Inference on the host status of feral ferrets (*Mustela furo*) in New Zealand for *Mycobacterium bovis* infection

Peter Caley

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Abstract

This thesis is about making inference on the host status of feral ferrets in New Zealand for *Mycobacterium bovis*, the aetiological agent of bovine tuberculosis. The central question addressed is whether the rate of intra-specific transmission of *M. bovis* among ferrets is sufficient for the disease to persist in ferret populations in the absence of external, non-ferret sources of infection (inter-specific transmission). The question is tackled in three parts—firstly using model selection to identify suitable models for estimating the force of *M. bovis* infection in ferret populations; secondly applying statistical hypothesis testing to the results of planned manipulative field experiments to test the relationship between *M. bovis* infection in brushtail possums and that in ferrets; and thirdly using modelling to estimate intra-specific disease transmission rates and the basic reproductive rate (R_o) of *M. bovis* infection in ferrets.

The model selection approach clearly identified the hypothesis of oral infection related to diet was, as modelled by a constant force of infection from the age of weaning, the best approximation of how *M. bovis* infection was transmitted to ferrets. No other form of transmission (e.g., during fighting, mating, or routine social interaction) was supported in comparison. The force of infection (λ) ranged from 0.14 yr^{-1} to 5.77 yr^{-1}, and was significantly higher (2.2 times) in male than female ferrets.

Statistical hypothesis testing revealed transmission of *M. bovis* to ferrets occurred from both brushtail possums and ferrets. The force of *M. bovis* infection in ferrets was reduced by 88% (λ=0.3 yr^{-1} vs. λ=2.5 yr^{-1}) at sites with reductions in the population density of sympatric brushtail possum populations. A smaller decline in the force of infection resulting from the lethal cross-sectional sampling of the ferret populations was also demonstrated.

The modelling approach estimated the basic reproductive rate (R_o) of *M. bovis* infection in ferrets in New Zealand to vary from 0.17 at the lowest population density (0.5 km^{-2}) recorded to 1.6 at the highest population density (3.4 km^{-2}) recorded. The estimates of R_o were moderately imprecise, with a coefficient of variation of 76%. Despite this imprecision, the R_o for *M. bovis* infection in ferrets was significantly less than unity for all North Island sites surveyed. Hence it is inferred ferrets are spillover hosts ($0<R_o<1$) for *M. bovis* infection in these environments. That is, *M. bovis* infection will progressively disappear from these ferret populations if the source of inter-specific transmission is eliminated. The estimates of R_o for *M. bovis* infection in South Island ferret populations were above one (the level required for disease establishment) for a

number (5/10) of populations, though the imprecision made it impossible to ascertain whether R_o was significantly greater than one. The estimated threshold population density (K_T) for disease establishment was 2.9 ferrets km^{-2}. It is inferred that, given sufficient population density (>K_T), the rate of intra-specific transmission of *M. bovis* among ferrets is sufficient for the disease to establish in ferrets in the absence of inter-specific transmission. In these areas, ferrets would be considered maintenance hosts for the disease. Active management (e.g., density reduction or vaccination) of ferrets would be required to eradicate *M. bovis* from ferret populations in these areas, in addition to the elimination of sources of inter-specific transmission, particularly brushtail possums.

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Chapter 1

Introduction

This thesis makes inference on the role of feral ferrets (*Mustela furo*) in New Zealand as hosts of *Mycobacterium bovis*, the causative (aetiological) agent of bovine tuberculosis. Emphasis is placed on using the results of past and/or current observational studies to develop *a priori* hypotheses of *M. bovis* infection in ferrets. These hypotheses are then compared with independent empirical data by assessing model fit, and the best model(s) is subsequently used in manipulative field experiments to estimate parameters of interest, especially the force of infection (λ) and basic reproductive rate of disease (R_o). The central hypothesis to be evaluated is that *M. bovis* infection will not establish in ferret populations in the absence of an external (non-ferret) source of infection.

The thesis is structured as follows. An overview of methods for making inference on the disease host status is given in Chapter 1, with particular reference to *M. bovis* infection in wildlife. Methods for estimating the instantaneous incidence of *M. bovis* infection (termed the 'force of infection') from field data are developed in Chapter 2. The relationships between the population densities of ferrets and brushtail possums (*Trichosurus vulpecula*) and the force of *M. bovis* infection are examined experimentally in Chapter 3. A modelling approach is employed in Chapter 4, using results described in Chapters 2 and 3 to estimate the basic reproductive rate (R_o) of *M. bovis* infection in feral ferret populations, so that inference can be made on the host status of ferrets for *M. bovis* infection. Finally, an overall discussion of the results is presented in Chapter 5.

1.1 Background of *M. bovis* infection in New Zealand

Mycobacterium bovis has probably been present in New Zealand since the first shipments of domestic cattle in 1840. By the 1940s, *M. bovis* infection was formally recognised as a serious problem in New Zealand dairy herds that reduced productivity and put human health at risk (Jamieson 1960). In 1945, a voluntary scheme involving a diagnostic caudal fold skin test using tuberculin (a purified protein derivative of *M. bovis*) to screen cattle for the disease was introduced for 'town milk' supply dairy herds.

This scheme became compulsory for all town supply dairy herds in 1956, and for all dairy herds in 1961 (O'Neil & Pharo 1995). A national scheme to eradicate *M. bovis* infection from all cattle herds in New Zealand started in 1968. Initial progress in the scheme was promising, with *M. bovis* infection eradicated from most herds. However, in the late 1960s and early 1970s, it became apparent that on the West Coast of the South Island, the central part of the North Island and southeast of the North Island in particular, the scheme was failing to eradicate *M. bovis* infection from cattle. This provided evidence of a non-cattle reservoir of infection, as *M. bovis* infection is readily eradicated from cattle herds using the tuberculin test provided there is no reinfection from other sources (Cousins *et al.* 1998). A search in Buller County on the West Coast for a likely reservoir in wild animals identified *M. bovis*-infected brushtail possums (hereafter simply 'possums') coexisting with several chronically infected local cattle herds.

M. bovis infection was first identified in free-ranging possums in New Zealand in 1967 (Ekdahl *et al*. 1970), though it appears likely that possum populations were infected as early as 1955–1960 (Morris & Pfeiffer 1995). Soon after, infected possums were identified in other widely dispersed areas of New Zealand. In each instance, infected possums coexisted with disease problems in local cattle. An association between *M. bovis* infection in cattle herds and *M. bovis*-infected possums gradually unfolded. Control of possum populations usually resulted in reduction in the incidence of *M. bovis* infection in cattle (Barlow 1991a), and subsequent experiments have shown a causal link between *M. bovis* infection in possums and cattle (Caley *et al*. 1999). How possum populations came to be infected with *M. bovis* is unknown, although *M. bovis*-infected red deer (*Cervus elaphus*) have been postulated to be the principal initiator of infection in possum populations (Morris & Pfeiffer 1995). Indeed, there is an unconfirmed report of what appears to be a tuberculous wild red deer from the west coast of the South Island as early as 1954 (Coleman & Caley 2000).

Possum populations in many parts of New Zealand are now actively managed (usually by lethal forms of control such as poisoning or trapping) in an attempt to control *M. bovis* infection in possums, and hence reduce transmission to livestock. However, species other than possums may also play an important role in the epizootiology of *M. bovis*. One such species is the feral ferret. Ferrets were liberated in New Zealand starting in 1879 in an attempt at biological control of rabbits (*Oryctolagus cuniculus*) (Lavers & Clapperton 1990). Ferrets are specialist predators of rabbits; their population dynamics are closely linked to rabbit abundance (Barlow & Norbury 2001),

and in some situations they may play a part in suppressing wild rabbit populations (Robson 1993). Feral ferrets infected with *M. bovis* were first identified in the 1970s (de Lisle *et al.* 1993). However, they received little attention as a host for *M. bovis* until the early 1990s, when a high prevalence of infection was discovered in ferret populations from areas of the Mackenzie Basin where possums occurred at naturally low population density (Walker *et al.* 1993). At that time, however, *M. bovis*-infected possums had been found in some parts of the Mackenzie Basin. The ferret belongs to the Family Mustelidae, which served to increase suspicions about the role ferrets were playing, as another member of this family, the Eurasian badger (*Meles meles*), was considered to be a maintenance host of *M. bovis* infection in Great Britain (Cheeseman *et al.* 1988). However, scientific opinion remained divided about the significance of *M. bovis* infection in ferrets, and it became clear that better data were needed on the epizootiology of *M. bovis* infection in ferrets (Morris & Pfeiffer 1995), so that wildlife managers could effectively manage *M. bovis* infection in wildlife populations where ferrets were present.

1.2 Roles of wildlife as disease hosts

Whilst nearly all mammals are susceptible to *M. bovis* infection, not all play an important role in the epizootiology of the disease. Determining the role of wildlife as hosts of disease is one of the main issues in the study of diseases and vertebrate pests (Hone 1994a). This role is largely determined by the host status of the species for the disease in question. Commonly used terms to describe the host status of different species for disease include ‘reservoir host’, ‘maintenance host’, ‘spillover host’, ‘dead-end host’ and ‘vector’. It is useful to define these. A reservoir host is defined as ‘one in which an infectious agent normally lives and multiplies, and therefore is a common source of infection to other animals’ (Thrusfield 1997). The term reservoir host is often used interchangeably with maintenance host, though the latter’s definition of ‘An animal that maintains an infection in the latter’s endemic area’ (Thrusfield 1997) is more restrictive in terms of risk of transmission to other species. A reservoir host is essentially a maintenance capable of infecting species other than its own. A definition of a spillover host is apparently missing from epidemiological texts, but its common usage appears to describe a species that may be infected with the infectious agent, and have intra-specific transmission, though not at a sufficient rate for the disease to persist

in that species. Being a spillover host does not negate the possibility of transmission to a second species (inter-specific transmission). A dead-end host is defined as 'one that does not usually transmit an infectious agent to other animals', and is also called an accidental or incidental host (Thrusfield 1997). In the case of spillover and dead-end hosts, *M. bovis* infection will disappear progressively if the disease is eliminated from the species that is acting as the main source. Species are considered of much more importance to the cycle of *M. bovis* if they are maintenance or reservoir hosts of disease. A vector is defined as an 'intermediate host (e.g., arthropod) that transmits the causative agent of disease from infected to non-infected hosts' (Fenner *et al.* 1993). An intermediate host has been defined as 'a host in which a parasite lives for part of its life cycle, but in which it does not become sexually mature' (Holmes 1979). By common usage, vectors are defined as invertebrate animals that transmit infectious agents to vertebrates (Thrusfield 1997). Clearly no vertebrates are true vectors of disease, despite the common use of the term in New Zealand when referring to mammalian wildlife hosts of *M. bovis.*

The two most common questions concerning the management of disease in wildlife are whether species A (usually a wildlife species) transmits infection to species B (commonly humans, domestic livestock or another wildlife species), and whether the infection is cycling independently in the wildlife population in question. To transmit infection to cattle, species A could be either a spillover or reservoir host. For the infection to cycle independently in populations of species A, it must be either a maintenance or a reservoir host.

1.3 Making inference on disease host status

Whilst the definitions of the host status of a species for a particular disease are relatively clear, determining the host status of a species for a particular disease is not so straightforward. Testing causal hypotheses is fundamental to epidemiology (Weed 1986), and epidemiologists have long grappled with philosophical and methodological issues surrounding testing hypotheses of disease causation (e.g., Robert Koch's postulates from 1882 of causation between bacteria and disease (Cartwright & Biddiss 2000)). As a result, analytical methods (e.g., Cox's proportional hazards model for analysis of case-control studies Cox (1972)) and experimental designs (e.g., double-blind trials) have been developed for testing hypotheses of disease causation.

The epidemiological literature is much less helpful, however, when providing guidance for addressing the question 'what is the host disease status of a particular species?' This lack of clear guidelines has led to authors making inference on the disease host status of species using a variety of methods, with resulting lack of agreement among the inferences so made. For example, as early as 1982 some authors (e.g., Henderson 1982; Clifton-Hadley 1996) considered there was irrefutable evidence that *M. bovis* was cycling independently in badger populations in Great Britain, and that *M. bovis*-infected badgers were the cause of tuberculous cattle in south-west England (i.e. badgers were reservoir hosts for *M. bovis*). An apparent positive relationship was reported between badger density and the incidence of *M. bovis* in cattle (McAleer, 1990), and repeated interventions (badger culling) were followed by reductions in the incidence of *M. bovis* infection in cattle, which further supported the model (Clifton-Hadley *et al.* 1995; Clifton-Hadley 1996). This would be considered inductive logic. The veterinary profession considered all the data were consistent with transmission of *M. bovis* from badgers to cattle (the data fitted the model). However, Krebs *et al.* (1998) came to the conclusion there was no strong scientific proof badgers transmit *M. bovis* to cattle, and Hancox (1999) rather uncritically considered there was little evidence badgers were reservoir hosts for the disease. How could such dichotomy of opinion exist? The answer lies as much in the method used to make inference from the available data, as in the data themselves. Krebs *et al.* (1998) took a much stricter line on the inference that could be made with the available data, and basically said that although the model proposed (that badgers transmit *M. bovis* infection to cattle) was quite likely correct, no critical experiments (deductive logic) had been undertaken to test its validity. O'Mairtin *et al.* (1998a,b) subsequently have gone part way to providing such experimental data, supporting the conclusions of Clifton-Hadley *et al.* (1995) and Clifton-Hadley (1996).

Epidemiologists often construct a conceptual model of a disease process and hence host status based on a range of data types, some of which are easily quantifiable (e.g., disease prevalence), intangible (e.g., expert knowledge), highly descriptive though difficult to quantify (e.g., lesion pathology), or evidential (e.g., DNA fingerprinting). For example, despite being confronted with data showing a very high prevalence of *M. bovis* infection in feral pigs (*Sus scrofa*) from the floodplains in Northern Australia, Corner *et al.* (1981) concluded feral pigs were essentially dead-end hosts for *M. bovis* infection in that habitat. They inferred that the observed infection arose from feral pigs scavenging on the carcasses of dead water buffalo (*Bubalus bubalis*) and feral cattle

(*Bos* spp.) known to be infected with the disease (McCool & Newton-Tabrett 1979). This inference was based on the observed distribution of lesions in feral pigs being consistent with an alimentary route of infection, the low prevalence of generalised disease, and the absence of pulmonary (lung) lesions suggesting intra-specific transmission in the wild would be rare (note that intra-specific transmission of *M. bovis* occurs in closely-housed captive pigs (Ray *et al.* 1972)). History shows the inference of Corner *et al.* (1981) was largely correct, as following major reductions in the population density of feral water buffalo brought about during the Brucellosis and Tuberculosis Eradication Campaign (Freeland & Boulton 1990), the prevalence of *M. bovis* infection in feral pigs declined dramatically (McInerney *et al.* 1995). However, strictly speaking, McInerney *et al.* (1995) did not show feral pigs in the Northern Territory were dead-end hosts for *M. bovis* infection, and confirm the prediction of Corner *et al.* (1981). Feral pigs could well have been spillover hosts for the disease. Rather, McInerney *et al.* (1995) demonstrated that most, but not necessarily all the infection observed in feral pigs was derived from an association with infected water buffalo. They assumed that what little infection remained could probably be explained by transmission from the residual infection in the remnant population of buffalo.

Another example (this time involving domestic livestock and humans) of making inference about host status (that subsequently proved to be incorrect) is the bovine spongiform encephalothapy (BSE) epidemic in Britain (Lacey 1994). This was a debate about the disease host status of cattle for BSE. Firstly, regarding intra-specific transmission of BSE in cattle—was it sufficient for the disease to persist in cattle after BSE-contaminated food was removed from the food chain (i.e. were cattle maintenance hosts for BSE in their own right)? Secondly, regarding inter-specific transmission of BSE—were cattle essentially a dead-end host for BSE, or was there the potential for inter-specific transmission (to humans) via the consumption of cattle products? The first UK government committee to report on the BSE outbreak (Southwood 1989) reported, ‘From present evidence, it is likely that cattle will prove to be a ‘dead-end’ host for the disease and most unlikely that BSE will have implications for human health. Nevertheless, if our assessments of these likelihoods are incorrect, the implications would be extremely serious.’ Briefly, the three key conclusions to support the ‘dead-end’ host statement were: (1) BSE was a form of scrapie (the spongiform encephalopathy known in sheep for 100s of years); (2) changes in the processes for rendering cattle feed had allowed the scrapie agent to infect cattle; and (3) scrapie did not infect humans, so neither would BSE. History now shows these assumptions (and

hence the underlying inference) were incorrect—both intra-specific (though possibly not at a rate sufficient for cattle to be considered maintenance hosts) and inter-specific transmission (resulting in an epidemic of variant Creutzfeltdt-Jakob Disease in Britain) were occurring (Coghlan 2000).

A central underlying issue here is the use and interpretation of the term likely, and the related statistical (or model) likelihood as applied to scientific inference. On its own, the estimated statistical likelihood of a model is meaningless, and bears no relationship to the probability of whether a model is correct. Indeed, other than for defined populations (e.g., packs of cards in gaming theory), any concept of a model having a defined probability of being correct (quantitative measure of belief in a model) is flawed and should be rejected (Edwards 1992). However, when it comes to issues of relative support, measures of likelihood form the foundation of sound statistical inference (Edwards 1992). Hence, likelihood-based inference cannot be undertaken in the absence of competing models—a particular model may appear 'likely', but what is critical in terms of scientific inference, is the likelihood relative to other models. Likelihood is the obvious analytical tool for applying Chamberlain (1897)'s acclaimed 'Method of multiple working hypotheses' (an accessible complete reprint of this paper is to be found in Hilborn & Mangel (1997)). Note however, that Kuhn (1962) argues that when developing competing models we are constrained by the dominant scientific paradigm of the time. This was not the case in the BSE outbreak in Britain. Competing models of transmission, some of which in hindsight turned out to be much more likely, had existed from early in the outbreak (Lacey 1994). However, the data initially either did not exist or was not made available (Coghlan 2000) to assess the relative likelihood of these competing models.

These three examples demonstrate that scientific method and philosophy play a big role in how scientists make inference on disease host status, and that critical experiments are often not undertaken. Krebs (2000) suggests ecologists have largely ignored the advice of Popper (1963), who emphasized the importance of hypotheses being falsifiable. This is the cornerstone of what Platt (1964) termed 'strong inference' in science. Platt (1964) recommended that a starting point to making strong inference was to ask 'What experiment would disprove your hypothesis?' or 'What hypothesis does your experiment disprove.' It is useful to evaluate some examples of inference on the host status of wild animals for disease in this light. Chantrey *et al.* (1999) considered they had conclusively demonstrated bank voles (*Clethrionomys glareolus*) were a reservoir host for cowpox virus in Great Britain by showing: (1) A high sero-

prevalence of cowpox virus infection in bank voles; (2) PCR analysis pointing to the same strain of the virus infecting voles and domestic animals; and (3) A worldwide distribution of cowpox that correlated reasonably well with the distribution of voles. Clearly none of these pieces of evidence either individually or in combination would falsify the alternative hypothesis the bank voles were in fact spillover hosts for cowpox virus (or even a dead-end hosts for that matter). In another study, Cleaveland & Dye (1995) used the definition of a reservoir host to make several general predictions for determining the underlying reservoir species of rabies in the Serengeti. They specifically predicted: (1) reservoir host populations should show evidence of persistent infection; (2) cases should occur in the reservoir host in the absence of cases in other species, whereas the converse should not occur; and (3) outbreaks in other species should follow cases in the reservoir host population. These predictions were used to argue that domestic dogs are the likely reservoir of rabies in the Serengeti. Note that none of these predictions match any of the criteria put forward by Chantrey *et al*. (1999) for inferring reservoir host status. Observing the first prediction of Cleaveland & Dye (1995) would not falsify alternative hypotheses of spillover or dead-end host status, as there may be continual inter-specific transmission from the hitherto unrecognised true reservoir host, hence the first prediction is not of much use. Observing the second prediction of Cleaveland & Dye (1995), however, would indeed falsify the spillover or dead-end host status, providing absence of infection from other species can be demonstrated (never an easy task). Likewise for their third prediction, providing cases in the putative reservoir species were not introduced from an unknown source.

Is it possible to better quantify the host status of a species for disease? Greater application/estimation of the basic reproductive rate (R_o) of disease is one possible approach, and the study of Rhodes *et al*. (1998) for determining whether jackals (*Canis* spp.) in Zimbabwe are reservoir hosts for rabies is an example of this. The R_o is defined as the average number of secondary infections produced when one infected individual is introduced to a totally susceptible host population (Anderson & May 1991). By definition, it is predicted in a deterministic model that if R_o is greater than or equal to unity, the disease will establish in the population, and conversely, if R_o is less than unity, the disease will fail to establish (Anderson & May 1991). This concept is often referred to as the fundamental law of epidemiology (or as applied to the study of disease in wildlife—epizootiology), demonstrating the importance of R_o in understanding host/pathogen interactions. It seems logical to make the explicit link between R_o and

disease host status. The four disease host categories discussed previously (Section 1.2) can be quantified simply in terms of R_o (Table 1.1).

Table 1.1. Relationship between the basic reproductive rate (R_o) of a disease and commonly used disease host status categories.

	Host status			
	Dead-end	Spillover	Maintenance	Reservoir
R_o	0	$0<R_o<1$	≥ 1	≥ 1

The prediction of disease establishment ($R_o \geq 1$) does not necessarily equate with that of persistence, and hence maintenance or reservoir host status. High levels of disease-induced mortality, or recovery from disease with subsequent immunity, may result in disease 'fade-out' (Anderson & May 1991). This result is most likely for small, or isolated populations, so is really an issue of scale. Examples of fade-out in the literature include classical swine fever in wild boar (Hone *et al.* 1992), and measles infections in humans in small cities (Anderson & May 1991). On a larger scale, however, humans are clearly maintenance hosts for measles.

Estimating R_o quantitatively enables hypotheses regarding the host status of a species to be tested within a statistical framework, by enabling the relative likelihoods of possible outcomes to be examined. The use of statistical testing of hypotheses in wildlife research has been criticised in the past, and again more recently, particularly when testing point null hypotheses in descriptive studies (e.g., H_o: $\mu=\mu_o$ *vs.* H_1: $\mu\neq\mu_o$) (Cherry 1998; Johnson 1999). Johnson (1999) specifically criticised the testing of point null hypotheses when 'Certainly we knew before any data were collected that the null hypotheses being tested were false'. This rather cynical view of scientific method assumes reliable knowledge has been gained even before the experimenter has collected any data! This is contrary to the hypothetico-deductive scientific method which Popper (1963) showed to be so powerful for gaining knowledge, and greater use of which has been advocated (Romesburg 1981). Indeed, Peterson (1991) advocated more use of the hypothetico-deductive scientific method for gaining reliable knowledge of host-parasite interactions in wildlife species.

I consider that a greater emphasis on estimating R_o is warranted to determine the disease host status of wildlife as this provides an important link between theory and practice (in this case the fundamental law of epidemiology and resulting important theories such as threshold host population size for epidemic occurrence, and minimum vaccination or culling rates for disease eradication). Models that explicitly estimate R_o

are of great practical use for managing disease, as they can indicate how R_o may be lowered to less than unity by changing key biological parameters, and they can also show how much R_o needs to be changed (reduced). For example, this could involve reducing the susceptible wildlife host population, either by culling or vaccination, as modelled by Anderson *et al.* (1981) for fox rabies in Europe. In humans, for example, models of R_o may indicate the proportion of children who need be vaccinated to prevent measles epidemics (Anderson & May 1982).

Despite its fundamental importance to the understanding of host-pathogen interactions, R_o has historically received only limited theoretical treatment in standard ecological (e.g., Begon *et al*. 1989) or wildlife management (e.g., Caughley & Sinclair 1994; Wobeser 1994) texts. More recent texts (e.g., Begon *et al.* 1996; Krebs 2001) give a more detailed treatment of role of R_o in understanding host-pathogen interactions. Defining R_o is one thing, but estimating it is an entirely different matter. There is no discussion of estimating transmission coefficients in the 'gold standard' of ecological methods texts (Krebs 1989; Krebs 1999). Ecological modelling texts such as Gillman & Hails (1997) give precious little guidance on the practice of estimating R_o (though do give some theory). Hone (1994a), however, provides a useful summary of methods various authors have used for estimating disease transmission coefficients (needed for estimating R_o), and McCallum (2000) provides advice on how to estimate the parameters (especially the critical transmission coefficients) needed to estimate R_o. Briefly, McCallum (2000) describes how transmission coefficients may be estimated by relating the rate at which individuals acquire infection (the 'force of infection') to the number (or density) of infectious individuals, via an appropriate model of transmission.

1.4 Host status of New Zealand mammals for *M. bovis*

The host range of *M. bovis* is large in mammals (O'Reilly & Daborn 1995) and the pathogen has been isolated from a large number of free-living mammal species in New Zealand (Table 1.2). I expect the New Zealand list will continue to grow the harder people look, particularly in some of the deer species such as sambar (*Cervus unicolor*), rusa (*Cervus timorensis*) and white-tailed deer (*Odocoileus virginianus*).

Table 1.2. Free-living mammal species in New Zealand from which *M. bovis* has been isolated (excluding domestic cattle).

Species	Source
Brushtail possum	Ekdahl *et al.* (1970)
Brown hare (*Lepus europaeus occidentalis*)	Cooke *et al.* (1993)
European hedgehog (*Erinaceus europaeus occidentalis*)	Lugton *et al.* (1995)
European rabbit	Gill & Jackson (1993)
Fallow deer (*Dama dama*)	de Lisle & Havill (1985)
Feral cat (*Felis catus*)	de Lisle *et al.* (1990)
Feral ferret	de Lisle *et al.* (1993)
Feral goat (*Capra hircus*)	Allen (1987)
Feral pig	Ekdahl *et al.* (1970)
Red deer	de Lisle & Havill (1985)
Sheep (*Ovis aries*)	Davidson *et al.* (1981)
Sika deer (*Cervus nippon*)	de Lisle & Havill (1985)
Stoat (*Mustela erminea*)	Ragg *et al.* (1995a)

Morris & Pfeiffer (1995) considered that other than possums, wild deer (*Cervus spp.*) and feral ferrets, all other wild mammal species recorded as having been infected with *M. bovis* infection in New Zealand were either spillover or dead-end hosts for *M. bovis* infection. They considered it likely that wild deer are reservoir hosts, despite tuberculous possums being the source of most of the *M. bovis* infection in wild deer (Lugton *et al.* 1997a), but considered that lack of data prevented a definitive judgment of the host status of ferrets. Coleman & Cooke (2001), in a review of the literature, reached similar conclusions, though additionally categorized a number of species (e.g., feral pigs) as amplifier hosts. I will now consider in more detail the host status of possums, deer, ferrets and lagomorphs in New Zealand for *M. bovis* infection.

1.4.1 *Brushtail possums*

The common brushtail possum is considered to be the major wildlife reservoir of *M. bovis* in New Zealand. There now appears little scientific opposition in New Zealand to possums being considered maintenance (and reservoir) hosts for *M. bovis* infection, and scientific debate has centred on how best to control and/or eradicate *M. bovis* infection from possum populations (Barlow 1991a,b; Barlow 1993; Barlow 1994; Barlow 1996; Roberts 1996; Caley *et al.*1999). This acceptance of possums as reservoir hosts has occurred despite there being little or no rigorous testing of the hypothesis that

possums are indeed a reservoir host, and despite early speculation that cattle may be transmitting *M. bovis* to possums (Coleman 1988).

Two authors have estimated the R_o of *M. bovis* infection in possum populations, though neither provided any measure of uncertainty for the estimates. Barlow (1991b) estimated R_o to be in the range 1.8–2.0 (the variation arising from a range of choices for parameters used to calculated R_o, rather than an estimate of the uncertainty surrounding R_o), and Roberts (1996) estimated R_o to be 1.6. Both these authors estimated R_o using models that already assumed the disease was independently cycling (and persisting) in possum populations. In particular, prevalence was assumed to be both non-zero and at a steady state, and transmission from other species was ignored. Disease establishment therefore was already a given, so the resulting estimates of R_o could only be greater than or equal to unity. Only Morris & Pfeiffer (1995) explicitly addressed the question of the disease host status of possums, but did not attempt to estimate R_o. Instead, they listed evidence they considered to be conclusive that the possum met the requirements to be considered a reservoir host. Most of the evidence they presented is either descriptive or correlative, and the inference so made is essentially based on 'a coherent epidemiological pattern of spatial and temporal distribution of infection with specific restriction endonuclease types of tuberculosis in possums, consistent with the long-term maintenance of infection in groups of possums and the transfer of infection to other species, without maintenance in some of those secondary species (such as cattle).' I for one believe they are correct to choose a working model that has possums as maintenance hosts for *M. bovis* infection, as it adequately explains most of what we see. However, this inference is largely drawn from inductive rather than deductive logic. Epidemiology has largely neglected the latter, despite its obvious superiority in terms of strength of inference (Weed 1986).

1.4.2 *Wild deer*

At the high population densities achieved on deer farms, fallow and red deer may be maintenance hosts for *M. bovis*, with rapid spread among captive deer observed (e.g., Robinson *et al.* 1989) and repeated tuberculin testing (and the removal of identified infected individuals) is required for disease eradication (O'Neil & Pharo 1995). However, the population density of free-ranging wild deer species in New Zealand is several orders of magnitude lower than on deer farms, and it is unclear whether the disease will persist in deer at this low density, despite high disease

prevalence being observed in wild deer (Lugton *et al.* 1998). It appears the free-ranging white-tailed deer in the eastern USA are acting as maintenance hosts for *M. bovis* infection, and this is hypothesized to be a result of high deer densities caused by a removal of natural predators and supplementary feeding (Schmitt *et al.* 1997).

1.4.3 *Ferrets*

The host status of ferrets has been subject to considerable debate that has focussed on whether ferrets transmit *M. bovis* to livestock (i.e. they act as reservoirs of infection) (Walker *et al.* 1993; Sauter & Morris 1995; Ragg *et al.* 1995a) and whether *M. bovis* is capable of cycling independently in ferrets (i.e. they are maintenance hosts of *M. bovis*) (Morris & Pfeiffer 1995; Lugton *et al.* 1997b; Caley 1998; Qureshi *et al.* 2000; Caley *et al*. 2001b). The answers to these questions determine whether active management of ferret populations (and of what form) is needed for the long-term control and hoped for eventual eradication of *M. bovis* infection from New Zealand wildlife. Those from a practitioner's background (e.g., Walker *et al.* 1993) early on considered there was sufficient evidence implicating ferrets as both reservoir and maintenance hosts. In essence, they promoted use of the precautionary principle (Deville & Harding 1997)—that ferret populations should be managed until proven otherwise. Ragg *et al.* (1995a) echoed these thoughts. The precautionary principle is generally thought of as applying to issues of environmental protection (see definition in Deville & Harding (1997)); however, it is rapidly gaining currency in a range of fields, particularly food safety (see Anonymous (2000) for a recent example concerning BSE and food safety in Britain).

In a review of available evidence combined with their belief that the dominant paradigm of *M. bovis* transmission within maintenance host species (aerosol transmission) applied to ferrets, Morris & Pfeiffer (1995) considered ferrets were most likely spillover hosts (cf. maintenance hosts), but considered lack of data prevented a definitive judgement of their host status. Sauter & Morris (1995) considered *M. bovis*-infected ferrets posed a lower risk (per individual) to cattle than did *M. bovis*-infected possums (i.e. they were unlikely to act as reservoirs of infection).

Despite the presence of lively debate, few published studies have directly addressed the host status of ferrets for *M. bovis* infection. Ferrets infected with *M. bovis* have been found in many parts of New Zealand, with infection highly prevalent in many areas in both the North and South Islands (de Lisle *et al.* 1993; Walker *et al.* 1993;

Ragg *et al.* 1995a; Caley 1998). In nearly all cases, *M. bovis*-infected possums are also known to occur in the same general area (de Lisle *et al.* 1993). Ferrets scavenge extensively, and although their diet consists mainly of rabbit, dead and possibly live possums are also eaten (Roser & Lavers 1976; Smith *et al.* 1995; Ragg 1998a). As ferrets are highly susceptible to *M. bovis* infection, administered either orally or subcutaneously (Dunkin *et al.* 1929), their scavenging of *M. bovis*-infected carcasses, or predation of *M. bovis*-infected prey is very likely to result in infection. It is common to find *M. bovis* infection in carnivores or omnivores associated with endemic infection in other host species. For example, Bruning-Fann *et al.* (2001) isolated *M. bovis* from coyote (*Canis latrans*), red fox (*Vulpes vulpes*), bobcat (*Felis rufus*), raccoon (*Procyon lotor*) and black bear (*Ursus americanus*) populations in an area where the disease appears to be cycling in free-ranging white-tailed deer populations (Schmitt *et al.* 1997). Likewise, *M. bovis* has been isolated from predators and scavenging species such as lions (*Panthera leo*) (Keet *et al.* 1996) and chacma baboons (*Papio ursinus*) (Keet *et al.* 2000) from areas where African buffalo (*Synercus caffer*) are endemically infected with *M. bovis* (Rodwell *et al.* 2001).

The pathology of *M. bovis* infection in feral ferrets indicates that infection is predominantly via the alimentary route (Ragg *et al.* 1995b; Lugton *et al.* 1997c), suggesting that infection arises from ingestion of infective material (Ragg *et al.* 1995b; Lugton *et al.* 1997b). While *M. bovis* has been isolated from the mammary glands of a small proportion of infected lactating females (Lugton *et al.* 1997b), the general absence of infection in very young ferrets has been used to infer mother-to-offspring transmission via suckling (pseudo-vertical) is not a significant transmission pathway (Lugton *et al.* 1997b). Similarly, because pulmonary lesions are uncommon by comparison with lesions associated with the alimentary tract, respiratory transmission is considered to be rare. *M. bovis* bacilli can be isolated from the mouths and tracheal washes of infected individuals, and percutaneous infection originating from bites probably also occurs, but much less frequently than via the alimentary route (Lugton *et al.* 1997b). This led Lugton *et al.* (1997b) to consider *M. bovis*-infected possum carcasses to be the major source of *M. bovis* infection for ferrets. However, ferrets have been observed to scavenge the carcasses of dead ferrets (Ragg *et al.* 2000), and ferret hair has been found in ferret scats to support this observation (Ragg 1998a). Hence the alimentary route of infection is also consistent with intra-specific transmission through ferrets scavenging on *M. bovis*-infected ferret carcasses.

It is unclear whether the rate of intra-specific transmission is high enough for ferrets to be maintenance hosts for the disease (Lugton *et al.* 1997b; Caley 1998; Caley *et al.* 2001b). Ragg *et al.* (1995a) suggested ferret populations might be maintenance hosts of *M. bovis* in areas of high ferret population density. This suggestion is based on the implicit hypothesis that at high densities of ferrets, the prevalence of the disease is increased due to a higher *per capita* rate of intra-specific transmission. Also implicit in this suggestion is that ferrets are not maintenance hosts where ferret population density is low. This implies that R_o may be greater than unity in some habitats, though not others, which is analogous to habitat-related variability in an individual's net reproductive rate (R) (Krebs 2001). Qureshi *et al.* (2000) echoed the sentiments of Ragg *et al*. (1995a) after observing intra-specific transmission of *M. bovis* in captively housed ferrets (the perils of extrapolating from captive situations to the field should not require repeating!). In contrast, Lugton *et al.* (1997b) used a descriptive epidemiological study to conclude that current evidence and logic does not support the notion that ferrets are a reservoir host for bovine tuberculosis in New Zealand.

