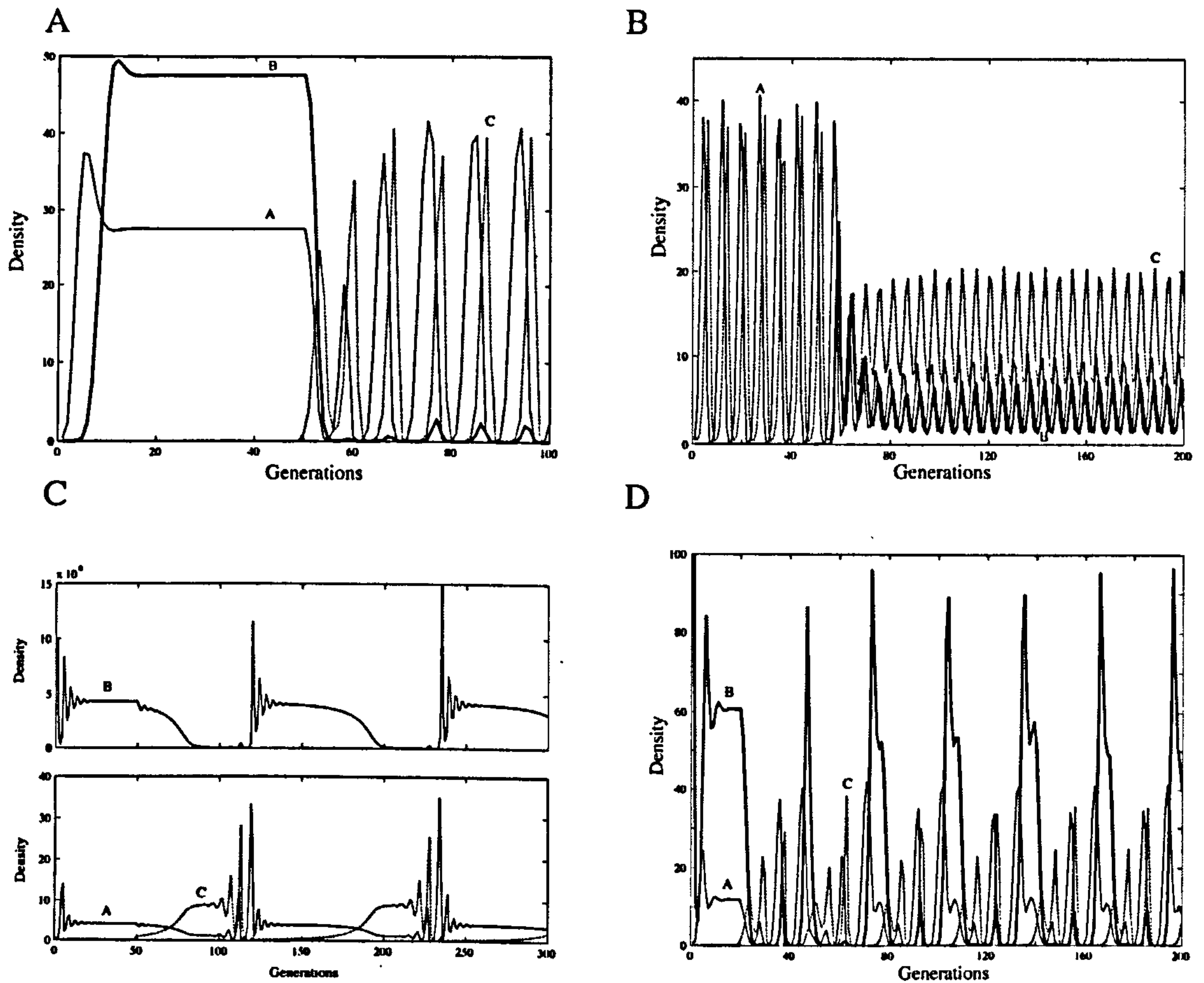


Figure 6.12. Population dynamics of the host, A (scaled by 25), and its two natural enemies, the pathogen, B (scaled by 1×10^5), and the parasitoid, C, which invades in each plot after 50 generations. A) High amplitude oscillations brought about by parasitoid invasion into stable host-pathogen equilibrium. B) Invasion of parasitoid into oscillating system reduces the amplitude of oscillations but not period. C) Periodic reinvasion of the parasitoid into a more stable host pathogen interaction. D) Period of the natural enemies become uncoupled with pathogen cycling at twice the wavelength than the host and parasitoid. A. $\beta=4 \times 10^{-8}$; B. $\beta=1 \times 10^{-7}$, C. $\beta=1 \times 10^{-7}$; $Lh=150$; D. $A=0.75$; $n=0.9$; $h=0.02$; $\beta=8 \times 10^{-8}$: all other parameters are $A=0.5$; $n=0.85$; $L=5$; $Lh=400$; $r=150$; $\delta=0.15$; $b=0.5$; $de=dl_1=dl_2=1 \times 10^{-3}$; unless otherwise stated.

