Parasites and the Costs of Reproduction in Soay Sheep

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Abstract

Over the lifetime of an individual, decisions are constantly being made. These arise as a result of trade-offs between life-history traits (e.g. lifespan and fecundity). Trade-offs may occur when two traits are limited by the same resource, such that one trait can be increased only at the expense of another. The cost of reproduction is the trade-off between current and future reproduction. An intermediate trade-off that can affect reproduction, is that between parasite resistance and reproduction. This thesis attempts to explore the interaction between parasites and the costs of reproduction in a wild population of Soay sheep on the Scottish island group of St Kilda (Chapter 2).

In domestic sheep, costs associated with lambing incur a temporary increase in parasite burden, termed the *peri-parturient rise.* Soay sheep that successfully rear a lamb also suffer from this increase in parasitism (Chapter 3), whereas non-lambing adult ewes do not. In contrast, non-lambing *yearling* ewes do suffer from an increase in parasite burden at this time, despite incurring only minimal costs of reproduction, probably as a consequence of poor body condition and a lack of acquired immunity to parasites. Male Soays also suffer an increase in parasitism during spring, even though they do not reproduce at this time, suggesting that the spring rise in parasitism is partly associated with poor body condition.

The effects of parasites on the lambing success of Soay ewes were explored using observational (Chapter 4) and experimental methods (Chapters 5 and 6). Ewes treated with an anthelmintic to temporarily remove their parasites over the winter were in better condition in the following spring. This did not affect their probability of lambing, but there was a trend for treated ewes to have a higher incidence of twinning than control ewes, and for their lightest lambs to have a higher probability of survival than those of control ewes. Early growth and development is not improved by treatment of the mother, despite these ewes being in a better condition. It is suggested that this may reflect a possible trade-off between current and future reproductive effort: ewes may not exceed the investment necessary to ensure survival of offspring, in anticipation of future breeding events.

Declaration

This thesis is a result of my own research, and no part of this thesis has been submitted in application for a higher degree at this or any other institution. All collaborative involvement has been duly acknowledged.

Signed..... ***........date...............*** 27/7/06

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List of abbreviations

FEC	**Faecal Egg Count**
PPR	**Peri-Parturient Rise**
GLM	**General Linear Model**
GLMM	**General Linear Mixed Model**

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Chapter 1

Introduction

1.1 Life history theory and the costs of reproduction

Over the lifetime of an individual, decisions are constantly being made that affect the fitness of the individual. These decisions arise, in part, as a result of *trade-offs* between life-history traits, such as reproductive effort and lifespan. Trade-offs may occur when two traits are limited by the same resource, i.e. one trait can only be increased at the expense of another (Bell & Koufopanou 1986, Stearns 1989). The 'principle of allocation' (Levins 1968) states that a resource, such as energy or time, can only be used as currency once, and must therefore be allocated between competing traits. For example, in the female bruchid beetle (*Callosobruchus maculatus*), resources can be allocated to either producing eggs or somatic maintenance, such that females that invest in offspring production tend to have shorter life spans than those females that are experimentally discouraged from laying eggs (Wilson 1989, see also Lessells 1991). Life history traits are those traits that directly or indirectly affect the survival or fecundity of the individual. These traits include age at first reproduction, the frequency of reproduction, and lifespan, but they also include others, such as growth rate and immunity to disease or parasites, that may form intermediate trade-offs that ultimately affect fitness (Stearns 1989, Lessells 1991).

Life history theory predicts that current reproductive effort can have detrimental effects on the fitness of the parent (Partridge and Harvey 1985, Partridge 1992). Several studies have experimentally demonstrated a cost of reproduction on parent survival. For example in the collared flycatcher (*Ficedula albicollis*), artificially increasing brood size to increase reproductive effort caused a decrease in parental survival (Nordling and Gustaffson 1998). In red deer hinds (*Cervus elaphus*) it was found that barren females and hinds that lost calves early had better survival than those hinds that reared calves (Clutton-Brock *et al.* 1983).

The suggestion that increasing current reproductive effort, by increasing offspring numbers and parental care, may cause a reduction in the resources available for future reproduction, was first made by Williams (1966). This major trade-off in life history theory between *current* and *future* reproduction is known as the 'cost of reproduction'. The classic method of testing this theory is by manipulating clutch size or offspring number. In the collared flycatcher (*Ficedula albicollis*) females that had their clutches experimentally enlarged showed reduced fecundity in the following year in terms of decreased clutch size, and less recruits (Gustafsson & Sutherland 1988, Gustafsson & Part 1990).

More recently, the trade-off between current and future reproduction was demonstrated by experimentally manipulating current reproductive effort in female eider ducks, *Somateria mollissima* (Hanssen *et al.* 2005). Costs of egg incubation were manipulated by varying clutch size. Females that incubated larger clutches had increased mass loss and reduced immune function, and although this did not adversely affect current reproduction, fecundity in the following year was reduced (Hanssen *et al.* 2005).

Another trade-off exists within current reproductive effort: the number of offspring produced versus the amount of resources available to provision those offspring. The two extremes of this scenario would be one, very well provisioned offspring, compared to the other end of the scale where there would be many poorly provisioned offspring. For example, in the barn swallow (*Hirundo rustica*), nestlings reared in an experimentally enlarged brood were generally in a poorer body condition (smaller body mass, lower immune function, and suffered from more ectoparasites) than nestlings reared in a small brood (Saino *et al.* 2002).

Life history theory predicts that increasing allocation of resources to one trait would necessarily reduce the resources available to invest in another, leading to a negative correlation between them. However, this pattern is not always observed, and often two life history traits have been shown to covary positively. For example, in nature, heavy female *Callosobruchus* beetles tend to produce large numbers of offspring *and* they also live a long time (Lessells 1991, Wilson 1994). The reason that life history traits sometimes positively covary, is because individuals tend to differ in the amount of resources available to them. Van Noordwijk and de Jong (1986) proposed that this variation in the *acquisition* of resources between individuals, leads to variation in the *allocation* of resources. In other words, although life history theory predicts that any individual has a limited resource that must be divided and allocated towards different aims, e.g. reproduction, growth, or immunity; certain individuals are 'richer' in that they have more resources to allocate. In a human analogy of this situation, Van Noordwijk and de Jong made a comparison with differences in family wealth: a rich family may be able to afford a big car and a big house, whereas a poorer family may only be able to afford a small car and a small house. Where there is more variation in resource acquisition, i.e. a greater range of 'rich' and 'poor' individuals, then positive correlations between life history traits can occur (van Noordwijk and de Jong 1986). Conversely, where there is less variation in acquisition and more in allocation, the negative correlations of life history theory are apparent. In other words, there is a greater limitation on resources, and the decisions on how and when to invest will vary depending on individual circumstance.

Because of natural variation in levels of 'resource acquisition', phenotypic correlations often fail to reveal life history trade-offs. One potential way around this problem is to experimentally manipulate individuals to expose the underlying trade-offs. One method researchers have often used is to experimentally manipulate individuals such that they reduce or increase their levels of reproductive effort, and in so doing, reveal a cost of reproduction. For example, manipulation of brood size revealed costs of reproduction in the collared flycatcher, *Ficedula albicollis* (Nordling and Gustaffson 1998), and in eider ducks, *Somateria mollissima* (Hanssen *et al.* 2005). Thus, in order to reliably examine potential life history trade-offs it is often necessary to take an experimental approach.

However, even experimental manipulations sometimes fail to reveal the expected trade-offs. In a modification to the original van Noordwijk & de Jong model, it has been argued that that this is because there is often genetic variation in resource acquisition (Spitze *et al.* 1991a, b, Reznick *et al.* 2000). A classic example of positive genetic relationships between traits is in *Daphnia pulex* (Spitze 1991). Populations of *Daphnia* under predation risk from *Chaoborus americanus* evolved towards larger body size, and reproduced earlier with an increase in fecundity, than those from control (predator-free) populations.

Why are the individuals that have greater acquisition of resources (termed 'super-phenotypes') not selected for? The answer appears to lie in the *costs* of acquisition (reviewed in Reznick *et al.* 2000). Super-phenotypes are only super when resource levels are high and there is no cost to the enhanced ability to acquire resources. In fact, when resource levels are low, they probably have lower fitness than the non-super individuals.

A possible cost of resource acquisition has been shown in species of *Daphnia* (Desmarais & Tessier 1999, Tessier *et al.* 2000). There is much variation in the size of *Daphnia* species/clones; some are small bodied (e.g. *Daphnia rosea*) and some are larger bodied (e.g. *Daphnia pulex*). Growth rate increases as resource levels increase, and larger size is advantageous in terms of fitness. However, large-bodied *Daphnia* are more sensitive to a decrease in resource concentration, such that at lower resources the smaller species have a higher growth rate. The smaller species are better at converting resources when these are scarce, whereas large *Daphnia* possessed a greater ability during abundant resources (Desmarais & Tessier 1999, Tessier *et al.* 2000). The cost of acquisition which allows some species to grow faster into larger size, and reap the fitness benefits this may allow, is traded off with their ability to survive at minimum resource availability.

In red deer, hinds in a good body condition are more likely to successfully rear a calf, but show reduced future fecundity and survival than non-successful hinds (Clutton-Brock *et al.* 1989). However, in years when resources are abundant, the hinds in the best condition ('super phenotypes') do not suffer costs of acquisition. Under these circumstances hinds of superior body condition have higher survival rates than the inferior hinds that were barren or lost their calf early, despite having greater energy expenditure. So, here we have another example of the costs of acquisition: the super-phenotypes only perform better when resources are high, and suffer from the costs of acquisition during periods of low resource when the inferior individuals surpass them.

1.2 Parasites and the costs of reproduction

There is evidence to suggest that parasites can have significant detrimental effects upon host survival, growth and fecundity (reviewed in Gulland 1995, Grenfell & Gulland 1995, Tompkins & Begon 1999). For example, comprehensive studies on red grouse have suggested that its parasite (*Trichostrongylus tenuis*) may drive population cycles; through direct effects on mortality and by limiting reproductive success (Hudson 1986, Hudson & Dobson 1989, Dobson & Hudson 1992, Hudson *et al.* 1998).

Mounting an immune defence to parasitism requires allocation of resources, which reduces those available for reproduction and creates a trade-off between current reproduction and parasite resistance. Experimental manipulation of brood size in the collared flycatcher (*Ficedula albicollis*) affected female ability to mount an effective immune response to Newcastle disease virus (NDV) (Nordling *et al.* 1998). Antibody production was lower in NDV immunized females when brood size was artificially increased; while a greater antibody response was observed in females with experimentally reduced brood size. Larger brood sizes also increased susceptibility to infection; females with large broods had increased levels of infection of blood parasites *Haemoproteus* spp., which are haemosporidians commonly encountered in wild birds. Females with high *Haemoproteus* spp. infections also suffered reduced survival (Nordling *et al.* 1998).

Another example of the trade-off between current reproduction and immunity to parasites was demonstrated in female bighorn sheep, *Ovis canadensis* (Festa-Bianchet 1989). Ewes that reared a lamb (especially a son, which are costlier than daughters), suffered an increased lungworm burden (*Protostrongylus spp*), but did not experience a decrease in reproductive success in the following year (Festa-Bianchet 1989).