Caley (1998) demonstrated a positive association (R^2=0.92) between the population density of possums (not necessarily tuberculous) and the prevalence of macroscopic *M. bovis* infection in feral ferrets. There was no association between the population density of ferrets and the prevalence of macroscopic *M. bovis* infection in feral ferrets. A simple linear regression model predicted it would be unlikely for macroscopic *M. bovis* infection to occur in ferrets in the absence of possums (i.e. solving the regression for x=0). Caley (1998) cautioned that the simple regression model used to identify the contribution of possums to *M. bovis* infection in ferrets may be inappropriate to assess their host status. In particular, there is no clear justification (either theoretical or empirical) as to what form (straight line, curve, etc.) the relationship between prevalence of macroscopic *M. bovis* infection in ferrets and possum abundance should take. The relationship between the prevalence of *M. bovis* infection in badgers and badger density has been modelled as a convex-up curve (Anderson & Trewhella 1985) (though note this is a one host/one pathogen system compared with the two host/one pathogen system that is of interest here). The inclusion of data additional to that of Caley (1998) changed (lowered) the slope of the resulting regression line substantially (though the regression remained highly significant (R^2=0.80, P<0.001), with an increase in the y-intercept (the predicted prevalence of macroscopic *M. bovis* infection in ferrets in the absence of possums) to above (1.2%), though not significantly different from, zero (95% CI –2.0–4.4%, P=0.47) (Caley *et al*.

2001b). This result was interpreted as meaning it is uncertain whether the extent of intra-specific transmission among ferrets is of any consequence. This is not to say that a low prevalence of disease implies ferrets are unlikely to be maintenance hosts, as the prevalence of disease in maintenance hosts may be very low (e.g., 2%), as for example in possums (Coleman & Caley 2000). Similarly, inferring the likely host status of ferrets (or any other species for that matter) based on a frequently observed high prevalence of disease may be flawed, as a high prevalence of disease does not imply maintenance host status—take *M. bovis* infection in feral pigs as an example (McInerney *et al*. 1995). Clearly, existing disease prevalence data are insufficient for making strong inference on the host status of ferrets for *M. bovis*.

Finally, the epidemiological 'picture' of *M. bovis* infection in feral ferrets appears to be very different from that of known tuberculosis maintenance hosts such as badgers, cattle, possums and humans (for *M. tuberculosis*) (Morris *et al.* 1994). For all these species, pulmonary involvement is high, and transmission is postulated to be predominantly via the respiratory route, with the lung considered the site of predilection (Morris *et al.* 1994; Gallagher *et al.* 1998). Indeed, belief in the importance of respiratory transmission could be considered the dominant paradigm governing the epidemiology of *Mycobacterium* infections with respect to intra-specific transmission in maintenance hosts. In contrast, the epizootiology of *M. bovis* infection in feral ferrets most closely resembles that in feral pigs, which are considered to be a spillover host (Morris & Pfeiffer 1995). Comparative logic would suggest that ferrets are spillover hosts also. However, McArdle (1996) cautions that logic does not rank far ahead of anecdote (the lowest form of evidence) in terms of the strength of inference that can be made from it. Logic does, however, help formulate hypotheses to be tested.

1.4.4 *Lagomorphs*

Lagomorphs (rabbits and hares) are not considered potential maintenance hosts for *M. bovis* (Morris & Pfeiffer 1995). However, they are the most important prey item of feral ferrets (Lavers & Clapperton 1990), and the pathology of *M. bovis* infection indicates ferrets are infected predominately via ingestion. It is reasonable therefore to consider *M. bovis*-infected rabbits and/or hares as a source of infection for ferrets. Spontaneous tuberculosis in laboratory rabbits is extremely rare, which is extraordinary given they are extremely susceptible to experimental *M. bovis* infection (Francis 1958). Worldwide there is only one recorded case of *M. bovis* in a free-living wild rabbit—you

guessed it, from New Zealand (Gill & Jackson 1993)! Likewise, there is only one recorded case of *M. bovis* infection in the European brown hare (Cooke *et al*. 1993)—again from New Zealand. Clearly, however, the number of cases will be highly dependent on how hard people have looked, and the saying 'absence of evidence is not evidence of absence' may have some currency here. However, during the early 1990s, several thousand rabbits in the Mackenzie Basin were examined without success for evidence of *M. bovis* infection (R. Walker, personal communication), whilst trying to source the high prevalence of *M. bovis* infection in sympatric feral ferrets. Likewise, in my own work I have examined hundreds of rabbits without finding any visible (macroscopic) signs of *M. bovis* infection. The counter argument, of course, is that despite possums being a maintenance host of *M. bovis*, it is common to examine hundreds, and sometimes thousands of possums before finding the first *M. bovis* infected case! This is a result of the highly spatially aggregated distribution of the disease in possums (Coleman & Caley 2000), and its typical low prevalence over a broad scale (e.g., Pfeiffer *et al.* (1995) recorded 1.3% of 6083 possums examined as being macroscopically infected with *M. bovis*).

The pathology of the *M. bovis*-infected rabbit reported by Gill & Jackson (1993) precluded determination of the route of infection. Cooke *et al*. (1993) suggested that the remarkable absence of authenticated cases of *M. bovis* infection in free-living lagomorphs must be caused by species-specific and ecological factors that prevent effective contact between them and diseased animals. At this stage, I think it is reasonable to assume that *M. bovis* infection is uncommon enough in lagomorphs to be ignored, at least for the time being, as a source of *M. bovis* infection for ferrets. I realise that dismissing lagomorphs as a significant source of *M. bovis* infection for ferrets is done in the absence of sufficient data to assess the relative likelihood of this model (ferret/rabbit/*M. bovis*) of inter-species transmission.

1.5 The current study

There is clearly a need to better determine the host status of ferrets in New Zealand for *M. bovis* infection. Quantification of R_o of *M. bovis* infection in ferret populations is one way of achieving this, and this is attempted in this thesis by a combination of observations, field estimates of disease transmission rates in a large-scale experiment, and modelling. Such a linking of theory and data has not been clearly

demonstrated for *M. bovis* infection in other wildlife hosts, with the possible exception of *M. bovis* infection in badgers. Estimating transmission rates and associated model parameters of microparasites is an essential component of understanding host-pathogen dynamics, and remains a great challenge in ecology (McCallum *et al.* 2001), often resulting in a paucity of data (DeLeo & Dobson 1996). This thesis aims to test the null hypothesis that feral ferrets are dead-end (R_o=0) hosts of *M. bovis*. The alternative, or working, hypotheses are that ferrets are spillover ($0<R_o<1$) or maintenance ($R_o \geq 1$) hosts. The issue of whether *M. bovis*-infected ferrets transmit *M. bovis* to livestock (act as reservoirs of infection) is not addressed.

Chapter 2

Estimating the force of *Mycobacterium bovis* infection in feral ferrets

2.1 Introduction

This chapter is concerned with estimating the force of *M. bovis* infection in feral ferret populations in New Zealand from age-prevalence data, and using the data to infer the likely pattern of disease transmission. These data are needed for assessing the host status of ferrets (Chapter 4). The bulk of this chapter is contained in Caley & Hone (2002), shown in Appendix 6.8. Estimating rates of disease spread and associated model parameters of microparasites is an essential component of understanding host-pathogen dynamics, and remains a great challenge in field ecology (McCallum *et al.* 2001). Estimating 'the force of infection, λ' (Muench 1959), and relating it via a model to host density and the relative population density of susceptible and infected animals, in combination with other demographic parameters, is one practical approach for estimating transmission rates (McCallum *et al*. 2001). In New Zealand, *M. bovis* infection is prevalent in many feral ferret populations (Caley 1998). The brushtail possum is a known maintenance host for the disease in New Zealand (Coleman & Caley 2000), in a similar role to that played by the Eurasian badger in Britain (Cheeseman *et al*. 1989). However, it is unclear whether ferrets are maintenance hosts for the disease (i.e. the disease is capable of cycling independently in ferret populations in the absence of external [non-ferret] sources of infection), or whether the observed disease is simply a spillover from possum populations (see Chapter 1). Determining the host status of ferrets requires estimating disease infection rates. Hence methods for estimating the force of *M. bovis* infection in ferrets are needed.

Previous inference regarding *M. bovis* infection in feral ferrets has been based on estimates of point prevalence, which is simply the proportion of animals diagnosed as being infected at the time of survey. For example, Caley (1998) observed the prevalence of macroscopic *M. bovis* infection in ferrets from 11 sites in New Zealand to range from 0 to 31.6%, and reported a positive correlation between the prevalence of infection in ferrets and possum population density but no correlation with ferret population density. However, there are limitations to the utility of point prevalence estimates alone for making epidemiological inference. The prevalence of *M. bovis* infection in ferrets is highly age-specific, with a higher proportion of adults infected

than juveniles (Lugton *et al*. 1997b). *M. bovis* infection in ferret populations can be better quantified by using age-prevalence data to estimate the instantaneous rate at which feral ferrets acquire *M. bovis* infection. In epidemiological terms, λ is the *per capita* rate of acquiring disease (Anderson & May 1991). In survival analysis, λ is analogous to the hazard rate (the hazard being becoming infected). Observing how the prevalence of disease changes with increasing age provides a starting point for estimating the rate of disease transmission, as age can be used as a surrogate for time (Grenfell & Anderson 1985). Indeed, age-prevalence data are considered of great value in the determination of the net force of infection within host communities (Anderson & Trewhella 1985). Furthermore, different forms of transmission (e.g., vertical vs. horizontal) may result in the prevalence of infection changing with age in different ways (different shaped curves), which can be related to different underlying hazard models. Thus for some diseases it should be possible to use observed age-specific prevalence data to screen for adequate candidate models of disease transmission.

The development of an *a priori* set of candidate models before undertaking any data analysis and model fitting has been recommended (Hilborn & Mangel 1997; Burnham & Anderson 1998). Following this approach, this chapter develops an *a priori* set of hypotheses of how *M. bovis* is transmitted to ferrets. Transmission of *M. bovis* to ferrets has been postulated to occur by routes including pseudo-vertical through suckling (as opposed to true vertical transmission across the placenta) (Lugton *et al*. 1997b), horizontal-direct through routine social activities (den-sharing, etc.) (Ragg 1998b), horizontal-direct through fighting (Lugton *et al*. 1997b), and scavenging on *M. bovis* infected carcasses (Ragg *et al.* 1995b; Lugton *et al*. 1997b; Ragg *et al*. 2000). These possible routes of transmission can be thought of as *a priori* hypotheses of the underlying transmission mechanisms of *M. bovis* among ferrets. Not explicitly stated by any author, but one commonly considered in the transmission of disease is that of environmental contamination. The hypotheses, none of which are mutually exclusive, are spelt out as follows.

Hypothesis 1 (H1): Transmission occurs from mother-to-offspring (pseudo-vertically) during suckling until the age of weaning, which occurs at 1.5–2.0 months of age (Lavers & Clapperton 1990).

Hypothesis 2 (H2): Transmission occurs during mating and fighting activities associated with it, from the age of 10 months when the breeding season starts (Lavers & Clapperton 1990).

Hypothesis 3 (H3): Transmission occurs during routine social activities such as sharing dens simultaneously from the age of independence, estimated to be at 2.0–3.0 months (Lavers & Clapperton 1990).

Hypothesis 4 (H4): Transmission occurs through scavenging/killing tuberculous carrion/prey from the age of weaning (1.5–2.0 months of age).

Hypothesis 5 (H5): Transmission occurs through environmental contamination from birth.

These hypotheses correspond to various hazard functions, where the hazard represents the instantaneous probability of becoming infected (schematically shown in Fig. 2.1), and equate directly to λ. The possible combinations of five hypothesized underlying hazards (assumed to be additive as they are not mutually exclusive) yields many possible hypotheses for how the force of *M. bovis* infection may vary with age (H5–H12, Fig. 2.1). Note that H5 may also potentially arise through combinations of either H1 and H3 or H1 and H4. Just how many hypotheses there are depends on the relative size of the different baseline hazards (Note that when plotting the combined hazard functions, I have not distinguished between Hypotheses 3 & 4). For example, depending on whether the hazard for H1 is higher or lower that H3 or H4 determines whether the resulting hypothesized hazard function takes on the shape of H5, H7 or H8. These various hypotheses may be represented by different mathematical models of infection that fulfil the requirement of Burnham & Anderson (1998) that candidate models must make biological sense. I personally prefer the approach of Hilborn & Mangel (1997) of using models to represent hypotheses, compared with the reverse as suggested by Underwood (1997) for example.

The models can be fitted to (or 'confronted with' as Hilborn & Mangel (1997) would put it) the data as a 'test' of the competing hypotheses, although this is not hypothesis testing in the usual falsification sense. It is an exercise in determining which model is most likely, given the data and the number of parameters fitted, not which model is truly correct, as there can never be a fully correct model, only a best approximating model (Burnham & Anderson 1998). This model-fitting approach for making inference has been termed 'post-Popperian' science, and contrasts with classical reductionist experiments with emphasis on falsification, which for some workers in the biological sciences, can seem too constraining (Walker 1998) (though note that Edwards (1992) muses that his 'Method of Support' [the foundation of likelihood-based approaches to inference] is not greatly at variance with the views of Popper (1959)). I then go on to use the best model as a means of comparing the force of *M. bovis*

infection in ferrets between sites and sexes, as a precursor to using the model to estimate inter- and intra-specific transmission rates of *M. bovis* to ferrets from manipulative studies (Chapter 4).

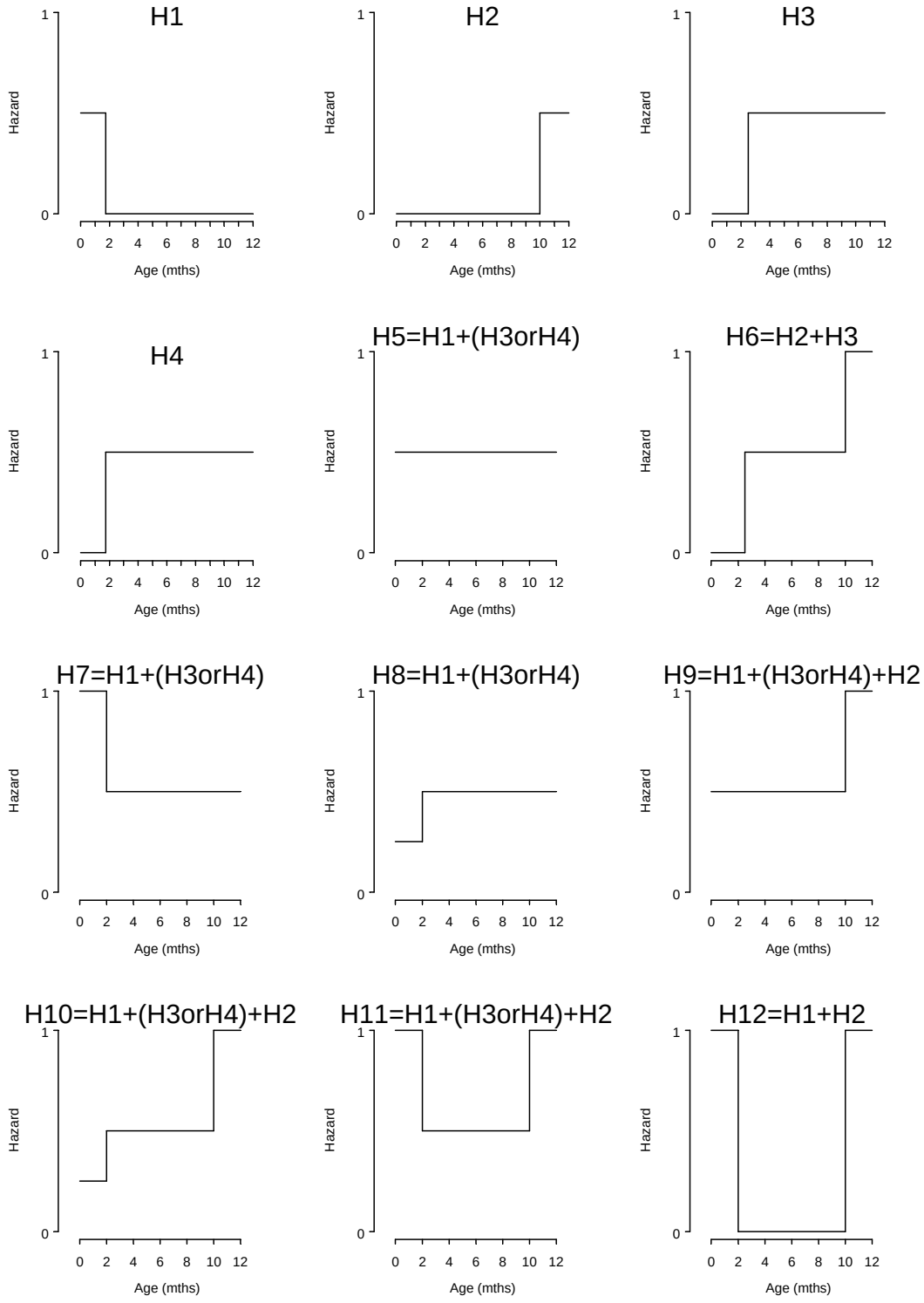

Figure 2.1. Schematic representation of hypotheses 1 to 5 (H1–H5) for transmission of *M. bovis* infection to feral ferrets, in terms of baseline hazard functions. Hypotheses 6 to 12 (H6–H12) represent composite hazard functions arising from the baseline hazard functions. The scaling of the y-axis is arbitrary.

2.2 Methods

2.2.1 *Selection of study sites*

This chapter uses data collected from cross sectional surveys of *M. bovis* infection in feral ferrets at five sites in New Zealand (Fig. 2.2). Sites were primarily selected for survey on the basis that *M. bovis* occurred in wildlife. This was inferred either from previous wildlife surveys undertaken at these sites, or from tuberculin testing of cattle herds at the sites. In the absence of buying-in *M. bovis*-infected stock, persistent infection of cattle herds with *M. bovis* despite regular (yearly or more frequent) testing (with removal of infected individuals) provides strong evidence of a non-bovine source of infection. In the known absence of such sources of infection, such a testing regime causes the rapid eradication of *M. bovis* from cattle (Cousins *et al*. 1998). Sites were deliberately chosen to sample a range of possum and ferret densities. In the case of possum population density, this was low at Lake Ohau in the Mackenzie Basin that has a naturally sparse population of possums, moderate at Scargill Valley in North Canterbury, and high at Awatere Valley in Marlborough and the Castlepoint and Cape Palliser study sites in the coastal Wairarapa. Possum and ferret population density are in general inversely related (Caley 1998). In New Zealand, ferrets occur at highest densities in semi-arid regions where their principal prey species (rabbits) are most abundant, whereas possums tend to be more abundant in areas of at least moderate rainfall (P. Caley, unpublished data). The Scargill Valley site was subject to intensive culling of ferrets following initial cross-sectional surveys (Chapter 3). As the effect of culling was unknown (though see Chapter 3), only data collected during the initial surveys were included for analysis. For other sites that were subjected to repeated surveys (e.g., Castlepoint), the numbers of ferrets removed in each survey were considered insignificant (this is addressed further in Chapter 3); hence data from all surveys were included for analysis. The Castlepoint and Scargill Valley sites were subject to intensive possum control from 1998 (see Chapter 4). To avoid confounding, only data collected before the possum control intervention were included in the analysis. For the purpose of analysis, factors considered to possibly influence the force of *M. bovis* infection in ferrets (specifically site) were assumed to be constant over time. Becker (1989) points out that from a single cross-sectional survey, any effects of age and time on λ are confounded, so whether λ is age-dependent (i.e. $\lambda(a)$), time-dependent (i.e. $\lambda(t)$) or both (i.e. $\lambda(a,t)$) cannot be determined.

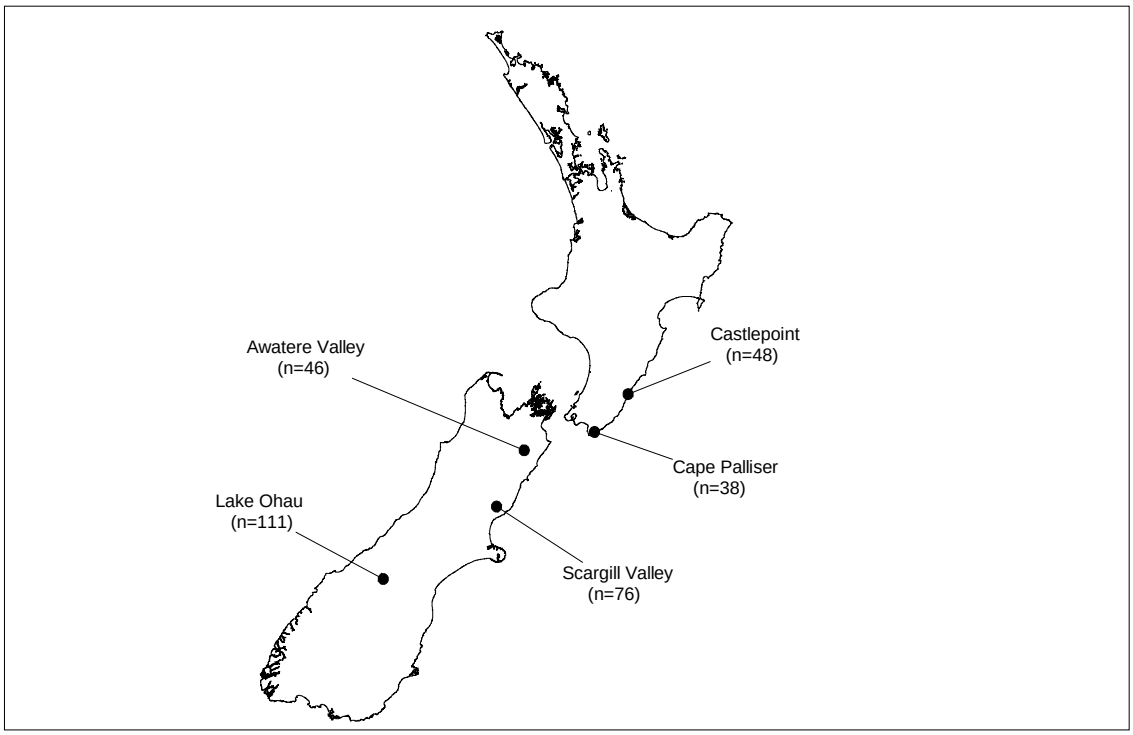

Figure 2.2. Location of sites of cross-sectional surveys of *M. bovis* infection in feral ferrets in New Zealand. Sample sizes are shown in parentheses.

2.2.2 *Data collection*

Ferrets were captured in Victor Soft-Catch® leg-hold traps (size 1½) baited with fresh rabbit, hare or domestic chicken meat. Traps were set at approximately 200-m intervals, usually for 5–10 nights, and checked daily. Animals were humanely killed at the trap site where they were captured. Bait was replaced as needed. Traps were located in all areas of each study site thought most likely to be frequented by ferrets, particularly areas of highest rabbit density. All fieldwork procedures were approved by the Landcare Research Animal Ethics Committee (Approval Project No: 98/10/4).

2.2.3 *Diagnosis of M. bovis infection*

At the time of the surveys, there was no reliable (high specificity and high sensitivity) non-lethal diagnostic test for *M. bovis* infection in feral ferrets (Chinn *et al*. 1995). From each ferret caught, the jejunal (mesenteric), both caudal cervical (prescapular), and both retropharyngeal lymph nodes were collected. All other major peripheral lymph nodes and internal organs were examined, and a portion of any suspect lesion was added to the lymph node pool, which was stored frozen. Diagnosis of *M. bovis* infection in ferrets was made from bacterial culture of the pooled lymph

node samples, whatever the animal's apparent disease status. There is an unknown period between infection and positive diagnosis based on the bacterial culture of pooled lymph nodes. However, because of the high sensitivity of modern bacterial culture techniques, and the collection of all the lymph nodes considered to be the sites of predilection, this period was assumed to be negligible (G. de Lisle, personal communication).

2.2.4 *Estimating ferret age*

Each winter, ferrets were assumed to lay down a dense cementum layer, which becomes visible as a distinct band from spring onwards. Ferret age was initially estimated to the nearest year by counting cementum annuli in sections of a lower canine tooth (Grue & Jensen 1979). The age of each animal was then calculated to the nearest month, from the date of capture and seasonality of breeding, with all ferrets assumed to have been born on 30 October. This date was arrived at by estimating the median birth date of juveniles caught during February trapping sessions using the growth curve for European polecats (*Mustela putorius*) (Shump & Shump 1978).

2.3 Model specification

Mathematical models were used to represent the various hypotheses of disease transmission among ferrets, and model selection used as a method of choosing the best hypothesis (or best working model) (Hilborn & Mangel 1997; Burnham & Anderson 1998). For each hypothesis, I consider candidate mathematical models first with, and then without disease-induced mortality (α). Few data exist on the disease-induced mortality rate of *M. bovis* infection in feral ferrets. Disease-induced mortality rates arising from *M. bovis* infection in other wildlife species are variable. Though not measured, *M. bovis* infection in feral pigs is considered to cause negligible mortality, as the disease is usually reasonably well contained and rarely spreads to vital body organs (Corner *et al*. 1981). Disease-induced mortality in badgers arising from *M. bovis* infection was estimated to be negligible in animals infected with but not excreting *M. bovis* organisms, significantly higher for individuals excreting *M. bovis* organisms (indicative of more generalised disease), and higher in males compared with females (Wilkinson *et al*. 2000). In chacma baboons, disease-induced mortality arising from *M.*

bovis infection is very high (Keet *et al*. 2000). Lugton *et al*. (1997b) document a radio-collared feral ferret surviving at least one year with tuberculosis infection, and suggest that the time of survival after infection probably ranges from several months in a few cases, to in excess of a year in many cases.

The hazard functions for the 12 hypotheses are nested within four general shapes of hazard function, which are based on variations of the exponential step-hazard model (Lee 1992). For α equal to zero, H1 may be modelled by the exponential model by allowing transmission only during the suckling period (*s*), (Hazard Function 1 and Model 1.1; Table 2.1). For non-zero values of α, H1 may be modelled based on the model of Cohen (1973) (see below) (Model 1.2, Table 2.1).

Hypotheses H2, H3, H4 and H5 may be modelled by the exponential model, modified to allow for a period when ferrets are not exposed to infection, here termed *g* (Hazard Function 2, Table 2.1). This is analogous to the concept of a guarantee time in survival analysis (Lee 1992). In epidemiological studies it commonly arises when individuals are protected from disease for a period after birth due to the presence of maternal antibodies (for mycobacterial infections such as *M. bovis*, immunity is cell-mediated only, hence there is no maternally-derived immunity). The value of *g* was set to specify each relevant hypothesis (10, 2.5, 1.75 or 0 months for H2, H3, H4 and H5 respectively). For α equal to zero, the age-prevalence solution is Model 2.1 (Table 2.1). For non-zero α, the age-specific prevalence for hypotheses H2–H5 can be obtained from the solution of Cohen (1973), modified as before to include the term *g*, and omitting the disease latent period term (Model 2.2, Table 2.1).

To represent hypotheses H6–H12 (Fig. 2.1), the hazard function needs be able to take different values (not just 0 or λ) over anything up to 3 age classes—say λ_1, λ_2, and λ_3. For hypotheses with a single step in the hazard function at g_1 (H7, H8, H9), this is represented by Hazard Function 3 (Table 2.1). For α equal to zero, the age-specific prevalence for H7, H8 and H9 is modelled as Model 3.1 (Table 2.1). For non-zero α, the resulting age-specific prevalence for hypotheses H7–H9 can be obtained from the solution below (Model 4.2, Table 2.1) with g_1 set to zero.

Hypotheses H6, H10, H11 and H12 that have two steps in the hazard function (say at g_1 and g_2) are modelled by Hazard Function 4 (Table 2.1). For α=0, the age-specific prevalence for H6, H10, H11, and H12 is given by Model 4.1 (Table 2.1), with λ_1 constrained to equal zero for H6, and λ_2 constrained to be zero for H12. For non-zero α, there are considerable complications in finding solutions of the age-specific prevalence. For reasons made clear in the results, solutions with a non-zero force of

infection up until the age of weaning were not needed. For the piece-wise constant exponential model with $\lambda_1 = 0$ (H6), the age-specific prevalence including disease-induced mortality (G. Fulford, personal communication) is given by Model 4.2 (Table 2.1).

As an alternative to the piece-wise smooth exponential models being used to account for λ varying with age, the exponential models can be generalised to the Weibull model (Lee 1992). The Weibull model contains an additional shape parameter γ, with λ now termed a scale parameter. Lambda (now age-dependent) and including a guarantee time, *g*, is given by Hazard Function 5 (Table 2.1), and increases with age when $\gamma>1$, and decreases with age when $\gamma<1$, hence the Weibull hazard may model an increasing, decreasing or constant λ. The age-specific solution is given by Model 5 (Table 2.1). Setting γ equal to 1 simplifies the hazard function to the exponential case ($\lambda(a) = \lambda$). The flexibility of the Weibull model, modified to include a guarantee time, enables it broadly to represent all the hypotheses except those that are U-shaped (H11 & H12) (Fig. 2.3). It does not, however, represent any of the hypotheses explicitly. There is no explicit solution for the Weibull model with a non-zero disease-induced mortality rate (G. Fulford, personal communication).

Finally, I fitted the polynomial hazard function (of order *k*) (Model 6; Table 2.1) of Grenfell & Anderson (1985) because it has the flexibility to fit many shaped curves, including those that the Weibull model is unable to. Grenfell & Anderson (1985) allowed $\lambda(a)$ to be zero below a lower threshold age (denoted *L* in their study but replaced here with *g* to avoid confusion), which is analogous to the guarantee time used in this study. As for the Weibull model, analytical solutions for the polynomial models including disease-induced mortality do not exist (G. Fulford, personal communication), except for the case with $k=0$ (equivalent to Model 2 version of the exponential model). A possible disadvantage of fitting models of this type containing polynomial terms is that $\hat{\lambda}$ is not constrained to be non-negative (other than for $k=0$, the Model 2.1 version of the exponential model)—hence the best fitting hazard functions are not always biologically plausible, considered a key requirement of a model for making inference (Burnham & Anderson 1998). Indeed, it is difficult to put a biological interpretation on the coefficients estimated, other than visually comparing the estimated hazard functions. The polynomial hazard functions are difficult to constrain to have the similar shapes, making direct quantitative comparisons difficult. Ades & Nokes (1993) extend the ideas of Grenfell & Anderson (1985) by using exponential polynomial hazard functions, which are constrained to be positive. As for the Weibull model, analytical solutions for

the polynomial models including disease-induced mortality do not exist (G. Fulford, personal communication), except for the case with k=0 (equivalent to the exponential model).

Table 2.1. Details of each Hazard Function (HF) in terms of the age-specific force of infection ($\lambda(a)$) for various age classes (Age), and the age-specific disease prevalence model without (α=0) and with (α>0), disease-induced mortality. The suckling period is s, and the guarantee time g. Model numbers are given to the right of brackets.

HF	Age	$\lambda(a)$	Age-specific disease prevalence (α=0)		(α>0)	
1	$a \le s$	λ	$1-e^{-\lambda a}$	1.1	$\frac{\lambda(1-e^{(\alpha-\lambda)a})}{\lambda-\alpha e^{(\alpha-\lambda)a}}$	1.2
	$a > s$	0	$1-e^{-\lambda s}$		$\frac{\lambda(1-e^{(\alpha-\lambda)s})}{\lambda-\alpha e^{(\alpha-\lambda)s}}e^{-\alpha(a-s)}$	
2	$a \le g$	0	0	2.1	0	2.2
	$a > g$	λ	$1-e^{-\lambda(a-g)}$		$\frac{\lambda(1-e^{(\alpha-\lambda)(a-g)})}{\lambda-\alpha e^{(\alpha-\lambda)(a-g)}}$	
3	$a \le g_1$	λ_1	$1-e^{-\lambda_1 g_1}$	3.1	*	3.2
	$a > g_1$	λ_2	$1-e^{-\lambda_1 g_1}e^{-\lambda_2(a-g_1)}$		*	
4	$a \le g_1$	λ_1	$1-e^{-\lambda_1 g_1}$	4.1	0	4.2#
	$g_1 < a \le g_2$	λ_2	$1-e^{-\lambda_1 g_1}e^{-\lambda_2(a-g_1)}$		$\frac{\lambda_2(1-e^{(\alpha-\lambda_2)(a-g_1)})}{\lambda_2-\alpha e^{(\alpha-\lambda_2)(a-g_1)}}$	
	$a > g_2$	λ_3	$1-e^{-\lambda_1 g_1}e^{-\lambda_2(g_2-g_1)}e^{-\lambda_3(a-g_2)}$		$1-\frac{(\lambda_2-\alpha)(\lambda_3-\alpha)E_1E_2}{(\lambda_2-\alpha)(\lambda_3-\alpha)+\alpha(\lambda_3-\alpha)(1-E_1)+\alpha(\lambda_2-\alpha)E_1(1-E_2)}$	
W	$a \le g$	0	0	5	No explicit solution	—
	$a > g$	$\lambda\gamma(a-g)^{\gamma-1}$	$1-e^{-\lambda(a-g)^{\gamma}}$			
P	$a \le g$	0	0	6	No explicit solution	—
	$a > g$	$\sum_{i=0}^{k} b_i a^i$	$1-e^{\left(-\sum_{i=0}^{k}\frac{b_i(a^{i+1}-g^{i+1})}{(i+1)}\right)}$			

* Solution nested within Model 4.2.

Applicable only for $\lambda_1 = 0$, where $E_1 = e^{(\alpha-\lambda_2)(g_2-g_1)}$ and $E_2 = e^{(\alpha-\lambda_3)(a-g_2)+\alpha(g_2-g_1)}$

W—Weibull; P—Polynomial

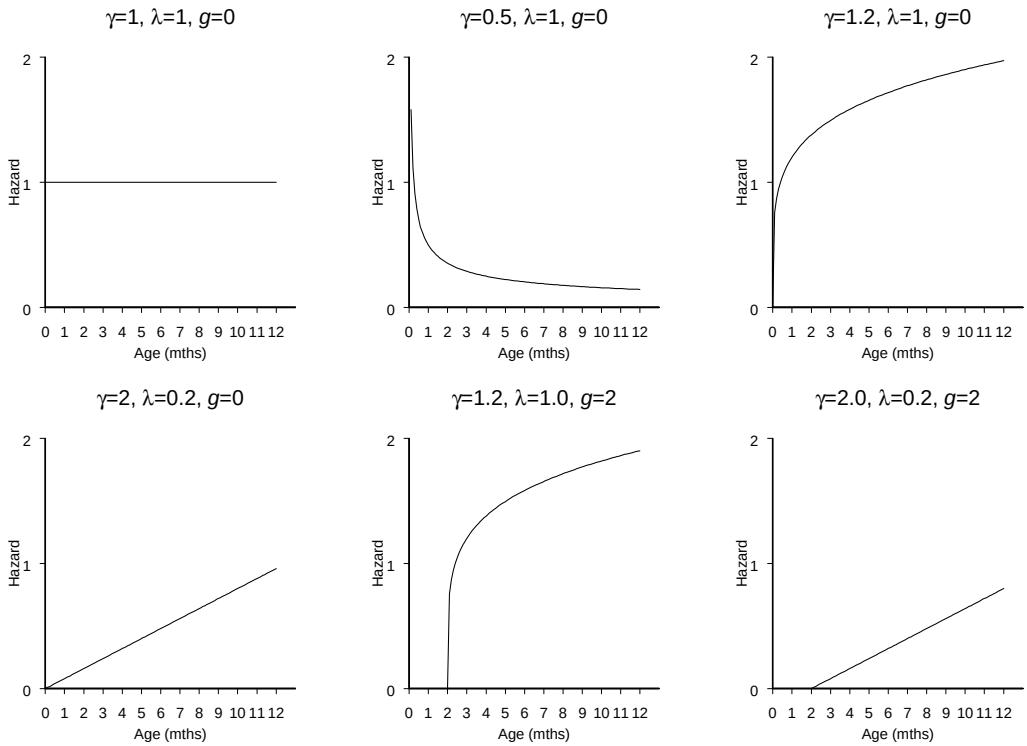

Figure 2.3. A sample of possible hazard functions of the Weibull distribution as a function of age for different values of the scale (γ), shape (λ), and guarantee time (*g*) parameters. The scaling on the y-axis is arbitrary.