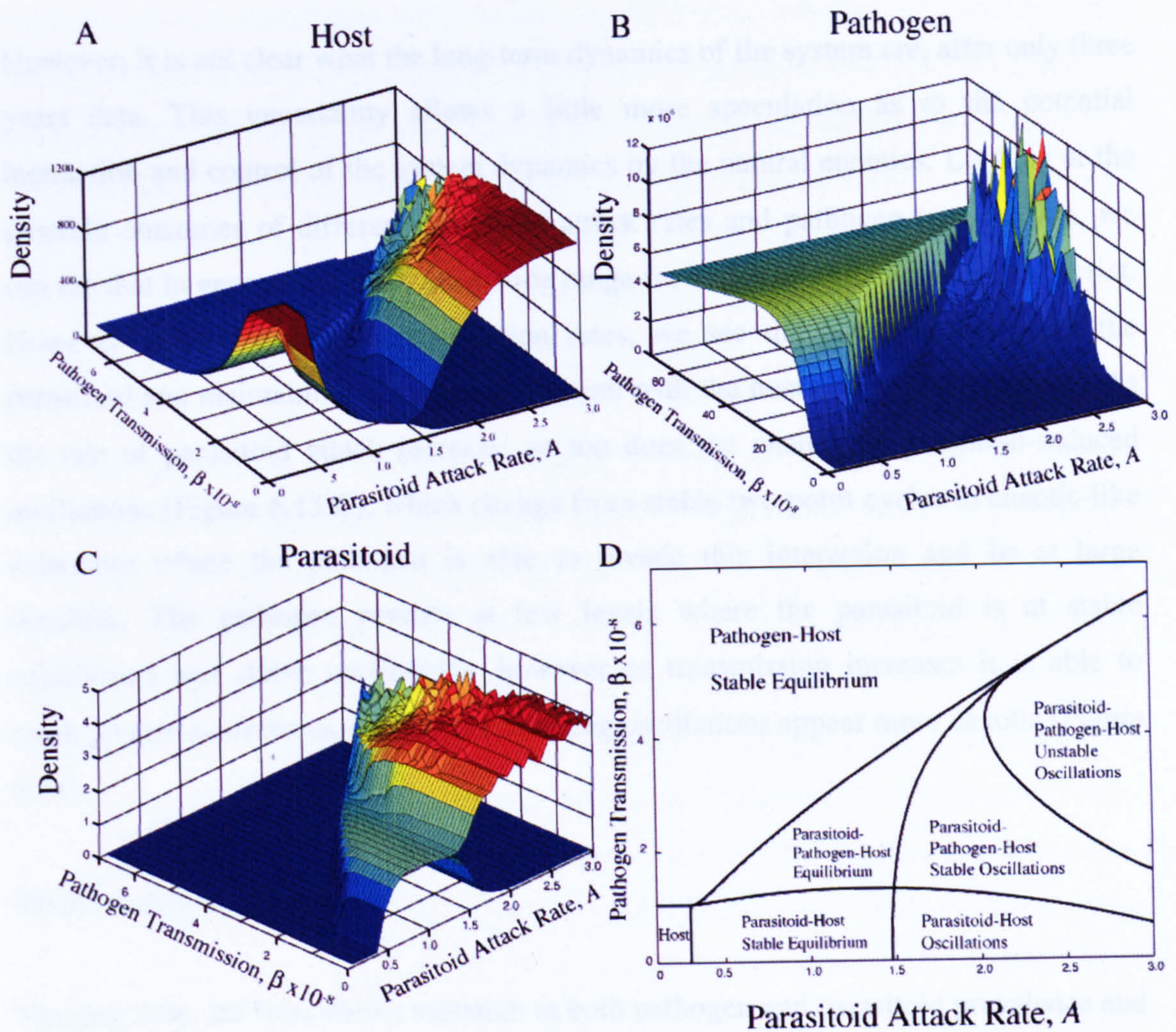


Figure 6.13. Plots of dynamics and invasion boundaries of both natural enemies with altering transmission in the pathogen and attack rate in the parasitoid. A. Host densities, showing maximum and minimum values in the cycles produced. B. Pathogen densities and dynamics. C. Parasitoid densities and dynamics. D. Contour plot of species interaction and dynamics. $n=0.9$; $L=5$; $Lh=200$; $r=150$; $\Omega=0.1$; $\delta=0.15$; $b=0.5$; $de=dl_1=dl_2=1 \times 10^{-3}$; unless otherwise stated.

However, it is not clear what the long-term dynamics of the system are, after only three years data. This uncertainty allows a little more speculation as to the potential interaction and control of the system dynamics by the natural enemies. Looking at the possible outcomes of different parasitoid attack rates and pathogen transmission, we can see that in general there are a wide range for which all three species can persist. However at high pathogen transmission rates, we see the pathogen excluding the parasitoid and maintaining a stable equilibrium with the host (Figure 6.13 A & B). As the rate of parasitoid attack increases, so too does the chance of parasitoid-induced oscillations (Figure 6.13 C), which change from stable two point cycles to chaotic-like behaviour where the pathogen is able to invade this interaction and be at large densities. The pathogen persists at low levels where the parasitoid is at stable equilibrium and stable oscillations, however as transmission increases it is able to reach greater densities and makes the resulting oscillations appear more chaotic (Figure 6.13).

Model with field predictions

The data from the field shows variation in both pathogen and parasitoid prevalence and also host densities, therefore it would be expected that the system is positioned in parameter space where all three persist and are undergoing oscillations. This would suggest from the model that the parasitoid might exert high attack pressure to produce oscillations that are destabilised further by the interaction with the pathogen.

6.6 Discussion

We can see complex dynamics arising purely from this three-species system, which uses the knowledge of the specific host-parasitoid-pathogen field system of the winter moth, *Operophtera brumata*, in Orkney. The model produces good qualitative fits to the within season field data showing that the pathogen interaction with the host can reproduce the changes in prevalence throughout a season. This then may allude to the patterns seen in the field being driven by the NPV interaction with the winter moth host. However, the range of possible complex dynamics is not unsurprising as

other models that have taken into account the difference in biology of parasites and pathogens tend to show these dynamics in simple multispecies systems (Hochberg *et al.* 1990). The action of the parasitoid is also competently qualitatively modelled. Possible inter-season changes in the parasitoids density and how they may effect the host population in both density and stability are demonstrated. However, with inadequate data it is necessary to investigate further the parasitoid behaviour regarding oviposition rate, dispersal and total egg loads.

How each natural enemy can interact with and control the host separately is shown, this adds to the understanding already gained from many species interactions concerning host-pathogen (Anderson & May 1981) and host parasitoid models (Bonsall *et al.*, 2002; Briggs *et al.*, 1999; Murdoch *et al.*, 1986; Wearing *et al.*, 2004). The results of an introduction of one of the species have a number of possible outcomes: the invading species can be competitively repulsed and unable to establish, it can invade and lead to a stable or unstable three species interaction, or it can exclude the other resident natural enemy. Of course, these outcomes have been demonstrated in many other multi-species models (Hochberg & Holt, 1990b; Holt & Pickering, 1985; May & Hassell, 1981). However, as only Hassell *et al* (1990) have explicitly taken into account the differences of pathogens and parasitoids in a model, we built on their work by incorporating a more detailed within-season interaction based on our knowledge of the winter moth system. This within-season model enables the direct competition of both pathogen and parasitoid within a season, whilst infecting a stage-structured host in a delay differential model. The model structure used here is novel and also essential in modelling the dynamics of a temperate host. Most other models of invertebrate hosts describe systems with overlapping generations. The discrete-continuous framework accurately describes and is suitable to describe many temperate univoltine invertebrate systems. However the increased detail in the model reduces the analytical convenience, hence simulations are required to fully explore the complexities of the model.

One aim was to determine if the parasitoid and/or pathogen could produce population cycles and if so, how the length of cycles is affected by their parameters and

interaction. These insights are important to understand how damaging outbreaks of winter moth may occur, as well as their frequency and causation. When oscillations do occur, the periodicity is altered by the change in natural enemy parameters. In general the better that either natural enemy is at parasitizing the host, the longer the periodicity will be. Thus it is more likely that parasitoids with high attack rate will cause oscillations, but also that the resultant peaks are spread further apart. The pathogen is seen to produce highly unstable oscillations with peaks in host density being inconsistent. Pathogens have been shown to be important in causing host cycles in theoretical studies previously. The study concludes that the effects of the self-regulation of the host and the action of the pathogen showed is inseparable and either or both can cause the resultant dynamics (Bowers et al., 1993). The study also suggested that the cycles produced could not reach densities close to the carrying capacity, unlike many outbreaks species that are found to severely damage their food source (Myers, 1988). Bowers *et al.* (1993) suggested that failings in the simple model could be remedied with some time-delay self-regulation of the host, with the carrying capacity then being better able to simulate natural systems.