One mechanism for reducing the impact of parasites on host fitness, other than avoidance tactics or mounting an immune defence, is for the host to modify their life-history tactics (Forbes 1993, Forbes 1996, Agnew *et al.* 2000). In fact, in any unfavourable environment (e.g. exposure to parasites or predators) where the prospects of future fecundity or survival are impeded, strategies to reduce their impact should be selected for.

An example of a phenotypically plastic response to an unfavourable environment includes shifting reproduction to an earlier age, known as 'fecundity compensation' (Thornhill *et al.* 1986, Polak & Starmer 1998, Krist 2001). There is experimental evidence that *Daphnia pulex* produce greater numbers of smaller offspring at an earlier stage in life in the presence of a size-selective predator; the water bug *Notonecta glauca,* which selectively predates larger sized *Daphnia* (Luning 1992). In contrast, another *Daphnia* clone under predation risk from a size-selective predator (the midge larvae *Chaoborus flavicans*) which prefers small prey; delayed age at first reproduction, and produced fewer but larger offspring (Luning 1992). In these cases, current reproductive effort has been altered as a strategy to reduce the impact of predation and increase future survival prospects.

Fecundity compensation also occurs in response to exposure to parasites that may either sterilize their hosts (Minchella & Loverde 1981, Krist 2001) or where parasites decrease survival or future reproduction (Polak & Starmer 1998, Chadwick & Little 2005). Increased reproductive effort was observed in male *Drosophila nigrospiracula* in response to a higher mortality risk imposed by a parasitic mite, *Macrocheles subbadius* (Polak & Starmer 1998). Experimental manipulation of the microsporidian parasite *Glucoides intestinalis* in the crustacean *Daphnia magna*, clearly demonstrated parasite-mediated fecundity compensation in *Daphnia* life history trade-offs (Chadwick & Little 2005). Fecundity was measured in female *Daphnia* exposed to either a high or low dose of parasite spores, and in controls where there was no exposure to parasites. *Daphnia* exposed to high levels of parasitism increased early reproductive effort, producing up to 39% more offspring in their first clutch than controls (Chadwick & Little 2005).

There are, however, costs to shifts in life history strategy. For example, the cost of increased early reproductive effort was demonstrated in the snail, *Biomphalaria glabrata* (Minchella & Loverde 1981). Fecundity was measured (eggs produced per day) in response to exposure to the parasite, *Schistosoma mansoni,* which eventually causes sterilization and increased mortality in infected snails. Egg production was increased earlier in snails exposed to the parasites, compared to controls. However there was a cost to this increased early reproductive effort, as not all exposed snails developed infection. The parasite caused infected snails to cease egg production at around 4 weeks after exposure, and therefore the increase in early reproductive effort in week 2 was successful in reducing the impact of the sterilizing parasite. In contrast, snails that were exposed to parasites but did not develop infection, also increased their early reproductive effort, but paid the price in terms of their future reproduction, as egg output was much reduced after week 6 in comparison with controls (Minchella & Loverde 1981).

In blue tits, ectoparasites such as the hen flea *Ceratophyllus gallinae*, cause low nestling weight. However, several experimental studies have shown that blue tits with nests heavily infested by ectoparasites increase food provisioning to their offspring to compensate for this (Richner and Tripet 1999, Tripet *et al.* 2002, Bouslama *et al.* 2002). As predicted by life history theory, however, this increase in current reproductive effort does not come without a cost, and these parents are less likely to breed in the next year, and have lower subsequent reproductive success (Richner and Tripet 1999).

A similar trade-off between current and future reproduction was observed in male alpine swifts (*Apus melba*), when nests were experimentally infested with ectoparasites (*Crataerina melbae*) (Bize *et al.* 2004). Males increased allocation of resources to current reproduction by increasing the brood rearing period, in comparison to de-parasitized males. However, this negatively impacted upon their reproductive success in the following year; as the number of offspring produced was lower (Bize *et al.* 2004).

Increased allocation of resources to current reproductive effort in response to parasites, at the expense of future survival or fecundity, has also been observed in mammals. In an experimental infection of female wild-derived house mice (*Mus musculus*) with the intestinal nematode *Heligmosomoides polgyrus*, parasitized mice allocated more resources to their offspring (Kristan 2004). Average litter sizes in parasitized mice were bigger (+2.8 pups per litter) than the unparasitized mice, and after accounting for litter size, pups were also larger by 1-2 % (Kristan 2004).

1.3 Parasitism in Soay sheep (*Ovis aries* L.) on St Kilda

A population of Soay sheep have inhabited the island St Kilda, situated off the North-west of Scotland, unmanaged and without competitors or predators, for at least one thousand years (Campbell *et al.* 1974). The Soay sheep on St Kilda suffer from a range of parasites, including gastrointestinal parasites (Cheyne *et al.* 1974, Wilson *et al.* 2004). The population is unstable, and the number of sheep fluctuates as a result of density- dependent mortality incidents caused by severe food shortage (Clutton-Brock *et al.* 1991, Grenfell *et al.* 1992, Grenfell *et al.* 1998, Coulson *et al.* 1999). The pathogenic affects of gastrointestinal parasites exacerbate the effect, and are a contributory factor to death (Gulland 1992).

1.4 Costs of reproduction and parasitism in Soay sheep

An experimental study on St Kilda has shown that removal of parasites by anthelmintic treatment causes an increase in daily survival in Soay sheep, (Gulland 1992, see Figure 1.1), and several other studies have confirmed that high levels of parasitism are correlated with lower survival in Soays (Illius *et al.* 1995, Coltman *et al.* 1999, Milner *et al.* 1999).

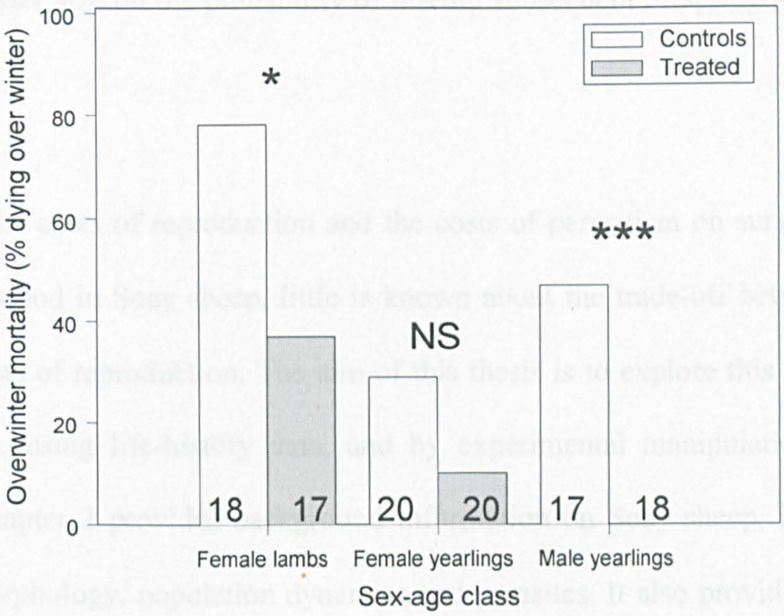


Figure 1.1: Over-winter mortality (1991-1992) of Soay sheep on St Kilda treated with anthelmintic bolus compared with an untreated control group, in different sex and age classes (from Wilson *et al.* 2004, see also Gulland *et al*. 1993). The numbers in the base of the bars indicate sample size. Significance levels: NS - $p>0.05$, * - $p<0.05$, *** - $p<0.001$.

In addition to this, there is evidence of age-related costs of reproduction on future survival (Clutton-Brock *et al.* 1996, Tavecchia *et al.* 2005). In years when overall mortality is high, the mortality of juvenile ewes is lower in those that do not produce a lamb. Furthermore, juvenile ewes prevented from breeding by hormonal intervention, survive better than untreated ewes (Gulland 1991, Tavecchia *et al.* 2005). Among adult ewes, when overall mortality is high, survival is lower in ewes that produce twins, than in ewes that produce single lambs, or do not have a lamb (Clutton-Brock *et al.* 1996). In contrast, in years where resources are abundant and overall mortality is low, these costs of reproduction on survival disappear in both juvenile and adult ewes, and in fact it is the ewes that do not breed at all that are more likely to die

(Clutton-Brock *et al.* 1996). In terms of future reproduction, there appear to be no effects of litter size on the probability of rearing subsequent offspring (Clutton-Brock *et al.* 1996).

Although the costs of reproduction and the costs of parasitism on survival are fairly well understood in Soay sheep, little is known about the trade-off between parasites and the costs of reproduction. The aim of this thesis is to explore this relationship in Soay ewes, using life-history data, and by experimental manipulation of parasite burden. Chapter 2 provides background information on Soay sheep, including their habitat, morphology, population dynamics and parasites. It also provides information about methodology in the field (on St Kilda) and statistical methods used. Chapter 3 examines the well-known phenomenon of the spring rise in parasitism at around the time of lambing. By making comparisons between females in different reproductive states, and across the sexes, this chapter attempts to gain a better understanding of a phenomenon that is also prevalent in domestic sheep. Chapter 4 considers the traits that affect reproductive success in Soay ewes, and should be considered as an introduction to the following two experimental chapters. The aim of this chapter is to establish whether there is a correlation between parasitism and reproductive success in Soay sheep. However, because of variation between individuals in resource acquisition (van Noordwijk & de Jong (1986) the only reliable way to establish whether parasites directly impact on reproductive success and fitness is to perform an experimental manipulation of parasitism and/or reproduction. Thus, Chapter 5 reports an experiment carried out in 2004, to test the effects of treatment to remove parasites from adult ewes on their subsequent body condition and fecundity. Chapter 6 examines the effects that of the same experimental manipulation on the lambs produced by those ewes. Together, these two chapters take an experimental approach to examining the costs of parasitism in terms of reproductive success. Finally, in Chapter 7, the findings of this study are summarised, and the implications for future research are discussed.

Chapter 2

Background and General Methodology

2.1 St Kilda

St Kilda is an archipelago consisting of four islands, situated north-west of the Outer Hebrides in Scotland (57° 49' N, 08° 34' W), 160km from the UK mainland (Figure 2.1). The study area encompasses Village Bay (175ha), which comprises the south-eastern area of the largest island, Hirta (638ha). A population of Soay sheep (*Ovis aries L.*) resides on Hirta, their numbers fluctuating from approximately 600 to 2000 individuals (Grenfell *et al.* 1992, Coulson *et al.* 1999). Approximately one third of the island population live within the study area, and of these more than 95% are known individuals (Clutton-Brock *et al.* 1991). The smallest island in the archipelago is Dun (32ha), which lies adjacent to Hirta and forms the southern edge of Village Bay. The island of Soay (99ha), from where the sheep were originally introduced onto Hirta, is situated on the north-west tip of Hirta. Finally, there is the island of Boreray (77ha), which lies 7km north east of Hirta.

The climate on St Kilda can be harsh, with wet and windy winter months a common occurrence (Campbell 1974, Clutton-Brock *et al.* 2004). Vegetation on Hirta consists mostly of grassland, however there are also bog and heather communities (Campbell 1974). Famously, St Kilda supports vast numbers of sea birds, in particular puffin, fulmar and gannet colonies; and is also home to indigenous species of wren and wood mouse. There are no other grazing competitors, or predators to the Soay sheep, however occasionally birds (including great skuas, *Catharacta skua skua*, and black-backed gulls, *Larus marinus*) may attack and kill weak lambs (Clutton-Brock *et al.* 2004).