2.3.1 *Model fitting*

All models were fitted by maximum likelihood. The likelihood (*L*) to be maximised was the binomial likelihood (Equation 2.1) where p_i is the modelled probability of infection, and y_i is the number of *M. bovis*-infected individuals out of a total n_i in each age-class *i*. There are *m* age classes in total.

$$L = \prod_{i=1}^{m} \binom{n_i}{y_i} p_i^{y_i} (1 - p_i)^{n_i - y_i} \qquad \text{(Equation 2.1)}$$

The binomial log-likelihood (ln*L*) is:

$$\ln L = \sum_{i=1}^{m} \left\{ \ln\binom{n_i}{y_i} + y_i \ln p_i + (n_i - y_i) \ln(1 - p_i) \right\}. \qquad \text{(Equation 2.2)}$$

Maximising *L* was achieved by numerically minimising the negative log-likelihood (Equation 2.2) with respect to $\hat{\lambda}$ (a separate estimate for each site), gender effect (single multiplicative factor), and $\hat{\alpha}$ (if applicable), after substituting for p_i from the relevant model. Gender and site were fitted as multiplicative factors, though only acting on the elevation (cf. slope) of the hazard function in question. The slope was considered a

constant for all sites and sexes (e.g., γ held constant for Weibull models, and sex and site factors multiplied on b_0 only for Grenfell & Anderson (1985)'s polynomial hazard model). When undertaking numerical minimisation, biological ($\hat{\alpha}$ and $\hat{\lambda}$ were constrained to be positive for all models) and hypothesis-generated bounds were placed on the values for parameters. Hypothesis-generated bounds were: H6—$\hat{\lambda}_1 = 0$ and $\hat{\lambda}_2 \le \hat{\lambda}_3$; H7—$\hat{\lambda}_1 \ge \hat{\lambda}_2$; H8—$\hat{\lambda}_1 \le \hat{\lambda}_2$; H9—$\hat{\lambda}_1 \le \hat{\lambda}_2$; H10—$\hat{\lambda}_1 \le \hat{\lambda}_2 \le \hat{\lambda}_3$; H11—$\hat{\lambda}_1 \ge \hat{\lambda}_2 \le \hat{\lambda}_3$; H12—$\hat{\lambda}_2 = 0$. The computer package S-PLUS (Data Analysis Products Division, MathSoft, Seattle) was used for all analyses.

2.3.2 *Model selection*

Akaike's Information Criterion corrected for sample size (AIC_c) (Burnham & Anderson 1998) was used to compare models, where AIC_c is calculated as:

$$AIC_c = -2\ln(L(\hat{\theta})) + 2K + \frac{2K(K+1)}{(n-K-1)} \qquad \text{(Equation 2.3)}$$

Here, $L(\hat{\theta})$ is the maximised binomial log-likelihood function (Equation 2.2) of the current model, K is the number of parameters fitted to the model, and n is the sample size. As a rule of thumb, Burnham & Anderson (2001) suggest that models having ΔAIC_c (difference in AIC_c scores) within 1–2 of the best model have substantial support. Models within about 4–7 of the best model have considerably less support, while models with $\Delta AIC_c > 10$ have essentially no support. Plots of Pearson residuals (Collett 1991) were used to further assess model fit. For the chosen model, confidence intervals for $\hat{\alpha}$ were calculated by profile likelihood (McCallum 2000). Confidence intervals for $\hat{\lambda}$ were not estimated here. Rather, Model 2.1 was used to test for the relative differences between sites and sexes in $\hat{\lambda}$, as this model can be fitted as a Generalised Linear Model (GLM), making estimates of standard errors for parameters relatively straightforward (see 2.3.3 below).

As this chapter aimed to estimate the absolute rate at which ferrets encounter *M. bovis* infection, Cox's proportional hazards model (Cox 1972) was not considered, despite its popularity for many epidemiological investigations. Cox's model is primarily concerned with estimating the proportional effects of different factors on the hazard rate, rather than the baseline hazard function, which in the current study is the variable of intrinsic interest.

2.3.3 *Hypothesis testing for effects of sex and site*

The Weibull Model may be linearised into the form of a Generalised Linear Model (GLM) (Equation 2.4), and this provides a convenient method for testing the relative effects of gender and site on the force of *M. bovis* infection in ferrets. The simplest exponential model (Model 2.1) is nested within this model by setting γ to one.

$$\ln(-\ln(1-p(a))) = \ln\lambda + \gamma\ln(a-g) \qquad \text{(Equation 2.4)}$$

Equation 2.4 was fitted to the data using the software package GLIM4 (Francis *et al*. 1993). *M. bovis* prevalence data were classified by sex, site and age. The error structure of *p(a)* was specified as binomial with the response variable the number of animals infected, and the binomial denominator the total number of individuals in that age group. The link function was specified as complementary log-log. For fitting Model 2.1, γ was fixed equal to one. This was achieved by specifying ln(*a*–*g*) as an offset (fixing the slope of the regression equal to 1) (Collett 1991). The statistical significance of sex and site on $\hat{\lambda}$ was assessed using the deletion test (Crawley 1993), which assesses the change in model deviance arising from the removal of a parameter from the model. The interaction between sex and site was not examined, as there was no *a priori* reason for doing so. Following the notation of Collett (1991), the model deviance (*D*) is given by:

$$D = -2[\ln\hat{L}_c - \ln\hat{L}_f], \qquad \text{(Equation 2.5)}$$

where $\hat{L}_c$ is the maximised likelihood under the current model (i.e. fitted values for p_i substituted into Equation 2.2), and $\hat{L}_f$ is the maximised log-likelihood under the full or saturated model, in this case the observed proportions of individuals infected in each age class. The adequacy of the fit of the chosen model was examined by testing the significance of the residual model deviance (Collett 1991).

2.4 Results

2.4.1 *Comparison of candidate age-specific prevalence models*

Hypothesis 4 (dietary related transmission), as represented by Model 2.2 (exponential model including disease-induced mortality with *g*=1.75 mths), had the lowest AIC_c of all the models fitted (Tables 2.2 and 2.3). It also had the highest

likelihood (best fit to the data). As neither the Weibull nor the Polynomial Hazard models represent any one hypothesis explicitly, their ranks were not calculated; however, their ΔAIC_c scores are shown in Table 2.3. Great difficulty was had in finding starting parameters for the polynomial model that led to likelihood function convergence for *k* greater than or equal to 2. As the AIC_c scores all worsened (got larger) going from $k = 0$ to $k = 1$, the process was abandoned for $k \geq 2$.

Two additional models have substantial support—H4 as represented by Model 2.1 (exponential with no disease-induced mortality and g=1.75 mths) (ΔAIC_c =0.6; Table 2.2), and the Weibull Model (Model 5 with g=1.75 mths) (ΔAIC_c= 0.7; Table 2.3). This Weibull model estimated γ to be 0.94, that equates with $\hat{\lambda}$ decreasing slightly with increasing age. This is possibly an artefact of disease-induced mortality causing a lower prevalence than expected for a given age if $\hat{\gamma}$ was equal to unity. The hypothesis that matched the best performing Weibull hazard function was H4. Several other Hypotheses (as represented by models) had $\Delta AIC_c < 3$, notably those relating to H6 and H8. However, in both cases there was either no support for a step (increase) in the hazard function (H6, $\hat{\lambda}_2 = \hat{\lambda}_3$) or, in the case of H8, $\hat{\lambda}$ in the second time interval ($a > g$) was 10-fold greater than that in the first time interval ($a \leq g$). So, essentially both hypotheses were trying to 'emulate' H4 (hence with similar likelihoods), and the observed difference (*c*. 2) in the AIC_c score was simply caused by the effect of increasing the number of parameters, as opposed to any difference in the statistical likelihood. Hence it can be concluded that H4 has considerably more support than the other candidate models.

Model 2.2 estimated α to be 1.4 yr^{-1} (95% C.I. –1.1–4.4 yr^{-1})—the wide confidence interval (including biologically unrealistic negative values) showing that the likelihood function with respect to $\hat{\alpha}$ must be very flat indeed (note that α is an instantaneous rate so can be greater than one). Indeed, H4 as represented by either Model 2.1 or Model 2.2 appeared to fit the data well over the range of ages sampled, with the residuals reasonably evenly spread when plotted against ferret age (Fig. 2.4). This demonstrates that the hazard function of this simple model has captured the key components of the disease transmission processes that shape the age-specific prevalence of disease—this statement could be considered a 'leap of faith' of the kind McCallum (1995) described.

Table 2.2. Akaike's Information Criterion (AIC_c) scores and differences in AIC_c (ΔAIC_c) scores of candidate hypotheses for the transmission of *M. bovis* infection to feral ferrets, as represented by various models fitted to age-specific *M. bovis* infection prevalence data. Steps in the hazard functions are given by g_1 and g_2. Disease-induced mortality rate = α. All models have sex and site fitted as factors (assumed multiplicative).

Hypothesis	Model	g_1 or s (mths)	g_2 (mths)	α	AIC_c	ΔAIC_c	Rank
H1	1.1	s = 1.75	—	0	154.4	51.4	22
	1.2	s = 1.75	—	≥0	NF	—	—
H2	2.1	10	—	0	1987.2	1884.2	23
	2.2	10	—	≥0	1987.4	1887.4	24
H3	2.1	2.5	—	0	142.1	39.1	21
	2.2	2.5	—	≥0	138.2	35.2	19
H4	2.1	1.75	—	0	103.6	0.6	2
	2.2	1.75	—	≥0	103	0	1
H5	2.1	0	—	0	105.9	2.9	7
	2.2	0	—	≥0	108.3	5.3	10
H6	4.1	1.75	10	0	105.6#	2.6	3
	4.2	1.75	10	≥0	105.7#	2.7	4
	4.2	2.5	10	≥0	140.9#	37.9	20
H7	3.1	0	1.75	0	108.5#	5.5	12
	3.2	0	1.75	≥0	111.0#	8	15
H8	3.1	0	1.75	0	105.8	2.8	5
	3.1	0	2.5	0	106.6	3.6	9
	4.2	0	1.75	≥0	106	3	8
	4.2	0	2.5	≥0	105.8	2.8	5
H9	3.1	0	10	0	108.5#	5.5	12
	4.2	0	10	≥0	111.0#	8	15
H10	4.1	1.75	10	0	108.4*	5.4	11
	4.1	2.5	10	0	109.2*	6.2	14
H11	4.1	1.75	10	0	111.2$	8.2	17
	4.2	1.75	10	≥0	NF	—	—
H12	4.1	1.75	10	0	132.8	29.8	18
	4.2	1.75	10	≥0	NF	—	—

$\hat{\lambda}_1 = \hat{\lambda}_2$ (i.e. hit bound); * $\hat{\lambda}_2 = \hat{\lambda}_3$ (i.e. hit bound); $ $\hat{\lambda}_1 = \hat{\lambda}_2 = \hat{\lambda}_3$ (i.e. hit bound); NF—not fitted.

Table 2.3. Akaike's Information Criterion (AIC_c) scores and differences in AIC_c (ΔAIC_c) scores for the Weibull and Polynomial hazard models (Model 5 and Model 6) with respect to the best performing exponential model (Model 2.1; see Table 2.2).

Model	g_1	AIC_c	ΔAIC_c
Weibull	0	108.2	5.2
	1.75	103.7	0.7
	2.5	138.7	35.7
	10	1985.8	1882.8
Polynomial (k=1)	0	108.3	5.3
	1.75	106.1	3.1
	2.5	144.6	41.6
	10	1989.5	1886.5

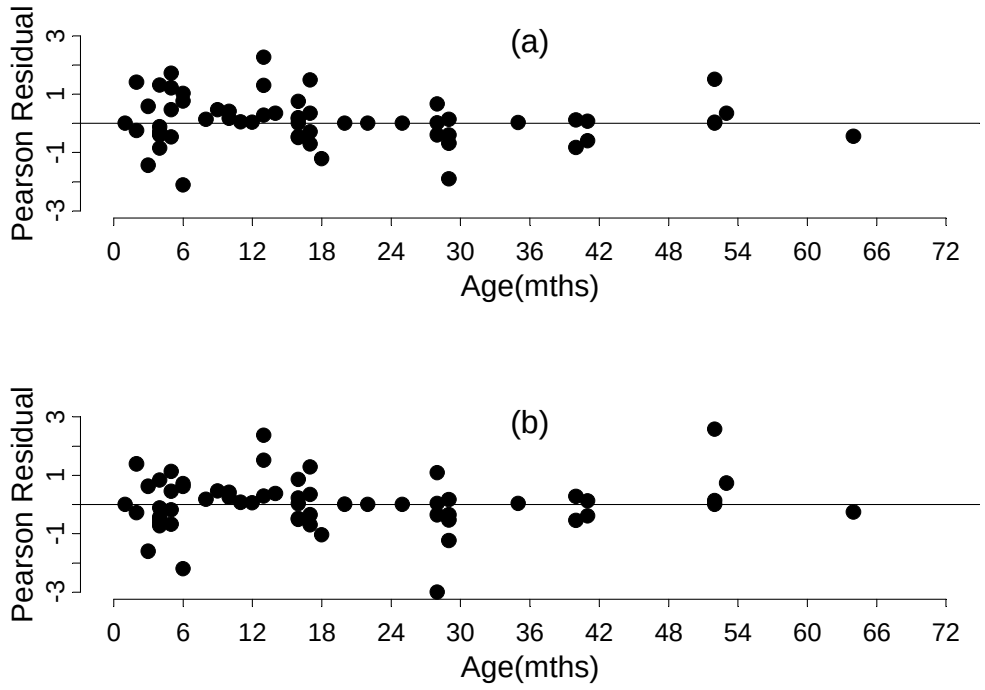

Figure 2.4. Pearson residuals for (a) Model 2.1 with g=1.75 mths and α=0 yr^{-1}; and (b) Model 2.2 with g=1.75 mths and α=1.4 yr^{-1}, plotted against ferret age.

2.4.2 *Force of infection*

Models 2.1 and 2.2 differed in their estimates of λ. The effect of ignoring disease-induced mortality was to lower $\hat{\lambda}$ significantly for Model 2.1 relative to Model 2.2 (Fig. 2.5). This discrepancy became more pronounced as $\hat{\lambda}$ became less. Despite the wide confidence interval around $\hat{\alpha}$, I choose H4 as represented by Model 2.2 as a working model for estimating the force of *M. bovis* infection in ferrets, as it seems biologically more plausible that some disease-induced mortality should occur. For this

model, $\hat{\lambda}$ in males was 2.2 times that in females. Ferrets at Castlepoint encountered *M. bovis* infection at about six times the rate of ferrets at Scargill Valley, and about 40 times the rate of ferrets at Lake Ohau (Table 2.4), resulting in a major difference in the age-specific disease prevalence (Fig. 2.6).

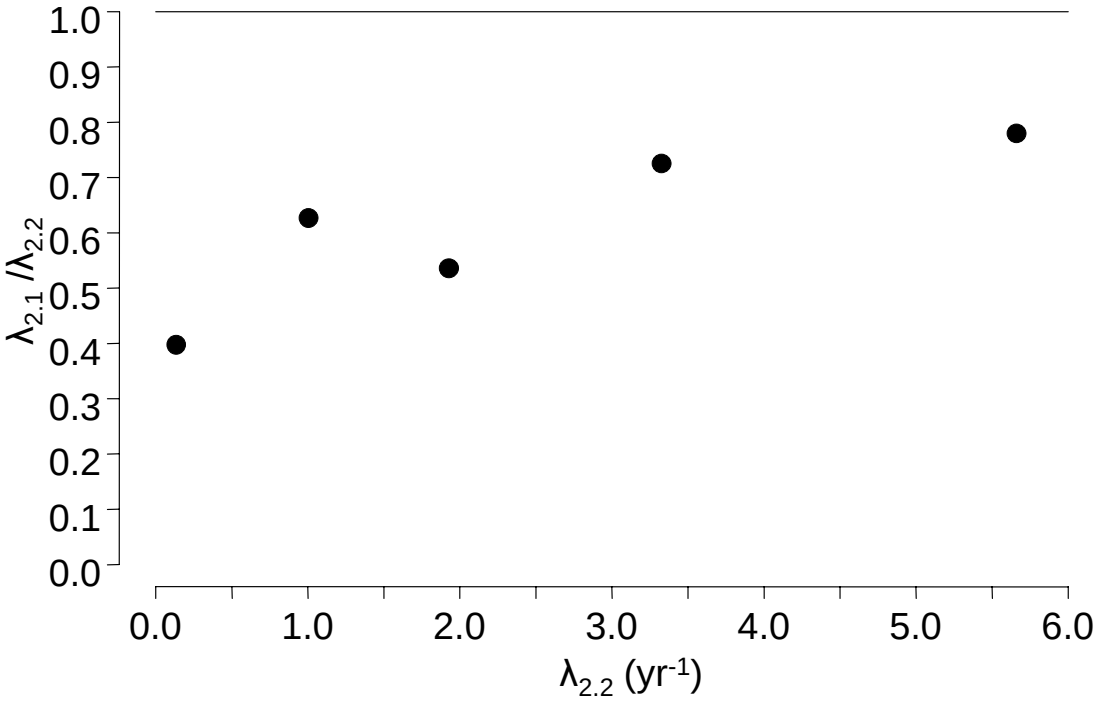

Figure 2.5. The estimated force of *M. bovis* infection in ferrets estimated using Model 2.1 ($\lambda_{2.1}$), expressed as a proportion of that estimated using Model 2.2 ($\lambda_{2.1}/\lambda_{2.2}$), plotted against the force of *M. bovis* infection (averaged over sexes) estimated using Model 2.2 ($\lambda_{2.2}$). Each point represents a different study site. The horizontal line represents the relationship if the model estimates were equivalent.

Table 2.4. The estimated force of *M. bovis* infection ($\hat{\lambda}$) in feral ferrets from five sites in New Zealand as determined from modelling age-specific disease prevalence using a modified exponential model including disease-induced mortality at 1.4 yr^{-1} and a guarantee time of 1.75 months (Model 2.2—see text for details).

Site	Sex	No. examined	No. Infected	$\hat{\lambda}$ (yr^{-1})
Lake Ohau	Male	57	3	0.19
	Female	54	2	0.09
	Sexes combined	111	5	0.14
Scargill Valley	Male	37	5	1.40
	Female	39	8	0.65
	Sexes combined	76	13	1.02
Cape Palliser	Male	15	11	2.69
	Female	23	10	1.24
	Sexes combined	38	21	1.97
Castlepoint	Male	27	21	7.90
	Female	21	10	3.65
	Sexes combined	48	31	5.77
Awatere Valley	Male	24	16	4.64
	Female	22	12	2.15
	Sexes combined	46	28	3.40

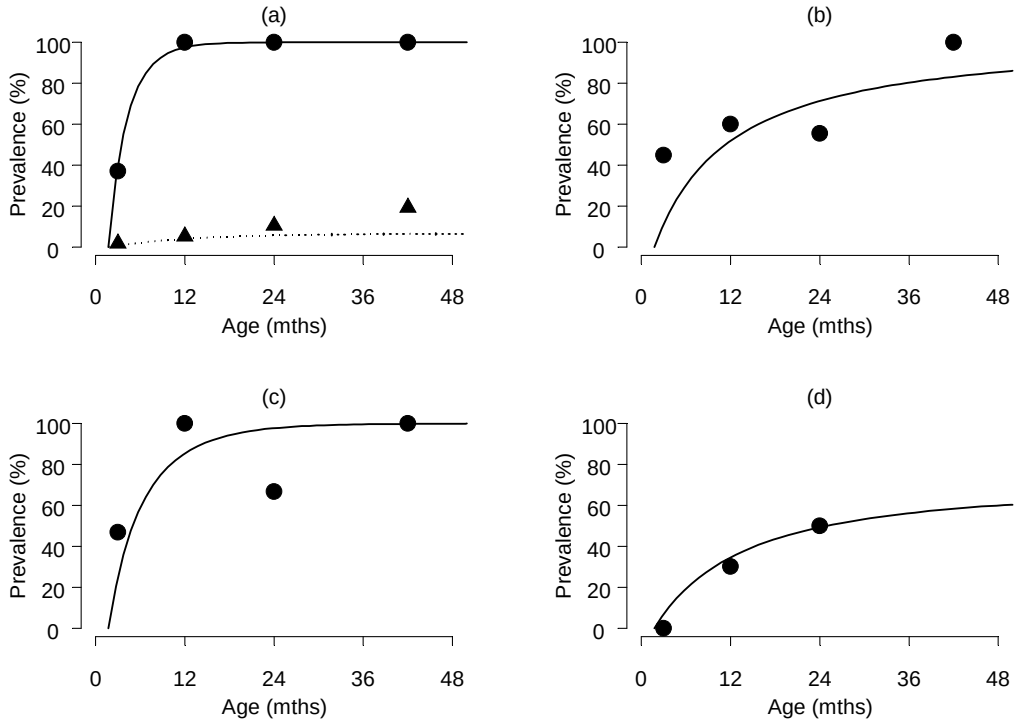

Figure 2.6. Observed age-specific prevalence of *M. bovis* infection in ferrets from: (a) Castlepoint (•) and Lake Ohau (▲); (b) Cape Palliser; (c) Awatere Valley; and (d) Scargill Valley. Fitted lines are for the exponential model including disease-induced mortality (Model 2.2) using the mean estimates of λ for males and females from Table 2.4. Data are pooled over age classes (cf. Fig. 2.4) for illustrative purposes—for an assessment of model fit see text and Figure 2.4.

2.4.3 *Testing site and gender effects on* λ

Because of its far superior ease for undertaking hypothesis testing, H4 as represented by Model 2.1 was used formally to test for differences (as opposed to estimating differences) between the sites and sexes. This model explained 76% of the overall binomial deviance of the data, and the model-fit was very good (χ^2=52.2, d.f.=56, *P*=0.62), which was not surprising given the even spread of residuals (Fig. 2.4 (a)). Lambda was significantly higher for males compared with female ferrets (χ^2=8.4, d.f.=1, *P*<0.001) and differed significantly among sites (χ^2=153.4, d.f.=4, *P*<0.001) (Table 2.5).

Table 2.5. Parameter estimates and standard errors (S.E.) of the effect of sex and site on the natural logarithm of the yearly force of *M. bovis* infection in feral ferret populations estimated using Model 2.1 (see text for details). The estimate of the intercept is the value of the natural logarithm of $\hat{\lambda}$ for male ferrets from the Scargill Valley site. Each additional parameter estimate represents its added contribution to the estimate of $\log_e(\hat{\lambda})$ for the intercept.

Parameter	**Estimate**	**S. E.**	***t***[a]	**Probability**[b]
Intercept (Male, Scargill Valley)	0.1	–	–	
Female	–1.0	0.26	–3.8	<0.001
Castlepoint	2.0	0.38	5.2	<0.001
Awatere Valley	1.3	0.38	3.6	<0.001
Cape Palliser	0.5	0.39	1.3	0.10
Lake Ohau	–2.5	0.53	–4.6	<0.001

[a] The observed *t* values are distributed with infinite degrees of freedom (*Z* scores).
[b] Significance of the difference of each parameter estimate from that of the intercept (Sex = male, Site = Scargill Valley).

2.5 Discussion

The use of age-prevalence curves for estimating the rate of disease spread in vertebrate wildlife has been limited. Anderson & Trewhella (1985) estimated the force of *M. bovis* infection in badger populations by fitting an age-structured version of a deterministic model to prevalence data. Dobson & Meagher (1996) presented age-specific prevalence data for *Brucella abortus* infection in bison (*Bison bison*), though they did not use it to estimate any parameters. They did, however, make some qualitative inferences from the data, concluding that the absence of any tendency for the prevalence to increase with age suggests that there is no significant mortality associated

with brucellosis infection in bison. This is not strictly correct, as disease-induced mortality may only cause a plateau in prevalence with increase age, as inferred by Anderson & Trewhella (1985). Caley *et al*. (1994) used age-prevalence data to make inference on the transmission mechanisms of porcine parvovirus amongst feral pigs. Packer *et al.* (1999) used age-prevalence data to make inference on the habitat-related rate of infection of lions (*Panthera leo*) with feline immunodeficiency virus (FIV). Lastly, Hudson & Dobson (1997) estimated the force of infection for the nematode *Trichostrongylus tenuis* in the red grouse (*Lagopus lagopus scoticus*) by modelling age-intensity curves. Here, intensity referred to parasite burden (cf. probability of individual host being infected), hence the force of infection measures the rate at which parasites are acquired, compared with the rate at which infection is acquired. Note that Medley *et al*. (1993) (and probably many other authors) consider it erroneous to call the *per capita* rate at which individuals become infected the force of infection. This highlights a slight difference in terminology between epizootiology applied at an individual level (where the level of parasite burden is of great interest) compared with a population level (where the proportion of individuals infected is often of most interest). I think both definitions of the force of infection are useful, and can happily coexist, providing it is clear what is being measured.

The general lack of use of age-prevalence data for estimating disease incidence in vertebrate wildlife is in contrast to the study of disease incidence in humans, where analysis of age-prevalence data is used routinely (e.g., Cohen 1973; Griffiths 1974; Grenfell & Anderson 1985; Farrington 1990; Ades 1992). The difficulties in obtaining adequate sample sizes in studies of disease in wildlife is one reason for this disparity, along with difficulties in the diagnosis of infection and accurate ageing of animals. This study has demonstrated that estimating the force of infection from age-prevalence data is possible, and this can assist in discriminating between alternative hypotheses about routes of disease transmission.

The model selection exercise undertaken here has identified that the hazard function underlying the observed age-specific prevalence of *M. bovis* infection in ferrets is adequately modelled by a constant λ from the age of weaning, supporting Hypothesis 4 (dietary-related transmission). Other candidate hypotheses, of which a reasonably exhaustive number representing all hypothesized or combinations of hypothesized transmission mechanisms were tested, were unsupported in comparison. Notably, the data gave no support for transmission occurring in the suckling period before weaning (H1). Neither did the data support an increase in λ once ferrets became socially

independent (H3), sexually mature (H2), nor λ being a constant from birth due to environmental contamination (H5). Hence I conclude that transmission during mating, suckling and routine social activities must be insignificant compared with dietary related transmission, in agreement with the observations of Lugton *et al*. (1997b).

Using a model selection approach can increase knowledge of the underlying processes of disease transmission, knowledge that otherwise would not be obtained by fitting a single model only. The key requirement here is that the scientific investigator needs to consider a range of biologically plausible hypotheses (preferably developed *a priori*). Burnham & Anderson (1998) describe the AIC model selection approach as being based on a formal relationship between Kullback-Leibler information (the dominant paradigm in information and coding theory) and maximum likelihood (the dominant paradigm in statistics). The concept of likelihood is very much data-based, as exemplified by the following quote from Edwards (1992):

> The probability model, the set of statistical hypotheses, and the data, form a triplet which is the foundation of statistical inference. Of the many outcomes, each with a specified probability given the hypothesis, which could have occurred on the basis of the accepted model, one has occurred—*the* data. What can they reveal about the hypothesis.

The data may have the final say; however, the final outcome of model selection techniques will only be as good as the candidate models considered (and of course the data themselves). Herein lies a lingering problem, that despite casting our minds far and wide, our hypotheses will be constrained (for the most part) by the dominant paradigm from within which we operate (Kuhn 1962).

Model selection procedures will not always select a single 'best' model. In this study, the similar AIC_c scores of the exponential model used to represent Hypothesis 4 (dietary related transmission) with (Model 2.2) and without (Model 2.1) disease-induced mortality provide an example of the difference between model selection as opposed to hypothesis testing of parameter estimates. The similar AIC_c scores cannot be taken to mean that there is no disease-induced mortality arising from *M. bovis* infection (especially when the parameter estimate is large). Rather, they can be interpreted as meaning that the age-specific prevalence of *M. bovis* infection in ferrets can be adequately modelled using either model. Whether or not disease-induced mortality occurs would be considered an estimation, rather than a model selection problem (Burnham & Anderson 1998). Because of its ease of fitting within a statistical

framework, Model 2.1 provides a logical starting point for comparing the incidence of *M. bovis* infection in feral ferret populations, though the estimates are probably biased downwards. Model 2.2, however, probably provides more accurate estimates of λ, and hence is more useful for obtaining parameter estimates needed for subsequent modelling of disease transmission (Chapter 4). There is a need to obtain independently a more reliable estimate of the rate of disease-induced mortality caused by *M. bovis* infection in ferrets.

A dietary-related working hypothesis for *M. bovis* transmission to ferrets must account for λ being two-fold higher in males than females. Possible causes consistent with dietary-related transmission supported by Hypothesis 4 include dietary composition (male ferrets being more prone to scavenge tuberculous carcasses than females), immunological (males being more susceptible to becoming infected) and ecological (larger male home-range having a greater probability of including a source of *M. bovis*) reasons (Lugton *et al*. 1997b). Ragg (1998a) reported no intra-specific differences in diet in the species postulated to be the main source of infection for ferrets, making the dietary composition hypothesis unlikely. However, whilst no inter-sexual differences may exist in the composition of ferret diet, due to pronounced sexual dimorphism (Lavers & Clapperton 1990; male $\bar{x}$ wt = 1187 g, female $\bar{x}$ wt = 627 g), male ferrets need to consume significantly more food than females, and hence could be exposed to a greater risk of encountering *M. bovis*-infected carcasses simply through greater dietary intake. Gender differences in the susceptibility of ferrets to *M. bovis* infection have not been evaluated; but such differences appear to occur in other species. For example, male badgers appear more susceptible than females to disease progression and have a higher rate of disease-induced mortality (Wilkinson *et al*. 2000). Hence the immunological hypothesis should not be ruled out. Home ranges of male ferrets are consistently larger than females, though the estimated size of the differences varies from small (21%) (Alterio *et al*. 1998), to medium (34%) (Norbury *et al*. 1998a) to large (*c*. 100%) (Caley & Morriss 2001). The distribution of *M. bovis* infection in possums is typically highly spatially aggregated (Caley 1996), hence it seems plausible that the observed differences in home range size could result in an elevated λ in male ferrets.

This chapter has identified the consumption of tuberculous carrion/prey as the most strongly supported hypothesis for the transmission of *M. bovis* infection to feral ferrets, and has identified a suitable model for estimating the force of *M. bovis* infection in feral ferrets. However, I have not identified the source of this infection. As well as accounting for the difference in λ between the sexes, a dietary-related working

hypothesis for *M. bovis* transmission to ferrets must also allow for λ differing by an order of magnitude between sites. Although the diet of ferrets consists mainly of lagomorphs (Ragg 1998a), they also scavenge extensively, and will readily eat possum and ferret carcasses (Ragg *et al*. 2000). *M. bovis* infection has been recorded, though at a very low prevalence, in common prey items of ferrets including the rabbit (Gill & Jackson 1993), hare (Cooke *et al*. 1993), hedgehog (Lugton *et al*. 1995), and of course ferrets themselves. For all these species other than for ferrets, *M. bovis*-infected possums are considered the underlying reservoir of infection. The highest λ appeared to occur at sites (e.g., Castlepoint; Awatere Valley) with the highest densities of possums, based on the incidental catch rate of possums caught in traps targeted at ferrets (this observation is quantified in Chapter 3). Hence the hypothesis that *M. bovis* infection in ferrets is simply a spillover from possum populations is an obvious candidate hypothesis for critical testing. However, the hypothesis of dietary-related transmission is not inconsistent with intra-specific transmission through ferrets scavenging on *M. bovis*-infected ferret carcasses. If this occurs at a high enough rate, it could enable *M. bovis* to cycle independently in ferret populations, irrespective of the contribution from possums. The critical experiments required to answer this are described in the next chapter.

Chapter 3

A test of the relationship between *M. bovis* infection in feral ferrets and brushtail possums

3.1 Introduction

Consumption of *M. bovis*-infected carrion/prey from the age of weaning is the most strongly supported hypothesis for the transmission of *M. bovis* infection to feral ferrets in New Zealand (Chapter 2). Although it is clear infection in ferrets is acquired orally, the analysis did not identify the source of this infection. The working hypothesis, however, is that the force of *M. bovis* infection in ferrets is influenced by the population density of possums (Chapter 2). That is, there is a significant amount of inter-specific transmission of *M. bovis* from possum populations. Here in this Chapter I explore the most parsimonious working model, namely a two-host one-pathogen model (possum/ferret/*M. bovis*).

Few studies have critically examined inter-specific disease transmission rates between vertebrate wildlife hosts. One experimental approach for testing whether inter-specific transmission is occurring is to quantify the effect of controlling disease in one wildlife host on the incidence of the disease in a second wildlife host. In one such study, McInerney *et al*. (1995) quantified changes in the prevalence of *M. bovis* infection in feral pigs following the control of *M. bovis*-infected feral water buffalo populations in the floodplain habitats of Northern Australia. Alternatively, rates of inter-specific transmission may be estimated using a modelling approach. For example, Begon *et al.* (1999) estimated the rate of transmission of cowpox virus between sympatric populations of bank voles (*Clethrionomys glareolus*) and wood mouse (*Apodemus sylvaticus*) populations in Britain, although they did not attempt to test critically whether inter-specific transmission was in fact occurring. For this type of study, however, model selection procedures (e.g., AIC) could be used to compare models with and without inter-specific transmission, as a way of making inference on whether inter-specific transmission occurs. This model selection approach is considered strong inference by some (e.g., Burnham & Anderson 2001), particularly for ecological studies.

Transmission of disease from wildlife to livestock or humans is often of more concern than transmission between wildlife. Hence estimates of inter-specific transmission often pertain to a wildlife/livestock/pathogen system or a wildlife/human/pathogen system. A pathogen in a wildlife species may be controlled in an attempt to reduce inter-specific transmission causing an unacceptably high incidence of disease in domestic livestock. For example, control of *M. bovis*-infected possum populations in New Zealand aims to reduce the incidence of *M. bovis* in cattle (Caley *et al.* 1999), as did control of *M. bovis*-infected badger populations in Britain (Clifton-Hadley *et al*. 1995). Wildlife/livestock/*M. bovis* systems such as these are highly analogous to the possum/ferret/*M. bovis* system in question here—particularly in reference to testing for a causative link (or demonstration of a non-zero rate of inter-specific transmission) between *M. bovis* infection in wildlife and livestock. In the possum/cattle/*M. bovis* model, the cattle population is effectively transformed into a population of spillover hosts, as infected animals are identified by regular (yearly or more frequent) tuberculin testing and are removed (sent to slaughter) from the population before their infection progresses to the infectious stage, eliminating intra-specific transmission. Transmission from cattle back to possums appears inconsequential (Caley *et al*. 2001a), hence analysis of the effect of manipulating possum population density (the *M. bovis* reservoir) on the incidence of *M. bovis* in cattle is relatively straightforward (see Caley *et al*. 1999). Likewise, in the possum/ferret/*M. bovis* system, transmission of *M. bovis* from ferrets to possums is considered unlikely, as possums are infected via the aerosol route (Jackson *et al.* 1995a) and the pathology of *M. bovis* infection in ferrets shows they do not readily excrete aerosols (Lugton *et al*. 1997b).