The time-series data are important in understanding how prevalence changes within a season, demonstrating different pulses of infection in the pathogen and the cumulative increase of parasitoid infection. The parameter values that give prevalence similar to those found from the field data suggest that there would be a stable interaction between host and parasitoid and host and pathogen, and thus we would not expect that these two causes would be leading suspects in the cycling of the winter moth. However, this is from data collected from three sites during 2004, and thus only provides a very limited snapshot of the possible dynamics that may be occurring. We then therefore must not resist the possibility, given the knowledge of other prevalence data from sites taken in one off samples, that these time series data may mislead us to understand that the stable interaction is the case. We can therefore speculate that if there are indeed cycles occurring in the system, then the time series-data could be any where within those cycles.

The deterministic predictions of prevalence of the OpbuNPV against time show how it is possible to gain two different bimodal shapes. Figure 6.2 D shows how this may occur as the inter-year survival of the pathogen occlusion bodies largely determines the shape of the prevalence trace in a season. With high proportion of occlusion body survival that remain accessible to the larvae in the next season that is seen in Figure 6.1 A, we see large prevalence in the first peak and a smaller amount in the second, whereas in the Figure 6.1 B, there is lower inter-season survival but higher transmission, and the second peak has the largest prevalence. The inter-season survival will then of course not only affect the prevalence over the season, but also will then affect the numbers of host that are available for the parasitoid to infect. Once a host is infected by the pathogen, the parasitoid is unable to superinfect it. The model showed that the change in the prevalence curve throughout a season is can be dependent on either climatic effects on pathogen availability for the start of a new season but also by pathogen induced dynamical oscillations.

The prediction by the model of the accumulation of parasitoids followed the increase in prevalence found in the time series data from the pooled data sets from the two years where it was taken. However after day 34, we see a sudden reduction in the prevalence of the parasitoid; this is where the parasitoids emerge from the 5th instar larvae and pupate in the field system. In the model, this change is not modelled, as it is not necessary for tracking the dynamics. We model the parasitoid at a constant density within each season; this density is dependent on the number of hosts infected by the parasitoid in the preceding season. Using the knowledge that inverse density dependence is apparent in the parasitoid from the field data to give the parasitoid a functional response that would produce a similar effect that, in this instance, is a Hollings Type II response. The functional response leads to the instabilities in the interaction of host and parasitoid, as the parasitoid is unable to fully control the host when the host reaches high densities.

We do not however factor into the parasitoid behaviour and changing in its searching model, and thus we presume that the parasitoid searches at random, as is the case for the pathogen also. Therefore the interaction between the two natural enemies means

coexistence is determined by their respective extrinsic and intrinsic factors. The extrinsic factors are attack rate and saturation point of the parasitoid and the transmission rates and the scaling with pathogen density, as well as the survival rates of both. The intrinsic factors are the within host competition and also the timing of host attacks. It has been seen in other studies that high clumping of parasitoid attacks can lead to coexistence even when parasitoid is a better extrinsic and intrinsic competitor (Hochberg *et al* 1990). Also in general some sort of heterogeneity increases the changes of coexistence and persistence of competitors, such as spatial and temporal differences. In this model we use transmission and attack rates that are stage-specific. Thus there are the early instars that are more susceptible to pathogen infection than the late instar sizes, whilst the parasitoid attack rate increases in the late instars. This difference in temporal competition is seen to allow coexistence. However in models of parasitoids, where one attacks host eggs and another parasitoid attacks larvae, if the egg parasitoid was unable to be superinfected, the exclusion of the second larval parasitoid occurred, even if the first egg parasitoid did not suppress the host as much as the other parasitoid would (Briggs, 1993). It was only when the larval parasitoid could infect already-parasitized hosts that coexistence occurred. In our model with both natural enemies able to parasitize each stage, albeit at differing intensities at each stage, a wider area of coexistence is realised. But undoubtedly as the parasitoid was unable to superinfect hosts infected by the pathogen, it was at a disadvantage to the pathogen, which can infect hosts infected by the parasitoid.

Here we see that the host in its range of oscillations with its interaction with the pathogen is able to reach densities that approach the carrying capacity using this model structure. Something that other models have been shown not to do (Bowers *et al.*, 1993). Indeed that is also true for the parasitoid. In this model, the parasitoid is unable to maintain a stable equilibrium at high attack rates and thus it shows that for reasonable levels of parasitism by either natural enemy may indeed cause cycles that will intermittently mean high damaging densities of the host, i.e. the winter moth. There are of course many ecological reasons that have been suggested for the outbreak of Lepidopteron species, including host plant effects (Abbott & Dwyer,

2007; Hogstedt *et al.*, 2005) as well as effects of predators, parasitoids and pathogens and indeed the interaction between host plants and natural enemies (Cory & Hoover, 2006). Cory & Hoover (2006) discuss how amongst other factors the plant quality has an important role in how susceptible the insect herbivore is to disease, but also how this also effects the reduction in the effectiveness of entopathogens in killing the insect. Outbreaks have been explained through theoretical models by food limitation that show that after an outbreak and severe defoliation, the host plant can recover and be abundant in inter-outbreak periods while herbivore levels remain very low (Abbott & Dwyer, 2007). Of course there are many factors that can drive population cycles, different systems must be affected by all these factors to differing degrees. The system studied here only looked in respect to the natural enemies, and thus no bottom-up control from the plant was investigated and also hence no tritrophic interactions investigated. We do show however possible oscillations produced purely from the interaction of the natural enemies without added factors. Whether or not these factors are the main driving force is nearly impossible to separate from other possible factors without studying the system for a longer period to gain a more comprehensive time series data set. It has already been shown that the food source on which *O. brumata* feeds does have an effect on the effectiveness of the NPV strain which is specific to the plant food source. Previous works shows that OpbuNPV persists least well on *Calluna vulgaris* due to little shade from foliage to protect the occlusion bodies from U/V light (Raymond *et al.*, 2005). Also as heather is in itself low in nutrient content (Vanbergen *et al.*, 2003; Wint, 1979), probably the quantity of heather eaten will have a greater effect than other plant species to gain the same body condition and hence with be more likely to consume more occlusion bodies.

The system described here has the potential to give greater insight specifically into the dynamics of the populations of winter moth on Orkney. It would be necessary to design better experimental studies to gain more accurate parameter estimates for the model. Further development would be to understand the linkage between different sites of winter moth. The linkage would describe their dispersal and how the sites are linked by parasitoids. The spatial structure of the populations on Orkney could possibly have a large impact on the systems dynamics. Spatial affects have been

shown to both stabilising with host-parasite interactions (Chesson & Murdoch, 1986; Hassell, 1978; Murdoch & Stewart-Oaten, 1989) and destabilising when exploring the spatial change in demographic factors of the system (Holt & Hassell, 1993). The winter moth system is a group of populations that may be linked through parasitoid search, immigration and also viral translocation via vectors (possibly the parasitoid wasps). It would then be interesting to see how linked particular populations are. The model itself, seemed to predict infection peaks of both parasitoid and pathogen well and thus with more years of data and better within season knowledge of pupae and pathogen survival this model would be a good predictor of this systems dynamics. Many studies cite pupal predation as a key ingredient in stability of system dynamics in univoltine Lepidopteron systems and have studied it in similar species (Buckner, 1969; East, 1974; Tanhuanpaa *et al.*, 1999). It is therefore concluded with increased parameter power this model may lead to predictions as to how the winter moth may be behaving dynamically on the Orkney Isles.