Figure 2.1: The St Kilda archipelago comprising the four islands of Hirta, Soay, Dun and Boreray. The shaded line shows the boundary of the study area (from Stevenson 1994). The inset shows the location of St Kilda relative to the west coast of Scotland.

2.2 Soay sheep

2.2.1 Sheep population on St Kilda

Soay sheep were introduced to Hirta from neighbouring island Soay. However, this, the most primitive domestic breed of sheep in Europe, is thought to have resided on St Kilda for at least 1000 years (Campbell 1974). It is thought that they were originally confined to Soay, and have only inhabited Hirta since the transfer of 42 tups (22 of which were castrates) and 65 ewes in 1932 (Campbell 1974). Since then, the population on Hirta increased rapidly so that by 1939 there were reports of approximately 500 sheep, and in 1952, when the first organised census took place, a total population of 1114 sheep were counted on Hirta (Boyd & Jewell 1974). Since the start of the current project in 1985, population numbers on Hirta have fluctuated from approximately 600-2000 individuals (Grenfell *et al.* 1992, Coulson *et al.* 1999). These fluctuations in the population are caused by density-dependent mortality episodes, when a high proportion of the animals die; this is often referred to as a 'crash' in the population (Figure 2.2). Mortality is largely due to starvation, as the population exceeds the carrying capacity of the island and vegetation biomass becomes severely depleted (Clutton-Brock *et al.* 1991, Grenfell *et al.* 1992, Grenfell *et al.* 1998, Coulson *et al.* 1999). The pathogenic effects of gastrointestinal parasites exacerbate problems associated with food shortages, and are a contributory factor to death (Gulland 1992).

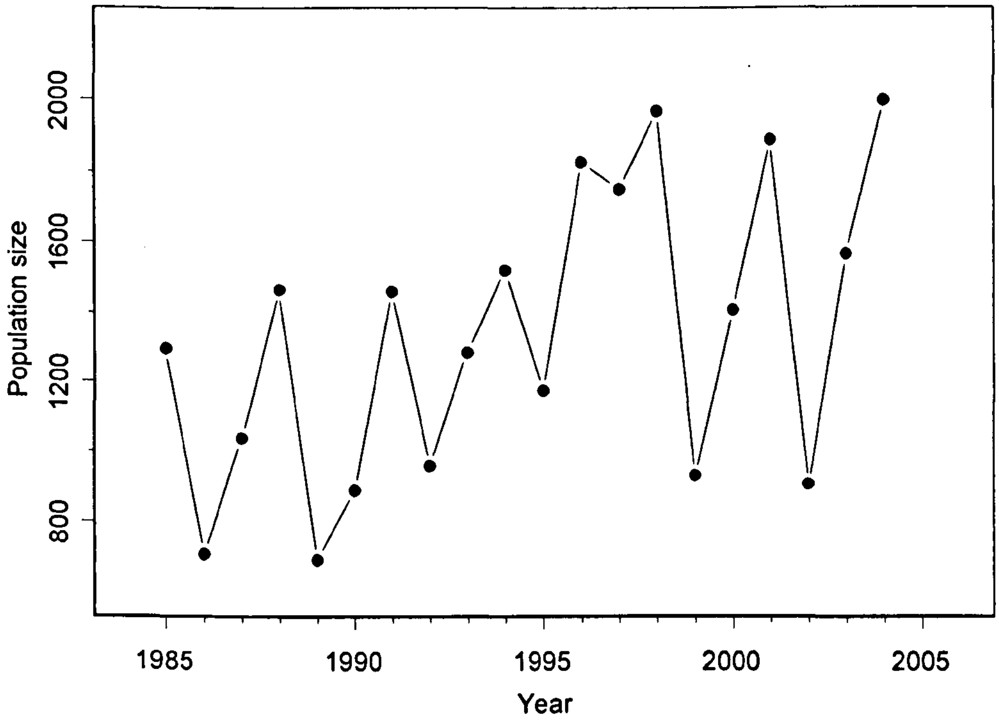


Figure 2.2: Yearly fluctuations in population size on the island of Hirta (1985-2004).

2.2.2 *Morphology*

Soay sheep are small in comparison to other wild and domestic breeds (Doney *et al.* 1974). Compared to Scottish blackface sheep, leg length is shorter (95%), and they are narrower (75% hip width) and with a shorter body length (77%) (Doney *et al.* 1974). Soays are sexually dimorphic, with adult males (tups) reaching an average August weight of 38kg, while adult females (ewes) are lighter with an average August weight of 24kg (Campbell 1974, Clutton-Brock *et al.* 1996, Milner *et al.* 1999).

The coats of Soay sheep on St Kilda vary from a dark brown colour to a light chestnut brown. There are four recognised morphs: dark wild, dark self, light wild and light self. The 'dark' morphs are dark brown in colour, whereas the 'light' morphs are chestnut colour. 'Wild' morphs have white facial markings (similar to Mouflon) and white bellys, in comparison to the 'self' coloured morphs, which have no white markings (Coltman & Pemberton 2004).

Soay sheep have horns, which are larger and more developed in males. Most males (85%) develop large spiral horns ('normal'), while the remainder develop malformed, stunted horns ('scurred'). In females, horns are smaller and generally less impressive than males, with 35% of females having normal horns, 35% with scurred horns, and 30% are polled (no horns). Horn type is largely genetically determined (Clutton-Brock *et al.* 1997, Clutton-Brock *et al.* 2004, Coltman & Pemberton 2004). There is evidence to suggest that horn type influences male and female breeding success (Clutton-Brock *et al.* 1997, Stevenson *et al.* 2004).

2.2.3 Reproduction

The mating or 'rutting' season occurs in November on St Kilda, when males compete to gain access to oestrus females (Grubb & Jewell 1973, Stevenson *et al.* 2004). Gestation lasts approximately 151 days, with the first lambs born at the end of March or beginning of April, and the median lambing date ranging around mid-April (Clutton-Brock *et al.* 1992, Clutton-Brock *et al.* 2004). Most adult ewes produce lambs every year. Juvenile Soays can come into oestrus at seven months old. However, the proportion of juveniles that produce lambs varies with population density, ranging from over 80% in years when autumn population density is low, to under 10% when autumn population density is high and weather conditions are adverse (Clutton-Brock *et al.* 2004, Tavecchia *et al.* 2005). Females that conceive in their first year of life do not produce twins, whereas in older ewes, the incidence of twinning is approximately 15%. However, the probability of adult ewes bearing twins is affected by population density and their weight (Clutton-Brock *et al.* 1991, Clutton-Brock *et al.* 1996).

2.2.4 Parasites

The Soay sheep on St Kilda harbour a range of parasites (Table 2.1) including protozoa, ectoparasites, lungworms, and gastrointestinal parasites (Cheyne *et al.* 1974). Most of these are also common to domestic sheep on the mainland although there are some exceptions (see Wilson *et al.* 2004). There are several species of protozoa on St Kilda, including *Eimeria* spp. (see Craig 2005, for more details).

The ectoparasites found on St Kilda are keds (*Melophagus ovinus*), which are wingless flies and lice (*Damalina ovis*). Both are highly prevalent, although tend to be more common among younger animals, particularly lambs (Wilson *et al.* 2004). Keds live in the wool and feed on blood, which can cause anaemia and a loss of body condition when infestation is heavy. Lice feed on wool and dead skin and, like keds, cause much irritation to their host (including disruption of normal behaviour patterns; sleeping and feeding) (Soulsby 1982).

There are two species of lungworm present on St Kilda, *Dictyocaulus filaria* and *Muellerius capillaris*. *D. filaria* has a direct life-cycle. Adult worms inhabit the trachea and bronchi of the host, and cause bronchitis, and occasionally pneumonia (Soulsby 1982). *D. filaria* is predominantly found in lambs, which can often be observed coughing up parasite eggs onto the pasture (Wilson *et al.* 2004). The other lungworm present, *M. capillaris,* is more commonly found in adult sheep. Adult worms live in the alveoli, and eggs coughed up onto the pasture require ingestion by an intermediate molluscan host before sheep can be re-infected (Soulsby 1988).

The presence of the tapeworm *Taenia hydatigena* on St Kilda is somewhat unexpected, since the life cycle is not via direct transmission, and requires an intermediate host. It is thought that *T. hydatigena* may be brought to the islands via scavenging birds from the mainland (Torgerson *et al.* 1992, Torgerson *et al.* 1995). The prevalence of *T. hydatigena* is estimated at between 30-50% of adult Soays infected (Gulland 1992, Torgerson *et al.* 1992, Torgerson *et al.* 1995), however like the other tapeworm present on St Kilda, *Monezia expansa,* it is not considered to be an important source of morbidity or mortality (Torgerson *et al.* 1992).

The other gastrointestinal parasites present in Soay sheep on St Kilda are nematodes, and include *Teladorsagia* spp., *Trichostrongylus* spp., *Nematodirus* spp., *Bunostomum trigonocephalum, Capillaria longipes*, *Chabertia ovina*, *Strongyloides papillosus* and *Trichuris ovis* (Cheyne *et al.* 1974, Gulland 1992). Among *Teladorsagia* spp. (also known as *Ostertagia* spp) three species had previously been identified: *T. circumcincta, T. davitiana,* and *T. trifurcata* (Gulland 1992).

Table 2.1: Parasites associated with Soay sheep on St Kilda (from Wilson *et al.* 2004).

Taxon	Location	Species
Protozoa	Small intestine	*Cryptosporidium parvum* *Giardia duodenalis* *Eimeria ahsata* *Eimeria bakuensis*
	Small intestine/large intestine	*Eimeria crandallis* *Eimeria faurei* *Eimeria intricata* *Eimeria ovinoidalis* *Eimeria pallida* *Eimeria weybridgensis*
	Unknown	*Eimeria granulosa* *Eimeria marsica* *Eimeria parva*
Flies	Wool	*Melophagus ovinus*
Lice	Wool	*Damalinia ovis*
Tapeworms	Small intestine	*Moniezia expansa*
	Abdominal cavity	*Taenia hydatigena*
Nematodes	Lungs	*Dictyocaulus filaria* *Muellerius capillaris*
	Abomasum	*Teladorsagia circumcincta* *Teladorsagia trifurcata* *Teladorsagia davtiana*
	Abomasum/small intestine	*Trichostrongylus axei* *Trichostrongylus vitrinus*
	Small intestine	*Capillaria longipes* *Strongyloides papillosus* *Nematodirus battus* *Nematodirus filicollis* *Nematodirus helvetianus* *Bunostomum trigonocephalum*
	Large intestine	*Trichuris ovis* *Chabertia ovina*

However, recent work on mitochondrial and nuclear DNA sequences found no evidence to separate these as three distinct species (Braisher 1999), and so they are now considered as one species, *Teladorsagia circumcincta.*

The pathogenic consequences of gastrointestinal nematode infection in ruminants include reduction in food intake, weight loss, diarrhoea and endogenous protein losses (Holmes 1985, Symons 1985, reviewed in vanHoutert & Sykes 1996). *T. circumcincta* is considered the most important of the gastrointestinal nematodes, as it is the most pathogenic and dominant species (Gulland & Fox 1992, however see Craig 2005), especially in adults (Craig 2005). During the crash of 1989, post mortems revealed that the sheep were emaciated and had abomasal lesions caused by *T. circumcincta* (Gulland 1992). These findings were consistent with death by protein-energy malnutrition, exacerbated by the pathogenesis of *T. circumcincta* (Gulland 1992). Therefore, it is this species that most parasitological studies on St Kilda are focussed upon, including this current study.