Three of the previous examples have purported to demonstrate inter-specific *M. bovis* transmission between hosts (a causative link in other words), however their conclusions have been subjected to widely differing levels of scrutiny and skepticism (e.g., Krebs *et al.* 1998 for *M. bovis* infection in badgers). Possible reasons for this, particularly in respect to experimental design, are examined further in the Discussion in the light of the results of the current study.

This Chapter aims to test experimentally whether inter-specific transmission of *M. bovis* from possums to ferrets occurs. It presents an alternative treatment of the topic to that presented by Caley *et al*. (2001b), though using an enlarged dataset. Key findings from Caley *et al.* (2001b) included the confirmation of the positive relationship between the prevalence of macroscopic *M. bovis* infection in ferrets and possum

abundance (Fig. 3.1a). For a given level of ferret abundance, sites where possums had been controlled had a lower prevalence of macroscopic *M. bovis* infection in ferrets than sites where possum populations were left uncontrolled (Fig. 3.1b). Experimentally reducing possum abundance reduced the odds of macroscopic *M. bovis* infection in ferrets by 80% in the years immediately following possum control (Odds Ratio=0.23, *P*=0.003).

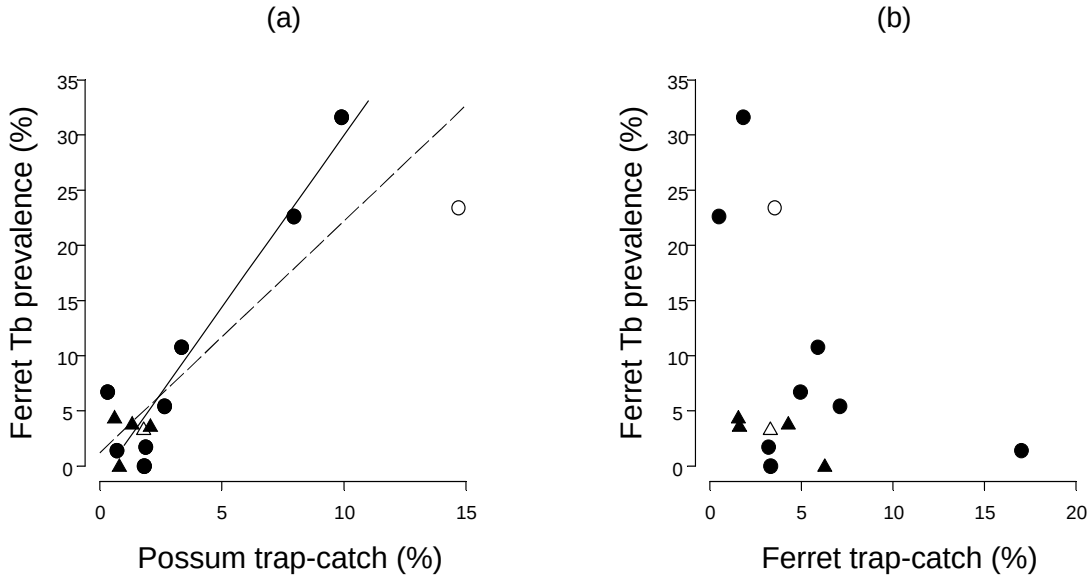

Figure 3.1. The prevalence of macroscopic *M. bovis* infection in ferrets in relation to (a) possum abundance, or (b) ferret abundance. Abundance is indexed as the mean number of possums or ferrets captured per 100 trap-nights in traps intended for ferrets. Triangles denote data points from sites subject to intensive possum control. Circles denote data points from surveys at sites with no possum control. The solid line represents the original weighted least-squares regression line of best fit presented by Caley (1998) for datapoints represented by filled symbols only (*Y = –1.22 + 3.12X*). The dashed line represents the new least-squares regression line (*Y = 1.19 + 2.08X*) including all data points. New survey data (since Caley (1998)) are denoted by open symbols (Awatere Valley (O) & Rangitikei (Δ)). Adapted from Caley *et al.* (2001b).

The response variable used in the analysis of Caley *et al*. (2001b) was the prevalence of macroscopic (visible) lesions resembling *M. bovis* infection (with subsequent bacteriological confirmation). This is a reasonably coarse measure of disease, as a variable proportion of infected individuals do not exhibit macroscopic lesions, and no attempt was made to correct for the large effect of ferret age (and hence

time of exposure to potential infection) on the prevalence of disease (Chapter 2). A much better response variable for analysis is the force of infection (λ), which ultimately determines the observed age-specific prevalence of disease. Methods for estimating the force of *M. bovis* infection in ferrets have been developed (Chapter 2). Anderson & Trewhella (1985) modelled changes in the force of *M. bovis* infection in badgers arising from badger removal operations in the Gloucestershire area. The force of *M. bovis* infection in badgers was estimated to decrease from 0.28 yr^{-1} to 0.12 yr^{-1} following badger removal operations, though the statistical significance of this decrease was not presented (Anderson & Trewhella 1985).

In this chapter, a manipulative experiment is described where the population density of possums is reduced, and changes in the force of *M. bovis* infection in ferrets are quantified and compared with sites where possum population density is left unchanged. Emphasis is placed on manipulating the population density of sympatric possum populations (cf. other possible prey items). The analysis tests for an experimental effect of reducing the population density of possums on the force of *M. bovis* infection in feral ferret populations, thus demonstrating inter-specific transmission. It also addresses whether there is intra-specific transmission of *M. bovis* in ferret populations by estimating the effect of lethal ferret sampling (akin to culling) on the force of *M. bovis* infection in feral ferret populations. Similar time-dependent epidemiological models have previously been fitted to age-specific disease data of humans to look for time-dependent changes in the force of infection, arising from changed epidemiological circumstances (treatments), for example hepatitis A in Europe (Schenzle *et al*. 1979), and toxoplasmosis in England (Ades & Nokes 1993). These studies have not had to account for the added complication of the sampling affecting host population density. The focus of the current chapter is very much on issues of hypothesis testing. The following chapter focuses on issues of estimation (with regard to transmission coefficients).

3.2 Methods

3.2.1 *Study sites*

This Chapter uses data collected from cross sectional surveys of *M. bovis* infection in feral ferrets at nine sites in New Zealand (Fig. 3.2), including the five sites

used in Chapter 2. The four additional sites (Hohotaka, Rangitikei, Waipawa & Tiromoana/Mt Cass) had all been subjected to possum control before the surveys, hence survey data from these four sites were not considered in the age-prevalence modelling analysis in Chapter 2. All sites lie within what are termed 'Vector Risk Areas', as wildlife are considered to be infected with *M. bovis* in these areas, and to pose a risk of infection to livestock. Indeed, *M. bovis*-infected possums are known from within seven of the nine sites, or nearby for the remaining two sites (Table 3.1). In all sites, DNA fingerprinting (Collins & de Lisle 1985) reveals a REA (restriction endonuclease analysis) match between at least one of the strains of *M. bovis* found in ferrets and that found in possums (Table 3.1). These data provide clear evidence of inter-specific transmission between wildlife, though give no clue to what species are involved in the transmission (e.g., is transmission from possum-to-ferret or from possum-to-deer-to-ferret) or the direction of transmission (e.g., possum-to-ferret or ferret-to-possum).

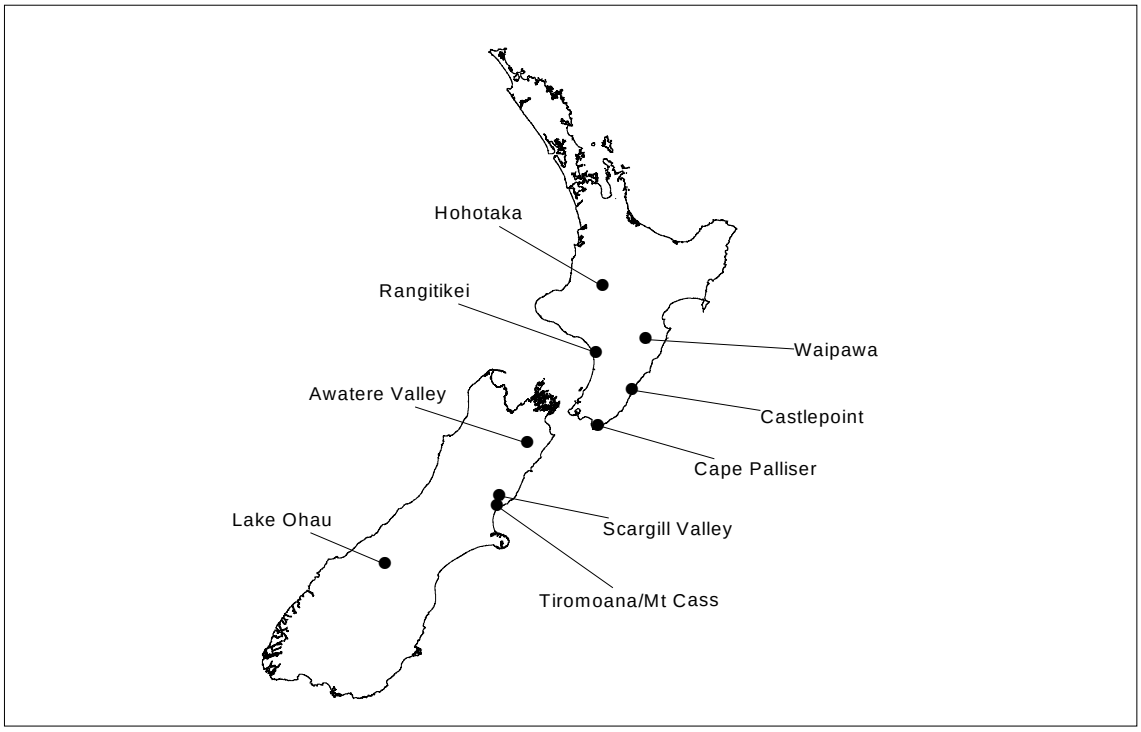

Figure 3.2. Sites of cross-sectional surveys of *M. bovis* infection in feral ferrets in New Zealand, including those used in Chapter 2.

Table 3.1. Effective trapping area (Area) of study sites along with the *M. bovis* infection status of possums from sites where ferrets were surveyed. Whether a restriction endonuclease analysis (REA) match was found between at least one of the strains of *M. bovis* in ferrets and that found in possums is also indicated. Area is calculated from methods in Appendix 6.3. For sites where multiple surveys were undertaken the value of Area represents an average.

Site	Area (km^2)	*M. bovis*-infected possums inside site?	*M. bovis* isolated from possums in general area?	REA match
Lake Ohau	24.1	No	Yes	Yes
Waipawa	33.0	Yes	Yes	Yes
Tiromoana/Mt Cass	53.2	No	Yes	Yes
Scargill Valley	59.9	Yes	Yes	Yes
Cape Palliser	61.2	Yes	Yes	Yes
Castlepoint	29.7	Yes	Yes	Yes
Hohotaka	36.6	Yes	Yes	Yes
Rangitikei	15.5	Yes	Yes	Yes
Awatere Valley	35.7	Yes	Yes	Yes

3.2.2 *Experimental design*

The timing of experimental interventions (reduction in possum population density) and observations (lethal cross-sectional surveys) to the study sites are shown in Table 3.2, following the notation of Manly (1992). These data may be analysed in many ways. Two approaches are used here. The first and simplest is a CI (control, intervention) design that compares estimates of λ from sites with no history of possum management (experimental control treatment) with those from sites following a sustained reduction in possum population density (experimental intervention treatment) similar to that described by Caley *et al*. (1999). The CI design would be described as having simultaneous experimental control (Hone 1994a), and the experimental intervention would be considered a 'press' rather than a 'pulse'. The Castlepoint, Cape Palliser, Awatere Valley, Scargill Valley, and Lake Ohau sites made up the experimental control treatment whilst Hohotaka, Rangitikei, Tiromoana/Mt Cass and Waipawa sites made up the experimental intervention treatment. Note that the Castlepoint and Scargill Valley sites included in the experimental control treatment (Table 3.2) were subsequently subjected to the experimental intervention treatment, and so also form part of the BACI design (see below). For the CI analysis, only survey data collected from these sites before the experimental intervention were considered for analysis.

Table 3.2. Summary of the application of experimental interventions (X) and observations (O) of *M. bovis* infection in feral ferrets (following the notation of Manly (1992)). The experimental intervention is the sustained reduction of possum population density ('press' cf. 'pulse'). Observations are cross-sectional surveys of the ferret population. Numbers in parentheses are sample sizes.

	Site*								
Year	HH	RA	CP	CPR	AWA	SCAR	TIRO	LO	WAIP
Pre-94	X						X		X
1994			O (15)						
1995	O (22)		O (2)			O (78)†	O (19)†		
1996						†	†		
1997		X				O (50)†	O (50)†	O (72)	O (28)
1998	O (55)		O (31)	O (19)		O (33)†	O (39)†		
			X			X			
1999			O (27)	O (14)		O (58)			O (4)
2000		O(30)	O (14)	O (7)	O (47)	O (62)		O (40)	
2001			O (8)	O (1)	O (42)	O (85)			

* HH—Hohotaka; RA—Rangitikei; CP—Castlepoint; CPR—Cape Palliser; AWA—Awatere Valley; SCAR—Scargill Valley; TIRO—Tiromoana/Mt Cass; LO—Lake Ohau; WAIP—Waipawa.

† Both the Scargill Valley and Tiromoana/Mt Cass sites were subjected to intensive ferret control during this period (1995–1998), with 779 and 753 ferret removed respectively (including those shown here). Further details are given by Caley *et al*. (1998a).

The second approach follows a BACI (before vs. after, control vs. intervention) design (Green 1979), which inferentially is considerably stronger than a simple CI design. For example, for some sites used in the CI design, although a possum population density reduction 'treatment' had been applied, and possum population density was indeed low following this, there was little supporting information on the population density before the 'treatment' was applied. Hence there is uncertainty as to exactly what treatment (in terms of a reduction in possum population density) was applied. The BACI design may also be described as having sequential and simultaneous experimental controls (Hone 1994a). Four sites were used in the BACI design, these being Castlepoint (experimental intervention), Cape Palliser (experimental control), Scargill Valley (experimental intervention) and Awatere Valley (experimental control) (Table 3.2). These sites were originally chosen to be matched (as practicably as possible) for possum population density (in the absence of experimental intervention), ferret population density, and the force of *M. bovis* infection.

3.2.3 *Possum control*

Possum control over a 6400-ha area encompassing the Scargill Valley survey area started in winter/spring of 1998 using leg-hold traps, cyanide paste and encapsulated cyanide (Feratox®). Maintenance control to maintain the possum population at the lowered post-control population density was undertaken using encapsulated cyanide in 1999 and 2000. Possum control over a 6510-ha area encompassing the Castlepoint survey area started during the summer/autumn of 1998 using leg-hold traps and encapsulated cyanide, with further maintenance control in 1999. *M. bovis*-infected possums had been found at the Awatere Valley, Cape Palliser, Scargill Valley and Castlepoint sites, and reducing the population density of possums at the latter two sites can be reasonably expected to reduce the density of *M. bovis* infected possums (Caley *et al*. 1999). Indeed, a number of macroscopically *M. bovis*-infected possums were removed during trapping at Castlepoint during 1998.

3.2.4 *Estimating possum population density*

Two indices of possum population density were obtained. The first was based on the number of possums caught incidentally in traps targeted at catching ferrets, using a modified version of Leslie's Removal Method (Seber 1982). The measure of abundance was the estimated number of possums per trap (compared with population density). This was done as home-ranges of possums are in general small compared with the distance between traps. Exact details of the method used are provided in Appendix 6.3. These data were collected from all sites, and provide a standardised index of possum population density enabling comparisons between surveys at all sites.

The second index of possum population density was based on the nationally recognised residual trap-catch (*RTC*) methodology (NPCA 2001). At the time of the study, the *RTC* method for monitoring changes in possum population density involved catching possums on lines of 20 leg-hold traps, with the starting point of each line randomly selected within available possum habitat (stratified random sampling). For repeated yearly surveys, a new random sample of starting points was selected each year. The 20 traps in each line were spaced at 20-m intervals, ran in a North–South direction (Magnetic North), were lured with a mix of flour (5 parts) and icing sugar (1 part), and set for 3 fine nights. The trap-catch statistic for each line was calculated as the average number of possums caught per trap per night. The *RTC* method was used to monitor changes in the population density of possums at Scargill Valley and Castlepoint

resulting from possum control and to monitor natural fluctuations in the population density of possums at Cape Palliser and Awatere Valley. Possums captured at Scargill Valley and Castlepoint point during *RTC* monitoring were killed, whereas those captured at Cape Palliser and Awatere Valley were released. Possums captured during ferret trapping (see below) were treated similarly.

3.2.5 *Sampling ferret populations*

Ferrets were captured in Victor Soft-Catch® leg-hold traps (size 1½) as described in Chapter 2. Methods used to diagnose *M. bovis* infection in ferrets and estimate ferret age are presented in Chapter 2. All ferret carcasses were either incinerated or disposed of in covered offal pits. Lethal cross-sectional sampling of ferret populations is essentially a form of control or culling. There has been no examination of the effect of ferret control on the force of *M. bovis* infection in feral ferrets. Sampling ferret populations infected with *M. bovis* (where sampled ferrets are physically removed from the population) should decrease the force of *M. bovis* infection in ferret populations by reducing the density of *M. bovis*-infected carcasses available to be scavenged by ferrets. Sampling may also reduce the force of infection in other ways, by for example, reducing population density if disease transmission is density-dependent. Ferret population density was estimated in each trapping session at each site using a modified version of Leslie's Removal Method (Seber 1982). Details are provided in Appendix 6.3. Possible changes in the population density of ferrets arising from sampling were tested for by regressing the natural logarithm of population density on time, and testing using a *t*-test (Sokal & Rohlf 1995) whether the instantaneous rate of increase (r) estimated as the slope of this regression (Caughley & Sinclair 1994) was significantly less than zero. The test is one-tailed, as I expected *a priori* that ferret sampling should decrease rather than increase ferret population density. This approach to hypothesis testing is in line with one of the recommendations of Krebs (2000), who argued there should be much more use of one-tailed tests in ecology, especially in the case of planned experiments.

3.2.6 *Analysis of Control-Intervention design*

To avoid any effect of ferret sampling, only λ estimated during the first survey from each site is used for this analysis. For the experimental control sites, estimates of λ using Model 2.2 presented in Chapter 2 were used. For the experimental intervention sites, estimates of λ were made using the same model, though with the effect of sex (2.2 increased hazard for males) and disease-induced mortality (α=1.4 yr^{-1}) fixed at previous estimates from Chapter 2. Differences in the mean λ and possum population density between the treatments were compared using a *t*-test (Sokal & Rohlf 1995). Again the *t*-tests were one-tailed, as I hypothesised *a priori* that the possum control intervention treatment would reduce both possum population density and λ. Any differences in the population density of ferrets between the treatments was compared also using a *t*-test (two-tailed this time).

3.2.7 *Analysis of Before-After Control-Intervention design*

The first problem encountered when analysing this type of observation-intervention-observation data is that some animals spend time in both treatments. During the first sampling session, all animals captured have been subject to one treatment only, making estimation of λ up to this point relatively easy (see Chapter 2). However, in subsequent sampling sessions, some individuals have been subject to both treatments, or only the second treatment (estimation of λ is again straightforward for these animals), as shown schematically in Figure 3.3. Dealing with animals that have spent time in more than one treatments is problematical. One way around this problem is to exclude these individuals from the analysis. This approach is undesirable as it wastes information.

Alternatively, if the time period an animal has spent before capture is divided into two treatment periods, no ferret sampling (Treatment 1), and after the start of ferret sampling (Treatment 2), the prevalence of infection can be expressed as a function of the respective forces of infection in each treatment and the time spent by each individual in each treatment. More specifically, if the times spent in Treatment 1 and Treatment 2 are t_1 and t_2 respectively, then:

P(infected at capture) = 1 – P(not infected during t_1)P(not infected during t_2).

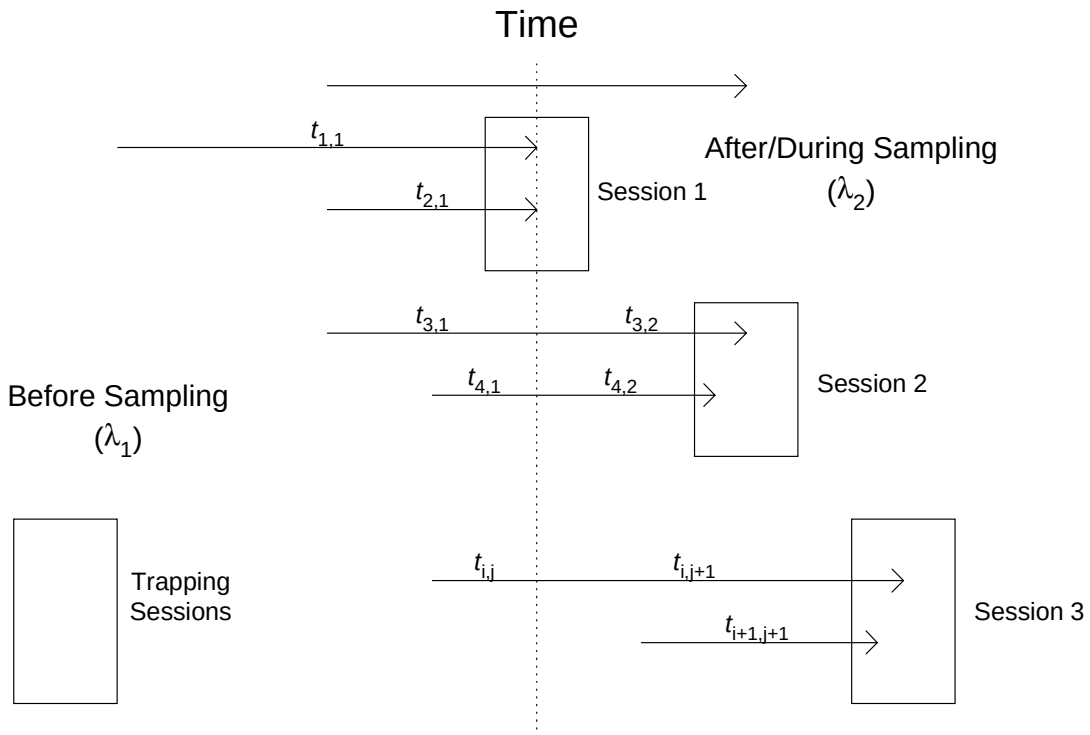

Figure 3.3. A schematic representation of how sampled ferrets have spent different times in the 'sampling' treatments with force of infection λ_1 before sampling and λ_2 after sampling. The start of each line indicates of the time of birth on the time axis (moving from left to right), whereas the end of the arrow represents sampling and death. For example, during Session 1, ferret number 1 spends a period $t_{1,1}$ during Treatment 1 (before sampling), whilst during Session 2, ferret number 3 spends a period $t_{3,1}$ during Treatment 1, and $t_{3,2}$ during Treatment 2 before capture. In general, $t_{i,j}$ represents the time spent by the i^{th} ferret in the j^{th} treatment.

An exponential model ignoring disease-induced mortality is adequate for modelling the force of *M. bovis* infection in feral ferrets, and is much more tractable than the exponential model with disease-induced mortality (Chapter 2). This is the approach taken here. To avoid confusion, from now on I denote the force of infection estimated assuming no disease-induced mortality (α=0, Model 2.1; Chapter 2) as λ'. Assuming a constant force of infection during each treatment period (λ_1' during t_1, λ_2' during t_2), the prevalence of infection at capture for ferrets who have spend all their time in either the first or second treatments is given by Model 2.1 (Chapter 2, Table 2.1), with λ_1' replacing λ, and t_1 or t_2 replacing a. Using the results of the previous short foray into probability theory, the prevalence of infection at capture for ferrets that spend time in both treatment periods can be modelled as:

$$p(t_1,t_2)=1-e^{-\lambda_1' t_1}e^{-\lambda_2' t_2} \qquad \text{(Equation 3.1)}$$

Combining these results gives a model of the prevalence of infection in a system where the force of infection takes on two time-dependent values (Model 2.3) (I have kept the numbering of models in line with Chapter 2, where Model 2.1 & 2.2 pertained to the dietary-related transmission of *M. bovis* [Hypothesis 4]).

$$\left.\begin{array}{ll} p(t_1,t_2)=1-e^{-\lambda_1' t_1} & t_1>0,\ t_2=0 \\ p(t_1,t_2)=1-e^{-\lambda_1' t_1}e^{-\lambda_2' t_2} & t_1>0,\ t_2>0 \\ p(t_1,t_2)=1-e^{-\lambda_2' t_2} & t_1=0,\ t_2>0 \end{array}\right\} \text{(Model 2.3)}$$

Expressions for the age-specific prevalence in Model 2.3 are all nested within Equation 3.1, which makes calculations simple. Rearranging Equation 3.1 and taking the natural logarithm of both sides gives the prevalence of *M. bovis* infection as a function of the λs in the different treatments, and the time spent in each treatment (Equation 3.2).

$$\ln(1-p) = -\lambda_1' t_1 - \lambda_2' t_2 \qquad \text{(Equation 3.2)}$$

The aims of this chapter are to test whether λ_1' differs from λ_2', and to estimate the size of the effect. If sampling ferrets reduces the force of infection by an amount τ, then $\lambda_2' = \lambda_1' - \tau$. Substituting for λ_2' in Equation 3.2 yields the prevalence as a function of the unknown parameters λ_1' and τ (Equation 3.3).

$$\begin{aligned} \ln(1-p) &= -\lambda_1' t_1 - (\lambda_1' - \tau)t_2 \\ &= \lambda_1'(t_1+t_2) + \tau t_2 \end{aligned} \qquad \text{(Equation 3.3)}$$

This equation may be fitted to the data using a generalised linear model (GLM) with the response variable $q=(1-p)$ distributed as binomial with a logarithmic link function (Crawley 1993). An estimate of λ_1' is made by adding the term (t_1+t_2) to the model and estimating its regression coefficient. The magnitude and significance of τ is then estimated by adding t_2 to the model and estimating its regression coefficient. Testing whether τ differs from zero determines if $\hat{\lambda}_2'$ differs from $\hat{\lambda}_1'$. The appropriate test is one-tailed, as I expect τ to be positive. That is, I am testing the null hypothesis $\tau=0$ against the working hypothesis $\tau>0$. To remain consistent with dietary-related transmission (Chapter 2) requires that the guarantee time (g) is subtracted from either t_1 or t_2 (as determined by the circumstances of each individual). As in Chapter 2, g is set to 1.75 months. Note this model ignores any sex effects on λ'. It does so in the interests of utility. The model described should be adequate for testing the question at

hand (does sampling reduce the force of *M. bovis* infection in ferrets), whereas the effect of gender on the λ (note lack of a prime) has been addressed previously in Chapter 2. This assumption is valid assuming the sex ratio of the necropsied sample is independent of treatment, otherwise it may introduce bias.

The model used previously for two treatments (Model 2.3) can be extended to three treatments (or as many as you like), to estimate the additional effect of possum control on the force of infection:

$$p(t_1,t_2,t_3) = 1 - e^{-\lambda_1' t_1} e^{-\lambda_2' t_2} e^{-\lambda_3' t_3}, \qquad \text{(Equation 3.4)}$$

where λ_3' is the force of infection during the period t_3 that the animal is subjected to treatment 3 (here, a reduction in possum population density). Let Δ be the reduction in λ' over and above that observed after the start of ferret sampling, hence:

$$\begin{aligned} \lambda_3' &= \lambda_2' - \Delta \\ &= \lambda_1' - \tau - \Delta \end{aligned} \qquad \text{(Equation 3.5)}$$

Substituting for λ_2' and λ_3' into Equation 3.4 and rearranging yields:

$$\ln(1-p) = -\lambda_1'(t_1 + t_2 + t_3) + \pi t_2 + (\tau + \Delta) t_3 \qquad \text{(Equation 3.6)}$$

As before, Equation 3.6 can be used to estimate λ_1', τ and (τ+Δ) using a GLM (again subtracting g from t_1, t_2 or t_3 as appropriate). Estimates of Δ and its standard error are then calculated as:

$$\hat{\Delta} = (\hat{\tau} + \hat{\Delta}) - \hat{\tau}, \text{ and} \qquad \text{(Equation 3.7)}$$

$$s.e.(\hat{\Delta}) = \sqrt{\text{var}(\hat{\tau} + \hat{\Delta}) + \text{var}(\hat{\tau})}. \qquad \text{(Equation 3.8)}$$

For each site, testing whether $\hat{\Delta}$ or $\hat{\tau}$ differed from zero was undertaken using a one-tailed *t*-test. A meta-analysis approach was used to combine the results from the different sites within the North Island (Castlepoint and Cape Palliser) and South Island (Awatere Valley and Scargill Valley). The probabilities arising from the *t*-tests (examining whether the treatments 'ferret sampling' or 'possum control' influenced λ') from the different sites were combined using the formulae presented by Fisher (1935) (cited by Underwood (1997)):

$$C = -2\sum_{i=1}^{k} \log_e P_i \qquad \text{(Equation 3.9)}$$

Here, P_i is the probability associated with the i^{th} site, and k is the number of sites. C is distributed as χ^2 with $2k$ degrees of freedom.

3.3 Results

3.3.1 *Control-Intervention Analysis*

The population density of possums was significantly (t= –2.2, d.f.=7, P=0.013, one-tailed test) and substantially (89% reduction) lower at experimental intervention sites ($\bar{x}$ =0.10 possums trap^{-1}) than experimental control sites ($\bar{x}$ =0.89 possums trap^{-1}). Likewise, the estimated force of *M. bovis* infection (with non-zero α) in ferrets was significantly (t= –1.9, d.f.=7, P=0.049, one-tailed test) and substantially (88% reduction) lower at experimental intervention sites ($\bar{\lambda}$ =0.30 yr^{-1}) than experimental control sites ($\bar{\lambda}$ =2.50 yr^{-1}) (Fig. 3.4). Ferret population density did not differ (t=0.16, d.f.=7, P=0.88, two-tailed test) between experimental intervention sites (2.4 ferrets km^{-2}) and experimental control sites (2.2 ferrets km^{-2}).

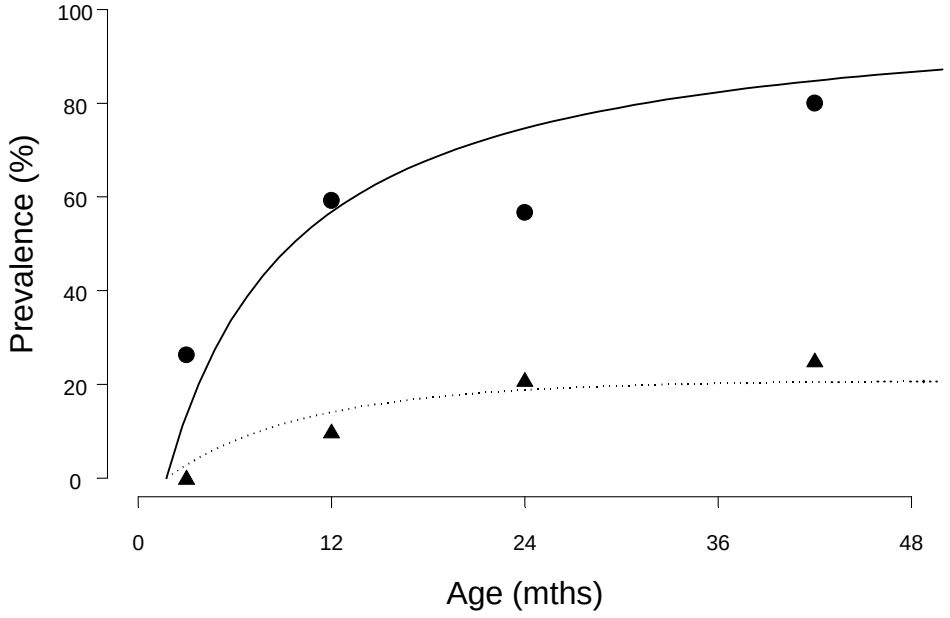

Figure 3.4. Age-specific prevalence of *M. bovis* in ferrets from experimental control (no possum control) sites (solid circles and solid line) compared with experimental intervention (possum control) sites (triangles and dotted lines). Data have been pooled over sites and ages.

3.3.2 *Before-After Control-Intervention Analysis — changes in possum population density*

Possum control at Scargill Valley significantly reduced the possum trap-catch from 13.1% (95% C.I. 9.9–16.3%) pre-control (1998) to 1.2% (95% C.I. 0.5–1.9%) in post-control year 1 (1999), and 0.12% (95% C.I. 0.0–0.24%) in post-control year 2 (2000). Likewise, possum control at Castlepoint significantly reduced the trap-catch from 31.2% (95% C.I. 20.6–41.8%) pre-control (1998) to 0.7% (95% C.I. 0.0–1.4%) in post-control year 1 (1999), and 1.2% (95% C.I. 0.1–2.3%) in post-control year 2 (2000). At the Cape Palliser site where there was no possum control over the same period, the incidental catch rate of possums in traps targeted at ferrets was 9.9% in 1998, 8.4% in 1999 and 6.5% in 2000, indicating a slight decline in population density. Standard trap-catch monitoring of possums at Cape Palliser estimated the trap-catch to be 23.8% (95% C.I. 18.3–29.3%) in 1999 and 20.0% (95% C.I. 13.6–26.4%) in 2000. At the Awatere Valley site, the possum trap-catch decreased from 16% in 2000 to 9% in 2001, however the index based on possums caught in traps targeted at ferrets (based on a much larger sample than the *RTC* estimate) increased from 1.5 possums trap^{-1} to 1.9 possums trap^{-1} over the same period, indicating no significant change in possum population density occurred over this period.

3.3.3 *Before-After Control-Intervention Analysis — changes in ferret population density*

While ferret population density was reduced at the Scargill Valley site in response to the intensive control from 1995 to 1998, when the intensity of sampling was eased after 1998, the population density increased (Fig. 3.5). The estimated rate of increase (0.01 yr^{-1}) over the duration of the study did not differ from zero (t=0.01, d.f.=5, P=0.95). At the other South Island site used in the BACI analysis (Awatere Valley), no change in ferret population density was evident following the start of sampling (Fig. 3.5), though there were too few data points for regression analysis. In contrast, at the two North Island sites used in the BACI analysis, sampling led to a decline in ferret population density (Cape Palliser: r = –1.4 yr^{-1}, t= –4.8, d.f.=2, P=0.02, one-tailed test; Castlepoint: r= –0.4 yr^{-1}, t= –2.2, d.f.=2, P=0.08, one-tailed test). This was particularly so at the Cape Palliser site, where the ferret population steadily declined to near extinction as a result of the sampling (Fig. 3.5). There was no evidence ferret population density was affected by possum control (Fig. 3.5).