7. Discussion

This section will highlight the contributions made by this thesis to the theory of multiple parasite interactions. Chapter 2 demonstrates how a purely vertically transmitted parasite, even when it has a virulent effect on the host, may persist in a system, largely by protecting the host from another virulent, horizontally transmitted parasite. This finding is similar to others that have looked at related questions (Lipitch et al., 1996; Lively et al., 2005). The protecting parasite provides a system



Stones of Stenness, Sunset, Orkney. 2004

7.1 Conclusions and general perspectives

This section will highlight the contributions made by this thesis to the theory of multiple parasite interactions. Chapter 2 demonstrates how a purely vertically transmitted parasite, even when it has a virulent effect on the host, may persist in a system, largely by protecting the host from another virulent, horizontally transmitted parasite. This finding is similar to others that have looked at related questions (Lipsitch *et al.*, 1996; Lively *et al.*, 2005). The protecting parasite provides a system with greater stability at higher levels of transmission from the horizontal parasite. In a system where the horizontal parasite is free-living, the point at which a Hopf bifurcation occurs are at higher levels of horizontal transmission the greater the protection affect of the vertically transmitted parasite. This highlights the increased stability in a system containing vertically transmitted parasites that persists through protection. It also showed that the vertically transmitting parasite was more likely to persist if the horizontally transmitting parasite was a functional predator, whereby the parasite kills the host before the host can reproduce (Boots 2004). The study also demonstrated similar results to that of Lively *et al.*, (2005) whereby the persistence of the protecting vertically transmitting parasite increases total host density through a greater number of hosts having increased life reproductive success through lower infection by the horizontal parasite.

With the ecological dynamics of vertically and horizontal transmitted parasites in a system described in Chapter 2, Chapter 3 and 4 both extend the study by exploring the evolutionary dynamics of these possible interactions. These two chapters highlight new theories on the interaction between vertically and horizontally transmitted parasites and show how both parasites can alter the selection of certain life-history traits in the other. Chapter 3 investigates the evolutionary dynamics of a horizontal parasite and its interaction with a vertically transmitting parasite that persists through feminisation. The vertical parasite is assumed to maintain itself in the host population purely through increasing fecundity in the host it infects by increasing the number of females producing offspring. It assumes that the vertical parasite reduces the host lifespan in which it is present. There is no direct interaction

between the two parasites and there is no protection or interference evident. Therefore the horizontal host is able to infect either the uninfected host or the host infected with the vertically transmitted host at an equal rate. In a horizontally transmitted parasite with a trade-off in its transmission and its virulence, high horizontal transmission and high virulence were selected when the vertical parasite had high feminisation, intermediate virulence and high vertical transmission efficiency. This selection is due to the increased number of susceptible hosts at these levels. In one sense, the vertical transmitted parasite can simply be considered as a form of the host with greater reproductive and death rates, which leads to parallels with the work of Gandon *et al.*, (2002), who showed that host reproductive effort also increases parasite virulence.

The vertical parasite was allowed to evolve using two different trade-off strategies. The first strategy had a link between feminisation and virulence and the second linked vertical transmission ability and virulence. Using the first strategy, high feminisation was found at low horizontal transmission and high virulence and showed lowest feminisation to be at intermediate levels of castration from the horizontal parasite. When using the second strategy of vertical transmission traded-off with virulence, high horizontal transmission, high castration and high virulence select in general for a high level of vertical efficiency. The increase in vertical virulence is due to each of these horizontal parasite parameters increasing the cost of being infected by the horizontal parasite, therefore increasing vertical transmission efficiency and reducing vertical parasite life-span.

Chapter 3 also investigated the coevolution of both the vertical and horizontal parasite. These results showed that each of the vertical parasites could have CoESS points with the horizontally transmitted parasite. In general, virulence in both parasites increased while host life-span decreased. This is a common assertion in classical life-history theory, but it is contrary to some host-parasite models that use resistance to show a decrease in parasite virulence as host life-span decreases (Restif & Koella, 2003; Gandon *et al.*, 2001).

Chapter 4 used a similar approach to look at two types of systems where the vertically transmitted parasite directly interferes with the horizontal parasite to aid in the maintenance of the vertically transmitting parasite. The first system dealt with the evolutionary dynamics of the model in Chapter 2. Chapter 4 looked at how protection from a vertical parasite could affect the evolution of the horizontal parasite and vice versa. Vertical parasite protection at intermediate levels selected for maximum horizontal parasite transmission and virulence. At high levels of protection, a high proportion of the population is protected from the horizontal parasite. Thus horizontal transmission is selected against. However at intermediated levels of protection, there is a large number of susceptibles, and selection for high horizontal transmission. The horizontal parasite selects for high protection at high horizontal transmission and low protection at high virulence. Protection strategy was not affected greatly by a change in horizontal parasite castration. However, if the vertical parasite had a level of feminisation, evolutionary bistabilities occur. Therefore from some areas a stable ESS for protection could not be reached due to evolutionary repellors.

The second strategy was that of sabotage. We based the system on Haine *et al.*, (2005) and developed it to allude to possible evolutionary outcomes of parasite interaction. Our system contained a vertically transmitted microsporidian reducing the geotaxis behaviour of the host, which is induced by a horizontally transmitted acanthocephalan that uses the manipulation to increase its transmission by increasing predation rate of the host. The horizontal parasite had a trade-off between transmission ability and its induced behaviour, which was seen as induced death rate. Therefore as transmission rate increased, the acanthocephalans' behaviour manipulation decreased. This trade-off showed that in a system with a highly sabotaging or feminising vertical parasite, the horizontal parasite increases its induced death rate to combat it. As in both cases, the level of sabotage present in the system increases, whether it is by each individual sabotaging more or a greater proportion of the population sabotaging. The vertically transmitting parasite trades off between strategies that allow it to be maintained in the system. Feminisation and sabotage showed that with increasing behavioural induced death by the horizontal parasite, a vertical parasite with high sabotage is selected for. Castration by the

horizontal parasite complicates selection, it seems to maximise sabotage at intermediate levels castration.

With the advance of molecular techniques available for use in describing the plethora of vertical parasites hitherto unnoticed (Terry *et al.*, 2004), there is an increased interest in how these parasites both persist and transmit. More interestingly, as we have shown here, we can observe how their interactions may affect the evolution of other more overt parasites. Chapters 3 and 4 both highlight possible outcomes of evolution between these differently transmitting parasites.