The direct life-cycle of *Teladorsagia circumcincta* is typical of other trichostrongylids (Soulsby 1982). Adult females produce eggs, which are voided in the faeces after development to the morula stage. Emergence of first-stage larvae (L1) can occur within 24 hours, however depending on environmental conditions, eggs can survive on the pasture for several months. First-stage larvae on the pasture undergo two moults; to second-stage (L2) larvae, and then on to the infective third-stage (L3). The period of transition from egg to L3 is sensitive to environmental conditions, although the L3 is afforded some protection through retention of the L2 cuticle, which acts as a protective sheath (Soulsby 1982).

The L3 larvae are ingested by the host and, after passing through the abomasum, they migrate to the gastric glands after two or three days. In the glands, L3 larvae moult to early fourth-stage larvae (EL4), before moulting to the fifth stage (L5), which is the immature adult stage. Most larvae will become mature adults at around 12 days post-ingestion, and will emerge from the glands at day 16, when they attach to the abomasum wall. The worms copulate, and the females lay eggs, which are voided in the faeces of the host 17-18 days post-infection. During the EL4 stage, larval development can become arrested in the mucosa, which is known as hypobiosis (Armour *et al.* 1969). This can last for several months, and de-arrestment often coincides with lambing in the spring (Wilson *et al.* 2004).

There are two seasonal peaks of infective larvae (L3) on the pasture on St Kilda (Wilson *et al.* 2004). The first occurs in the spring, during May and June, and is thought to be due to the development of eggs deposited by immunocompromised peri-parturient ewes. The second, larger peak, occurs mid-summer, and is due to the development of eggs deposited by immunologically naïve lambs (Figure 2.3, Wilson *et al.* 2004). The magnitude of this second peak depends on the size of the lamb population born the previous spring (Wilson *et al.* 2004).

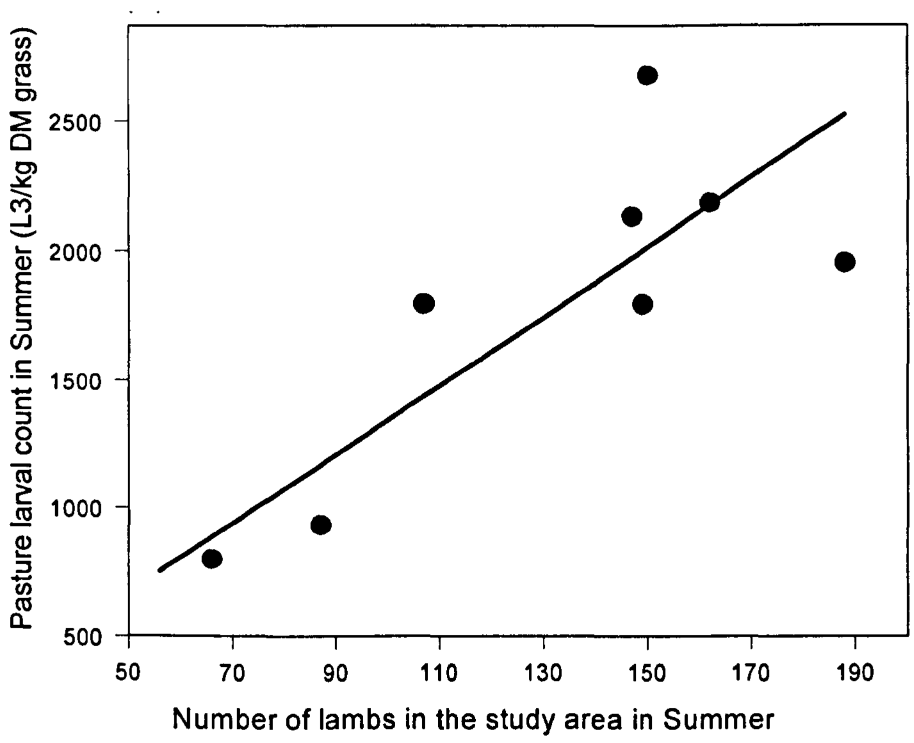


Figure 2.3: Relationship between the number of lambs in the study area and the number of infective larvae (L3) on the pasture across years (1988-1997). The correlation between number of lambs and L3 count is high ($r^2 = 0.64$, $F_{1,6} = 10.61$, $p<0.01$). Figure from Wilson *et al.* 2004.

2.3 General methodology

2.3.1 Core data

The study population in the Village Bay area (175 ha) comprises around one third of the island population, and more than 95% of these sheep are known individuals, monitored throughout their lives and identified with an individually identifiable plastic ear tag (Clutton-Brock *et al.* 1991). During the spring, around 95% of new lambs in Village Bay are caught, weighed and tagged within 3 days of birth (Robertson *et al.* 1992). In each August, approximately half of the sheep in the study area are caught, weighed, and other morphometric measurements recorded (Clutton-

Brock *et al.* 1992). Daily weather data are recorded using three weather stations in the study area, providing accurate information on multiple weather variables including temperature, sunshine, wind speed and rainfall. Ambient temperature is measured in °C at a height of 1.5m every 30 seconds, and an average is calculated over every 24h hour period. Wind speed (m/s) is measured every second at a height of 2.25m, and an average calculated over 24 hours. Daily rainfall (precipitation) is measured in mm using a tipping bucket (at resolution 0.2mm). Sunshine is measured as the approximate number of hours sunshine per day (measured using Campbell's algorithm, Campbell 1974).

2.3.2 Parasite data

Throughout this thesis various terms are used to describe the extent or magnitude of the degree of parasitism experienced by individual sheep. *Prevalence* refers to the proportion of animals infected with a given parasite, whereas *intensity* refers to the average number of parasites per host. Parasite *burden* or *load* is used here in a looser sense, to refer to either the prevalence or intensity of parasitic infection. In much of the parasitological literature, these terms are restricted to the actual number of parasites harboured by individual hosts, but often actual numbers are hard to establish without first killing the host. In these instances, indirect measures are usually used (Wilson *et al.* 2002). One such measure, which is used throughout this thesis is Faecal Egg Count or FEC. This is the density of strongyle parasite eggs per gram of faeces (wet weight).

Faecal samples were collected from known individuals (identified from their ear-tag) after direct observation of defaecation. Parasite burden was measured by counting the number of parasite eggs per gram (epg) of faeces, using a modification of the McMaster technique (MAFF 1971). This involved measuring 3g wet weight of each faecal sample, which was then homogenised through a tea strainer using a pestle, whilst suspended in a bowl containing 87ml of saturated salt solution. A pipette was then used to fill both chambers of a McMaster slide. Using a compound microscope (10x magnification) parasite eggs under the grid of both chambers were identified and counted, before being multiplied by 100 to obtain the number of eggs per gram of faeces. The parasite taxa were classified into strongyles (including *Teladorsagia* spp., *Trichostrongylus* spp., *Bunostomum trigonocephalum, Chabertia ovina* and *Strongyloides* spp), *Capillaria longipes*, *Monezia expansa*, *Nematodirus* spp., and *Trichuris ovis*. Faecal egg counts (FEC) used as a measure of parasitism in this thesis refer exclusively to strongyle eggs. Previous studies have shown that FEC provides a good measure of parasite worm burden in Soay sheep (Gulland 1992, Boyd 1999, Wilson *et al.* 2004, see Figure 2.4).

2.4 Bolussing experiment

2.4.1 August 2003 - August 2004

During the annual August catch-up of 2003, 51 female Soay sheep from age four to seven, were caught and processed as normal, and assigned to either the Bolus (treated) or Control (not treated) group. Within each age group, ewes were assigned randomly to either Bolus (25) or Control (26) group using a predetermined sequence, which had been created using a random number generator (MS Access). The ewes in the Bolus group were administered with an anthelmintic bolus (Captec slow-release bolus; active ingredient Albendazole) which removes parasite burden for at least 100 days.

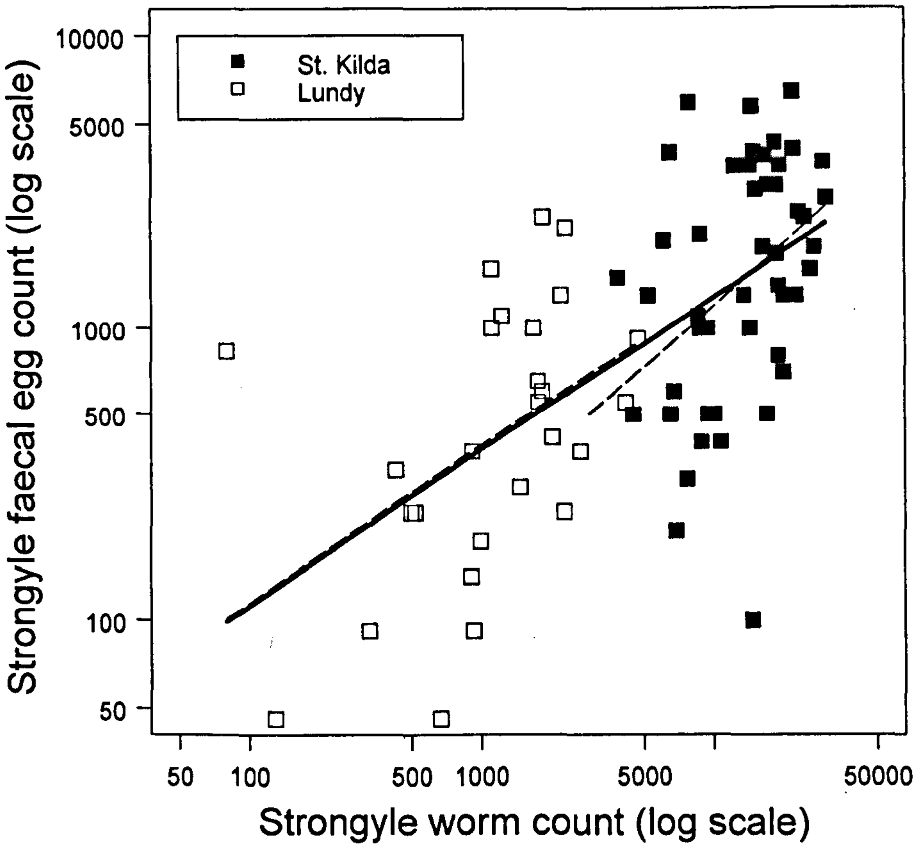


Figure 2.4: Relationship between strongyle faecal egg count (FEC, measured prior to death) and strongyle worm burden (assessed by autopsy) in Soay sheep on St Kilda (natural deaths), and Lundy (culled). The dashed lines represent the model fit for separate datasets; the solid line represents the model fit for the combined dataset. The correlation between log FEC and log worm burden is reasonably high ($r^2 = 0.392$, $F_{1,73} = 47.05$, $p<0.0001$). Figure from Wilson *et al.* 2004.