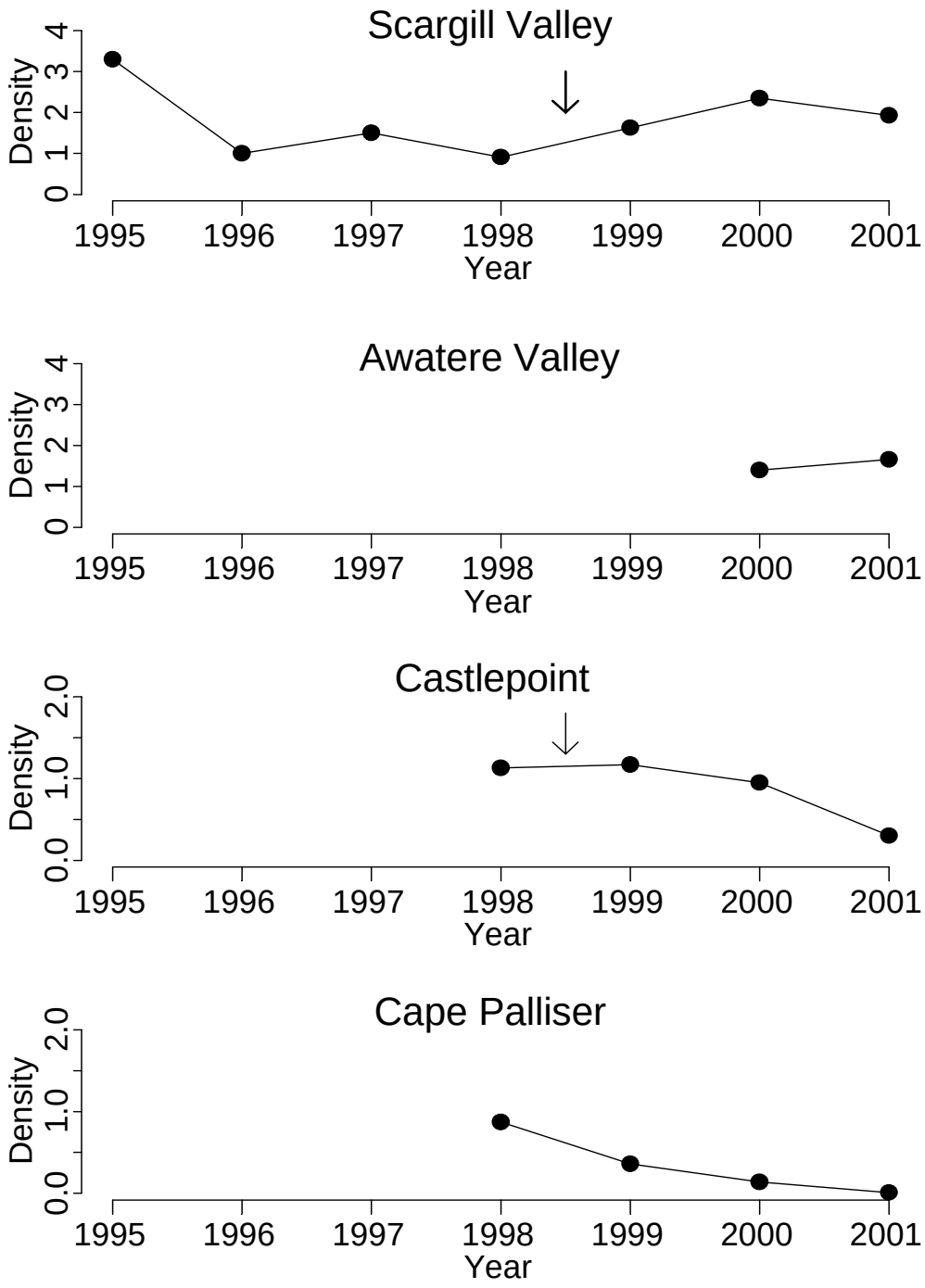

Figure 3.5. Trends in the population density (km^{-2}) of ferrets at experimental intervention sites (Scargill Valley & Castlepoint) and experimental control sites (Awatere Valley & Cape Palliser). Arrows indicate when the experimental intervention (possum control) started. Note the difference in scale of y axes. Estimates of population density were obtained during May of each year using methods detailed in Appendix 6.3.

3.3.4 *Before-After Control-Intervention Analysis — changes in* λ'

At the two North Island sites (Castlepoint & Cape Palliser), λ' was unaffected by ferret sampling, with $\hat{\tau}$ small biologically and statistically non-significant (Table 3.3). In contrast, the effect of reducing possum population density ($\hat{\Delta}$) was both large (94% reduction) and statistically significant (P<0.001; Table 3.3). These results were inferred as demonstrating negligible intra-specific transmission though substantial inter-specific (possum-to-ferret) transmission in these ferret populations.

At the two South Island sites (Awatere Valley & Scargill Valley), it appears lethal sampling of ferret populations reduced $\hat{\tau}$ by biological meaningful amounts (33% and 40 % respectively). Although not statistically significant on their own (P<0.05; Table 3.3), combining the probability values using Equation 3.9 indicates it is likely that a sampling effect exists (χ^2=9.6, d.f.=4, P=0.048). Again, the effect of reducing possum population density was statistically significant (Table 3.3), though the effect size was not as large (40% reduction) as for the North Island sites. These results were inferred as demonstrating that both intra-specific and inter-specific transmission were occurring in these populations.

Table 3.3. Estimates of the parameters λ_1' (force of infection before any treatment interventions), τ (additive effect [reduction] of ferret sampling on λ') and Δ (additive effect [reduction] of possum control on λ') from fitting the model $\ln(1-p) = -\lambda_1'(a-g) + \tau t_f + \Delta t_{f+p}$, where a is the age of ferrets, t_f is the time spent by ferrets in the ferret sampling treatment, and t_{f+p} is the time spent in combined ferret sampling and possum control treatments. The guarantee period is denoted by g (See text for full explanation). Note that figures are rounded.

Treatment (site)	Parameter	Estimate (mth^{-1})	Standard Error	T	Probability (one-tailed)
Experimental Control (Cape Palliser)	λ_1'	0.07	0.03	—[a]	—[a]
	τ	0.01	0.05	0.2	0.41
Possum Control (Castlepoint)	λ_1'	0.34	0.08	—	—
	τ	–0.03	0.30	0.1	0.54
	Δ	0.32	0.04	8.5	<0.001
Experimental Control (Awatere Valley)	λ_1'	0.16	0.04	—	—
	τ	0.06	0.05	1.2	0.11
Possum Control (Scargill Valley)	λ_1'	0.05	0.01	—	—
	τ	0.02	0.014	1.4	0.075
	Δ	0.02	0.01	1.9	0.027

[a] Testing whether λ_1' is significantly greater than zero would be trivial, as by definition λ_1' must be greater than zero, given that infection has already been observed.

3.4 Discussion

This chapter has provided strong inference that a significant level of inter-specific transmission of *M. bovis* occurs from possums to ferrets. Indeed, most of the force of *M. bovis* infection observed in ferrets can be explained by their association with possum populations. This supports the contention of Lugton *et al*. (1997b), and is consistent with the pattern in the prevalence of *M. bovis* in ferrets reported by Caley *et al*. (2001b). Key underlying assumptions supporting this inference include that *M. bovis*-infected possums existed in the study sites in the first place, that reducing the

population density of possums reduces the density of *M. bovis*-infected possums, and that reducing the density of possums reduced the density of *M. bovis*-infected possum carcasses. These assumptions seem entirely reasonable, as possums infected with *M. bovis* have been found at nearly all sites (see Table 3.1). A further important assumption of the analysis is that the experiment was not confounded (ferret population density reduced) by the method used to control possums. Two studies have reported on the effect of possum control on the population density of ferrets. Ground-laid 1080-poisoned jam baits (note this is not a currently approved control method) resulted in significant mortality of resident ferrets (Moller *et al*. 1996). In contrast, Caley *et al*. (1999) recorded no change in the year-to-year population density of ferrets at a site subjected to possum control using a variety of means, including 1080-jam baits (above ground), cyanide baits (above ground), aerially sown 1080-cereal baits, 1080-cereal baits in bait stations, broadifacoum cereal bait in above-ground bait stations, and leg-hold trapping. A further two studies have reported on the effect of controlling rabbits on the population density of ferrets. Rabbit poisoning using 1080-coated (0.02% wt/wt) carrot resulted in only low (*c*. 10%) mortality of resident ferrets (Heyward & Norbury 1999) (though clearly possum poisoning operations use a much higher [typically 4–fold] 1080 concentration in baits). High ferret mortality was recorded following a rabbit poisoning operation using brodifacoum to target rabbits (Alterio 1996). Hence ferrets appear highly susceptible to secondary poisoning from a chronic anticoagulant like brodifacoum. Five of the sites reported on here had been subject to possum control before being surveyed (Hohotaka, Scargill Valley, Waipawa, Rangitikei, Tiromoana/Mt Cass). Other than Hohotaka (for which no change in ferret population density was observed, see Caley *et al*. (1999)), none of these sites used anticoagulants as the method for either initial or maintenance control of possums, before our ferret surveys. Hence I reasoned possum control had not greatly influenced ferret population density at these sites, and this reasoning is strongly supported by the analysis here detecting no effect of possum control on ferret population density.

The experimental result supporting intra-specific transmission, at least in high population density habitats, has major implications for our understanding of the epizootiology of *M. bovis* infection in ferrets. At low ferret population density, there was little support for intra-specific transmission. Indeed, at the Cape Palliser site, the force of infection remained constant despite the ferret population being trapped nearly to extinction. This indicates that intra-specific transmission is likely to be density-dependent, which fits with the classical prediction (Kermack & McKendrick 1927) that

intra-specific transmission should be higher at higher population density. This may go part-way to explaining conflicting conclusions (e.g., Lugton *et al*. (1997b) vs. Ragg (1998b)) as to the importance of intra-specific transmission of *M. bovis* between ferrets. Lugton *et al*. (1997b)'s study was predominantly undertaken in North Island habitats (low ferret population density), whereas Ragg (1998b)'s study was undertaken in the lower part of the South Island in habitats supporting high densities of ferrets. Alternative explanations do exist for the observed reduction in the force of infection arising from lethal ferret sampling, including that the reduction in ferret population density arising from sampling resulted in higher *per capita* food availability of preferred prey (rabbits) and lower rates of scavenging. The data do not allow the relative support for these two hypotheses to be compared. A critical experiment could involve reducing the population density of ferrets by removing non-diseased ferrets only. This would require a highly sensitive and specific non-lethal diagnostic test, which currently does not exist. Ways of modelling density-dependent scavenging rates are discussed further in Chapter 5.

I now return to issues of inference for inter-specific transmission in host/pathogen systems mentioned in the Introduction. McInerney *et al*. (1995) inferred that a major reduction in the prevalence of *M. bovis* infection in feral pigs following a major reduction in the population density of *M. bovis*-infected feral swamp buffalo in the floodplains of the Northern Territory indicated feral pigs were spillover hosts for *M. bovis* in this habitat. Their inference (that feral pigs were a spillover host for *M. bovis*, and that control of the maintenance host [buffalo] *caused* the reduction in disease in feral pigs) has been accepted without question, despite the experimental design used (simply a 'before' vs. 'after' comparison) being generally considered weak in terms of strength (reliability) of inference (Caughley & Sinclair 1994). Why this is so deserves further thought. First, the experimental result was hypothesized *a priori* by Corner *et al*. (1981), based on a biological understanding of the host/pathogen system in question. This is deductive logic (most powerful). In contrast, simply observing that the prevalence of *M. bovis* infection in feral pigs had declined, and then hypothesizing in an *a posterior* manner that the near-removal of buffalo was the cause would be considered inductive logic. Second, the size of the effect was very large (there was no doubt the response variable 'after' was different from the response variable 'before'). Third, the scale of the experiment was large, with considerable replication. Finally, there was considerable supporting evidence. For example, the population density of buffalo had indeed declined following control (Bayliss & Yeomans 1989; Freeland & Boulton

1990), demonstrating an experimental treatment (here the reduction in population density of *M. bovis*-infected buffalo) had been applied in nature as well as name. Hone (1999) provides further discussion on the need for supporting evidence in experimental studies. Caley *et al*. (1999) essentially used a BACI design to test the effect of reducing the population density of possums on the incidence of *M. bovis* in sympatric domestic livestock. The reported experimental result (of a causative link between *M. bovis* in possums and *M. bovis* in cattle) has received little or no opposition — probably because the result was what most people already believed!

In contrast to the buffalo/ pig/*M. bovis* system, the inference on whether *M. bovis* in British cattle is a spillover from infected badgers, and hence whether reducing the population density of badgers results in a lowered incidence of *M. bovis* infection in cattle has been subjected to considerable scrutiny and scepticism (Krebs *et al*. 1998). Part of the reason for this was the experimental design of studies (e.g., Clifton-Hadley *et al*. 1995) purporting to demonstrate a link was weak (essentially 'before' vs. 'after' only), with additional issues of inadequate replication, small scale, and possible confounding (movement of cattle). There was supporting evidence as to why badger culling could potentially reduce the incidence of *M. bovis* in cattle, as Anderson & Trewhella (1985) demonstrated (though did not statistically test) a reduction in the estimated the force of *M. bovis* infection in badgers following badger removal operations in the Gloucestershire area. However, the design of Anderson & Trewhella (1985) was a 'before' vs. 'after' comparison. Subsequently, stronger inference has come to light from Ireland on the role of badgers in transmitting *M. bovis* to cattle (O'Mairtin *et al*. 1998a,b). The intense scrutiny of the evidence supporting inter-specific transmission of *M. bovis* from badgers to cattle, and hence management of badger populations is understandable, given the public affection for badgers (Neal & Cheeseman 1996)—also they are legally protected wildlife. In contrast, affection for possums in New Zealand, or feral pigs in the Northern Territory is near to non-existent (personal observation).

This chapter has focussed on issues of hypothesis testing. In particular, it has critically tested whether the control of possums influences the transmission of *M. bovis* to ferrets. I have not dwelt on the absolute sizes of the estimated changes in the force of infection arising from possum control and/or ferret sampling, as the estimator used for the force of infection is probably biased downwards (Chapter 2). The next chapter is concerned with issues of estimation. That is, how much of the *M. bovis* infection

observed in ferrets arises from inter-specific transmission (e.g., possum-to-ferret), as opposed to intra-specific (ferret-to-ferret) transmission.

Chapter 4

Estimating the basic reproductive rate of *Mycobacterium bovis* infection in feral ferrets

4.1 Introduction

The basic reproductive rate of a disease (R_o) is the most fundamental measure of a pathogen's ability to establish within a host population (Anderson & May 1981; 1991). The R_o is defined as the average number of secondary infections produced when one infected individual is introduced to a totally susceptible host population (Anderson & May 1991). By definition, if R_o is greater than or equal to unity, the disease will establish in the population, and conversely, if R_o is less than unity, the disease will fail to establish (Anderson & May 1991). This concept is sometimes referred to as the fundamental law of epidemiology, and it provides a quantitative approach to determining the host status of species for a given pathogen (Chapter1). A closely related epidemiological parameter of great importance is the threshold density for disease establishment, denoted K_T (Kermack & McKendrick 1927). However, few studies of disease (particularly those caused by micro-parasites) in free-living mammals (excluding humans), have estimated R_o for the express purpose of assessing host status. Rather, the prime motivation for estimating R_o has been to model (estimate) the control effort or strategy (culling, vaccination etc.) needed for disease eradication (e.g., Barlow1991a; Roberts 1996).

The key point here is that authors have assumed *a priori* disease is established and persisting, and hence R_o is greater than unity (e.g., Barlow 1991b; Roberts 1996; McCarty & Miller 1998)—a rather circular approach to estimating R_o. This does, however, facilitate simpler computation of R_o from steady state (equilibrium) prevalence. For example, Barlow (1991b) estimated R_o for *M. bovis* infection in possum populations in New Zealand to be in the range 1.8–2.0. The range presented does not represent any estimate of variability, rather it was estimated by varying combinations of parameter values (used in calculating R_o) in the model at hand to see which yielded reasonable (non-zero) prevalence and produced desired disease dynamics. Roberts (1996) used a similar approach to estimating R_o of *M. bovis* in possums (though used a different expression for it) by varying the value of the disease

transmission coefficient and disease mortality parameters to find those that produced a non-zero disease prevalence of the desired value. McCarty & Miller (1998) did similarly, fitting their model of *M. bovis* infection in white-tailed deer to ensure a non-zero, non-decreasing disease prevalence. There are exceptions. Hone *et al*. (1992) estimated R_o of classical swine fever in wild boar (*Sus scrofa*) populations without any *a priori* assumptions as to whether or not the disease was already persisting, though knowing it had established. Anderson & Trewhella (1985) used age-prevalence data to estimate λ, and went on to use this estimate in calculating R_o for *M. bovis* infection in badgers.

There has been no attempt to estimate the R_o of *M. bovis* in ferret populations. Estimates of R_o and K_T from studies of diseases of other vertebrate wildlife are shown in Tables 4.1 and 4.2, respectively, noting whether there was any estimate of the variance around the parameter. Of the studies listed in Tables 1 and 2 (18 in total), only one estimated the variability of either $\hat{R}_o$ or $\hat{K}_T$ (although some did undertake sensitivity analyses). It seems while estimating R_o and K_T is considered fundamental to the understanding of host/pathogen dynamics, estimating their variance is not so popular! The situation is not always quite so bleak for diseases of domestic animals (e.g., see Nodelijk *et al*. 2000), though Ferguson *et al*. (1999) omitted to estimate the variability around their estimate of $\hat{R}_o$ for BSE in British cattle—a little strange given that the express purpose of the study was to determine whether or not control measures (of food) had reduced R_o to below unity. Note, however, that Ferguson *et al.* (2001) calculated R_o and its variance for the 2001 outbreak of foot-and-mouth disease (FMD) in British cattle, with the express purpose of determining what form of management action would reduce R_o below unity.

Table 4.1. Estimates of the basic reproductive rate ($\hat{R}_o$) for wildlife host/pathogen systems, and whether the variance of the estimate [var($\hat{R}_o$)] was presented.

Species	Pathogen	$\hat{R}_o$	var($\hat{R}_o$)
Domestic dog (*Canis familiaris familiaris*)[a]	*Leishmania infantum*	Various	No
Possum[b]	*M. bovis*	1.6	No
Possum[c]	*M. bovis*	2.1	No
Possum[d]	*Leptospira interrogans*	1.5	No
Ring-necked pheasant (*Phasianus colchicus*)[e]	*Heterakis gallinarum*	1.2	No
White-tailed deer[f]	*M. bovis*	>1	No
Wild boar[g]	Swine fever virus	1.1–2.1	Yes

[a] Dye *et al.* (1992); [b] Roberts (1996); [c] Barlow (2000); [d] Caley & Ramsey (2001); [e] Tompkins *et al*. (2000); [f] McCarty & Miller (1998); [g] Hone *et al*. (1992).

Table 4.2. Estimates of the threshold population density or abundance ($\hat{K}_T$) for disease establishment for wildlife host/pathogen systems, and whether the variance of the estimate [var($\hat{K}_T$)] was presented. The units of $\hat{K}_T$ are individuals, unless otherwise stated.

Species	Pathogen	$\hat{K}_T$	var($\hat{K}_T$)
Bison[a]	**Brucella abortus**	200	No
Eurasian badger[b]	**M. bovis**	1 km^{-2}, 5 km^{-2}	No
Feral pig[c]	Foot-and-mouth disease (FMD)	2.3–14 km^{-2}	No
Feral pig[d]	FMD	0.027–0.037 km^{-2}	No
Feral pig[e]	FMD	0.6–2.0 km^{-2}	No
Feral pig[f]	FMD	Various	No
Feral pig[g]	Transmissible gastroenteritis	161–805	No
Harbour seals (*Phoca vitulina*)[h]	Phocine distemper	Various	No
Norway rat (*Rattus norvegicus*)[i]	*Yersinia pestis*	50000	No
Red fox[j]	Rabies	1 km^{-2}	No
Side-striped jackal (*Canis adustus*)[k]	Rabies	1.4 km^{-2}	No

[a] Dobson & Meagher (1996); [b] Anderson & Trewhella (1985);[c] Pech & Hone (1988); [d] Pech & McIlroy (1990); [e] Caley (1993); [f] Dexter (1995); [g] Hone (1994b); [h] Swinton *et al*. (1998); [i] Keeling & Gilligan (2000); [j] Anderson *et al*. (1981); [k] Rhodes *et al*. (1998).

To estimate R_o requires first estimating the disease transmission coefficient. Estimating the transmission coefficient is considered to be a very difficult problem (Anderson & May 1991), and remains a great challenge in field ecology today (McCallum *et al*. 2001). Indeed, Barlow (2000) suggests the most robust way of estimating transmission coefficients is by '… tuning models with repeated trial values to mimic observed disease behaviour.' This chapter estimates R_o for *M. bovis* infection of feral ferret populations, using a combination of modelling the relationship between

the force of *M. bovis* infection in ferrets (Chapter 2) and ferret and/or possum population density, and observational studies of ferret scavenging behaviour. The decision to additionally estimate R_o by modelling of ferret scavenging behaviour was made after recent studies (Ragg *et al*. 2000; McAuliffe 2001) were published, adding to my own data. Disease transmission coefficients are model-dependent, and an important issue is the form of the model for the scaling between host population density and parasite transmission rate (Grenfell & Bolker 1998; McCallum *et al*. 2001, and references therein). Hence I devote considerable time to discussing the most appropriate form of transmission for *M. bovis* infection to ferrets. As with most things, our scientific estimations are often conditional on what we believe is the most likely working model of the system (the paradigm of the moment, if you like). Here, the assumptions relate to the form of transmission (see below), and not the value of R_o.

4.2 Methods

4.2.1 *Modelling transmission*

A precursor to estimating R_o is identifying the appropriate model of disease transmission, and hence there is a model selection (Burnham & Anderson 1998) issue here. The default form of disease transmission for host/pathogen models has conventionally been homogeneous mixing between infectious and susceptible individuals, with the rate of new infections (transmission rate) equal to βSI, where β is the transmission coefficient, S is the density of susceptible individuals, and I the density of infectious individuals (Anderson & May 1979). This approximation of transmission (termed 'density-dependent') has been shown to be reasonable for many directly transmitted diseases, but may not be adequate for sexually transmitted diseases, for example, where the number of sexual partners (η) is independent of the absolute population size and hence no threshold population density exists (May & Anderson 1979). In this situation, the transmission rate may be approximated by $\beta\eta SI/N$ (May & Anderson 1979), or here by $\beta' SI/N$ (replacing $\beta\eta$ with β'). This model of transmission (termed 'frequency-dependent' transmission) is most appropriate for diseases transmitted through contacts that are largely density-independent (e.g., mating). However, De Jong *et al*. (1995) suggest frequency-dependent transmission may have wider application.

The β's for density-dependent and frequency-dependent transmission are not equivalent. When describing the frequency-dependent transmission model, Roberts &

Heesterbeek (1993) define β as the *per capita* contact rate for contacts that, if between a susceptible and an infective, would result in disease transmission. The β used by Anderson & May (1991) is a transmission coefficient, whilst that of Roberts & Heesterbeek (1993) and De Jong *et al*. (1995) is a simple disease contact rate. McCallum *et al*. (2001) seek to remove the confusion regarding modelling pathogen transmission. A key recommendation from their work is that transmission should be modelled using densities of individuals rather than absolute numbers.

Few studies have estimated transmission coefficients in free-living vertebrates, and compared forms of transmission. Begon *et al*. (1999) reported frequency-dependent transmission to be a superior descriptor to density-dependent transmission for the spread of cowpox virus in mixed populations of free-living rodents, and called into question the general assumption that transmission rate for non-sexually transmitted diseases is density-dependent. However, in contrast, Caley & Ramsey (2001) reported the transmission of *Leptospira interrogans* infection in brushtail possums to be best described by density-dependent compared with frequency-dependent transmission—given leptospirosis is a predominantly sexually transmitted disease of possums (Day *et al*. 1997), the reverse was expected (Caley & Ramsey 2001).

So which form of mixing is most appropriate for modelling *M. bovis* transmission in ferrets? I appeal to the biology of the situation for guidance. The biological model most strongly supported by the data is one of transmission to ferrets from the ingestion of *M. bovis*-infected carcasses (Chapter 2). These could be either *M. bovis*-infected ferrets or *M. bovis*-infected possum carcasses—ferrets show little difference in preference (Ragg *et al*. 2000). In this sense, the ferret/*M. bovis* system is highly analogous to the experimental laboratory system for Indian meal moth (*Plodia interpunctella*) and its granulosis virus (PiGV) described by Knell *et al*. (1998), whereby the transmission is largely by means of cannibalism of infectious cadavers. The form of intra-specific *M. bovis* infection among ferrets is also similar to the cycle of the nematode parasite *Capillaria hepatica* among feral house mice (*Mus domesticus*), whereby cannibalism and necrophagy are the main routes of transmission of the parasite eggs (McCallum & Singleton 1989).

A key result of the study of Knell *et al*. (1998) was that the assumption of density-dependent transmission was not valid, although a persistent problem in their experiments was the changing (decreasing) amounts of infectious material with time, and the possible confounding effect on the estimates of the transmission coefficient (in contrast I expect a more or less constant supply of infected carcasses). Their approach

to this of extrapolating backwards to zero to estimate the transmission coefficient is of concern, given that the data from which they are extrapolating are biased. However, this concern aside, their experiments show that over a large (four-fold) range of susceptible host densities, the estimated transmission coefficients assuming density-dependent transmission fell within a reasonably narrow range. Observed variability was ascribed to behavioural and physiological changes in susceptible hosts at high densities of susceptible hosts. Variations in the estimated transmission coefficient with changing density of infectious cadavers were more pronounced, with the estimated transmission coefficient decreasing with increasing abundance of infectious cadavers. Differential susceptibility among meal moth larvae was proposed as a reason for this (Knell *et al*. 1998).

The factors leading to violation of the density-dependent transmission assumption described by Knell *et al*. (1998) do not appear to occur in the ferret/*M. bovis* system. The diet of ferrets is predominantly rabbits, with potentially *M. bovis*-infected ferret and possum carcasses making up only a small proportion of the diet (Smith *et al*. 1995). Hence behavioural and physiological changes in susceptible hosts arising from changes in the ratio of susceptible hosts to infected carcasses could only be slight. The force of *M. bovis* infection in ferrets varies greatly but is less than about 5 yr^{-1} (Chapter 2). The time for ferrets to handle a possum or ferret carcass is short (maximum of 2–3 days). Hence there is little likelihood of saturation in food intake and therefore in the *per capita* functional response (to infected carcasses), since the carcass handling time is short compared with the expected time interval until the next infected carcasses becomes available to be encountered. The *per capita* functional response is a contact rate. The result of this gives little support for a non-linear contact rate function. In contrast, a density-dependent function $C(N) = \frac{N}{1-\varepsilon+\varepsilon N}$ for $\varepsilon \geq 0$ allows the relationship between the contact rate and population density to vary from linear to convex-up (Roberts 1996). Note, however, there is little empirical support for the convex-up contact rate function; though see Caley *et al*. (1998b) for an example of a convex-down relationship. Also, importantly, communal feeding by ferrets on carcasses occurs (Ragg *et al*. 2000)—there appears to be little or no interference competition.

As a starting point, I would expect the *per capita* rate of transmission to be directly proportional to the density of *M. bovis*-infected carcasses (possums or ferrets) for the range of data of interest. Ferrets do not appear to exhibit any strong intra-sexual

territoriality with associated spacing behaviour, but rather have a considerable amount of home-range overlap (Ragg 1998b; Norbury *et al*. 1998a), so 'mixing' is likely to be, at the very minimum, weakly homogeneous. Hence as a starting point for modelling, simple density-dependent transmission with a linear contact rate seems appropriate.

4.2.2 *An overview of models for estimating R_o of directly transmitted microparasites*

Anderson & May (1991) provide a number of steady-state solutions for the basic disease reproductive rate. Under Type I mortality (death rate consistently low until the older age classes), they derive the following expression for a steady-state system (Equation 4.1):

$$R_o = \frac{\lambda L}{1 - e^{-\lambda L}}, \qquad \text{(Equation 4.1)}$$

where L is the life expectancy (clearly disease-induced mortality [α] is assumed to be negligible). However, under Type II mortality, where life expectancy declines exponentially with increasing age (much more applicable to feral ferrets), they obtain (again under steady-state assumption and with negligible disease-induced mortality):

$$R_o = 1 + \lambda L. \qquad \text{(Equation 4.2)}$$

As λ is simply the reciprocal of the mean age of first infection (A), we can rewrite Equation 4.2 in terms of L and A (Equation 4.3):

$$R_o = 1 + \frac{L}{A}. \qquad \text{(Equation 4.3)}$$

Anderson & May (1991) also provide a general argument relating R_o for a microparasite in a homogeneously mixed host population to the overall fraction who are susceptible at equilibrium (x^*) (Equation 4.4). The parameter p in Equation 4.4 is the proportion of hosts that are infectious. Note that $x^* = \frac{S^*}{N^*}$, where S* and N* are equilibrium densities of the susceptible and total population respectively.

$$\begin{aligned} R_o &= \frac{1}{x^*} \\ &= \frac{1}{1 - p} \end{aligned} \qquad \text{(Equation 4.4)}$$

Equations 4.1, 4.2, 4.3 and 4.4 are clearly equal to or greater than unity for all nonnegative values of λ, L, A, or p. This seems a bit strange, for even if λ or p equal zero, R_o equals one (and by definition the disease establishes). This is because solutions for R_o in Equations 4.1, 4.2, 4.3 & 4.4 assume the system is in a steady-state with a non-

zero prevalence. Whatever the value of λ, the prevalence is assumed to be constant, so clearly the disease must be persisting, and hence R_o must be unity or greater. These equations are clearly inappropriate for estimating R_o when it is not clear whether the disease will establish.

Mollison (1995) provides a number of expressions for R_o, namely:

$$R_o = \beta N \tau_I, \qquad \text{(Equation 4.5)}$$

$$R_o = \frac{N}{S}, \text{ and} \qquad \text{(Equation 4.6)}$$

$$R_o = \frac{L}{A}, \qquad \text{(Equation 4.7)}$$

where β is the transmission coefficient (assuming density-dependent transmission), N is the total population size, τ_I is the mean infectious period, S is the equilibrium number of susceptibles, L the mean lifetime, and A the mean age of acquiring the disease. Mollison (1995) argues for the equivalence of 4.5, 4.6, and 4.7. Equation 4.6 is clearly always greater than or equal to one, as S must always be less than or equal to N. Equation 4.7 is perhaps the simplest and intuitively appealing. A heuristic interpretation of this idea is if on average ferrets die before they become infected, then clearly the disease will not persist. There is a flaw in this argument, however, because many diseases persist in populations at such a low prevalence that most individuals are never infected (i.e. $A >> L$). If we substitute for $\lambda = \frac{1}{A}$ in Equation 4.7, it yields $R_o = \lambda L$, that is clearly not equivalent to Equation 4.5, as $\lambda \neq \beta N$. It appears Equation 4.7 is an approximation suitable for when R_o is large—it should read $1 + \frac{1}{A}$ (equivalent to Equation 4.2). As with expressions 4.1–4.4, the estimated values of R_o from expressions 4.6 and 4.7 are always unity or greater—these expressions for R_o are clearly inappropriate for the task at hand. Equation 4.5, however, is not constrained to be greater than or equal to unity, and it is explored further (below).

Assuming the rate of conversion from the susceptible to the infected class is described by density-dependent transmission, βSI, with horizontal transmission only, the basic reproductive rate of the disease is given by (Anderson 1981):

$$R_o = \frac{\beta S}{\alpha + b + \gamma}, \qquad \text{(Equation 4.8)}$$

where β is the transmission coefficient, b is the natural mortality rate, S equals the number (or density) of susceptible animals, γ is the rate of disease recovery, and α is the rate of disease-induced mortality. The latent period is assumed equal to zero. Host

population dynamics assume exponential population growth, with the exponential rate of increase $r = a - b$, where a and b are the instantaneous *per capita* birth and death rates respectively. Most of the studies listed in Table 4.1 have estimated R_o using Equation 4.8 or variants of it. If host population growth follows the simple logistic model, the solution for R_o is essentially the same, although S may be replaced by K (population carrying capacity), and a replaces b, and a disease latency period $\left(\frac{1}{\sigma}\right)$ incorporated if required (e.g., Anderson *et al.* 1981; Pech & Hone 1988). Anderson & Trewhella (1985) used Equation 4.8 to estimate the R_o of *M. bovis* infection in badgers assuming generalised logistic growth. Equation 4.8 can be interpreted as one infected animal, equivalent to population density $I = \frac{1}{H}$ (where H is the home-range area), making $\frac{\beta S}{H}$ infectious contacts per unit area per unit time for its infectious life expectancy ($\frac{1}{\alpha + b + \gamma}$), over an area H. This term for life expectancy (whilst diseased) assumes α, b and γ are additive. This is equivalent to the mean infection period of infectiveness in Equation 4.5. Likewise, N in Equation 4.5 is equivalent to S in Equation 4.8, and hence Equations 4.5 and 4.8 are equivalent. For *M. bovis* infections, there is a general consensus that resolution of infection (cf. disease) is rare (the adage 'once infected, always infected' is commonplace in discussions with respect to *M. bovis* or *M. tuberculosis* infections, (though see Gallagher *et al*. (1998) and Bentil & Murray (1993)), hence γ is effectively zero, and Equation 4.8 may be simplified as:

$$R_o = \frac{\beta S}{\alpha + b}. \qquad \text{(Equation 4.9)}$$

For the frequency-dependent approximation of the transmission process, the maintenance of disease is independent of the population size, and occurs (May & Anderson 1979) when $\beta' > (b + \gamma + \gamma)$. It follows that the basic reproductive rate may be calculated (Roberts & Heesterbeek 1993; Heesterbeek & Roberts 1995) as:

$$R_o = \frac{\beta'}{\alpha + b + \gamma}. \qquad \text{(Equation 4.10)}$$

A heuristic explanation of Equation 4.10 is an infective individual meeting β susceptible individuals per unit of time, and it does this for a period of $\frac{1}{\alpha + b + \gamma}$ (Heesterbeek & Roberts 1995). Assuming local population density does not vary (and hence affect the contact rate), this expression for R_o is considered to be independent of population size (De Jong *et al*. 1995). This is also the case if local population density does vary;

however, individuals have a fixed number of infectious contacts per unit time (as may be the case for sexually transmitted diseases). This model is biologically unrealistic for the ferret/*M. bovis* system, hence is not explored further.

4.2.3 *Estimating R_o for M. bovis infection in ferrets*

The formulation of R_o for the ferret/*M. bovis* system needs to account for transmission occurring from *M. bovis*-infected carcasses, rather than living individuals. This requires the infectious life expectancy ($\frac{1}{\alpha+b+\gamma}$) is replaced by the viable life expectancy of a carcass ($\frac{1}{d}$), where d is the rate that *M. bovis* becomes non-viable in a carcass. The revised expression is:

$$R_o = \frac{\beta S}{d}. \qquad \text{(Equation 4.11)}$$

Obviously the carcass does not move and, strictly speaking, H should be zero! However, the way β is calculated assumes movement of both the susceptibles and the infectious animals (in this case carcasses).