Both Chapters 5 and 6 explicitly deal with the winter moth system. Chapter 5 gives details of the data collected over a number of years. The chapter demonstrates the responses of the two main natural enemies, the NPV and the parasitoid, to the density of the host. It shows the virus has a positive density-dependent relationship, whilst the parasitoid has a negative relationship with host density. Also, it describes instar dependent rates of parasitism and viral infection. The development times for each instar level were also calculated from the field data. These data were then used to construct and parameterise the discrete-continuous model in Chapter 6. This model built on previous models containing two natural enemies (Hassell and May, 1986; Dobson, 1985; Hochberg and Holt, 1990; Beddington and Hammond 1997) and was closely linked to the parasitoid-pathogen model by Hochberg *et al.*, (1990). This model had a good fit to the fieldwork data and highlighted that the pathogen (NPV) in the model was capable of inducing oscillations at prevalences similar to those found in the field data. Finally, the model also highlighted the limitations of the data, suggesting some parameters, such as attack rates of the parasitoids and pupal survival of the host between years are very important in the system but as yet unknown.

7.2 Future Work

Chapters 2, 3 and 4 are purely theoretical; they are based on inferences from vertically transmitting parasite systems in nature (Hurst & Jiggins, 2000; Hurst *et al.*, 1994; Hurst, 1991; Oliver *et al.*, 2006; Oliver *et al.*, 2003; Terry *et al.*, 1998).

However, the previous work on this topic does not explicitly explain either the issues of population dynamics or evolutionary implications investigated in the models. Further work in this area is necessary, predominantly through experimental studies, in order to provide evidence for the theory that has been established.

The main restriction of these theoretical studies is that they are all based on trade-offs between two life-history parameters. It is therefore necessary to be able to find indications as to whether the trade-offs used in this study are appropriate or indeed suggests trade-offs that are suitable. Describing trade-offs, although difficult, is essential particularly as we are starting to discover previously unnoticed vertically transmitted organisms as a result of improvements in molecular methods (Terry *et al.*, 2004). With the discovery of more and more vertically transmitted symbionts, describing their costs and benefits to the host will allow an understanding of their interactions with other parasites. The experimental systems must be easily manipulated. An obvious system to investigate further is that of the pea aphid – secondary symbiont bacteria – parasitoid (Oliver *et al.*, 2003, Oliver *et al.*, 2006). There is increasing literature on these secondary symbionts (Ferrari *et al.*, 2004; Moran *et al.*, 2005; Oliver *et al.*, 2003), and how they interact with the host. It may be possible for further work to focus specifically on validating the theory and possible trade-offs that maybe relevant. There are also other systems that have conflicting infections (Haine *et al.*, 2005) that may provide interesting insights into endorsing the predictions found in the theory proposed in this thesis.

Chapters 5 and 6 also deal with the specific system of the winter moth and its natural enemies. One important future step is to parameterise the model constructed in Chapter 6, most critically the parameters, attack rate and transmission rate of parasitoid and pathogen, and their respective functional density responses. An extension of this work would be the detection of possible inter-seasonal pupal predation counts that have been seen to be an important factor in univoltine moth dynamics (Frank, 1967; Raymond *et al.*, 2002b). There are also other parasitoids in the system, although not in as greater density and also a cytopolyhedrosis virus (CPV) in the system (Graham *et al.*, 2006). These may prove important in the

dynamics of the system, especially the transmission of the CPV and its interaction with the other virulent natural enemies such as the NPV and parasitoids. It is quite possible that the CPV largely transmits vertically and may indeed lead to conflicts with the other natural enemies, although this topic deserves further investigation.

This thesis has produced a good model for the winter moth system; however, more data is needed from previous years to examine both a time series of the dynamics and on specific natural enemies in the system. However, it is a good qualitative fit of the data collected so far, and therefore will prove useful to understanding the system with further parameter estimates. The thesis describes a new theory in the evolutionary dynamics of horizontally and vertically transmitting diseases and generates possible evolutionary outcomes of their interactions. These results are now available for further investigation and will lead to a greater understanding of multiple parasite interactions.

Appendix

A Chapters 3&4 Analysis

1. Conflict and Protection

Here I summarise the steady state and stability analysis for indirect and direct conflict (i.e. sabotage and protection) models.

Firstly I describe the analysis for the indirect conflict and protection models:

These are essentially the same models but in the model from chapter 3 we assume $\delta = 0$.

$$X' = (a - qH)(X + (1 - \kappa)Yx) + (1 - p)(af - qH)(V + (1 - \kappa)Yv) - (\beta Y + b)X \quad (1A)$$

$$V' = p(af - qH)(V + (1 - \kappa)Yv) - (1 - \delta)\beta VY - (\alpha_v + b)V \quad (2A)$$

$$Yv' = (1 - \delta)\beta VY - (\alpha_y + \alpha_v + b)Yv \quad (3A)$$

$$Yx' = \beta XY - (\alpha_y + b)Yx \quad (4A)$$

There are five equilibria

1. The first all species extinction

$$(X, V, Yv, Yx) = (0, 0, 0, 0)$$

The relevant eigen values are $-\alpha_y - b$, $a - b$, $-\alpha_y - \alpha_v - b$, $paf - b - \alpha_v$, and thus this is unstable if $a - b > 0$, $paf - b - \alpha_v$ (can be either sign)

2. The second is the uninfected host at its carrying capacity

$$(X, V, Yv, Yx) = \left(\frac{a - b}{q}, 0, 0, 0 \right)$$

1) $b - a, -\alpha_y - \alpha_v - b,$

Eigen values are 2) $p(af - (a - b)) - b - \alpha_v,$

$$3) \beta \frac{(a - b)}{q} - b - \alpha_y$$

The third (3) eigenvalue relates to $R_0 < 1$ for the HTP and the second (2) eigenvalue is similar to $R_0 < 1$ for the VTP. The host equilibrium is stable if $R_0 < 1$ for the HTP and VTP.

3. The third equilibrium is that of the host and VTP interaction

$$(X, V, Y_v, Y_x) = (X_3, 0, 0, Y_{x_3})$$

$$X_3 = \frac{\alpha_y + b}{\beta} \quad \theta = \beta(\kappa a - b) - qb - q\alpha_y - [\beta\alpha_y + q\kappa b + q\kappa\alpha_y],$$

Where

$$Y_{x_3} = \frac{\theta \pm \sqrt{\theta^2 + \sigma}}{\omega} \quad \text{and} \quad \sigma = 4(\alpha_y + b)q\kappa(\beta(a - b) - qb - q\alpha_y)$$

$$\omega = 2q\kappa\beta$$

Here if $\sigma > 0$ which occurs if $R_0 > 1$ for HTP then Y_{x_3} has one positive real value. Otherwise Y_{x_3} is negative and not biologically realistic.