Control ewes were untreated. A faecal sample was obtained from almost all experiment ewes within seven days, and faecal egg count (FEC) measured. In the lambing period during the spring of 2004, a small team collected data on the experiment ewes and their lambs. Data was collated on ewe lambing success, ewe parasite burden (collection of multiple FEC), lamb birth date and morphometric information, mother and lamb behavioural time budgets, and lamb growth rates. During the annual August catch-up in 2004, one year after bolussing, 25 out of 49 surviving experiment ewes, and 26 out of the surviving 51 lambs were re-caught. Their weights, and other morphometric measurements were recorded, and faecal egg counts obtained. More information on the methodologies implemented during this experiment can be found in Chapters 5 and 6.

2.4.2 Bolussing experiment 2001/2002

The bolussing experiment described above was the second attempt to determine the effects of parasites on ewe reproduction. A previous experiment was conducted in August 2001 (designed by O. R. Jones, see Jones 2004). In this experiment, 9 ewes were administered with an anthelmintic bolus to temporarily relieve parasite burden, and 8 ewes were assigned into an untreated Control group (all ewes were two-years old). During spring 2002, experimental ewes were to be monitored as described above (section 2.4.1). Unfortunately, much of this work had to be abandoned due to high over-winter mortality (see Figure 2.2): within the experimental group 66% of both Control and Bolus groups died. Although this turn of events was unfortunate, the data collected and experience gained proved vital as a pilot experiment to the 2003/2004 experiment.

2.5 Statistical methods

Data were analysed using the statistical package S-PLUS 2000 release 1 (*Mathsoft, Inc.*). General linear models (GLMs) and general linear mixed models (GLMMs), as well as some standard parametric and non-parametric tests were implemented in this thesis. General Linear Models include multiple regression, and analysis of variance (ANOVA) techniques in the analysis of data with normal (Gaussian) error distribution. Generalized Linear Models are an extension to standard GLMs, but allow the analysis of non-normal error distributions through the use of linearizing link functions (McCullagh and Nelder 1984). Wilson and Grenfell (1997) showed that the use of Generalized Linear Models in analyses of parasite data are a much more powerful tool than simply using classic linear regression of log-transformed data: reducing the probability of Type I and Type II errors. Hence, Generalized Linear Models have been used where possible, although in some analyses, it was necessary to log10 transform parasite data, before analysis with normal errors as a standard GLM.

General Linear Mixed Models allow for pseudo-replicated data, for example repeated measures of the same individual over time, by fitting individual identity as a random effect (Pinheiro and Bates 2000, Crawley 2002). Mixed models include both *fixed* effects and *random* effects as explanatory terms in statistical models. Fixed effects are explanatory terms or experimental treatments that might influence the mean of the response variable, whereas random effects are those that influence the variance of the response variable. The random effects structure in mixed effects models accounts for the non-independence of errors in repeated measures data. In S-PLUS, and in the chapters of this thesis, the model structure of GLMMs is written as follows:

y ~ xi + xii + xiii ..., random = ~ 1 | r

The response variable is **y**, the fixed effects are denoted by **x**, and the random effects structure is specified by: **random = ~ 1 | r** (where 1 is the intercept, and r is the variable explaining the grouping of the random effects, e.g. ID if the random effects are repeated measures of an individual).

Generalized Linear Mixed Models have also been implemented in this thesis, which are extension to GLMMs in that they model repeated measures of non-normal data using link functions. Paterson and Lello (2003) review the use of GLMMs as a powerful tool in analysis of *parasitological* data, which does not conform to a normal distribution, while still controlling for repeated measures.

Models were fitted as follows. All explanatory terms are fitted including interactions between up to second order interactions. Interactions including more than two terms (e.g. a third order interaction between three explanatory terms) were not permitted due to small sample sizes. This would reduce residual degrees of freedom, making the model unstable, and would also increase the risk of incurring both type I and type II errors (Crawley 2002).

Minimal models were obtained by step-wise deletion, removing the term that explained the least amount of variation. Significance of terms was tested by comparing two models using a χ^2 test, or an F-test if the model was over-dispersed. Terms that exceeded an α-value of 0.05, were considered non-significant and were omitted from the model. In some cases, due to small sample sizes, an α-value of 0.025 was used for interactions to account for the greater number of tests carried out (Crawley 2002). This approach was used instead of the familiar Bonferonni correction, in light of recent views that problems of low power can occur with this technique when many variables are measured, and when the sample size is small (Nakagawa 2004). The minimal model was obtained when no other terms could be removed without causing a significant loss of explanatory power. All deleted terms were re-tested against the minimal model.

Chapter 3

Spring rise in parasitism in Soay sheep

3.1 Introduction

The peri-parturient rise, or PPR, is a well known phenomenon that has been documented widely in domestic sheep (Connan 1968, O'Sullivan & Donald 1970,1973). It is the temporary increase in parasite burden, most often measured in nematode strongyle egg counts, around the time of lambing from April to June. Although subject to much scrutiny, the proximate causes of the PPR remain unclear, despite the fact that much headway has been made in the subject in the last fifty years. The most important gastro-intestinal nematode contributing to the PPR is widely regarded as *Teladorsagia* spp. (Morgan *et al.* 1951, Parnell *et al.* 1954, Crofton 1954, Dunsmore 1965, Connan 1968b, Herd *et al.* 1983, Bishop & Stear 2001), although *Haemonchus contortus* has been implicated as the dominant species in several studies (Field *et al.* 1960, Gibbs 1964, Connan 1967, Procter and Gibbs 1968, Herd *et al.* 1983). It is thought that ewes become immuno-compromised at lambing, which allows parasitism to increase in several possible ways. These include (a) an increase in establishment of newly acquired larvae (Morgan *et al.* 1951, Brunsdon 1970b, Jeffcoat *et al.* 1992); (b) the maturation of arrested larvae acquired some months previously (Naerland 1949, Spedding and Brown 1956, Field *et al.* 1960, Dunsmore 1965, Gibbs 1967, Connan 1968, Herd *et al.* 1983, Gibbs & Barger 1986, Fleming *et al.* 1988, Jeffcoat *et al.* 1992); (c) a decrease in the expulsion of developing and adult worms (Connan 1976, Gibbs & Barger 1986); (d) an increase in the fecundity of existing adult worms (Cushnie & White 1948, Naerland 1949).

The PPR appears to be a cost of reproduction; in domestic sheep it has been shown that it does not occur in ewes that fail to breed or have lost their lamb (Crofton 1958, Field *et al.* 1960, Brunsdon 1964, Procter & Gibbs 1968, Shubber *et al.* 1981, Gibbs

& Barger 1986). More specifically, the PPR appears to occur as a cost of lactation, probably mediated by the hormone prolactin. In support of this, several studies have demonstrated that ewes that have had their lambs removed at birth do not show the characteristic rise in parasite burden (Gibbs 1967, Connan 1968a,b, Salisbury & Arundel 1970) and some studies have shown that hormonally inducing lactation in non-breeding ewes can also elicit a rise (Gibbs 1967, Salisbury & Arundel 1970). In contrast, in a more recent study, non-pregnant ewes with artificially raised levels of prolactin did not show an increase in parasitism comparable to pregnant ewes and, in fact, demonstrated similar parasite burdens to barren ewes (Coop *et al.* 1990). In another study, chemical suppression of prolactin immediately post-partum did not prevent PPR, though neither did it prevent lactation as lamb growth rate was unaffected by this treatment (Jeffcoate *et al.* 1990). The role of the hormone prolactin in the PPR has been the subject of much debate, and it has been suggested that while prolactin may maintain the dip in immunity that leads to the peri-parturient rise, it may be acting in conjunction with another factor (e.g. progesterone - Fleming & Conrad 1989, adrenal corticoids - Connan 1976). In fact, it has also been proposed that the anti-parasite antibody IgA, and not hormones associated with parturition and lactation, may reduce immunity at that time (Jeffcoate *et al.* 1992). IgA in the gut provides immunity to nematodes and, during lactation, it is transferred to the mammary glands, where it is passed to the neonate in milk as maternally transferred immunity. This temporary dip in ewe immunity in the gut, due to the decline in levels of IgA, is believed to allow maturation of arrested larvae, and/or increase establishment of newly acquired larvae (Jeffcoate *et al.* 1992).

In Soay sheep, the peri-parturient rise appears to be expressed differently to domestic sheep. Firstly, it has been reported that the peak of the PPR occurs within the first ten days of lambing (Wilson *et al.* 2004) in contrast to the PPR of domestic sheep which occurs between 6-7 weeks after lambing (Crofton 1954, Brunsdon 1964). However, the main difference in Soays is that the magnitude of the PPR tends to be greater in yearling ewes that fail to raise a lamb, than yearling ewes that successfully raise a lamb (Wilson *et al.* 2004). This result is counter-intuitive when considering the previously discussed causes of the PPR (lactation), and so the focus of this chapter is to determine the factors that may be affecting the peri-parturient rise in Soay sheep.

Some early studies demonstrated the importance of other factors on the PPR, including malnutrition (Paver 1955). For example, following severe winters, increased spring parasite burdens were observed in both female and male sheep (Morgan *et al.* 1951, Wilson *et al.* 1953). On St Kilda, increased spring parasite burden (as measured by faecal egg count) was observed in both male and female Soay sheep after a winter when population density was very high (Gulland and Fox 1992). In light of these observations, parasite burden in tups was investigated, and compared with parasite burden in ewes.

3.2 Methodology

Parasite data (strongyle FEC) collected in the spring months was collated from the Soay sheep database spanning 1985-2004 (see Chapter 2 for more information of how FEC are obtained). As is typical of these type of data, the distribution of FEC was skewed with a negative binomial distribution; most individuals had low FEC, while only a few had very high FEC (see Figure 3.1). To account for this, data were log10-transformed (log10 (FEC+100)) before analysis. Data were analysed using the statistical package S-PLUS 2000 release 1 (*Mathsoft, Inc.*). To account for repeated sampling of FEC for individual sheep, data were analysed using general linear mixed models (GLMMs). Terms were tested up to second order interactions. Terms were tested by step-wise deletion using maximum-likelihood methods, and were only retained in the minimal model with an α-value of <0.05. All excluded terms were then re-tested against the minimal model using the same criteria.

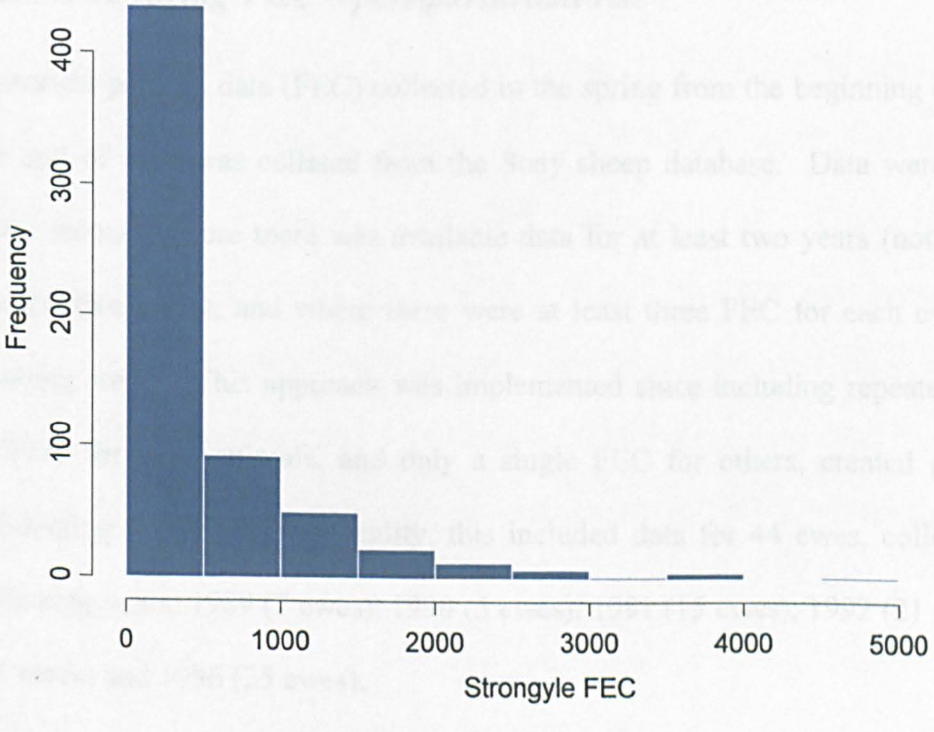


Figure 3.1: Histogram of ewe spring (March to May) strongyle faecal egg counts (FEC), measured as eggs per gram (epg) of faeces. Data represents FEC collected from 1989 to 1996.