Hence, to estimate R_o for a given population of ferrets requires estimates of S, β, and d (to answer 'Will *M. bovis* infection establish in a particular ferret population?'). To estimate R_o for differing values of S requires estimates of β and d. By setting R_o equal to unity in Equation 4.11, the threshold population density (K_T) for disease establishment is found (Equation 4.12) (This is to answer, 'At what level of mean population density will *M. bovis* establish in ferret populations?').

$$K_T = \frac{d}{\beta} \qquad \text{(Equation 4.12)}$$

4.2.4 *Estimating transmission coefficients from force of infection estimates*

As λ is the instantaneous *per capita* rate at which susceptible individuals acquire infection, in a population containing S susceptible ferrets, the rate of conversion from susceptibles to infecteds will be λS. Under density-dependent transmission for a single-species model, this must equate with the term βSI, where I is the density of infectious animals. That is, $\lambda S = \beta SI$, hence $\lambda = \beta I$. However, ferrets may be infected from several sources, hence the observed force of infection is the summation of the contribution of the different sources of infection. If, for simplicity, we assume random mixing not only

among ferrets (more specifically, between live and dead ferrets), but between ferrets and other species (*n* species in total including ferrets), the rate at which susceptible ferrets are infected may be represented by the sum of the 'mass-action' terms (Equation 4.13):

$$\lambda S_F = \sum_{i=1}^{n} \beta_i S_F I_i \,. \qquad \text{(Equation 4.13)}$$

Here (Equation 4.13), I_i is density of infectious individuals of species *i*, and S_F is the population density of susceptible ferrets. The term S_F is common to all terms on both sides of Equation 4.13; hence λ may be simply expressed as the product of the density of each infected species and the relevant transmission coefficient (Equation 4.14);

$$\lambda = \sum_{i=1}^{n} \beta_i I_i \,. \qquad \text{(Equation 4.14)}$$

For *M. bovis* infection in ferrets, I initially hypothesize ferrets acquire infection from one of two sources—either scavenging on *M. bovis*-infected carcasses of ferret (*i*=1) or possum (*i*=2). Disease transmission arises from intra-specific, or inter-specific (possum-to-ferret) contact, hence Equation 4.14 may be expressed (after replacing *I*s with *W*s as is more conventional when referring to abundance of cadavers) as:

$$\lambda = \beta_F W_F + \beta_P W_P + O\,, \qquad \text{(Equation 4.15)}$$

where:

W_F = the density of dead infectious ferrets

W_P = the density of dead infectious possums

β_F = ferret carcass-to-ferret disease transmission coefficient

β_P = possum carcass-to-ferret disease transmission coefficient

O = contribution to λ from other infectious species ($= \sum_{i=3}^{n} \beta W_i$)

The term *O* is included in the model as a way of assessing the two species assumption. An estimate of *O* significantly different from zero indicates bias. From Appendix 6.2, the density of *M. bovis*-infected ferret carcasses (W_F) containing viable bacilli is related to the density of *M. bovis*-infected ferrets (I_F) by:

$$W_F(t) \cong \frac{(\alpha + b) I_F}{d}, \qquad \text{(Equation 4.16)}$$

where *d* is the rate of decay of *M. bovis*-infected ferret carcasses. Hence Equation 4.15 may be rewritten as:

$$\lambda = \beta_F \frac{(\alpha + b)}{d} I_F + \beta_p W_P + O$$
$$= \beta'_F I_F + \beta'_P I_P + O. \qquad \text{(Equation 4.17)}$$

Equation 4.17 was fitted to data by linear least-squares regression, using estimates of I_F and I_P (as indexed by the estimated population density of possums), to obtain estimates of β'_F, and β'_P. The primes for the parameters signify the change in units.

This simple model has the *per capita* rate of transmission of *M. bovis* infection to ferrets (λ) as proportional to the sum of the density of *M. bovis*-infected ferrets (I_F), and the density of *M. bovis*-infected possums (I_P). This clearly assumes there is no competition for carcasses from other ferrets. There is certainly little if any direct behavioural competition at carcasses, as Ragg (1997) observed communal feeding by ferrets at carcasses. Competition by other scavenging species such as feral cats is possible; however, Ragg (1997) observed ferrets to be dominant over cats at carcasses.

A critical model assumption is that the density of infected ferrets is relatively constant throughout the year. The age-prevalence modelling (Chapter 2) strongly supports this assumption. I can further assess its validity by estimating seasonal variation in the density of *M. bovis*-infected ferrets. Ferrets are seasonal breeders (Lavers & Clapperton 1990), hence the population density of ferrets is cyclical, peaking in late January/early February following the emergence of recruits, and declining thereafter, until the first recruitment appears in mid- to late December (personal observation). The prevalence of *M. bovis*-infected ferrets is heavily influenced by the age structure of the population (see Chapter 2), hence it is cyclical, being lowest when population density is highest, and visa versa. I can examine whether the expected density of infected ferrets is a constant, by examining the sum of the products of the expected age-specific prevalence (from Chapter 2) and the expected proportion surviving in the population for each age cohort in the population. This was calculated from Table 4.3, assuming μ_1 (the instantaneous mortality rate in the first year of life) is 1.44 yr^{-1}, μ_2 (the instantaneous mortality rate thereafter) is 0.56 yr^{-1} (Appendix 6.4) and α=1.4 yr^{-1} (Chapter 2). This was undertaken assuming a maximum survival age of five years (the oldest recorded age from this study—see Appendix 6.4), and for the different forces of infection observed in Chapter 2.

Table 4.3. Cohort age, proportional survival, and *M. bovis* prevalence of ferrets as a function of the time (t) following the annual date of emergence from the natal den ($0 \leq t \leq 1$ year). Mortality rates are calculated following Appendix 6.4, and disease prevalence from Chapter 2. μ_1 is the instantaneous mortality rate during the first year of life post emergence, μ_2 is the rate thereafter, α is the rate of disease-induced mortality arising from *M. bovis* infection, and λ is the force of *M. bovis* infection.

Cohort age (years)	Proportion of cohort surviving	Prevalence
0–1	$e^{-\mu_1 t}$	$\frac{\lambda(1-e^{(\alpha-\lambda)t})}{\lambda-\alpha e^{(\alpha-\lambda)t}}$
1–2	$e^{-\mu_1-\mu_2 t}$	$\frac{\lambda(1-e^{(\alpha-\lambda)(t+1)})}{\lambda-\alpha e^{(\alpha-\lambda)(t+1)}}$
2–3	$e^{-\mu_1-\mu_2(t+1)}$	$\frac{\lambda(1-e^{(\alpha-\lambda)(t+2)})}{\lambda-\alpha e^{(\alpha-\lambda)(t+2)}}$
3–4	$e^{-\mu_1-\mu_2(t+2)}$	$\frac{\lambda(1-e^{(\alpha-\lambda)(t+3)})}{\lambda-\alpha e^{(\alpha-\lambda)(t+3)}}$
4–5	$e^{-\mu_1-\mu_2(t+3)}$	$\frac{\lambda(1-e^{(\alpha-\lambda)(t+4)})}{\lambda-\alpha e^{(\alpha-\lambda)(t+4)}}$

The assumption that the density of *M. bovis*-infected ferrets is constant during the year appears reasonable for $\lambda = 2.0$ yr^{-1} or less (i.e. Lake Ohau, Scargill Valley and Cape Palliser site), though less supported for higher λ (e.g., Awatere Valley, Castlepoint) (Fig. 4.1). A further critical assumption, that *M. bovis* infection actually occurs in the possum population, indeed appears reasonable (Chapter 3), and the prevalence is reasonably independent of population density, as shown by Barlow (1991b) after analysing data collected by Pfeiffer *et al*. (1995).

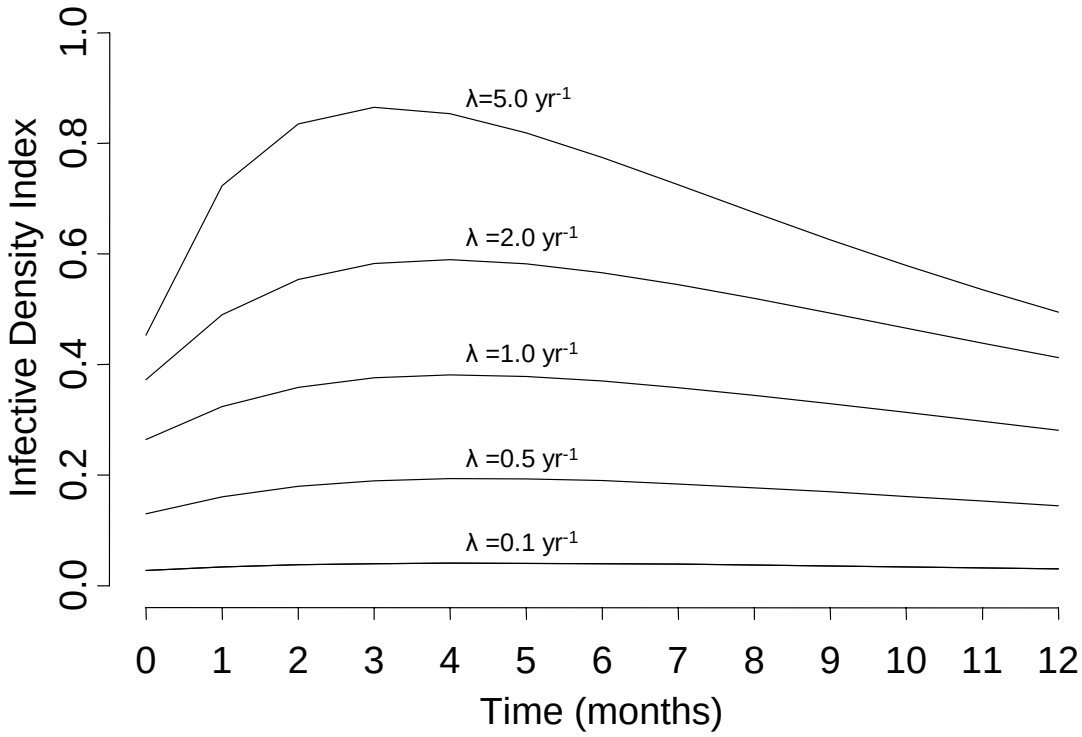

Figure 4.1. A relative index of the density of *M. bovis*-infected ferrets, plotted as a function of time since emergence, for varying values of λ. The index is calculated from age-specific survival and prevalence data presented in Table 4.3.

4.2.5 *Estimating λ from cross-sectional surveys*

Equation 4.17 requires estimates of λ. This was achieved using the Model 2.2 (exponential model including disease-induced mortality with g=1.75 months) presented in Chapter 2. Data came from 9 independent sites, including the 5 sites used in Chapter 2 (see Figure 3.1). At the four additional sites (Hohotaka, Rangitikei, Waipawa & Tiromoana/Mt Cass) there had been a substantial reduction in possum population density before ferrets were surveyed (Chapter 3). For the four additional sites, λ was estimated assuming the effect of sex (2.2 increased hazard for males) and disease-induced mortality (α=1.4 yr^{-1}) were fixed as done in Chapter 3.

Equation 4.17 was fitted to two datasets. First, it was fitted to estimates of λ that were unbiased by ferret sampling (see Chapter 3) and hence should give the most unbiased estimates of coefficients. This was the λ estimated from the first survey of all sites (9 points in total). Second, Equation 4.17 was fitted with additional estimates of λ from Scargill Valley and Castlepoint following possum control, and from the repeated survey at Lake Ohau (3 years separated from first survey—assumed reasonably

independent) (12 points in total). The rationale behind doing this was to utilize as much of the available data as possible to maximise precision.

4.2.6 *Estimating ferret and possum population density during cross-sectional surveys*

Ferret population density was estimated in each trapping session at each site using a modified version of Leslie's Removal Method (Seber 1982) (details provided in Appendix 6.3). In addition, on two sampling occasions (May 1999 and May 2000) at the Scargill Valley site, a known number of radio-collared ferrets were present as part of a study of ferret movements (Caley & Morriss 2001). During each day of trapping, the number of radio-collared ferrets at risk of being captured (i.e. inside the trapping grid) was ascertained by an observer independent of the people servicing the traps. Radio-collared ferrets were deemed to have been at risk of being trapped if at any time during the trapping period they were located within the trapping grid, or sequential location indicated they must have traversed the grid. This provided an opportunity to estimate the absolute population density of ferrets based on the proportion of radio-collared ferrets known to be at risk of capture (within the study area during the period traps were set) that were caught in the sample using the Petersen Estimator (Krebs 1989), modified for small samples as recommended by Seber (1982). An index of the population density of possums in each site in each trapping session was also based on Leslie's Removal Estimator, only expressed as the estimated number of possums per trap (compared with population density), as home-ranges of possums are in general small compared with the distance between traps.

4.2.7 *Estimating disease-induced (α) and natural (b) mortality rates*

It appears unlikely b and α are additive (Appendix 6.4), and they are difficult to estimate separately. As α and b occur together as a term ($\alpha + b$) in all model equations, a better approach (and more realistic) is to estimate the observed mortality rate from sites with a very high (>50%) prevalence of infection, and low mean age (<5 months) of first infection, as the observed mortality rate is an approximation of the combined rates ($\alpha + b$). The Castlepoint and Awatere Valley sites fit these criteria, and using the methods presented in Appendix 6.4, $\alpha + b$ is estimated to be 1.21 ± 0.19 yr^{-1} (± S.E.).

4.2.8 *Estimating transmission coefficients from scavenging probabilities*

Estimates of the proportion of ferret carcasses scavenged by ferrets are given by Ragg *et al*. (2000), McAuliffe (2001), and this study, by monitoring the fate of dead ferrets (as identified by mortality sensors on radio-collars during movement studies; Caley & Morriss 2001). This allows direct estimation of transmission coefficients and R_o, providing the population density of ferrets (D) is known at the time scavenging rates were measured, and assuming that live ferrets and ferret carcasses mix in at least a weakly homogeneous manner. If ferret carcasses are encountered and scavenged on at a rate βD, then the expected time to scavenging follows an exponential distribution (Lee 1992). Using this result, in the time period $\left(\frac{1}{d}\right)$ that *M. bovis* bacilli would be expected to remain viable in a ferret carcass (where d is the decay rate of *M. bovis* bacilli), the proportion of carcasses scavenged (p_s) is:

$$p_s = 1 - e^{-\frac{\beta_F D}{d}}. \qquad \text{(Equation 4.18)}$$

The term β_F is of particular interest. Conveniently, nearly all scavenging of carcasses occurs while the carcasses are reasonably fresh (McAuliffe 2001), and hence within the time period $\left(\frac{1}{d}\right)$ *M. bovis* bacilli would be expected to remain viable. This simplifies things considerably, and negates the need to estimate d separately from β_F. Rearranging Equation 4.18 yields an expression for $\frac{\beta_F}{d}$ in terms of the observed probability of scavenging (p_s) and the population density of ferrets (D):

$$\frac{\beta_F}{d} = \frac{-\ln(1 - p_s)}{D}. \qquad \text{(Equation 4.19)}$$

Estimates of D at the study site of Ragg *et al*. (2000) were available from Cross *et al*. (1998). Likewise, the current study (see below) provided estimates of D pertinent to the scavenging study of McAuliffe (2001). Estimates of $\frac{\beta_F}{d}$ were calculated from observed scavenging probabilities using Equation 4.19, for use in Equation 4.24 (see below).

4.2.9 *Estimating R_o*

Two estimates of R_o were calculated, the first derived from a transmission coefficient based on estimates of λ (Equation 4.17), and the second from observed scavenging probabilities (p_s) (Equation 4.19). These equations estimate different quantities (combinations of parameters), so my approach is to calculate $\hat{R}_o$ and its variance separately for each method (denoted R_1 when derived from λ and R_2 when derived from p_s), then calculate a mean value of $\hat{R}_o$ with pooled variance (assuming estimates are independent—which they should be).

The expression for R_1 in terms of the parameters measured to estimate it (by substituting for β_F in terms of β'_F into Equation 4.11) is:

$$\hat{R}_1 = \frac{\beta'_F d}{(\alpha + b)} \frac{S}{d} = \frac{\beta'_F S}{(\alpha + b)}. \qquad \text{(Equation 4.20)}$$

The variance of $\hat{R}_1$ can be approximated using the delta method (Seber 1982), assuming correlations between the estimated values S, β and ($\alpha + b$) are zero, as:

$$\text{var}(\hat{R}_1) = \hat{R}_o^{\,2} \left(\frac{\text{var}(\hat{S})}{\hat{S}^2} + \frac{\text{var}(\hat{\beta}'_F)}{\hat{\beta}'^{\,2}_F} + \frac{\text{var}(\hat{\alpha} + \hat{b})}{(\hat{\alpha} + \hat{b})^2} \right). \qquad \text{(Equation 4.21)}$$

Clearly, $\hat{R}_1$ will vary depending on population density, so for a given density of susceptibles (S specified hence $\text{var}(\hat{S}) = 0$),

$$\text{var}(\hat{R}_1) = \hat{R}_o^{\,2} \left(\frac{\text{var}(\hat{\beta}'_F)}{\hat{\beta}'^{\,2}_F} + \frac{\text{var}(\hat{\alpha} + \hat{b})}{(\hat{\alpha} + \hat{b})^2} \right). \qquad \text{(Equation 4.22)}$$

Similarly, K_T (from Equation 4.12) can be expressed (and renamed K_1) in terms of β'_F:

$$K_1 = \frac{d}{\frac{\beta'_F d}{(\alpha + b)}} = \frac{\alpha + b}{\beta'_F}. \qquad \text{(Equation 4.23)}$$

The expression for $\hat{R}_2$ is simply:

$$\hat{R}_2 = \frac{\hat{\beta}_F}{d} S, \qquad \text{(Equation 4.24)}$$

and its variance (once again assuming S is measured without error):

$$\text{var}(\hat{R}_2) = S^2 \frac{\hat{\beta}_F}{d}. \qquad \text{(Equation 4.25)}$$

The relevant estimate of K_T is as shown in Equation 4.12, though renamed K_2 (more people have climbed its mountainous namesake than estimated it!). For a given density of susceptible ferrets, the mean value of $\hat{R}_o$ was simply calculated as:

$$\hat{R}_o = \frac{\hat{R}_1 + \hat{R}_2}{2}, \qquad \text{(Equation 4.26)}$$

and its variance as:

$$\text{var}(\hat{R}_o) = \frac{1}{2^2}\left(\text{var}(\hat{R}_1) + \text{var}(\hat{R}_2)\right). \qquad \text{(Equation 4.27)}$$

4.2.10 *Testing host status*

The relationships between the possible host status of ferrets and the epidemiological parameters of importance (R_o, β, λ) are shown in Table 4.4. The order of hypothesis testing is as follows. The initial null hypothesis is ferrets are end-hosts for *M. bovis* infection (i.e. R_o=0), with the working hypotheses ferrets are either spillover hosts or maintenance hosts. Hence the first test is R_o=0 versus R_o>0. It is clearly a one-sided test, as by definition R_o cannot be less than zero.

Table 4.4. Relationship between the host status of ferrets for *M. bovis* infection and parameters of interest.

Host status	R_o	β, λ
Maintenance	≥1	>0
Reservoir	≥1	>0
Spillover	0<R_o<1	>0
Dead-end	0	0

Should the null hypothesis be rejected (we accept the working hypothesis that R_o>0), the next step is to test the new (revised) null hypothesis ferrets are spillover hosts (0<R_o<1) against the working hypothesis that ferrets are maintenance hosts (R_o≥1). There is an obvious danger of making a Type II error (accepting the null hypothesis that ferrets are spillover hosts when in fact they are maintenance hosts), as the probability of making a Type II error is not controlled for. The precautionary principle (in a management sense) would assume ferrets are maintenance hosts until proven otherwise.

Finally, having calculated $\hat{R}_o$ and $\hat{K}_T$ in terms of mean ferret population density, I need to be able to express observed seasonal population densities in terms of their equivalent $\hat{K}_T$. Typically, the population of ferrets during February (peak population density) consists of 80% juveniles and 20% adults (Appendix 6.4). By applying the mortality rates of Appendix 6.4, I can estimate the relative population size by month,

and provide conversion factors for expressing an observed monthly population density in terms of an average yearly population density (Table 4.5).

Table 4.5. The proportion of a ferret population surviving by month, how this relates in relative terms to the yearly mean population density (Ratio to yearly mean), and the Conversion factor to calculate yearly mean population density from observed population density. Figures are calculated assuming a juvenile instantaneous mortality rate of 1.44 yr^{-1}, adult instantaneous mortality rate of 0.56 yr^{-1}, and juveniles making up 80% of the population at the month of peak population density (February). See Appendix 6.4 for exact details.

Month	Proportion Surviving	Ratio to yearly mean	Conversion factor
February	1.00	1.71	0.58
March	0.90	1.54	0.65
April	0.81	1.39	0.72
May	0.73	1.25	0.80
June	0.66	1.13	0.88
July	0.60	1.02	0.98
August	0.54	0.93	1.08
September	0.49	0.84	1.19
October	0.44	0.76	1.31
November	0.40	0.69	1.45
December	0.37	0.63	1.60
January	0.33	0.57	1.75

4.3 Results

4.3.1 Ferret population density

There was good agreement between the Petersen and Removal Estimates of population density for both May 1999 (1.6 km^{-2} vs. 1.7 km^{-2}) and May 2000 (2.5 km^{-2} vs. 2.4 km^{-2}). The highest recorded population density was 4.7 km^{-2} at Lake Ohau, and the lowest 0.6 km^{-2} at Cape Palliser (Table 4.6).

4.3.2 Estimating transmission coefficients from estimates of λ

A summary of data used is given in Table 4.6. Results of fitting Equation 4.17 to data are shown in Table 4.7. Including repeated surveys at sites following possum control changed the transmission parameters little, though improved the precision of estimates considerably (Table 4.7). Notably, the intercept did not differ significantly from zero for either dataset, and there is little doubt the possum-to-ferret transmission coefficient (β_P) is greater than zero (P<0.001; Table 4.7). As for the most critical

parameter of all, β_F, statistically speaking it was not significantly different from zero (Table 4.7; P=0.15). Using the estimate of β_F calculated from all the available data $\frac{\beta'_F}{(\alpha+b)}$ (needed for calculating $\hat{R}_1$ from Equation 4.20) is estimated to be 0.55 ± 0.63.

Table 4.6. Summary of data used to estimate R_0; the force of *M. bovis* infection in ferrets ($\hat{\lambda}$), prevalence of *M. bovis* infection in ferrets ($\hat{p}$), ferret population density ($\hat{D}$), and index of possum population density ($\hat{I}_P$).

Site	Year	$\hat{\lambda}$ (yr^{-1})	$\hat{p}$ (%)	$\hat{D}$ (km^{-2})	$\hat{I}_P$
Hohotaka	1998	0.19	5.5	3.1	0.05
Rangitikei	2000	0.10	3.3	2.0	0.04
Waipawa	1997	0.12	3.6	1.2	0.20
Castlepoint	1998	4.80	48.4	1.1	1.64
Castlepoint*	1999–2000	0.70	12.8	1.1	0.35
Cape Palliser	1998–2000	2.10	59.4	0.6	1.12
Awatere Valley	2000	3.40	61.7	1.4	1.51
Scargill Valley	1995	1.02	16.7	3.3	0.28
Scargill Valley*	1999–2001	0.25	7.3	2.0	0.04
Tiromoana/Mt Cass	1995	0.80	22.7	2.5	0.12
Lake Ohau	1997	0.13	4.2	4.7	0.06
Lake Ohau	2000	0.15	5.0	2.0	0.06

* Following intensive possum control

Table 4.7. Disease transmission parameters, their estimates and associated standard errors (S.E.) from fitting Equation 4.17 to two different datasets (see text for explanation).

Dataset	Parameter	Estimate	S.E.	*t*	*d.f.*	Prob.
One observation per site	Intercept (O)	–0.11	0.28	–0.4	1	0.69*
	β'_F	0.71	0.82	0.9	6	0.21^+
	β'_P	2.25	0.35	6.5	6	$<0.001^+$
Multiple observations per site	Intercept (O)	–0.07	0.18	–0.4	1	0.67*
	β'_F	0.65	0.62	1.1	9	0.15^+
	β'_P	2.25	0.28	8.1	9	$<0.001^+$

* two-tailed test; $^+$ one-tailed test.

4.3.4 *Relationship between force of infection and density of possums and/or ferrets*

Lambda was, in general, negatively (r= –0.57, d.f.=7, P=0.057) related to ferret population density (Fig. 4.2(a)), and positively (r=0.96, d.f.=7, P<0.001) and strongly related to possum population density (Fig. 4.2(b)). The data points from repeated surveys at sites following possum control do not appear to be outliers in any way, giving comfort to the previous decision to include them when estimating transmission coefficients.

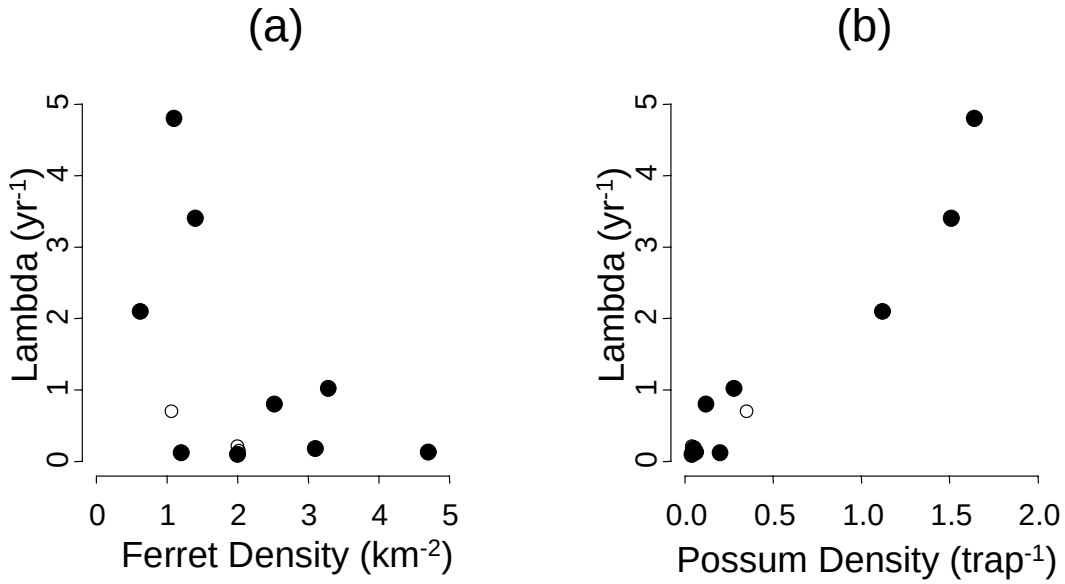

Figure 4.2. The relationship between the estimated force of *M. bovis* infection (λ) in ferrets and: (a) population density of ferrets; and (b) population density of possums as indexed by the estimated number of trappable possums per trap. Solid circles are data from first surveys only at each site. Open circles include repeated surveys after the possum control treatment.

4.3.5 Estimating transmission coefficients from scavenging probabilities

The probability of a ferret carcass being scavenged by ferrets was quite variable between sites, being highest at the Palmerston site. This site also had the highest ferret population density of those surveyed (Table 4.8). The mean value of $\frac{\hat{\beta}_F}{d}$ (needed for calculating $\hat{R}_2$ from Equation 4.24) was 0.13 ± 0.05 (S.E).

Table 4.8. Summary of observed proportion of ferret carcasses scavenged on by ferrets (p_s), the density of potentially susceptible ferrets (S), and the value of $\frac{\hat{\beta}_F}{d}$, obtained using Equation 4.19.

Site	p_s	S	$\frac{\hat{\beta}_F}{d}$	Source
Palmerston	0.63 (5/8)	5.3	0.19	Ragg *et al*. (2000)
Hurunui	0.13 (7/52)	2.7	0.05	McAuliffe (2001)
Scargill Valley	0.33 (3/9)	2.7	0.15	This study

4.3.6 Estimates of R_o

From Equation 4.20, $\hat{R}_1$=0.55*S*, whereas from Equation 4.24, $\hat{R}_2$=0.13*S*, hence from Equation 4.26, $\hat{R}_o = 0.34S$ and $\hat{K}_T$ = 2.9 ferrets km^{-2} (setting $\hat{R}_o$ to one and solving for *S*). Using Table 4.3, this mean threshold population density corresponds to a peak (February) population density of 5.0 ferrets km^{-2}. The coefficient of variation (CV) around the coefficient (0.34) used to estimate $\hat{R}_o$ was 76%, with the vast majority of this arising from imprecision in $\hat{R}_1$. The relationship between $\hat{R}_o$ and the mean density of susceptible ferrets (*S*) is shown in Figure 4.3, including 95% confidence intervals. The mean density of susceptible ferrets corresponding to the upper 95% confidence interval for $\hat{R}_o = 1$ is 1.2 ferrets km^{-2} (Fig. 4.3). Using Table 4.3, this corresponds to a peak (February) population density of 2.1 ferrets km^{-2}.

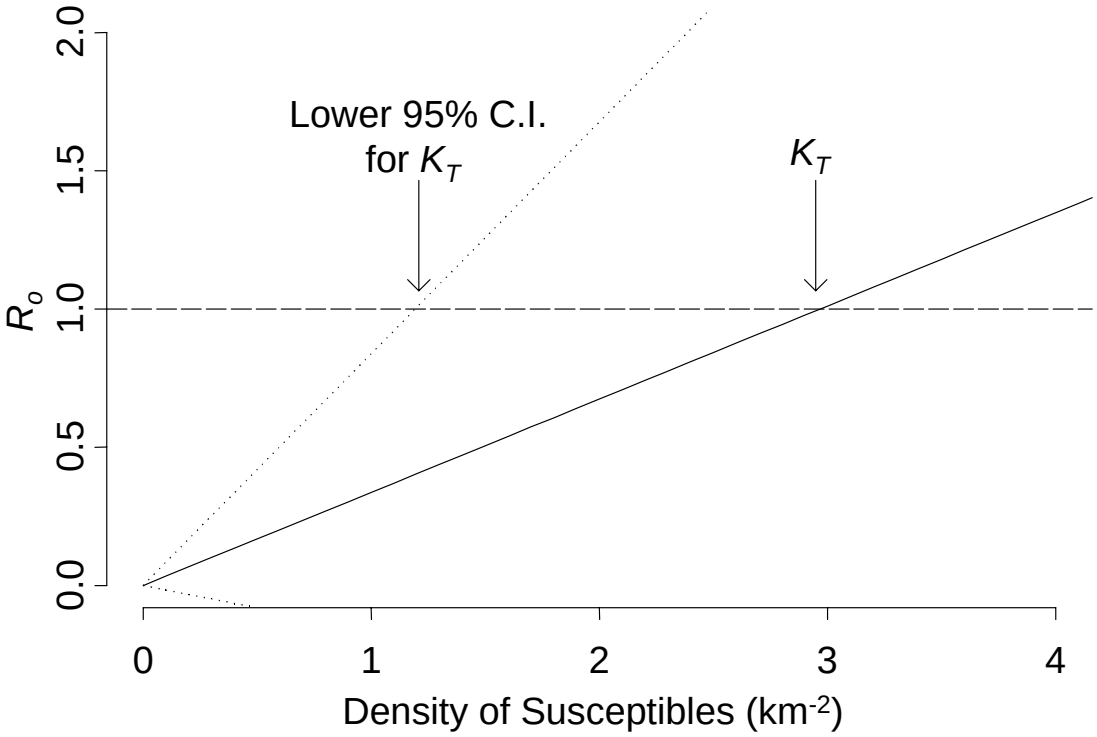

Figure 4.3. The relationship between the estimated basic reproductive rate (R_o) of *M. bovis* infection in feral ferrets and the mean population density of susceptible ferrets. Dotted lines are 95% confidence limits around $\hat{R}_o$. The dashed line is for R_o =1. The point on the dashed line where $\hat{R}_o = 1$ corresponds to a value of 2.9 ferrets km^{-2} that is the estimated threshold population density for disease establishment (K_T—marked with arrow). The lower 95% C.I. for K_T (1.2 km^{-2}) is also indicated by an arrow.

4.3.7 Implications of estimates of R_o for disease host status

The initial null hypothesis of R_o being zero (and ferrets being dead-end hosts) is not rejected (as the confidence intervals around $\hat{R}_o$ including zero). The alternate null hypothesis (ferrets are spillover hosts, $0<R_o<1$) is clearly accepted for ferret population densities less than 1.2 ferrets km^{-2} (upper 95% C.I. <1.0). Indeed, nowhere in the North Island did $\hat{R}_o$ approach unity, and in most (5/6) cases it was significantly ($P<0.05$) less than unity (Table 4.9). Hence I conclude that in these habitats, feral ferrets are spillover hosts for *M. bovis.* The situation in the South Island sites was less clear, with $\hat{R}_o$ less than unity (though not significantly so) for half (5/10) the surveys and greater than unity for the remainder (Table 4.9). The data do not, however, reject the revised null hypothesis for these sites (ferrets are spillover hosts).

Table 4.9. Estimates of ferret population density ($\hat{D}$) in New Zealand for the North and South Island (sorted by increasing latitude), the month of survey, equivalent mean population density ($\hat{\bar{D}}$; from Table 4.5), and $\hat{R}_o$ (assuming population density was measured without error). Unless otherwise indicated, data are from the current study.

Island	Site	Year	Mth	$\hat{D}$ (km^{-2})	$\hat{\bar{D}}$ (km^{-2})	$\hat{R}_o$
North	Hohotaka	1995	Feb.	0.8	0.5	0.17*
	Hohotaka	1998	Mar.	3.1	2.0	0.67
	Rangitikei	2000	Feb.	2.0	1.2	0.40*
	Waipawa	1997	Mar.	1.2	0.8	0.27*
	Castlepoint	1998	Feb.	1.1	0.6	0.20*
	Cape Palliser	1998	Apr.	0.9	0.7	0.22*
South	Awatere Valley	2000	Mar.	1.4	0.9	0.30*
	Scargill Valley	1995	May	3.3	2.6	0.88
	Reeces Road[a]	1996/97	All	–	3.7	1.25
	Tiromoana/Mt Cass	1995	May	2.6	2.1	0.70
	Lake Ohau	1997	Apr.	4.7	3.4	1.15
	Lake Ohau	2000	Mar.	2.0	1.3	0.44
	Grays Hills[b]	1994	Various	3.6	3.0	1.05
	Earnscleugh[b]	1994	Various	2.4	2.0	0.69
	Bendigo[b]	1994	Various	5.7	4.8	1.64
	Palmerston, Otago[c]	1997	April	5.3	3.8	1.28

* Significantly (P<0.05) less than unity.
[a] Morley (1999); [b] Norbury *et al*. (1998b); [c] Cross *et al*. (1998).

4.4 Discussion

This study is one of the few to estimate R_o for a wildlife host/pathogen system with the view to using the estimate in a hypothesis-testing framework. However, the confidence intervals around $\hat{R}_o$ were frightfully large—perhaps this is a reason why few authors choose to present them! The precision was high enough for the $\hat{R}_o$ still to have extremely useful management implications. In particular, there is a very low probability ferret populations at the North Island sites are capable of acting independently as a maintenance host of *M. bovis* (i.e. they are spillover hosts in North Island habitats). Thus the assertion of Lugton *et al.* (1997b) is supported for these habitats. In contrast, the modelling estimates that there is significant intra-specific transmission of *M. bovis* among high density ferret populations in parts of the South Island. In some situations (5/10), the number of secondary infections (R_o) at some sites

is estimated to be just enough for ferrets to act independently as a reservoir for the pathogen. This does not support Lugton *et al*. (1997b), but supports the speculation of Ragg *et al*. (1995a) that ferrets may be maintenance hosts for *M. bovis* in areas within New Zealand where they are most abundant. Caley *et al*. (2001b) suggested that whether or not *M. bovis* would occur in ferrets when possums were absent, as predicted from the regression model when possum abundance was set to zero, remained unclear. This statement can be quantified in terms of $\hat{R}_o$ using Equation 4.4, assuming the estimated prevalence of *M. bovis* infection in ferrets (c. 2% after correcting for the expected proportion of *M. bovis*-infected ferrets not exhibiting macroscopic infection; Lugton *et al*., 1997c) in the absence of possums represents a steady state prevalence. The estimated R_o is 1.02 (Upper 95% C.I.=1.07). I did not calculate the lower confidence interval as the prevalence corresponding to the lower confidence interval for the intercept is negative. Within the context of Equation 4.4, this implies the susceptible proportion at equilibrium is greater than one—this is nonsensical. The lower 95% C.I. would, however, be less than one; hence the interpretation of Caley *et al*. (2001b) regarding host status essentially remains unchanged (uncertainty whether $R_o \geq 1$).