For stability it helps to re-order the variable. The Jacobian J can be decomposed as follows. We concern ourselves with the top 2x2 to see if the interaction between host and HTP is stable.

$$J = \begin{pmatrix} J_{11} & J_{12} & x & x \\ J_{21} & J_{22} & x & x \\ 0 & 0 & J_{33} & J_{34} \\ 0 & 0 & J_{43} & J_{44} \end{pmatrix}$$

It is not trivial (since this is not a usual SI type model since the κ term complicates matters) that

$$\text{Trace}(J(\text{top}2 \times 2)) = -q(X + (1 - \kappa)Y_x) + a - q(X + Y_x) - \beta Y_x - b = A$$

Which is sign equivalent to AX_3 provided $X_3 > 0$. Now

$AX_3 = AX_3 - 0 = AX_3 - \zeta = -qX^2 - (1 - \kappa)Y_{x_3}(a - qY_{x_3}) < 0$ if $a - qY_{x_3} > 0$ which is true provided $Y_{x_3} < K$ which it is. So $\text{Trace} < 0$.

Here $\zeta = \text{RHS}(\text{equation}(1)) = 0$ for steady state.

By A similar argument $\text{Det}(J(\text{top}2 \times 2))$ is sign equivalent when multiplied by Y_{x_3} and is therefore positive if $a - b - qX_3 > 0$ which is also true provided $X_3 < K$ which it is. So the top 2x2 of the Jacobian has negative eigenvalues provided $X_3, Y_{x_3} > 0$ which requires $R_0 > 1$ for the HTP.

The bottom 2x2 of the Jacobian determines whether the VTP can invade the HTP equilibrium.

$$\text{Trace} = p(af - q(X + Y_x)) - (1 - \delta)\beta Y_x - \alpha_v - b - \alpha_y - \alpha_v - b$$

$$\text{Det} = -(\alpha_y + \alpha_v + b)(p(af - q(X + Y_x)) - (1 - \delta)\beta Y_x - \alpha_v - b)$$

$$- (1 - \delta)(1 - \kappa)\beta Y_x(p(af - q(X + Y_x)))$$

If $\text{det} > 0$ then $\text{trace} < 0$ is satisfied automatically and the VTP cannot invade.

The VTP can invade if

$$(p(af - q(X + Yx)) - (1 - \delta)\beta Yx - \alpha_v - b) + \frac{(1 - \delta)(1 - \kappa)\beta Yx(p(af - q(X + Yx)))}{(\alpha_y + \alpha_v + b)} > 0$$

4. The fourth equilibrium occurs with just the host and the VTP infected host

$$(X, V, Y_v, Y_x) = (X_4, V_4, 0, 0)$$

Where

$$X_4 = \frac{(afp - \alpha_v - b)(\alpha_v - b)(1 - p)}{qp^2(af - \alpha_v - a)}$$

positive if $(afp - \alpha_v - b - p(a - b)) > 0$

$$V_4 = \frac{(afp - \alpha_v - b)(afp - \alpha_v - b - p(a - b))}{qp^2(af - \alpha_v - a)}$$

This has a similar structure to the Jacobian as equilibrium (3)

$$\text{Trace(top } 2 \times 2) = \frac{-[(afp - \alpha_v - b - p(a - b)) + (paf - \alpha_v - b)]}{p}$$

$$\text{Det(top } 2 \times 2) = \frac{(afp - \alpha_v - b - p(a - b))(paf - \alpha_v - b)}{p}$$

So top 2x2 has negative eigenvalues provided $X_4, V_4 > 0$.

The bottom 2x2 tells us whether the HTP can invade the host VTP equilibrium.

$$\text{Trace (bottom } 2 \times 2) = [(1 - \delta)\beta V - \alpha_y - \alpha_v - b] + [\beta X - \alpha_y - b]$$

$$\text{Det(bottom } 2 \times 2) = ((1 - \delta)\beta V - \alpha_y - \alpha_v - b)(\beta X - \alpha_y - b) - \beta X(1 - \delta)\beta V$$

For HTP to invade then it seems just one or other of the square bracketed terms needs to become positive (since with a little algebra we can prove that this makes $\text{det} < 0$, i.e. $(1 - \delta)\beta V - \alpha_y - \alpha_v - b > 0$; or $\beta X - \alpha_y - b > 0$).

5. Thus the fifth equilibrium is where all three coexist, this being where both VTP and HTP are able to invade each other.

$$(X, V, Y_v, Y_x) = (X_5, V_5, Y_{v5}, Y_{x5})$$

Evolution conflict and protection

Firstly we evolve the vertically transmitted parasite.

Equations (1-4) become:

$$X' = (a - qH)(X + (1 - \kappa)Yx) + (1 - p)(af - qH)(V + V_1 + (1 - \kappa)(Yv + Yv_1)) - (\beta Y + b)X \quad (5A)$$

$$V' = p(af - qH)(V + (1 - \kappa)Yv) - (1 - \delta)\beta VY - (\alpha_v + b)V \quad (6A)$$

$$Yv' = (1 - \delta)\beta VY - (\alpha_y + \alpha_v + b)Yv \quad (7A)$$

$$Yx' = \beta XY - (\alpha_y + b)Yx \quad (8A)$$

$$V_1' = p(af - qH)(V_1 + (1 - \kappa)Yv_1) - (1 - \delta_1)\beta V_1Y - (\alpha_{v_1} + b)V_1 \quad (9A)$$

$$Yv_1' = (1 - \delta_1)\beta V_1Y - (\alpha_y + \alpha_{v_1} + b)Yv_1 \quad (10A)$$

Then assuming the $(X, V, Yv, Yx, 0, 0)$ equilibrium is stable equilibrium the invasion success of the mutant VTP depends upon the bottom 2x2 matrix from the

$$\begin{aligned} \text{Trace (2x2)} &= [p(af - qH)V_1 - (1 - \delta_1)\beta Y - (\alpha_{v_1} + b)] - [(\alpha_y + \alpha_{v_1} + b)] \\ \text{Det(2x2)} &= -[p(af - qH)V_1 - (1 - \delta_1)\beta Y - (\alpha_{v_1} + b)](\alpha_y + \alpha_{v_1} + b) \\ &\quad - (1 - \delta_1)\beta Y p(af - qH)V_1(1 - \kappa) \end{aligned}$$

If $f > 1$ then the $(af - qH) > 0$

Here for no invasion to occur, requires $\text{Tr} < 0$ and $\text{Det} > 0$. The $\text{det} > 0$ can only be true if

$$p(af - qH)V_1 - (1 - \delta_1)\beta Y - (\alpha_{v_1} + b) < \frac{-(1 - \delta_1)\beta Y p(af - qH)V_1(1 - \kappa)}{(\alpha_y + \alpha_{v_1} + b)}$$

So the term on the left has to be negative and so $\text{Tr} < 0$ is automatically satisfied.

So as $\text{Det} > 0$ term fails the mutant VTP can invade. As seen in Chapter 3 Equation (8).