Three analyses were carried out, examining spring parasite burden in ewes (1) and (2), and in tups (3):

1) Analysis of multiple FEC collected from ewes during the springs of 1989-1996.
2) Analysis of multiple FEC collected during spring 2004 from adult ewes treated with an anthelmintic bolus to temporarily remove their parasite burden.
3) Analysis of single monthly FEC collected in spring in different years in tups.

3.2.1 Ewe spring FEC – peri-parturient rise

Historical parasite data (FEC) collected in the spring from the beginning of March to the end of May was collated from the Soay sheep database. Data were limited to those animals where there was available data for at least two years (not necessarily consecutive years), and where there were at least three FEC for each ewe within a lambing season. This approach was implemented since including repeated measures of FEC for most animals, and only a single FEC for others, created problems in conducting the analysis. In reality, this included data for 44 ewes, collected in the following years: 1989 (7 ewes), 1990 (5 ewes), 1991 (15 ewes), 1992 (21 ewes), 1993 (31 ewes) and 1996 (25 ewes).

To account for repeated sampling of FEC for each ewe within a year, and across years, data were analysed using general linear mixed models (GLMMs). Random effects were relative day of sampling within ewe ID:

log10 (FEC+100) ~ a + b + c ..., random = ~ sample day | ewe ID

Fixed terms tested in the model were: ewe age (categorised into 1 year old (yearlings), 2 years old, and 3+ years old; these age classes were chosen in light of previous statistical analyses of age-related differences in ewe August FEC (Wilson *et al.* 2004)); weight in the previous summer (kg); coat type (DW- Dark Wild, DS - Dark Self, LW - Light Wild, LS - Light Self); horn type (scurred, polled, or normal-horned); day of sampling relative to the day of parturition (lambing day = day zero). In the cases where ewes failed to lamb, the relative day of sampling (RDS) was calculated around the mean parturition date for ewes that did lamb in that particular year. The mean lambing day (and range) for lambing ewes in each year was: 1989 - 18th April (10th April - 23rd April); 1990 - 1st May (25th April – 15th May); 1991- 19th April (8th April – 28th April); 1992 – 17th April (1st April - 22nd April); 1993 – 19th April (5th April – 28th April); 1996 – 24th April (6th April – 9th May). As year of collection and population density alias each other, both terms were tested separately in the model to determine which provided a better description of the variation in the data. Population density is the population entering the previous winter (e.g. for FEC sampled in 1990, population density entering the winter 1989/1990 was considered).

Ewe reproductive success or fecundity, was described as a 3 – level factor; 'Live lamb' (L), 'Dead lamb' (D) and 'No lamb' (N). Ewes that successfully reared at least one lamb which was known to be alive at 5 months old, were classified as 'L'. Ewes that failed to lamb were categorised into level 'N', and ewes with lambs that died within 5 months of birth were assigned to group 'D'. In reality, 98% of lambs that die in their first summer die within the first month; 93% of those that die within a month actually die within the first week; and of these, 91% are dead within the first two days. In effect, ewes in group 'L' incurred costs of gestation, parturition, and lactation, while ewes in group 'D' incurred costs of gestation and parturition but only minimal costs of lactation. It is probable that some of the ewes that failed to lamb (group N) may have been pregnant up until an unknown time before abortion of the foetus, and so it must be assumed that they incurred some costs of gestation.

To analyse the impact of litter size on PPR, it was necessary to consider only those ewes that lambed. If ewes that did not lamb were included, then there would be aliasing between factors 'Litter size' and 'Fecundity', as both would have the level 'No lamb'. Therefore, a separate analysis was carried out following the same criteria as outlined above, using a new dataset that included only ewes that lambed. In this dataset, a new factor 'Litter size' was used, specifying whether ewes had single or twin lambs.

3.2.2 PPR in ewes treated with an anthelmintic bolus

In August 2003, 51 female Soay sheep (aged four to seven) were caught and processed as normal, and randomly assigned to either the Bolus (treated) or Control (not treated) group. The ewes in the Bolus group were administered with an anthelmintic bolus (Captec slow-release bolus; active ingredient Albendazole) which removes the parasite burden for at least 100 days. Measurement of FEC of ewes within a few days of starting the experiment in August 2003, confirmed that the treatment had significantly reduced the parasite burden in the bolussed ewes. During the lambing period of 2004, experimental ewes were monitored from April 1st until May 31st. Multiple faecal samples were collected, with an average of eight samples per ewe (ranging from 2-14 samples). Parasite burden was measured by obtaining faecal egg counts (FEC). For a full description of this experiment, see Chapter 5.

Data were analysed using general linear mixed models (GLMMs) to account for repeated measures. Random effects were relative day of sampling within ewe ID:

$$\log 10\ (\text{FEC}+100) \sim a + b + c \ldots, \text{random} = \sim \text{sample day} \mid \text{ewe ID}$$

Data were available for 43 of the experimental animals, 21 of which were in the Bolus group, and 22 in the Control group. Fixed terms tested in the model included: treatment group (Bolus or Control); weight in the previous summer (kg); coat type (DW- Dark Wild, DS - Dark Self, LW - Light Wild, LS - Light Self); horn type (scurred, polled, or normal-horned); day of sampling relative to the day of parturition (the mean lambing date (and range) for all ewes was 16th April 2004 (2nd April – 24th April)); and litter size (single or twin lambs). As all ewes in this experiment were adults of 4-7 years old, age was not categorised in this analysis (in contrast to the other analyses, see sections 3.2.1, and 3.2.3), and was described as a covariate. Fecundity was categorised as in the previous analysis (section 3.2.1). However as none of the ewes in this dataset failed to lamb, ewes were categorised either as having successfully reared at least one lamb ('L'), or having lost their lamb(s) ('D'). Note that only two of the ewes were in this ('D') category.

3.2.3 Tup spring FEC

Single FEC measured in the spring months were collated for tups in 1989 (39 tups), 1990 (30 tups), 1994 (98 tups), 1995 (37 tups), 1996 (44 tups), 1997 (71 tups) and

1998 (54 tups). To account for repeated sampling of FEC for each tup, data were analysed using general linear mixed models (GLMMs). The random effects structure specified tup ID:

$$\log10\ (\mathrm{FEC}+100) \sim a + b + c \ldots, \text{ random} = \sim 1 \mid \text{tup ID}$$

Fixed terms tested in the model were: tup age (categorised into 1 year old (yearlings), 2 years old, and 3+ years old); weight in the previous summer (kg); coat type (DW-Dark Wild, DS - Dark Self, LW - Light Wild, LS - Light Self); horn type (scurred or normal-horned) and month of data collection (January, February, March, April or May). As year of collection and population density entering the previous winter alias each other, both terms were tested separately in the model to determine which provided a better description of the variation in the data.

3.3 Results

3.3.1 Ewe spring FEC – peri-parturient rise

There were significant differences in the peri-parturient rise (PPR) between different ewes, shown by the random effects structure which accounted for 24.5% of the variance in the data ($\chi^2_3 = 59.89$, $p<0.0001$). The characteristic rise and fall in FEC of the PPR is hump-shaped and was best described by a quadratic relationship (relative day of sampling^2 (RDS^2): $\chi^2_1 = 31.82$, $p<0.0001$; Table 3.1). The age of the ewe affected FEC, with ewes in the youngest age category (yearlings) having a much higher peak in PPR than older ewes (Figure 3.2). The magnitude of the PPR was lower in 2 year old ewes, and lower still for the oldest ewes (3 years and older)

Table 3.1: General linear mixed model of ewe spring FEC (log10-transformed).

Terms	Coefficient	St. Error	χ^2	df	p- value
(Intercept)	3.6067	0.1621	-	-	-
Age (2yo)	-0.1068	0.1023			
Age (3yo+)	-0.3424	0.1008	**64.43**	**2**	**<0.0001**
Fecundity (L)	0.2925	0.0914			
Fecundity (N)	0.2049	0.0912	**13.21**	**2**	**0.0014**
Relative Day Sampling (RDS)	-0.0043	0.0021	**24.45**	**1**	**<0.0001**
RDS ^2	-0.0004	0.0001	**31.82**	**1**	**<0.0001**
Weight	-0.0214	0.0079	**7.23**	**1**	**0.0072**
Year (1990)	-0.4156	0.0976			
Year (1991)	-0.3585	0.0822			
Year (1992)	-0.2657	0.0731			
Year (1993)	-0.4232	0.0859			
Year (1996)	-0.4025	0.0968	**28.29**	**5**	**<0.0001**
Fecundity (L): RDS	0.0109	0.0023			
Fecundity (N): RDS	0.0067	0.0031	**28.44**	**2**	**<0.0001**
Fecundity (L): RDS^2	0.0003	0.0001			
Fecundity (N): RDS^2	0.0002	0.0002	**6.06**	**2**	**0.0482**
Age (2yo) : Fecundity (L)	-0.3942	0.1219			
Age (3yo+) : Fecundity (L)	-0.2831	0.1197			
Age (2yo) : Fecundity (N)	-0.5535	0.1631			
Age (3yo+) : Fecundity (N)	-0.3736	0.1529	**16.15**	**4**	**0.0028**

(χ^2_2 = 64.43, p<0.0001; Table 3.1). In yearlings, the average maximum FEC that occurred at the peak of the PPR was 2100 epg (number of strongyle eggs per gram of faeces – see section 3.2.2). In 2 year old ewes, the average maximum was much lower at 560 epg, while in ewes of 3 years and older, this was just 280 epg. Statistically, there was no difference in the shape (i.e. the curve) of the peri-parturient rise between age categories (interaction term RDS: age category χ^2_2 = 3.17, p=0.20);