My failure to reject the revised null hypothesis of ferrets being spillover hosts (and accept the alternative working hypothesis that they are maintenance hosts) could be due to the low precision of $\hat{R}_o$ with its attendant low power (in which case there has been a Type II error). Undoubtedly, the power of the analysis was low to detect $\hat{R}_o$ as being greater than unity. When confronted with incomplete data (here, uncertainty around $\hat{R}_o$), wildlife managers should make decisions based on the most appropriate model of a system, though always being mindful the model may not be correct, and in continual need of improvement (Walker 1998). Applying the precautionary principle (Deville & Harding 1997) as it would apply to risk-averse management (cf. acceptance of the null hypothesis) indicates ferrets should be considered potential maintenance hosts for *M. bovis* when at high population densities ($>K_T$). In addition, having R_o less than one for a pathogen does not necessarily mean that species (e.g., ferrets) is inconsequential as a host for that pathogen (despite having spillover host status), especially if R_o is close to one (say >0.75). In this situation there will be a considerable number of secondary infections, and only occasional transmission from the true maintenance host (e.g., possums) will be required for there to be a high prevalence in

the spillover species, with possible undesirable consequences of inter-specific transmission to other species (e.g., domestic livestock).

The imprecision around the estimate of R_o is disappointing, given the large effort put into collecting the data for the current study, and more precise estimates of R_o should be pursued. It should be noted that in the current study, estimates of disease transmission coefficients (and hence R_o) differed depending on the estimation method used, being higher when estimated from modelling the force of infection compared with modelling observed scavenging probabilities. Alternative methods for estimating R_o are discussed in Chapter 5. Of a more positive note was the broad-scale nature of the research—the estimates of λ are genuinely independent. This gives me confidence that the estimated variability is valid, compared with when data are pseudo-replicated (and precision is over-estimated). Studies that have estimated the precision of $\hat{R}_o$ (e.g., Hone *et al*. 1992) have done so for one population only.

It is notable that most surveys where the estimated population density was sufficient for *M. bovis* to persist in ferret populations occurred during or before 1997, following major increases in rabbit populations in many South Island locations (e.g., Caley & Morley 2002). Since the introduction of rabbit haemorrhagic disease virus (RHDV) virus to New Zealand in late 1997, rabbit population density over the South Island has decreased on average by c. 50% (Parkes *et al*. 2002). Given the relationship between ferret population density and rabbit population density (Barlow & Norbury 2001), it is reasonable to assume ferret densities over the South Island have been significantly reduced as a result of RHDV infection in rabbits. Indeed, this was observed at the Lake Ohau site in this study (Table 4.9). The likelihood of ferrets acting as maintenance hosts has therefore been reduced for many areas.

This chapter has estimated the basic reproductive rate of *M. bovis* infection in feral ferret populations in New Zealand—the central aim of the thesis. The next and final chapter reviews the results of this and previous chapters, and examines the implication for the control of *M. bovis* infection in feral ferrets.

Chapter 5

Synthesis and review

5.1 Inference on the host status of ferrets for *M. bovis*

This research began with the aim of answering a management-driven question whether feral ferrets were maintenance hosts for *M. bovis*. I proposed estimating the R_o of *M. bovis* infection in ferret populations as the best approach to address this question. A prediction of this study is R_o for *M. bovis* infection of ferrets is density-dependent, hence ferrets may be maintenance hosts for *M. bovis* infection in some habitats where ferret population density is high, though not others where ferret population density is low, as suggested by Ragg *et al.* (1995b). The hypothesis can therefore be refined from the original null hypothesis 'Ferrets are dead-end hosts' versus the working hypotheses 'Ferrets are spillover hosts' or 'Ferrets are maintenance hosts' to 'Ferret populations are capable of acting as maintenance host of *M. bovis* above a threshold population density (K_T)'. This revised hypothesis would be more in line with the recommendations of Cherry (1998), Johnson (1999) and Anderson *et al.* (2000); however, Krebs (2000) would strongly argue it should be tested in a critical manner. This thesis has gone partway to addressing the revised hypothesis, by estimating at what population density (<2.9 km^{-2}) ferrets are not maintenance hosts of *M. bovis* ($\hat{R}_o$<1). Further, I have estimated the population density (<1.2 km^{-2}) where it is unlikely (P<0.05) that ferrets are maintenance hosts of *M. bovis*. This result is of considerable practical use to the many wildlife managers whose ferret populations are below this density. However, the estimates of R_o were too imprecise to determine at what population density ferrets definitely are maintenance hosts of *M. bovis* (i.e. $\hat{R}_o \geq 1$ with P<0.05).

The prediction that intra-specific transmission of *M. bovis* via scavenging of carcasses may be sufficient for ferrets to be maintenance hosts for the disease (albeit in limited areas) is in conflict with the respiratory transmission paradigm for wildlife reservoirs of *M. bovis* (Morris *et al.* 1994). Transmission by scavenging of carcasses/cadavers, however, is observed in insect (e.g., Knell *et al.* 1998) and vertebrate (e.g., McCallum & Singleton 1989) host/pathogen systems. Barlow (2000) argued insect host/pathogen and/or host/parasitoid models had much to offer vertebrate host/pathogen models in terms of how mixing between susceptible and infectious

individuals is treated. I suggest that there might be more similarities in the actual form of transmission (rather than simply how it is modelled), especially for systems such as the ferret/*M. bovis* one, where scavenging seems to play such an important role in transmission.

The implication of the R_o of *M. bovis* in ferrets being density-dependent is that management need only target ferret populations where R_o is greater than unity to eradicate the disease from the greater ferret population (assuming, of course, that transmission from other hosts such as possums has been eliminated). This requires identifying boundaries of the ferret distribution in New Zealand with $R_o \geq 1$ (or $D \geq K_T$) as opposed to $R_o < 1$ ($D < K_T$). The best approach to identifying where the density of ferrets is above K_T may be to first map the distribution and population density of rabbits, then relate this to the expected density of ferrets using a model (e.g., Barlow & Norbury 2001). A similar approach was taken by Wilson *et al.* (1982), who estimated the seasonal and regional variation in the R_o of common liver fluke (*Fasciola hepatica*) in sheep in England.

The dividing line between areas with $R_o \geq 1$ as opposed to $R_o < 1$ is analogous to the 'edge of the range' concept of Caughley *et al*. (1988) for population rate of increase. The edge of the range refers to the area where a population's intrinsic growth rate (r_m) is greater than or equal to zero, and hence the species will occur. Outside the edge, where r_m is less than zero, the species will be unable to persist. Population growth rate (as represented by r) is argued to be the key unifying variable linking the various facets of population ecology (Sibly & Hone 2002). R_o could be argued to be of similar importance, and following Hone (1992), the analogous measure of the intrinsic rate of increase for the number of infected cases (here denoted r_o) would be:

$$r_o = \frac{\ln R_o}{T}, \qquad \text{(Equation 5.1)}$$

where T is the generation length (whilst infected) of the host. Anderson & May (1986) used a similar measure for the intrinsic growth rate of sero-positive cases of human T lymphotrophic virus (HTLV-III), the etiological agent of acquired immunodeficiency syndrome (AIDS).

5.2 Estimating disease transmission and R_o

A critical test of the hypothesis *M. bovis* will not establish in ferret populations in the absence of inter-specific transmission requires the total removal of non-ferret sources of infection, along with complete prevention of immigration of *M. bovis*-infected species of any kind. This approach is not feasible, as one can never be sure (with current technology) the total removal of non-ferret sources of infection (and hence inter-specific transmission) has been achieved. An alternative approach is required, and hence in this thesis I have used modelling to estimate the intra-specific disease transmission coefficient needed to estimate R_o. The precision of $\hat{R}_o$ for *M. bovis* infection in ferrets was disappointingly low (c. 76%), though this is not surprising given that the various parameters making up $\hat{R}_o$ had to be estimated (rather than simply measured). I think the fundamental problem of imprecision arises from the number of parameters that must be estimated to partition the observed transmission into inter-specific and intra-specific components, in relation to the amount of data. Single species host/pathogen systems do not suffer from this complication, allowing more robust statistical treatment (e.g., Finkenstädt & Grenfell 2000).

Lack of sufficient replication for proper statistical analysis is the norm in large-scale (and often small-scale also) field experiments of host/pathogen systems (McCallum 1995). Additional surveys should increase the precision of estimated transmission coefficients and hence also $\hat{R}_o$ for *M. bovis* in ferrets. Alternatively, large-scale field experiments can be analysed without any comparison between sites but, rather, with parameter estimation (e.g., transmission coefficients) occurring within sites. This assumes the site is large enough for the parameter estimates to be applicable to the wider world, requiring a 'leap of faith' of the kind described by McCallum (1995). The time-series analysis by Begon *et al*. (1999) of a detailed longitudinal dataset of cowpox virus in sympatric bank vole and wood mouse populations from two sites in England is a good example of this. Estimates of intra-specific transmission coefficients were very precise, and although estimates of inter-specific transmission were imprecise, they were so small as to indicate that inter-specific transmission was inconsequential to the modelling of cowpox virus in either species (Begon *et al.* 1999). Likewise, from four sites (2 treatment, 2 experimental control), Caley & Ramsey (2001) obtained reasonably precise (coefficient of variation c. 20%) estimates of the transmission coefficient for *Leptospirosis interrogans* serovar *balcanica* infection (a bacterial disease transmitted

predominantly during mating contacts) in brushtail possums. However, the precision was insufficient to detect as being statistically significant an estimated 28% increase in the transmission coefficient of the disease at treatment sites where females had been tubally ligated (and hence expected to have a higher transmission coefficient through a higher frequency of oestrus and increased mating contacts).

The final approach is to consider alternative methods of estimating transmission coefficients. Direct experimentation is one such option, involving the introduction of *M. bovis*-infected ferrets into susceptible populations, and estimating through observation the number of secondary cases. This clearly requires the release of a novel strain of *M. bovis*, to avoid potential confounding with pre-existing strains, and, it is hoped, enable the untangling of inter-specific from intra-specific transmission. A study of this type would be politically difficult to undertake, though not without precedent in New Zealand, as there is an instance of a novel strain of *M. bovis* being deliberately introduced into a possum population to aid the study of intra-specific transmission of *M. bovis* in possums (L. Corner personal communication). The value of possibly letting a bit of disease 'get away', would be more than compensated for in terms of strength of inference, given that *M. bovis*-infected wildlife already occurs over about one-third of New Zealand (as of 1998, *M. bovis*-infected possums occupied *c*. 24% of New Zealand (Coleman & Caley 2000), with no apparent slowdown in the rate of expansion). A study of this nature would benefit greatly from a highly sensitive, highly specific, and non-lethal diagnostic test for *M. bovis* infection in feral ferrets (currently not available to my knowledge). McCallum (2000) refers to this approach as the 'ideal experiment'.

Given the difficulty in estimating transmission coefficients directly (McCallum *et al*. 2001), it is no surprise alternative indirect methods of estimation have been explored. For example, Dobson & Meagher (1996) use the allometric equation developed by DeLeo & Dobson (1996) relating minimum transmission coefficients for microparasite establishment to body mass when estimating the disease transmission coefficient for brucellosis infection in bison. Similarly, the DeLeo & Dobson (1996) equation can be applied to the ferret/*M. bovis* system. Assuming density-dependent transmission, a mean bodyweight of 0.9 kg, and a disease-induced mortality of 1.4 yr^{-1}, (hence the *m* used in the allometric equation of DeLeo & Dobson (1996) equals 0.4), the estimated minimum transmission coefficient (β_{min}) for disease spread is 0.009. This estimate is about two orders of magnitude lower than estimated here (with the same units of measurement — host density [km^{-2}], and time [years]). This should come as

little surprise, as the errors around predicted values from allometric relationships can be large (McCallum 2000), and should be borne in mind if contemplating this approach.

Imprecision of estimates may also be caused by the use of an inappropriate model. For example, the postulated method by which ferrets interact with *M. bovis*-infected ferret and/or possum carcasses may not be an adequate approximation. An obvious issue to explore would be the efficiency of ferrets in locating carcasses ('search efficiency'), and factors that influence the probability of ferrets scavenging on carcasses they locate. Search efficiency for carcasses may be influenced by 'relative food shortage', as indexed by the ratio of ferrets to rabbits (preferred food source). In times of relative food shortage, ferrets may be more likely to search for carcasses, and more likely to scavenge carcasses they find. This hypothesis could be modelled by the inclusion of a searching efficiency parameter, and the transmission coefficient modified accordingly, in a way analogous to the bubonic plague/flea/rat/human system described by Keeling & Gilligan (2000). In that system, Norway rats are the reservoir host for bubonic plague (aetiological agent the bacterium *Yersinia pestis*), fleas the vector, and humans are spillover hosts. Outbreaks in humans are caused by hungry fleas (hunger possibly caused by plague-induced rat deaths lowering rat population density) biting humans. In the *M. bovis*/possum/ferret system, possums are the reservoir, with spillover from possums into ferret populations. Alternatively, the structure of mixing could be modelled differently. For example, Knell *et al*. (1998) reported their *P. interpunctella*/PiGV host/pathogen model system could be better described using a negative binomial term for transmission $\left[k\ln(1+\frac{\beta I}{k})\right]S$, where k is an index of aggregation and β the transmission coefficient. Subsequently, Barlow (2000) observed transmission of this type better described *M. bovis* infection in possums, and suggested heterogeneous mixing models of this type (phenomenological in nature) should seriously be considered as the default form for wildlife disease systems. The expression for R_o assuming transmission of the negative binomial type is not quite as 'neat' as when assuming density-dependent transmission (Equation 5.2), as the home-range term (H) is not cancelled out of the expression as previously (Chapter 4).

$$R_o = \frac{k\ln\left(1+\frac{\beta}{kH}\right)HS}{\alpha+b+\gamma} \qquad \text{(Equation 5.2)}$$

The rationale for this expression is a solitary infected ferret $\left(I = \frac{1}{H} \right)$ makes secondary infections at a rate $k \ln\left(1 + \frac{\beta}{kH}\right) S$ over an area *H* for a period $\frac{1}{\alpha + b + \gamma}$.

Of course, the fitting of more parameters to a model may or may not (initially at least) increase the precision of the parameter estimates. Fitting too many parameters (over-fitted model) invariably results in model parameter estimates with excessively high variance, especially when the number of parameters estimated is high in relation to the number of data points (Burnham & Anderson 1998).

5.3 Controlling *M. bovis* infection in ferret populations

Before attempting to control an agent of disease (in this case *M. bovis*), we ideally need to understand the mechanisms that normally enable the disease agent to persist (Yorke *et al*. 1979). This thesis predicts that in areas where ferret population density is lower than the K_T, the mechanism by which *M. bovis* infection persists in ferret populations is inter-specific transmission from sympatric possum populations. Control of *M. bovis* infection in ferrets in these areas can be achieved by controlling inter-specific transmission alone. In areas where ferret population density is higher than K_T, the mechanism by which *M. bovis* infection persists in ferret populations is intra-specific and possibly inter-specific transmission of *M. bovis* from sympatric possum populations (assuming they are infected with *M. bovis*). Control of *M. bovis* infection in ferrets in these areas will require controlling intra-specific, and probably also inter-specific transmission. Hence the recommendation of Caley *et al*. (2001b) that eradication of *M. bovis* from possum populations was a good working model for managing *M. bovis* infection in ferrets appears over-simplistic.

In addition to knowing the mechanism by which disease transmission occurs, knowing R_o (or K_T) enables reasonably straightforward calculation of the proportion of individuals to be vaccinated or the rate of culling required for disease eradication (Anderson *et al*. 1981) (see below). Intuitively, a disease with a high R_o would be the most difficult to eradicate (Anderson 1984). This may well be true in general for diseases of humans, as the host populations involved often remain the same, or have similar attributes. However, for wildlife host/pathogen systems, host density and distribution may vary enormously, as might other factors critical to the success of a

disease eradication campaign, such as bait uptake rates (for delivering vaccines of lethal toxic baits), accessibility of terrain, etc. For example, the basic reproductive rate of rabies in foxes (as inferred from Anderson *et al*. (1981)) is equal to or greater than that of *M. bovis* in possums (Barlow 1991b). However, control of *M. bovis* in New Zealand possums over large areas has proved far more difficult (Coleman & Caley 2000) than control of rabies in foxes in Europe (Pastoret & Brochier 1998) and elsewhere (e.g., MacInnes *et al.* (2001)). Both campaigns have involved the distribution of millions of baits, containing a rabies vaccine in Europe, or a lethal toxin (commonly compound 1080) in New Zealand. Differences in the success of the two campaigns may be related to a number of the factors listed above, including host density (possums occur at about 100 times the density of red foxes), vaccine availability (there is currently no vaccine for *M. bovis* suitable for bait delivery), and habitat accessibility (*M. bovis*-infected possums often occur in inaccessible areas). My point is that before embarking on measures to control *M. bovis* in ferrets in areas where the estimated R_o is greater than one (or population density is greater than K_T), careful consideration is needed as to what form (e.g., density reduction, vaccination), and what intensity (e.g., rate of culling, proportion to be vaccinated) of control measures are required. Note here that deciding to manage *M. bovis* infection in ferrets assumes the precautionary principle (Deville & Harding 1997) has been applied — management of ferrets is instigated although it is unclear whether R_o for *M. bovis* infection in ferrets is greater than one.

So, assuming active management of ferret populations is needed for control of *M. bovis* in areas where population density is greater than K_T, how can this be achieved? Direct population reduction through trapping or poisoning is one approach, and is frequently attempted to control disease in wild animals (Wobeser 1994). On a cautionary note, Wobeser (1994) remarks that attempts to control wildlife disease through direct population reduction have met with highly variable success (mainly failure). Reasons for the lack of success include inadequate knowledge of population density or the degree of reduction required to achieve the desired effect. Potential control of *M. bovis* infection in ferrets should not suffer from this lack of information, as estimating population density is quite feasible (Cross *et al*. 1998; Appendix 6.3), estimates of K_T for *M. bovis* infection are available (Chapter 4), and the population dynamics of ferrets are increasingly being better understood (Barlow & Norbury 2001). So what level of culling would eradicate *M. bovis* from ferret populations where their density is above K_T? Assuming simple logistic growth of ferret populations and

$K_T > \frac{1}{2}K$, then the minimum culling rate (c) required for stable control of density of ferret populations below K_T is (following Anderson *et al*. 1981):

$$c > rK\left(1 - \frac{K_T}{K}\right). \qquad \text{(Equation 5.3)}$$

Here, K refers to the habitat carrying capacity and r the intrinsic (maximum) rate of increase (cf. observed rate of increase r_{obs}). Using a value of 1.3 yr^{-1} for r (the upper value of Barlow & Norbury (2001)), minimum culling rates for the theoretical eradication of *M. bovis* from ferret populations are shown in Figure 5.1. Caley *et al*. (1998a) reported that over a four year period, mean culling rates of 5.3 km^{-2} and 7.3 km^{-2} at the Scargill Valley and Tiromoana/Mt Cass sites in North Canterbury did not decrease the age-specific prevalence of macroscopic *M. bovis* in either ferret population. These culling rates are far in excess of what is predicted (Figure 5.1) to result in eradication of *M. bovis* from ferret populations. Inter-specific transmission of *M. bovis* from possums was postulated by Caley *et al*. (1998a) as the cause of this ongoing infection in feral ferrets in the face of intensive culling. Indeed, at the Scargill Valley site, subsequent control of possums reduced the age-specific prevalence of *M. bovis* in ferrets (Chapter 3), supporting this prediction. The other interesting results from the study of Caley *et al*. (1998a) is that ferret populations appear to be able to withstand a higher level of culling than predicted by Barlow & Norbury (2001). Investigating the role of immigration in the recovery of feral ferret populations would be a logical starting point in investigating this discrepancy, as the model of Barlow & Norbury (2001) was non-spatial.

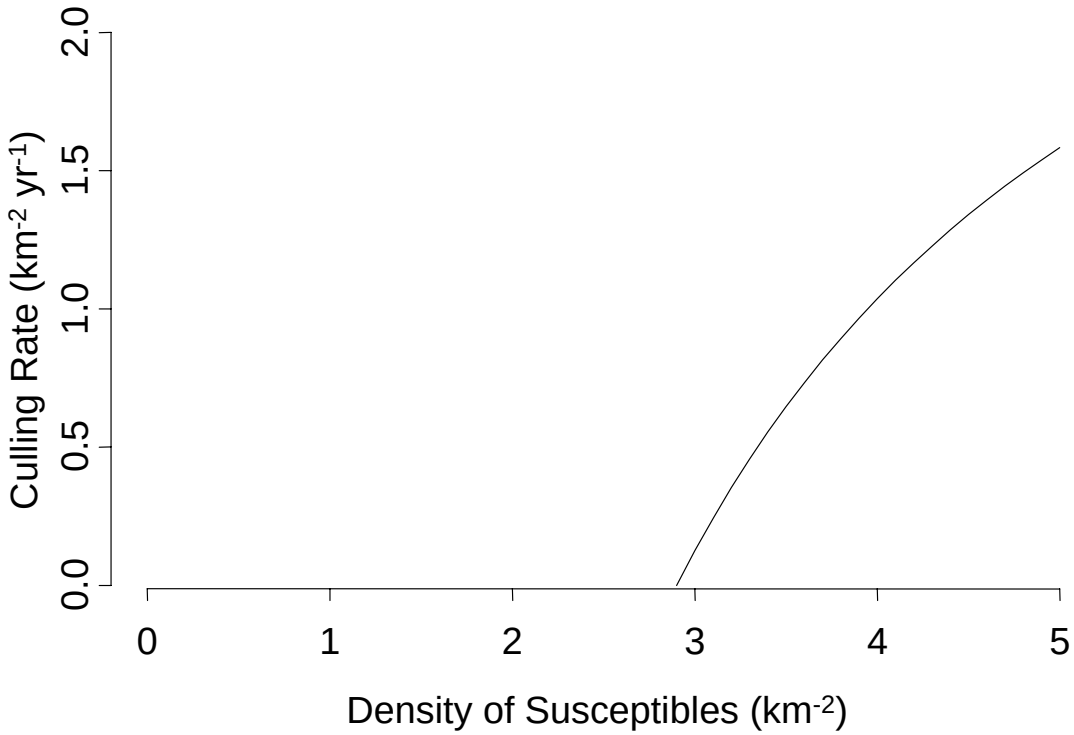

Figure 5.1. Estimated culling rate (ferrets removed km^{-2} yr^{-1}) required to reduce ferret population density (K) below K_T (2.9 ferrets km^{-2}) assuming logistic population growth with r=1.3 yr^{-1}. Estimates are calculated using Equation 5.3 (see text for details).

As scavenging on ferret carcasses appears to be the source of intra-specific transmission, control methods for ferrets that leave ferret carcasses available to be scavenged (e.g., poisoning) will be potentially less effective (for a given level of population suppression) in reducing R_o than those that allow the removal and safe disposal of carcasses (e.g., trapping). In the case of poisoning, the type of poison and its ability to cause secondary poisoning of ferrets will influence the ability of poisoned *M. bovis*-infected ferret carcasses to generate secondary infections. For example, ferrets lethally poisoned with cyanide would pose little or no risk of secondary poisoning but more risk of infection to other ferrets. In contrast, ferrets lethally poisoned with anticoagulants (e.g., broadifacoum) will pose a considerable secondary poisoning hazard (if scavenged), thus reducing the ability of a secondary infection to establish. Timing of lethal control of ferrets may also influence the number of secondary infections per *M. bovis*-infected ferret carcass, as the survival of the *M. bovis* bacilli is temperature-dependent (Jackson *et al*. 1995b). Most control of ferrets occurs over the summer/autumn, as they are most trappable then. Due to higher carcass decay rates at this time, the number of secondary infections would be at a minimum. In contrast, most

possum control operations using compound 1080 are undertaken during winter, as this generally results in the highest percentage kill. This is possibly a result of the toxicity of 1080 being temperature dependent (Veltman & Pinder 2001). Poisoning possums during winter (to reduce inter-specific transmission) potentially maximizes the number of secondary cases in ferrets (although the effect of lower ferret population density at this time of year needs to also be taken into account). Prey manipulation (in this case rabbits) should reduce the population density of ferrets. The level of rabbit control needed for a desired suppression in ferret population density could be calculated using the numerical response of Barlow & Norbury (2001).

Lastly, should a effective vaccine and delivery system become available for *M. bovis* infection in feral ferrets [bacillus Calmette & Guerin (BCG) is showing some potential in experimental pen trials (C. Mackintosh, personal communication)], the proportion of the population, *p*, that must be protected at any one time is (following Anderson (1984)):

$$p > 1 - \frac{1}{R_0}. \qquad \text{(Equation 5.4)}$$

Substituting for R_o in terms of ferret population density (D) from Chapter 4 yields:

$$p > 1 - \frac{1}{0.34D}. \qquad \text{(Equation 5.5)}$$

This is illustrated graphically in Figure 5.2 for a realistic range of ferret population densities. For densities of susceptibles higher than K_T, the proportion to be vaccinated to eliminate *M. bovis* infection in ferrets increases in a curvilinear, convex up manner. If K_T=2.9 ferrets km^{-2}, then at the highest recorded mean population density (*c*. 5.0 km^{-2}) (Chapter 4), about 40% of the ferret population would need to be vaccinated to eliminate *M. bovis* infection (Fig. 5.2). If, on the other hand, K_T=1.2 ferrets km^{-2} (the estimate corresponding to the lower 95% C.I.), then about 80% of a ferret population at a density of 5.0 km^{-2} would need to be vaccinated (Fig. 5.2). Note that these calculations assume inter-specific transmission has been eliminated.

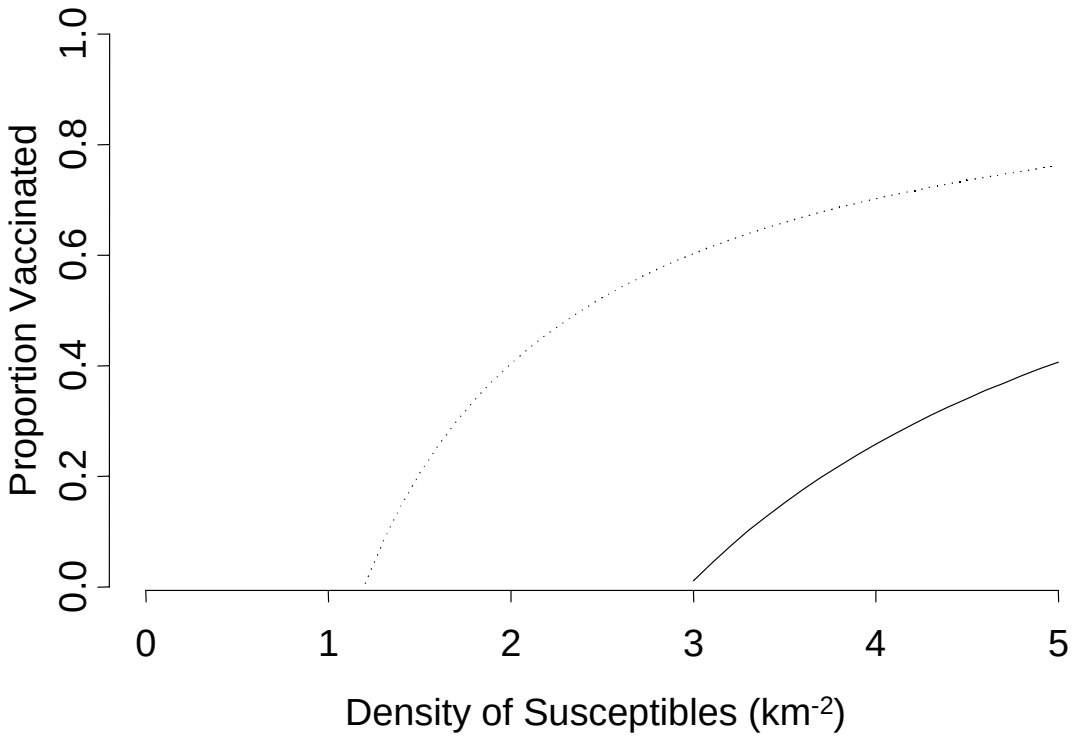

Figure 5.2. The minimum proportion of ferrets as a function of ferret population density that would need to be vaccinated to eliminate the *M. bovis* infection from feral ferrets. The solid line corresponds to the mean estimate of R_o, whereas the dotted line corresponds to the upper 95% confidence interval of R_o. Estimates are calculated using Equation 5.5 (see text for details).

5.4 Emerging wildlife reservoirs of *M. bovis*

The worldwide emergence of wildlife reservoirs of *M. bovis* is a very recent phenomenon (since the 1970s), and coincides with the emergence and/or recognition of a wide range of infectious diseases in wildlife with the ability to threaten domestic animal and human health (Daszak *et al*. 2000). It would be extremely foolish to assume we have seen the emergence of the last wildlife reservoir of *M. bovis*. It would certainly be useful if we could identify attributes that make a wildlife species capable of acting as a maintenance host for *M. bovis*, and use this information to identify 'at-risk' species. The key requirements for the establishment and maintenance of disease (with emphasis on *M. bovis*) are infection (as influenced by susceptibility), number of contacts (influenced by sociality and density), and the disease transmission of these contacts (influenced by environment). These attributes are tabulated in Table 5.1 for the currently confirmed wildlife maintenance hosts of *M. bovis* (excluding feral ferrets, as

maintenance host status remains doubtful for most ferret populations). Note that pulmonary involvement is a feature of *M. bovis* infection in all these species. I have included fur seals here in the absence of strong proof they are the maintenance for the unique *Mycobacterium* strain with which they are found infected. It is, however, clear there is at least one maintenance host for this pathogen, presumably amongst the pinnipeds, in the Southern Ocean.

Table 5.1. Attributes of known wildlife maintenance hosts of *M. bovis* (or in the case of fur seals, a closely related *Mycobacterium* spp.), in terms of susceptibility to *M. bovis* infection, population density, sociality and environment.

Species	Susceptibility	Density	Sociality	Environment
Eurasian badger	Very High	Very Low (*c*. 1 km^{-2})	Very High (tight social groups)	Very Closed (underground setts)
Brushtail possum	Very High	Very High (*c*. 1000 km^{-2})	Very Low (solitary)[a]	Closed (enclosed dens)
White-tailed deer	High	High (*c*. 50 km^{-2})	Moderate (groups)	Mod. Open ('yards')[b]
African buffalo	Moderate	High(herds in 1000s)	High (herds)	Very Open
Swamp buffalo	Moderate	High (*c*. 50 km^{-2})	High (groups, clans)	Very Open
Australian[c] (*Arctocephalus pusillus doriferus*) and New Zealand[d] (*A. forsteri*) fur seals	High	Very High (within colonies)	High (colonies, haul outs)	Very Open
Bison[e]	Moderate	High(herds in 1000s)	High (herds)	Very Open

[a] This ignores the prolonged period joeys spend with their mothers, facilitating 'pseudo-vertical' transmission.

[b] During periods of deep snow cover, white-tailed deer congregate (in large groups sometimes numbering in the 100's) in 'yards' under the protective cover of evergreen conifers.

[c] Hunter *et al*. (1998), [d] Woods *et al*. (1995), [e] Tessaro *et al*. (1990)

If examined in isolation, there is little consistency in population attributes of wildlife maintenance hosts for *M. bovis* listed in Table 5.1. While all species are susceptible, this is rather trivial, given the host range for *M. bovis* in mammals appears unlimited (O'Reilly & Daborn 1995). There appears to be a clear tradeoff between some attributes. For badgers, the high level of within-group sociality and an enclosed environment seem able to counter the effects of low density, whereas for brushtail possums, the very low levels of sociality are compensated for by very high density (Table 5.1), or possibly the long period of close association between mother and offspring. This implies that there must be a reasonable level of close social contact for a species to be a maintenance host for *M. bovis*, and this can be achieved by a number of combinations of density, sociality and environment.

Of the New Zealand mammal species, wild deer have the most potential to display some (or all) of the attributes listed in Table 5.1. The number of deer in New Zealand is increasing as their range expands through deliberate introductions, escapes from deer farms, and natural expansion through dispersal into previously uncolonised habitat (Fraser *et al*. 2000). There are also increases in potential habitat from expanding plantation forestry, and revegetation of extensive areas of uneconomic farmland. This is especially so for fallow deer, which are potentially susceptible to *M. bovis*, capable of achieving high densities, and highly social (forming large herds). The 'explosive' nature of the outbreak of *M. bovis* infection in a captive fallow deer herd (Robinson *et al*. 1989) should serve as a warning. The conditions leading to the unnaturally high densities of white-tailed deer in Michigan (succession of mild winters, lack of natural predation, supplementary feeding during winter) also occur (in varying degrees) in New Zealand. And one should not forget red deer. Indeed, red deer may have formed the original link between *M. bovis* infection in domestic livestock and possums (Morris & Pfeiffer 1995). The establishment of the captive deer industry involved the live capture of wild deer and their transport all over New Zealand (see Caughley (1983) for a fascinating account), and there is strong circumstantial evidence (e.g., de Lisle *et al*. (1995)) this resulted in the rapid geographic spread of *M. bovis* infection into wildlife.

Another species of potential importance is the feral goat. Feral goats are distributed widely in New Zealand (Parkes 1993), achieve very high densities where they are not controlled, and within endemic areas are commonly infected with *M. bovis* (Allen 1987; Sanson 1988; Lugton 1997). Continual exposure of feral goats to *M. bovis* infection clearly carries a risk of a new strain evolving whose pathology enables greater intra-specific transmission among goats and hence greater probability of establishment

and persistence. Indeed, in Spain there is a strain of *M. bovis* found only in domestic goats (at a high prevalence and widely distributed), and which appears to be highly pathogenic for goats (Liebana *et al*. 1998). It seems highly likely, then, that domestic goats in Spain are acting as a maintenance host for this strain of *M. bovis* — a disturbing tale, indeed!

In considering the current host status of feral ferrets (or any other species, for that matter) for *M. bovis* infection, it should be realised the situation is by no means static. To quote from Anderson & May (1986):

> In any consideration of the impact of a disease invasion it is of particular importance to remember that the association between host and pathogen is likely to evolve in a very dynamic manner. The often strong selective pressures exerted by pathogens on the host populations, and the invariably short generation times of parasites relative to those of their hosts, imply that evolutionary changes may occur fairly quickly after a successful introduction.