Evolution of the HTP

$$X' = (a - qH)(X + (1 - \kappa)(Yx + Yx_1)) + (1 - p)(af - qH)(V + (1 - \kappa)(Yv + Yv_1)) - (\beta Y + \beta_1 Y_1 + b)X \quad (11A)$$

$$V' = p(af - qH)(V + (1 - \kappa)(Yv + Yv_1)) - (1 - \delta)\beta V Y - (1 - \delta)\beta_1 V Y_1 - (\alpha_v + b)V \quad (12A)$$

$$Yv' = (1 - \delta)\beta V Y - (\alpha_y + \alpha_v + b)Yv \quad (13A)$$

$$Yx' = \beta X Y - (\alpha_y + b)Yx \quad (14A)$$

$$Yv_1' = (1 - \delta)\beta_1 V Y_1 - (\alpha_{y_1} + \alpha_v + b)Yv_1 \quad (15A)$$

$$Yx_1' = \beta_1 X Y_1 - (\alpha_{y_1} + b)Yx_1 \quad (16A)$$

Where $Y = Yx + Yv$ and $Y_1 = Yx_1 + Yv_1$

Then assuming the $(X, V, Yv, Yx, 0, 0)$ equilibrium is stable equilibrium the invasion success of the mutant HTP depends upon the bottom 2x2 matrix from the

$$\text{Trace (2x2)} = [\beta_1 X - \alpha_{y_1} - b] + [(1 - \delta)\beta_1 V - \alpha_{y_1} - \alpha_v - b]$$

$$\text{Det(2x2)} = [\beta_1 X - \alpha_{y_1} - b][(1 - \delta)\beta_1 V - \alpha_{y_1} - \alpha_v - b] - \beta_1 X (1 - \delta)\beta_1 V$$

Here if either of the terms in the Trace becomes positive the determinant condition will be violated. But it could be that the trace is satisfied but the determinant is not so the invasion condition for the HTP is that that the determinant condition is violated which can be re-written as the expression (6) in Chapter 3.

2. Sabotage

Now I deal with the Sabotage model from Chapter 4

$$X' = (a - qH)((X + (1 - \kappa)Yx) + (1 - p)(V + (1 - \kappa)Yv)) - \beta\Gamma X(Yx + (1 - \Delta)Yv) - bX \quad (17A)$$

$$V' = p(af - qH)(V + (1 - \kappa)Yv) - \beta\Gamma V(Yx + (1 - \Delta)Yv) - (\alpha_v + b)V \quad (18A)$$

$$Yx' = \beta\Gamma(Yx + (1 - \Delta)Yv)X - (\Gamma + b)Yx \quad (19A)$$

$$Yv' = \beta\Gamma(Yx + (1 - \Delta)Yv)V - ((1 - \Delta)\Gamma + b + \alpha_v)Yv \quad (20A)$$

where $H = X + V + Yx + Yv$.

Here I perform the same analysis.

1. The first all species extinction

$$(X, V, Yv, Yx) = (0, 0, 0, 0)$$

this is stable if $(a - b) < 0$ and $paf - b - \alpha_v < 0$

2. The second is the uninfected host at its carrying capacity

$$(X, V, Yv, Yx) = \left(\frac{a - b}{q}, 0, 0, 0 \right)$$

This is stable if $(a - b) > 0$, however it can be invaded by the VTP if $p(af - (a - b)) - b - \alpha_v$, and invaded by the HTP if $\beta\Gamma \frac{(a - b)}{q} - (\Gamma + b)$.

3. The third equilibrium is that of the host and VTP interaction

$$(X, V, Yv, Yx) = (X_3, 0, 0, Yx_3)$$

$$\text{Where } X_3 = \frac{\Gamma + b}{\beta\Gamma} \quad \theta = (q\kappa - \beta\Gamma - 2q)(\Gamma + b) + a\beta\Gamma(1 - v),$$

$$Yx_3 = \frac{\theta \pm \sqrt{\theta^2 + \sigma}}{\omega} \quad \text{and } \sigma = 4(\alpha_v + b)qk(\beta(a - b) - qb - q\alpha_v)$$

$$\omega = 2q(1 - \kappa)\Gamma\beta$$

Here if $\sigma > 0$ which occurs if $R_0 > 1$ for HTP then Yx_3 has one positive real value.

Otherwise Yx_3 is negative and not biologically realistic.

We solve the jacobian with the same procedure as described above and gain a trace and determinate as follows

$$\text{Trace}(J(\text{top}2 \times 2)) = -q(X + (1 - \kappa)Yx) + a - q(X + Yx) - \beta\Gamma Yx - b = A$$

Which is sign equivalent to AX_3 provided $X_3 > 0$. Now

$AX_3 = AX_3 - 0 = AX_3 - \zeta = -qX^2 - (1 - \kappa)Yx_3(a - qYx_3) < 0$ if $a - qYx_3 > 0$ which is true provided $Yx_3 < K$ which it is. So $\text{Trace} < 0$.

Here $\zeta = \text{RHS}(\text{equation}(1)) = 0$ for steady state.

By A similar argument $\text{Det}(J(\text{top}2 \times 2))$ is sign equivalent when multiplied by Yx_3 and is therefore positive if $a - b - qX_3 > 0$ which is also true provided $X_3 < K$ which it is. So the top 2×2 of the Jacobian has negative eigenvalues provided $X_3, Yx_3 > 0$ which requires $R_0 > 1$ for the HTP.

The bottom 2×2 of the Jacobian determines whether the VTP can invade the HTP equilibrium.

$$\text{Trace} = p(af - q(X + Yx)) - \beta\Gamma Yx - \alpha_v - b - \alpha_v - b$$

$$\text{Det} = -(\alpha_v + b)(p(af - q(X + Yx)) - \beta\Gamma Yx - \alpha_v - b) - (1 - \kappa)\beta\Gamma Yx(p(af - q(X + Yx)))$$

If $\text{determinante} > 0$ then $\text{trace} < 0$ is satisfied automatically and the VTP cannot invade.

4. The fourth equilibrium occurs with just the host and the VTP infected host and is the same as seen above in the conflict and protection models

$$(X, V, Y_v, Y_x) = (X_4, V_4, 0, 0)$$

however the trace and determinant for the HTP invasion is different.

$$\text{Trace (bottom } 2 \times 2) = [\beta\Gamma\Delta V - \alpha_v - b] + [\beta\Gamma X - \Gamma - b]$$

$$\text{Det(bottom } 2 \times 2) = (\beta\Gamma\Delta V - \Gamma - \alpha_v - b)(\beta X - \Gamma - b) - \beta^2\Gamma^2 X\Delta V$$

For HTP to invade then it seems just one or other of the square bracketed terms needs to become positive (since with a little algebra we can prove that this makes $\text{det} < 0$, i.e. $\beta\Gamma\Delta V - \Gamma - \alpha_v - b > 0$; or $\beta\Gamma X - \Gamma - b > 0$).

5. Thus the fifth equilibrium is where all three coexist, this being where both VTP and HTP are able to invade each other.

$$(X, V, Y_v, Y_x) = (X_5, V_5, Y_{v5}, Y_{x5})$$

Evolution.

Firstly we evolve the vertically transmitted parasite.