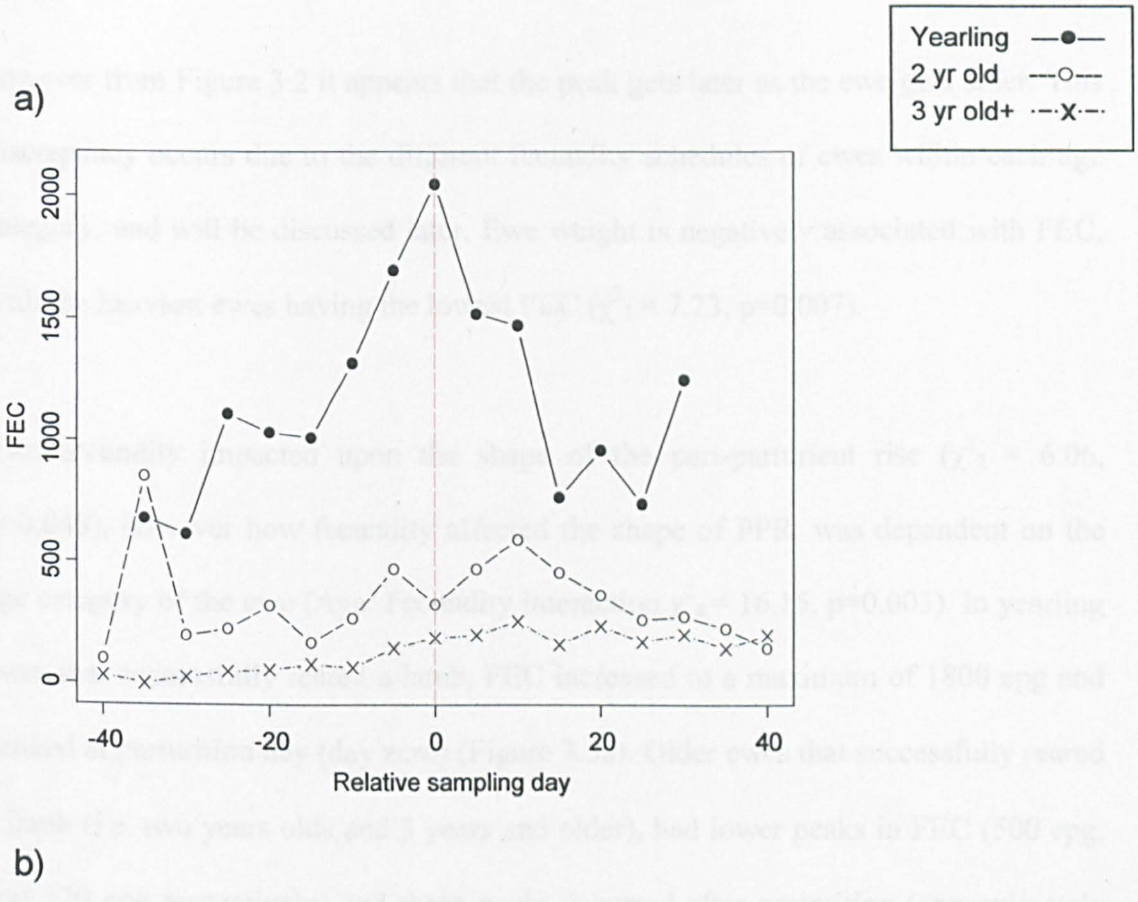


Figure 3.2: Faecal egg counts (FEC) relative to the day of parturition and age. FEC is the density of strongyle eggs per gram of faeces, represented as both the geometric mean FEC (a) and log10-transformed FEC (b). The red dashed line indicates day 0 (the day of lambing for those ewes that lambed or the median date of lambing for those that did not). The peri-parturient rise in FEC is shown for ewes in different age categories: Yearlings; Two-year olds; Three years old and over. Data points are grouped means for five day periods. Error bars are standard errors.

however from Figure 3.2 it appears that the peak gets later as the ewe gets older. This discrepancy occurs due to the different fecundity schedules of ewes within each age category, and will be discussed later. Ewe weight is negatively associated with FEC, with the heaviest ewes having the lowest FEC ($\chi^2_1 = 7.23$, p=0.007).

Ewe fecundity impacted upon the shape of the peri-parturient rise ($\chi^2_2 = 6.06$, p=0.048), however how fecundity affected the shape of PPR, was dependent on the age category of the ewe (Age: Fecundity interaction $\chi^2_4 = 16.15$, p=0.003). In yearling ewes that successfully reared a lamb, FEC increased to a maximum of 1800 epg and peaked at parturition day (day zero) (Figure 3.3a). Older ewes that successfully reared a lamb (i.e. two years olds and 3 years and older), had lower peaks in FEC (500 epg, and 320 epg respectively) and these peaks occurred after parturition (approximately 10 days and 30 days after parturition, respectively) (Figure 3.3a). In those ewes whose lamb died, PPR peaked at parturition day (except in yearlings where it occurred slightly earlier) before the FEC rapidly declined in all age categories (Figure 3.3b). In ewes that did not have a lamb, there was a rise in FEC that peaked just after the mean parturition day in yearlings, and a similar, smaller peak in ewes aged two years. However, in older ewes (aged 3 years and over), there was no obvious increase in FEC (Figure 3.3c).

In summary, ewes that lambed successfully showed a peak in FEC that occurred after parturition (Figure 3.3a). In contrast, ewes whose lambs died also showed a peak in FEC, however this occurred earlier and dropped sharply (Figure 3.3b). In ewes that did not lamb, only yearlings showed a clear peak in FEC; older ewes did not show such an increase (Figure 3.3c).

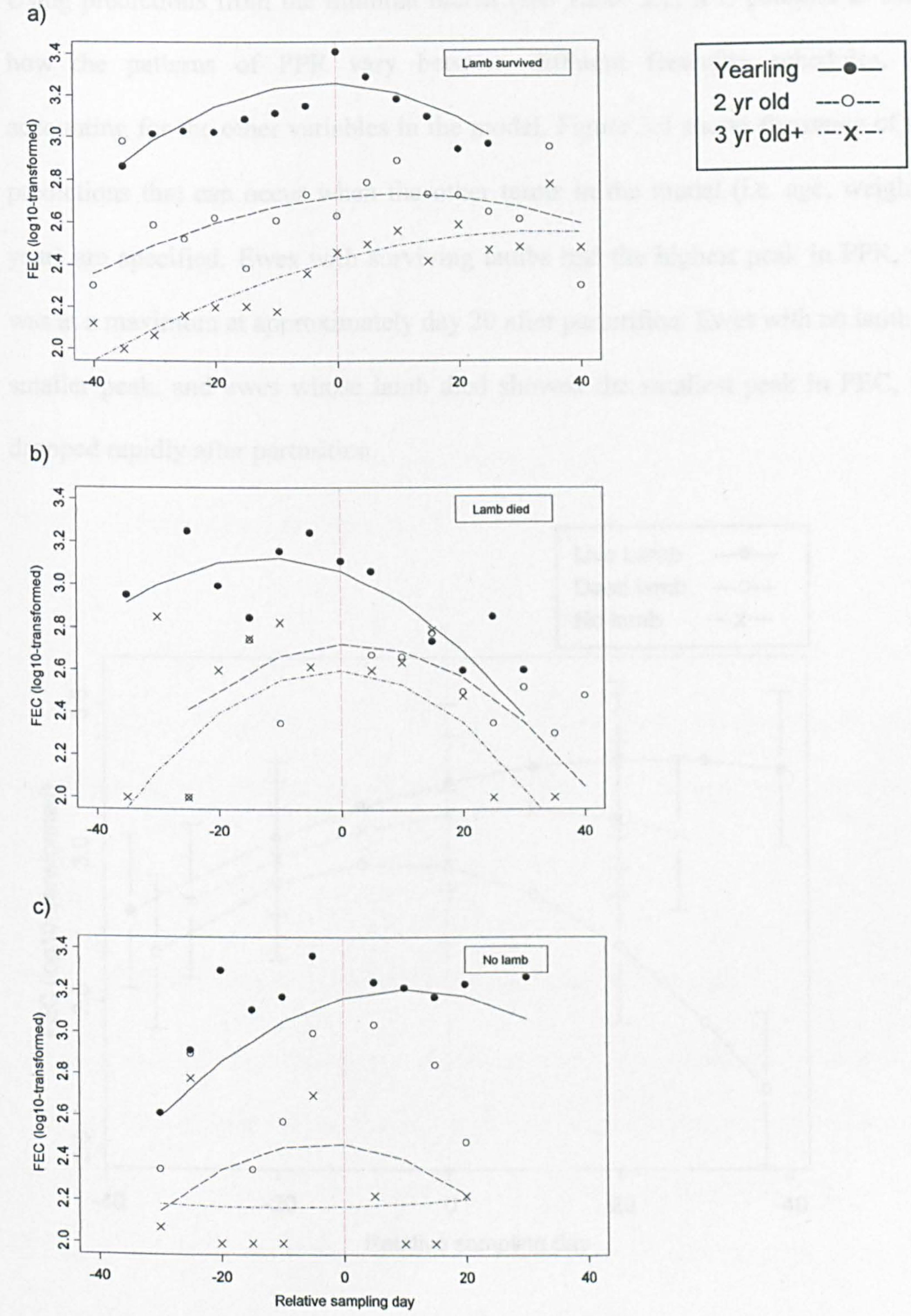


Figure 3.3: Faecal egg counts (FEC) relative to the day of parturition, age and lambing status. FEC is the log10-transformed density of strongyle eggs per gram of faeces, and the red dashed line indicates day 0 (the day of lambing for those ewes that lambed or the median date of lambing for those that did not). The peri-parturient rise in FEC is shown for ewes that had: a) a lamb that survived; b) a lamb that died; c) no lamb. Data points are grouped means for five day periods for yearling ewes, 2 year olds and ewes aged 3 years and older. Fitted lines are drawn from model predictions.

Using predictions from the minimal model (see Table 3.1) it is possible to examine how the patterns of PPR vary between different fecundity schedules, whilst accounting for the other variables in the model. Figure 3.4 shows the range of model predictions that can occur when the other terms in the model (i.e. age, weight, and year) are specified. Ewes with surviving lambs had the highest peak in PPR, which was at a maximum at approximately day 20 after parturition. Ewes with no lamb had a smaller peak, and ewes whose lamb died showed the smallest peak in FEC, which dropped rapidly after parturition.

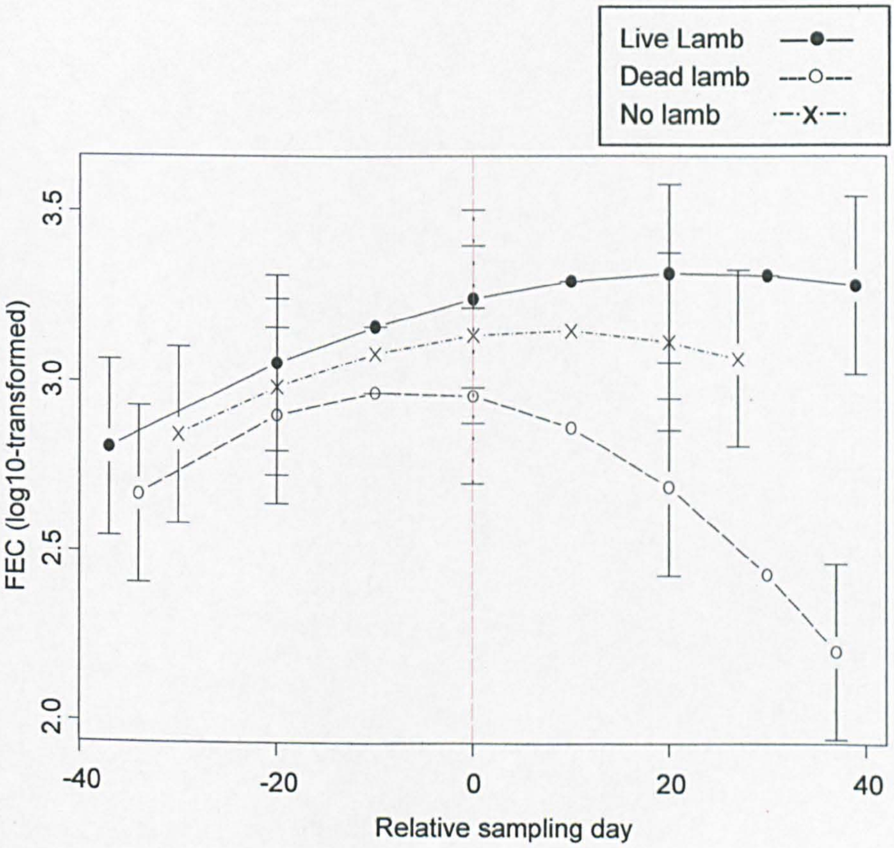


Figure 3.4: Faecal egg counts (FEC) relative to the day of parturition, and lambing status. FEC is the log10-transformed density of strongyle eggs per gram of faeces, and the red dashed line indicates day 0 (the day of lambing for those ewes that lambed or the median date of lambing for those that did not). The peri-parturient rise in FEC is shown for ewes that had: a lamb that survived; a lamb that died; no lamb. Data points and fitted lines are extracted from the model predictions, when other terms in the model (age, weight, and year) are specified and accounted for. Error bars are standard errors of the model predictions, from a range of model specifications.