It is inevitable *M. bovis* will be undergoing coevolution with possums, as observed in other host/pathogen systems (e.g., myxoma virus in Australian rabbit populations (May & Anderson 1983)). However, as *M. bovis* is a slow growing, slow acting pathogen compared with pathogens such as the myxoma virus, I would expect the rate of selection to be much slower. As a sink for *M. bovis* infection (at least in most habitats), ferret populations will be constantly providing any new strain of *M. bovis* with a potential host population to invade, thus turning the sink into a source and new reservoir for *M. bovis* infection. It appears that having a large reservoir of *M. bovis*-infected possums constantly infecting an entire assemblage of species ranging from small carnivores to large herbivores is akin to New Zealand sitting on an evolutionary disease time bomb. With such a large infected host population to fall back on, *M. bovis* can afford to be a phenotypic risk taker!

5.5 Paradigms for examining host status (and doing science)

The way we undertake scientific investigations is varied and subject to ongoing change (Ford 2000). For example, following the plea of Romesburg (1981) for greater use of the hypothetico-deductive method (Popper 1963) to gain reliable knowledge regarding wildlife science, several authors have argued that the hypothesis testing (with

associated probability values) component of this approach has been overdone. For example, Cherry (1998) and Johnson (1999) have deplored the overuse of statistical significance testing of null hypotheses of a trivial nature, and Anderson *et al*. (2000) suggested as an alternative information-theoretic methods based on likelihood. Guthrey *et al*. (2001) however, have subsequently contended that the likelihood approach differs in emphasis only from statistical significance testing. Krebs (2000) strongly recommended estimating the size and biological significance of observed effects. All authors seem to agree more *a priori* critical scientific thought (hypothesis formulation) is needed, as summarised by Anderson *et al*. (2001).

The contention of Guthrey *et al*. (2001) that the way forward in wildlife science may lie with astatistical methods is interesting, though far from convincing. Their example of a recent triumph of science over statistics, the counting of the number of genes in the human genome, seems to have little in common with wildlife science. For a start, it was simply an exercise in counting. If nature were completely 'observable' (as the human genome was able to be sequenced), methods that allow for the inherent variability in our observations (i.e. statistics), and would indeed be obsolete. Major advances in the understanding of host/pathogen systems such as Koch's germ theory, or epidemic theory (Kermack & McKendrick 1927), have indeed occurred in the absence of statistical hypotheses. However, Platt (1964) argued that many major scientific discoveries were made by people who applied what he calls a 'strong-inference' habit of thought, of which critical hypothesis testing (though not necessarily expressed in statistical terms) is a cornerstone. As we still cannot readily observe infection in wildlife occurring, there remains a need for rigorous (critical) hypothesis testing in this field. The bovine BSE epidemic in Britain (Lacey 1994) is a case in point. If the critical tests of the hypothesized model (that BSE was a form of scrapie, and hence unlikely to be transmissible to humans, as is the case for scrapie) had been undertaken sooner (rather than acceptance of what appeared at the time to be a plausible model given the available data), needless loss of life might have been averted (Coghlan 2000).

Kuhn (1962) argued that the way we undertake our science is constrained by the paradigm within which we operate, and this thesis is no exception. I have operated within the paradigm of the basic disease reproductive rate (R_o) and the closely related threshold density for pathogen establishment (K_T). Accepting this paradigm implies a few things—notably that I believe these parameters actually exist (and hence can be estimated), and are useful measures for understanding host/pathogen interactions. As for the next, or alternative paradigm—we must await the required revolution in ways of

thought that will arise from an increasing number of discrepancies not explained by the present paradigm. Some scientists would consider examining host status on a species-by-species basis is restrictive, and ignores that assemblages of species could 'combine' to act as reservoirs for wildlife disease (as discussed by Begon *et al*. (1999) for cowpox infection in sympatric populations of bank voles and wood mice). For example, could an assemblage of species, each of which individually has a R_o value less than unity, have a R_o value greater than unity when viewed as an assemblage? This is a question worthy of further exploration. Others may argue the deterministic assumptions implicit when estimating R_o ignore the fact that the distribution of secondary infections per infected individual may be highly skewed, with possibly only a few individuals responsible for most infections. The phrase 'super excretors' has been used to describe these individuals (Smith *et al*. 2001a,b). In this situation, the number of secondary infections per infected individual will be more influenced by traits of the infected individual (whether or not it is a super excretor), than the number of susceptible hosts available. The deterministic prediction of pathogen establishment when R_o is greater than one ignores the ubiquitous stochasticity of biological processes, and hence will not be true all the time. Lastly, as mentioned in Chapter 1, I did not seriously considered inter-specific transmission of *M. bovis* to ferrets from species other than possums. Given the importance of rabbit in the diet of ferrets, a closer examination of the occurrence of *M. bovis* infection in rabbits may be warranted. The model to assess the likelihood of this would be a ferret/possum/rabbit/*M. bovis* one.

This thesis has combined the use of model selection/information theoretic (Chapter 2), statistical significance testing (Chapter 3), and parameter estimation (Chapter 4) methods of ecological investigation, interlaced with some mathematics, and much observation during data collection. The approaches have by and large been complementary, suggesting there is a need for all forms of scientific method. There are conflicting views on what is the best form of scientific method, with much of the current tension arising from the widening gulf between theory (often expressed in complex mathematical models) and data (particularly of the field variety—laboratory datasets have enabled the fitting of far more complex models). McCallum (1995) argues all modes of ecological investigation at some point require a 'leap of faith' before applying the results to the real world (though he refrains from discussing whether some modes of investigation require bigger 'leaps' than others). Individual opinion as to who should be doing what when undertaking scientific research is influenced greatly

from where one stands, as the following viewpoints demonstrate. Mantel (cited by Ludwig & Cooke 1975) suggests:

> … that mathematicians should contribute themselves, not their mathematics. They should try and imbue themselves with the actual substance of epidemiological, biological, and medical investigations; then make use of both their mathematical abilities and their above-average intelligence to come up with answers.

This view is echoed by Bradley (1982), who emphasizes the importance of truly multidisciplinary approaches to problem solving (in this case for malaria) when making the oft-quoted statement 'For real progress, the mathematical modeller as well as the epidemiologist must have mud on his boots.' Krebs (2000) gives similar sentiments, though from the viewpoint of standing in the mud, with one of his key recommendations for ecologists (that may come as a surprise to some!) being to 'Use a mathematical model of your hypothesis to articulate your assumptions explicitly.' McNeil (1975) emphasizes the importance of statistics in assessing how well models fit with data, when saying:

> Turning to the problem of epidemic modelling, it seems to me that there are lots of epidemiologists who are collecting and analysing data, there are public health officials who are carrying out programs for controlling disease, and there are mathematicians who are developing models, but there are not enough people fitting statistical models to the data. As a result, the mathematicians are forced to invent models based on reflection and mathematical elegance, only some of which may be relevant to the actual data.

On the other hand, statisticians such as Box (1990) (cited by Chapman (1995)) have expressed the sentiment that statisticians should strive to become first-class scientists rather than second-class mathematicians, and states 'Statistics is, or should be, about scientific investigation and how to do it better.' I think this is cutting close to the chase, and for me, Hilborn & Mangel (1997) strike a chord with their philosophy that 'for the ecological detective [solving] the problem is paramount.'

5.6 Finale

To conclude, this thesis has made inference on the host status of feral ferret populations in New Zealand for *M. bovis* infection, so that more informed decisions could be made on whether active management of ferret populations was needed to achieve wildlife disease control objectives. Inference was made by combining model selection, hypothesis testing, and parameter estimation, as applied to data collected from manipulative experimental field studies. In doing so it has linked epidemiological and ecological theory with data. The key result of the work is that in low-density ferret populations in New Zealand the rate of intra-specific transmission of *M. bovis* infection alone is insufficient for the disease to establish in ferrets. It is inferred that ferrets in these habitats are spillover hosts for *M. bovis* infection. An effective management tactic for controlling *M. bovis* infection in feral ferrets in these areas (all the North Island and most of the South Island sites) is therefore to control *M. bovis* infection in sympatric brushtail possum populations. In areas of high density, however, it appears *M. bovis* may be just able to establish in ferret populations. It is inferred that ferrets may be maintenance hosts in these habitats, and that here active management of ferrets may be required to control *M. bovis* infection in ferret populations. There remains considerable uncertainty around this prediction, and more precise estimates of disease transmission rates will be required to reduce this uncertainty.

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Appendices

6.1 Derivation of the age-specific prevalence of disease with a constant force of infection with non-zero disease-induced mortality and no disease recovery.

Assume we start with a cohort of *N* animals of which *S* are susceptible and *I* are infected. Assume there are no births. Let the force of infection (the instantaneous rate at which individuals are infected) be λ, and the instantaneous rate of natural mortality be *b*, and the additional instantaneous mortality rate due to disease be α. The resulting differential equations are:

$$\begin{aligned}\frac{dS}{dt} &= -(\lambda + b)S \\ &= -(\lambda + b)(N - I),\end{aligned} \qquad \text{(Equation 6.1)}$$

$$\begin{aligned}\frac{dI}{dt} &= \lambda(N - I) - (b + \alpha)I \\ &= \lambda N - (\lambda + b + \alpha)I,\end{aligned} \qquad \text{(Equation 6.2)}$$

and

$$\begin{aligned}\frac{dN}{dt} &= \frac{dI}{dt} + \frac{dS}{dt} \\ &= -bN - aI.\end{aligned} \qquad \text{(Equation 6.3)}$$

I are inherently interested in the prevalence ($p = \frac{I}{N}$), and how it changes over time. Differentiating with respect to time (appealing to the Product Rule of differentiation (Swokowski 1979)) yields:

$$\frac{dp}{dt} = \frac{1}{N}\frac{dI}{dt} - \frac{1}{N^2}\frac{dN}{dt}. \qquad \text{(Equation 6.4)}$$

Substituting for $\frac{dI}{dt}$ and $\frac{dN}{dt}$ from Equations 6.2 and 6.3 into Equation 6.4 yields:

$$\begin{aligned}\frac{dp}{dt} &= \frac{1}{N}\big(\lambda N - (b + \alpha + \lambda)\big) - \frac{1}{N^2}(-bN - \alpha I) \\ &= \alpha p^2 - (\alpha + \lambda)p + \lambda \ .\end{aligned} \qquad \text{(Equation 6.5)}$$

Integrating with respect to time gives:

$$\begin{aligned}\int \frac{dp}{\alpha p^2 - (\alpha + \lambda)p + \lambda} &= \int_{t=0}^{t=a} dt \\ &= a + c\end{aligned} \qquad \text{(Equation 6.6)}$$

where a *is the age of the animal, and* c *is the constant of integration. I now appeal to the result (Anonymous 1980) that:*

$$\int \frac{dx}{ax^2+bx+c} = \frac{1}{\sqrt{b^2-4ac}} \ln\left(\frac{2ax+b-\sqrt{b^2-4ac}}{2ax+b+\sqrt{b^2-4ac}}\right) \qquad (\text{for } b^2 > 4ac)$$

$$= \frac{1}{\sqrt{4ac-b^2}} \arctan\left(\frac{2ax+b}{\sqrt{4ac-b^2}}\right) \qquad (\text{for } b^2 < 4ac) \quad \text{(Equation 6.8)}$$

$$= -\frac{2}{2ax+b} \qquad (\text{for } b^2 = 4ac)$$

In this case $a=\alpha$, $b=-(\alpha+\lambda)$, and $c=\lambda$, so $b^2>4ac$ as (note this b does not represent the instantaneous natural mortality rate as before):

$$\begin{aligned} b^2-4ac &= (-(\alpha+\lambda))^2 - 4\alpha\lambda \\ &= \alpha^2 + 2\alpha\lambda + \lambda^2 - 4\alpha\lambda \\ &= \alpha^2 - 2\alpha\lambda + \lambda^2 \\ &= (\alpha-\lambda)^2 \\ &> 0\,. \end{aligned} \qquad \text{(Equation 6.9)}$$

With initial conditions $p=0$ at $t=0$, the solution to Equation 6.6 in terms of p is:

$$p(a) = \frac{\lambda(1-e^{(\alpha-\lambda)a})}{\lambda-\alpha e^{(\alpha-\lambda)a}}\,. \qquad \text{(Equation 6.10)}$$

This is simply the solution of Equation (3) presented by Cohen (1973), with the 'force of defection' (this is the disease recovery rate, termed *b* by Cohen (1973), though subsequently more commonly termed γ) set to zero, *a* replaced with λ and ε replaced with α.

6.2 Estimating the density of *M. bovis*-infected ferret carcasses

Let:

I_F = density of *M. bovis*-infected ferrets

$\alpha+b$ = combined mortality of ferrets due to *M. bovis* infection and natural causes

d = rate at which *M. bovis* infection in carcasses becomes non-viable

W_F = density of ferret carcasses containing viable *M. bovis* organisms

β_F = ferret carcass-to-ferret disease transmission coefficient

D = ferret population density

The rate at which W changes with respect to time is:

$$\frac{dW_F}{dt} = -(d + \beta_F D)W_F + (\alpha + b)I_F . \qquad \text{(Equation 6.11)}$$

Rearranging in the form of a linear equation of order one (Rainville & Bedient 1981):

$$\frac{dW_F}{dt} + (d + \beta_F D)W_F = (\alpha + b)I_F , \qquad \text{(Equation 6.12)}$$

for which the solution with initial conditions $W_F(0)=0$ is:

$$W_F(t) = \frac{(\alpha + b)(1 - e^{-(d+\beta_F D)t})I_F}{d + \beta_F D} . \qquad \text{(Equation 6.13)}$$

For a system at equilibrium, *t* is large, hence:

$$W_F(t) \cong \frac{(\alpha + b)I_F}{d + \beta_F D} , \qquad \text{(Equation 6.14)}$$

which describes the ratio of loss of ferrets to loss of carcasses. However, the rate at which ferret carcasses are scavenged ($\beta_F D$) is not equivalent to the rate at which they are lost (in the sense of the disease modelling issue at hand), as communal feeding is possible, and most scavenging events result in the only partial "loss" of the carcass. Hence Equation 6.14 can be approximated further by ignoring the $\beta_F D$ term as:

$$W_F(t) \cong \frac{(\alpha + b)I_F}{d} . \qquad \textbf{(Equation 6.15).}$$

6.3 Removal estimates of population density when trapping effort is not constant

Estimating the size of ferret populations appears quite feasible using removal methods, whilst trap success appears to provide a reasonable relative index of abundance (Cross *et al*. 1998). Assuming a closed population, constant sampling effort, and equal probability of captures, data can be analysed by Zippin's Removal Estimator (Zippin 1958). If sampling effort varies, though the other assumptions hold, data may be analysed by, for example, Leslie's Method

(Seber 1982). The ferret surveys I have undertaken suffer from unequal sampling effort arising from the capture of numerous non-target species (e.g., brushtail possums, Australasian harriers (*Circus approximans*), hedgehogs etc.). Fitting Leslie's model soon illustrated that the relationship between the proportion of available traps catching ferrets and cumulative density of ferrets removed was not linear, but rather convex-up (Figure 6.1). This could be due to heterogeneity in capture probabilities, trap response, or competition for traps. A similar convex-up relationship caused by competition for traps was shown by Batcheler *et al*. (1967) for brushtail possums, where the proportion of traps catching possums on any one night was extremely high (>90%). Whilst the proportion of traps catching ferrets on a broad scale is never high (usually <30%), the distribution of traps catching ferrets tends to be highly skewed (most ferrets are captured from a few traps). Hence competition for traps is very real possibility.

One approach to addressing competition for traps is to model the way available traps and ferrets 'mix'—similar to how host/pathogen models treat the mixing between susceptibles and infectious individuals. This is the approach here, using parameters defined in Table 6.1. If we first assume traps and ferrets 'mix' homogeneously (obviously it is only the ferrets doing the mixing!), the density of ferrets (analogous) that encounter a trap (infective) and are removed (infected) on the first night is given by:

$$R_1 = \beta N t_1, \qquad \textbf{(Equation 6.16)}$$

and, the density of ferrets removed on the second night is by:

$$R_2 = \beta (N - R_1) t_2, \qquad \textbf{(Equation 6.17)}$$

and for the ith night:

$$R_i = \beta (N - \sum R_i) t_i. \qquad \textbf{(Equation 6.18)}$$

Rearranging in terms of p_i, yields:

$$p_i = \frac{R_i}{t_i} = \beta(N - \sum R_i). \qquad \textbf{(Equation 6.19)}$$

This is Leslie's Method as applied to the proportion of traps catching animals (in this case ferrets).

Table 6.1. **Parameters, their notation and units used in estimating ferret abundance.**

Parameter	Symbol	Units
Effective trapping area	A	km^2
Density of available traps on the i^{th} night	t_i	km^{-2}
Initial density of ferrets	D	km^{-2}
Mixing coefficient between available traps and ferrets	β	km^{-2}
Ferrets removed on i^{th} night	R_i	—
Proportion of available traps catching ferrets on i^{th} night	p_i	—

Alternatively, one can tackle the heterogeneous mixing problem in a phenomenological manner, such as that of Barlow (2000), who used a negative binomial transmission term. The analogy here is:

$$R_i = k \ln(1 + \frac{\beta(N - \sum R_i)}{k})t_i \qquad \textbf{(Equation 6.20)}$$

or:

$$p_i = k \ln(1 + \frac{\beta(N - \sum R_i)}{k}) \qquad \textbf{(Equation 6.21)}$$

Equation 6.21 often provided a better fit to observed catch-effort data (see Figure 6.1). However often it was sometimes difficult to find starting values for *k* and *β* that would lead to the non-linear regression converging, and sometimes impossible within reasonable time limits. This precluded using this method for estimating density for all trapping sessions, so I abandoned the idea of using it to estimate ferret population density for the time being.

Another approach is to assume ferrets search their home-range randomly at a rate λ km^{-2}, hence available traps encounter ferrets at a rate λ*D* per night. Hence, we can express the proportion of available traps on the ith night catching ferrets (*p*) as:

$$p_i = 1 - e^{-\lambda(D - \sum R_i)} \qquad \textbf{(Equation 6.22)}$$

Rearranging:

$$\ln(1 - p_i) = -\lambda(D - \sum R_i) \qquad \textbf{(Equation 6.23)}$$

Equation 6.23 gives near identical results to Equation 6.19 (Fig. 6.1), probably because the proportion of available traps catching ferrets is low. It can either be fitted by linear least-squares regression, or as a general linear model (GLM). The GLM would have the response variable $(1 - p)$ specified as binomially distributed, and the link function logarithmic. Problems are encountered on fitting this model as a GLM if during the iteration process the fitted values for p_i fall outside the range 0–1. Using linear least-squares regression does not suffer from this problem.

Calculating the density of ferrets removed, and hence density, requires an estimate of the effective trapping area. I used Kernel Estimators (Worton 1989) of effective trapping area. A combination of 250 m x 250 m bins with the 99% isopleth yielded realistically shaped effective trapping areas, with an average extension of c. 500 m around traps, as required. Clearly this is not the intended

use of Kernel estimators, however it satisfies a purpose.

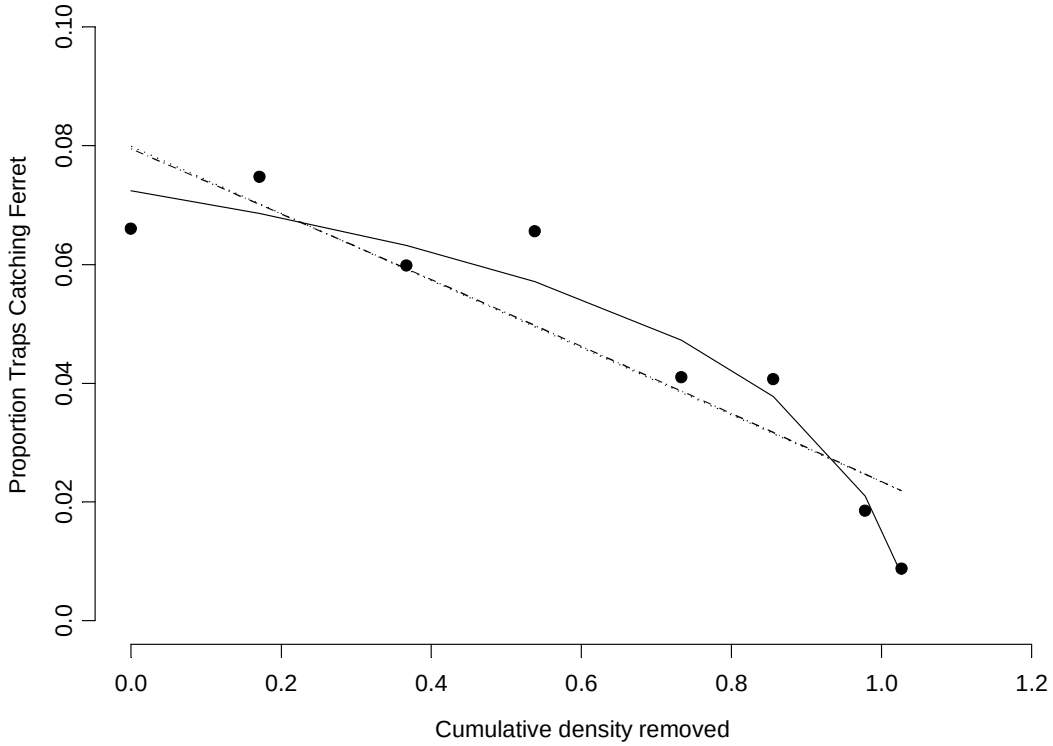

Figure 6.2. The relationship between the proportion of traps catching ferrets, and the population density of ferrets previously removed by trapping for the Awatere Valley site survey during 2000. The dotted line is the fitted proportion of traps catching ferrets, modelled assuming ferrets and traps 'mix' homogeneously—Leslie's Method (Equation 6.19). The solid line is the fitted proportion of traps catching ferrets, modelled assuming ferrets and traps mix heterogeneously (Equation 6.21). The dashed line (overlaying the dotted line) is calculated using Equation 6.23).

6.4 Estimating mortality rates of feral ferrets

If the natural mortality rate (μ) and the disease-induced mortality rate (α) are additive, then the total mortality rate is $\mu + \alpha$. If disease-induced mortality is perfectly compensated by changes in the natural mortality rate (increased survival) then the total mortality rate is μ. An example of the issue of additive versus compensatory mortality is described by Nichols *et al.* (1984). Here I use the analytical approach of Sibly *et al.* (1997) to estimate the instantaneous rate of mortality of ferrets. I compare models of mortality, starting with the simplest (mortality rate constant across ages and population

disease status) and increasing in complexity (mortality rate differs with age and disease status), and use model selection to identify which model adequately represents the observed mortality pattern in ferrets.

Data were collated from cross-sectional surveys of ferret populations undertaken at seven sites in the North and South Islands, New Zealand: Castlepoint, Cape Palliser, Awatere Valley, Hohotaka, Lake Ohau, Rangitikei and Waipawa. All surveys were undertaken in the summer/autumn period (February–April) during 1997–2000. Only data from one (the first if multiple surveys undertaken) survey per site were included for analysis. Ferret age was estimated to the nearest year by counting cementum annuli in sections of a lower canine tooth (Grue & Jensen 1979), with ferrets assigned to year age classes.

For the purpose of the analysis, sites were divided into two groups on the basis of their force of *M. bovis* infection, as estimated using the model in Chapter 2. The mean force of *M. bovis* infection is, in relative terms, very high at the Castlepoint, Cape Palliser and Awatere Valley sites, and very low at the Hohotaka, Lake Ohau, Rangitikei and Waipawa sites (Table 6.2). The mean age of first infection is hence low for the first three sites, and high at the last three sites (Table 6.2), as the mean age of first infection is simply the reciprocal of the force of infection (Grenfell & Anderson 1985), added to the 1.75 month 'guarantee time' during which ferrets are not exposed to infection prior to weaning (Chapter 2). Indeed the estimated mean age of first infection at the Lake Ohau, Rangitikei and Waipawa sites is very high (>7 years), indicating ferrets are rarely infected in their lifetime (maximum *c*. 5 years), so disease-induced mortality arising from *M. bovis* infection at a population level can effectively be ignored. Conversely, most ferrets at the Castlepoint, Cape Palliser and Awatere Valley sites are infected early in life, and consequently *M. bovis*-induced mortality (α) could add substantially to the natural mortality rate (μ).

I define μ as the natural instantaneous mortality rate, and α as the disease-induced instantaneous mortality rate due to *M. bovis* infection. For some data sets it was necessary to pool data over ages to ensure the observed survival rate was less than or equal to one (Table 6.3). This was modelled by modifying the model of Sibly *et al.* (1997) to account for differing times (e.g., Δt=1.0, 1.5 years) over which survival $S(\Delta t)$ was measured (Equation 6.24). When Δt=1.0, Equation 6.24 reduces to the equation of Sibly *et al.* (1997).

$$\log_e\left\{-\log_e\left[S(\Delta t)\right]\right\} = \lambda + \log_e(\Delta t) \qquad \text{(Equation 6.24)}$$

Equation 6.24 was fitted by specifying $\log_e(\Delta t)$ as an offset (Collett 1991). The parameter λ is the parameter of interest to be estimated from Equation 6.24, where $\lambda = \log_e(\mu)$, and $S(\Delta t)=e^{-\mu\Delta t}$ (Sibly *et al.* 1997).

Three models were considered here. The first (age-invariant) assumed no transition age in mortality rate. The second model (2-phase) assumed one transition age with juveniles (age <1 year) having a different mortality rate than adults. The third model (3-phase) had a further transition age (for senescence) at 2 years—ferrets older than 3 years are uncommon (Table 6.2). The effect of disease (categorised into low age of first infection cf. high age of first infection; Table 6.2) was fitted as a factor.

Table 6.2. Instantaneous rate (force of infection) at which ferrets at sites in New Zealand are infected with *M. bovis* infection ($\hat{\lambda}$) and the mean age of first infection ($\hat{A}$), from Caley & Hone (2002). Age of first infection category represents how $\hat{A}$ for each site was categorised to model the effect of disease.

Site	$\hat{\lambda}$ (yr^{-1})	$\hat{A}$ (yr)	Age of first infection (categorical)
Cape Palliser	2.0	0.6	Low
Castlepoint	5.8	0.3	Low
Awatere Valley	3.4	0.4	Low
Hohotaka	0.18*	5.7	High
Waipawa	0.12*	8.5	High
Lake Ohau	0.14	7.3	High
Rangitikei	0.10*	10.1	High

*Estimated using the model presented in Chapter 2.

There are a number of clear assumptions in my analysis, namely (1) no age bias in probability of capture; (2) no year-to-year variation in mortality and fecundity rates (i.e. stable age structure); and (3) α and μ are additive. I am also estimating mortality rates from the age juvenile ferrets emerge above ground in mid-summer and become available to be trapped (after birth in late spring/early summer). The adequacy of model fit was examined by analysis of deviance as used by Sibly *et al.* (1997), and plots of standardised Pearson residuals (Collett 1991). Analyses were undertaken using the software GLIM4 (Francis *et al.* 1993). The data analysed are presented in Table 6.3. A 2-phase model was fitted first, giving a deviance of 26.2 with 16 *d.f.*—indicating the data were over-dispersed (Collett 1991) (P = 0.05), as were data for subsequent models (Table 6.4). Hence F-tests were used, following Sibly *et al.* (1997), to compare models

rather than analysis of deviance.

Table 6.3. Frequency distribution for the age structure of feral ferret populations from 7 sites in New Zealand.

Site	Age of first infection	Age (years)					
		0–1	1–2	2–3	3–4	4–5	n
Cape Palliser	Low	13	2	2	0	1	18
Castlepoint	Low	25	3	0	0	0	28
Awatere Valley	Low	32	6	3	2	3	46
Sites combined	**Low**	**70**	**11**	**5**	**2**	**4**	**92**
Hohotaka	High	39	2	7	3	3	54
Waipawa	High	21	5	2	0	0	28
Lake Ohau	High	55	10	4	1	0	70
Rangitikei	High	18	7	4	1	0	30
Sites combined	**High**	**133**	**24**	**17**	**5**	**3**	**182**

The 3-phase model was clearly no improvement on the 2-phase (both models have same deviance—Table 6.4). The 2-phase model was a considerable improvement on the age-invariant model ($F_{1,16}$=14.4, P<0.01). Adding the disease factor to the 2-phase model made no significant improvement ($F_{1,15}$=0.35, P=0.56). The estimated mortality rate was 12% higher in sites with a low age of first infection, though this was statistically non-significant (Z=0.75, P=0.23, one-tailed test), demonstrating that overall mortality rates differed little, if at all, between areas of high and low disease prevalence. The fitted age structure using the 2-phase model matched the data reasonably well (Fig. 6.2), and a plot of residuals against ferret age was reasonably evenly spread (Fig. 6.3), suggesting the 2-phase model captured the key components of mortality acting in the populations.

Table 6.4. Deviances for models of mortality for *Mustela furo*. The 2-phase model has transition in mortality rate at 1 year, and the 3-phase has additional transition at 2 years. Disease refers to either a low or high age of first infection with *Mycobacterium bovis*.

Model	**Deviance**	***d.f.***	***P***
Age-invariant	49.7	17	<0.001
2-phase	26.2	16	0.05
3-phase	26.2	15	0.04
2-phase + Disease	25.6	15	0.04

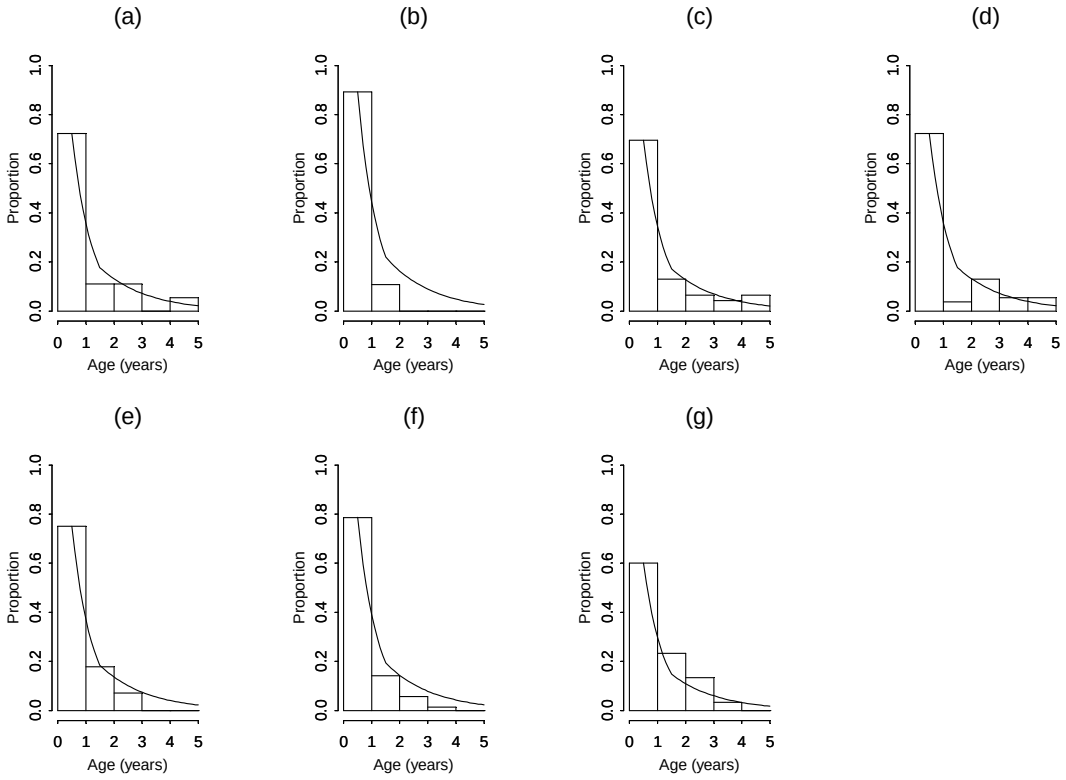

Figure 6.2. Observed (bars) and fitted (lines) age structure of feral ferret populations from: (a) Cape Palliser; (b) Castlepoint; (c) Awatere Valley; (d) Hohotaka; (e) Waipawa; (f) Lake Ohau; and (g) Rangitikei. Lines were fitted using a 2-phase version of Equation 6.24 (see text for details).

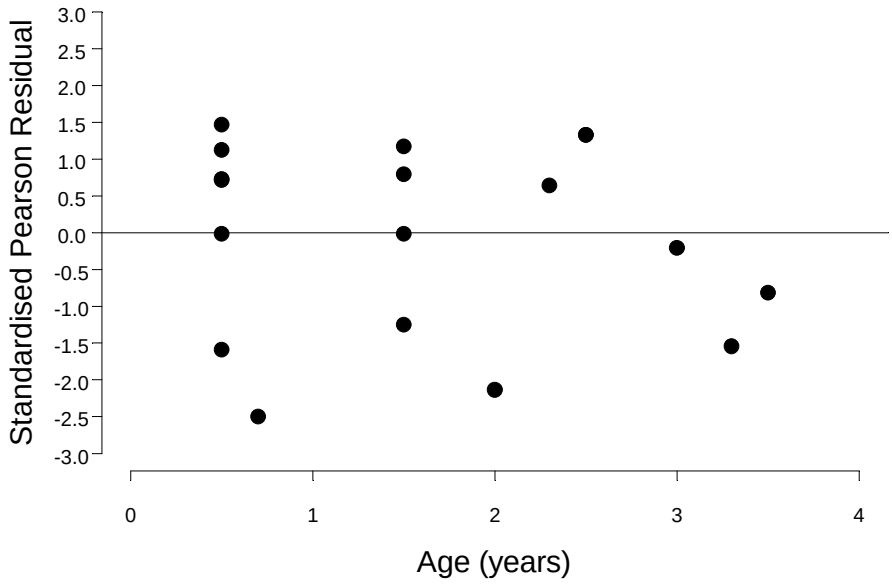

Figure 6.3. Standardised Pearson residuals for the estimated mortality rates of feral ferrets from 7 sites in New Zealand. The model used to estimate mortality rates was a 2-phase version of Equation 6.24 (see text for details).

The 2-phase model (preferred model) estimated μ to be 1.44 yr^{-1} (95% C.I. 1.2–1.7 yr^{-1}) for juveniles and 0.56 yr^{-1} (95% C.I. 0.4–0.9 yr^{-1}) for adults. These estimates are considerably more precise (narrower 95% C.I.) than those of Caley & Morriss (2001), and correspond to a survival probability of 0.25 yr^{-1} during the first year of life, rising to 0.55 yr^{-1} thereafter. The life expectancy (*L*) of ferrets subjected to a instantaneous mortality rate μ_1 until the transition age (τ), and instantaneous rate μ_2 thereafter is given by:

$$L = \int_0^{\tau} t\mu_1 e^{-\mu_1 t} dt + e^{-\mu_1 \tau} \int_{\tau}^{\infty} t\mu_2 e^{-\mu_2 (t-\tau)} dt \,. \qquad \text{(Equation 6.25)}$$

The values $\hat{\mu}_1$ = 1.4 yr^{-1}, $\hat{\mu}_2$ = 0.6 yr^{-1} and τ = 1.0 yr equate to an expected life expectancy (*L*) of 0.95 years or 11.5 months.

Finally, if disease-induced and natural mortality are additive, then the results demonstrate disease-induced mortality is negligible by comparison with natural mortality. Alternatively, if the mortality rates are compensatory, then we have learned little of the disease-induced mortality rate of *M. bovis* infection in ferrets. However, as the mortality rate in populations where nearly all individuals are infected is no different from that where few individuals are infected, the rate of disease-induced mortality must be less than or equal to the natural rate. Hence I can now put an upper bound on α (i.e. $\alpha \leq 1.4$ yr^{-1} for juveniles and $\alpha \leq 0.6$ yr^{-1} for adults).