Equations (1-4) become:

$$X' = (a - qH) \left((X + (1 - \kappa)Yx) + (1 - p)(V + V_1 + (1 - \kappa)(Yv + Yv_1)) \right) - \beta\Gamma X (Yx + (1 - \Delta)Yv + (1 - \Delta_1)Yv_1) - bX \quad (21A)$$

$$V' = p(af - qH)(V + (1 - \kappa)Yv) - \beta\Gamma V (Yx + (1 - \Delta)Yv + (1 - \Delta_1)Yv_1) - (\alpha_v + b)V \quad (22A)$$

$$Yx' = \beta\Gamma (Yx + (1 - \Delta)Yv + (1 - \Delta_1)Yv_1)X - (\Gamma + b)Yx \quad (23A)$$

$$Yv' = \beta\Gamma (Yx + (1 - \Delta)Yv + (1 - \Delta_1)Yv_1)V - ((1 - \Delta)\Gamma + b + \alpha_v)Yv \quad (24A)$$

$$V_1' = p(af_1 - qH)(V_1 + (1 - \kappa)Yv_1) - \beta\Gamma V_1 (Yx + (1 - \Delta)Yv + (1 - \Delta_1)Yv_1) - (\alpha_v + b)V_1 \quad (25A)$$

$$Yv_1' = \beta\Gamma (Yx + (1 - \Delta)Yv + (1 - \Delta_1)Yv_1)V_1 - ((1 - \Delta_1)\Gamma + b + \alpha_v)Yv_1 \quad (26A)$$

Then assuming the $(X, V, Yv, Yx, 0, 0)$ equilibrium is stable equilibrium the invasion success of the mutant VTP depends upon the bottom 2x2 matrix from the

$$\text{Trace (2x2)} = \begin{bmatrix} p(af_1 - qH)V_1 - \beta\Gamma (Yx + (1 - \Delta)Yv + (1 - \Delta_1)Yv_1) - (\alpha_v + b) \\ -[(\Gamma + \alpha_v + b)] \end{bmatrix}$$

$$\text{Det(2x2)} = \begin{bmatrix} - (p(af_1 - qH)V_1 - \beta\Gamma (Yx + (1 - \Delta)Yv + (1 - \Delta_1)Yv_1) - (\alpha_v + b))(\Gamma + \alpha_v + b) \\ - \beta\Gamma (Yx + (1 - \Delta)Yv + (1 - \Delta_1)Yv_1)p(af_1 - qH)V_1(1 - \kappa) \end{bmatrix}$$

If $f_1 > 1$ then the $(af_1 - qH) > 0$

Here for no invasion to occur, requires $\text{Tr} < 0$ and $\text{Det} > 0$. The $\text{det} > 0$ can only be true if

$$p(af_1 - qH)V_1 - \beta\Gamma (Yx + (1 - \Delta)Yv + (1 - \Delta_1)Yv_1) - (\alpha_v + b) < \frac{-\beta\Gamma (Yx + (1 - \Delta)Yv + (1 - \Delta_1)Yv_1)p(af - qH)V_1(1 - \kappa)}{(\Gamma + \alpha_v + b)}$$

So the term on the left has to be negative and so $\text{Tr} < 0$ is automatically satisfied.

So as $\text{Det} > 0$ term fails the mutant VTP can invade.

Evolution of the HTP

We use the same procedure as before and find the trace and determinate for the HTP mutant invasion to be

Then assuming the $(X, V, Y_v, Y_x, 0, 0)$ equilibrium is stable equilibrium the invasion success of the mutant HTP depends upon the bottom 2x2 matrix from the

$$\text{Trace (2x2)} = [\beta_1 \Gamma_1 X - \Gamma_1 - b] + [\beta_1 \Gamma_1 \Delta V - \Gamma_1 - \alpha_v - b]$$

$$\text{Det(2x2)} = [\beta_1 \Gamma_1 X - \Gamma_1 - b][\beta_1 \Gamma_1 \Delta V - \Gamma_1 - \alpha_v - b] - \beta^2 \Gamma_1^2 X \Delta V$$

Here if either of the terms in the Trace becomes positive the determinant condition will be violated. But it could be that the trace is satisfied but the determinant is not so the invasion condition for the HTP is that the determinant condition is violated which can be re-written as the expression (11) in Chapter 4.

B. Chapter 6 Derivation of Host Equilibrium

Where birth rate per individual is represented by r , the number of pupae surviving to reproduce is δ . I can find an expression for the steady state of the host without any natural enemies. I do this by assuming that the number of eggs produce in year τ is equal to those produced in year $\tau+1$, i.e. $E_{(\tau+1)} = E_{(\tau)}$, I then assume that the survivorship of the pupae and subsequent reproduction between the seasons is matched by the loss of individuals in the larvae within seasons. I therefore can express the rate of change of pupae in terms of the neonates surviving the larval stages

$$\frac{dP}{dt} = bE_{(t-TL_1-TL_2)} e^{-(d_1TL_1 + d_2TL_2)} \quad (1B)$$

To solve this we require the solution for $E_{(t)}$. This is found by integration by separation of the variables and partial fractions of equation (1), for the derivation see the appendix.

$$\frac{dE}{dt} = -bE_{(t)} - deE^2_{(t)} \Rightarrow$$

$$-\int_{E_{(0)}}^{E_{(t)}} dt = \int_{E_{(0)}}^{E_{(t)}} \frac{dE_{(t)}}{-bE_{(t)} - deE^2_{(t)}} \Rightarrow t = -\frac{1}{b} \int_{E_{(0)}}^{E_{(t)}} \frac{1}{E_{(t)}} - \frac{de}{(b - deE^2_{(t)})} dE_{(t)}$$

$$-tb = \ln \left(\frac{E_{(t)}(b + deE_0)}{E_0(b + deE_{(t)})} \right) \Rightarrow E_{(t)} = \frac{bE_0e^{-tb}}{b + deE_0(1 - e^{-tb})} \quad (2B)$$

In the above, E_0 is the number of neonates at time $t = 0$. Therefore substituting this expression for the number of neonates at time, t , into the expression for the rate of change in pupae, I can solve the equation through integration and I acquire an expression for the equilibrium value P^* ,

$$\frac{dP}{dt} = \frac{b^2 Eo e^{-(d_1 TL_1 + d_2 TL_2)} e^{-b(t - TL_1 - TL_2)}}{b + de Eo (1 - e^{-b(t - TL_1 - TL_2)})} \quad (3B)$$

which then solves to give

$$P_{(t)} = \frac{b e^{-(d_1 TL_1 + d_2 TL_2)}}{de} \left[\ln \left(\frac{b + de Eo (1 - e^{-b(t - TL_1 - TL_2)})}{b} \right) \right] \quad (4B)$$

As at the end of the season we fix $P_{(t)}$ it is possible to extend $t \rightarrow \infty$, and thus reduces the effect of the delays in the system and thus the equation above reduces to

$$P^* = \frac{b e^{-(d_1 TL_1 + d_2 TL_2)}}{de} \left[\ln \left(\frac{b + de Eo}{b} \right) \right] \quad (5B)$$

and hence

$$Eo = \frac{r \delta b \exp^{-(d_1 TL_1 + d_2 TL_2)}}{de} \left[\ln \left(\frac{b + de Eo}{b} \right) \right], \quad (6B)$$

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