The magnitude of the PPR differed between years, with FEC in some years being much higher than in others ($\chi^2_5 = 28.29$, $p<0.0001$). For example, FEC was high in 1989 (geometric mean of FEC during spring = 1100 epg) following a crash in the population, and low (mean FEC of 240 epg) in 1990 following a winter of low population density. However, this was not entirely due to the population size entering the previous winter, as the model was significantly better (tested by χ^2) with 'Year' included as a term, rather than 'Population density' ($\chi^2_4 = 15.82$, $p=0.003$), despite using up an additional 4 degrees of freedom. See section 3.3.3 and Figure 3.7, for further comments on this result, and for a comparison with tup spring FEC.

Litter size had no effect on the PPR; FEC was no different in ewes with single or twin lambs ($\chi^2_1 = 0.20$, $p=0.65$). Thus, whilst the presence/absence of successful reproduction affected the magnitude of the PPR, its magnitude (singleton/twins) did not.

3.3.2 PPR in ewes treated with an anthelmintic bolus

In order to determine the effects of summer parasite removal on the magnitude and timing of the peri-parturient rise in faecal egg counts, a comparison was made of the PPR profiles of ewes in the bolussing experiment conducted in August 2003 (see Chapters 5 and 6 for further details). The random effects structure of the GLMM identified variation in FEC between ewes, explaining 14.5% of the variance ($\chi^2_2 = 17.7$, $p=0.0001$). As in the previous analysis, the rise and fall of FEC during the PPR was hump-shaped and best described by a quadratic relationship ($\chi^2_1 = 41.0$, $p<0.0001$). The peak FEC during the PPR occurred at around day 10-15 after parturition and averaged 515 ± 1.2 epg (geometric mean ± s.e.). Treatment group was the only factor that appeared to influence the magnitude of the PPR (Figure 3.5), which was higher in Control animals (525 ± 1.1 epg), than in animals that had been bolussed seven months earlier (380 ± 1.1 epg) ($\chi^2_1 = 4.21$, p=0.040). There was no difference in the shape of the PPR between these two groups, (Treatment: RDS interaction $\chi^2_1 = 2.62$, p=0.27) and the PPR peaked similarly at approximately 10-15 days after parturition. Thus although the treatment reduced the PPR in bolussed ewes, it did not change the shape or pattern of the PPR. Ewe age did not affect the PPR ($\chi^2_1 = 0.35$, p=0.55), nor did fecundity ($\chi^2_1 = 0.37$, p=0.55).

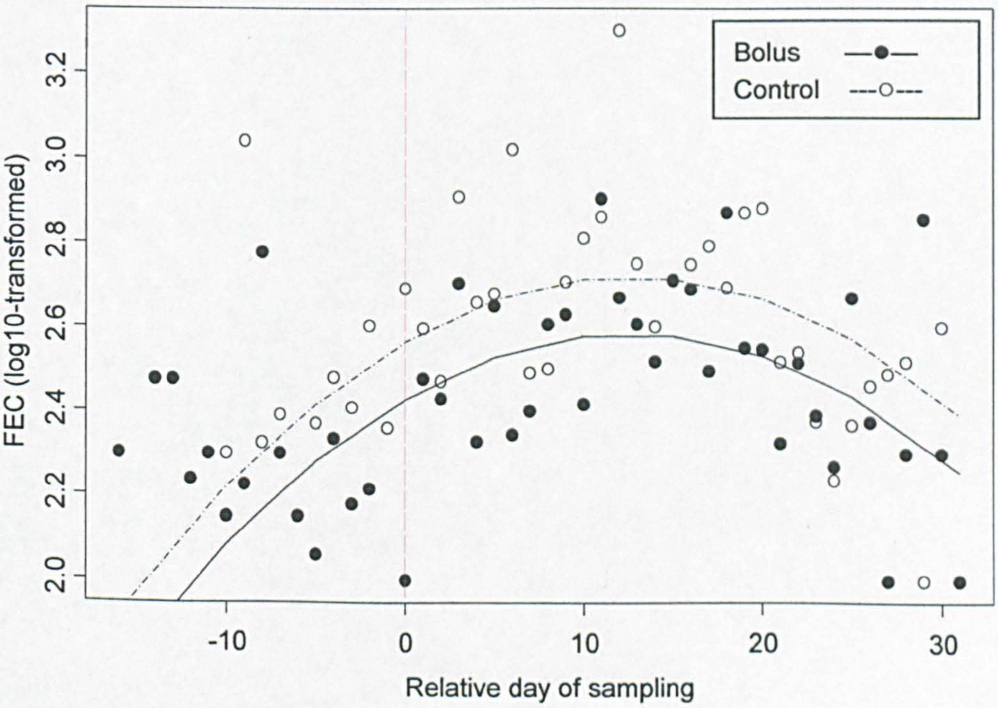


Figure 3.5: Faecal egg counts (FEC) relative to the day of parturition, and treatment group. FEC is the log10-transformed density of strongyle eggs per gram of faeces, and the red dashed line indicates day 0 (the day of lambing). The peri-parturient rise in FEC is shown for ewes in the Bolus and Control groups. Data points are daily grouped means. Fitted lines are drawn from model predictions.

3.3.3 Tup spring FEC

In order to determine if there are any other factors that may have an impact upon parasite burden during the spring months, spring FEC was also examined in tups. Variation in spring FEC between individual tups explained 14.5% of the variation in the data, however this was not statistically significant (χ^2_1 = 2.13, p=0.15). Nonetheless, tup ID was retained as a random effect in the minimal model, to account for repeated sampling of tups.

Spring parasite burden showed a peak in FEC that increased from January (370 ± 1.1 epg, mean ± s.e.) and was highest in April (735 ± 1.1 epg), before decreasing in May (355 ± 1.2 epg) (χ^2_4 =26.2, p < 0.0001) (Figure 3.6; Table 3.2).

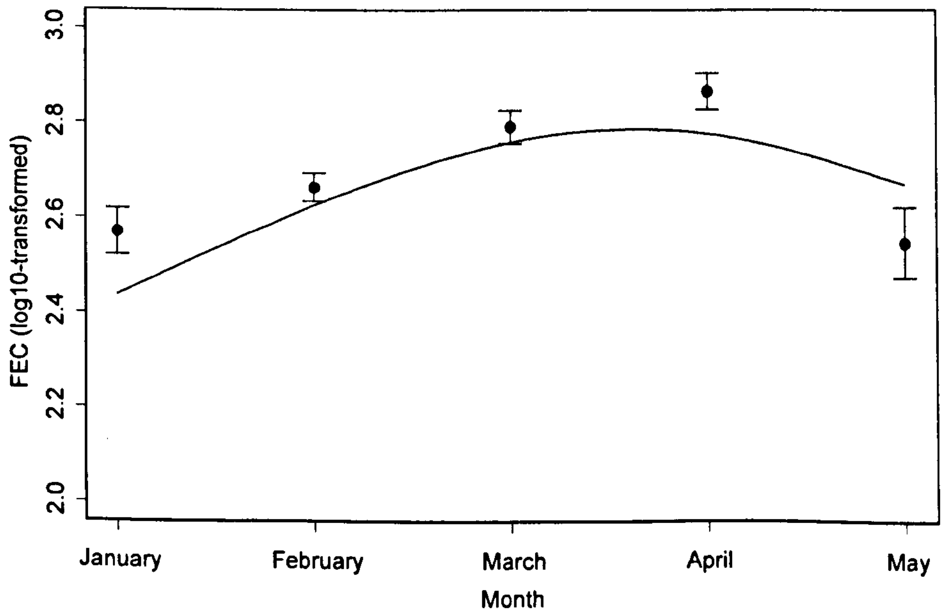


Figure 3.6: Average spring faecal egg counts (FEC) in tups. FEC is the average log10-transformed density of strongyle eggs per gram of faeces, from January to May. Fitted line is drawn from model predictions. Error bars are standard errors.

Table 3.2: General linear mixed model of tup spring FEC (log10-transformed).

Terms	**Coefficient**	**St. Error**	χ^2	**df**	**p- value**
(Intercept)	3.333	0.170	-	-	-
Age (2yo)	-0.262	0.060			
Age (3yo+)	-0.203	0.093	**19.10**	**2**	**0.0001**
Weight	-0.017	0.004	**15.34**	**1**	**0.0001**
Month (February)	0.164	0.139			
Month (March)	0.161	0.134			
Month (April)	0.269	0.135			
Month (May)	0.197	0.152	**26.18**	**4**	**<0.0001**
Year (1990)	-0.551	0.117			
Year (1994)	-0.074	0.004			
Year (1995)	0.108	0.011			
Year (1996)	-0.434	0.099			
Year (1997)	-0.014	0.081			
Year (1998)	0.144	0.091	**85.95**	**6**	**<0.0001**

Spring FEC varied greatly between years (χ^2_6 =86.0, $p < 0.0001$; Table 3.2), being highest in 1995 (930 ± 1.1 epg), and lowest in 1996 (335 ± 1.1 epg). Although FEC was highest in 1995, this was not the year when the population density entering the previous winter was highest. Therefore it appears that although FEC is affected by population density, it is not a linear relationship, and the variation in FEC between years is best described by the factor 'Year', rather than the population density that relates to that year (comparison of model including 'year' versus model including ' population density': χ^2_4 =33.7, $p < 0.0001$).

For graphical purposes, spring FEC is shown with population size in the *previous* winter (Figure 3.7). The change in population size over the years is shown in Figure 3.7a, where periods of population growth are followed by a large crash in the population (indicated by inverted arrow heads). Spring FEC tends to be highest in years following a winter of high population density, or in years just prior to a major crash in the population. This is most evident in tup spring FEC (Figure 3.7b), however there is a similar tendency in ewe spring FEC (Figure 3.7c) (see also section 3.3.2).

Spring FEC was highest in yearling tups (1 year old) and decreased with age ($\chi^2_2 = 19.1$, $p = 0.0001$). The average FEC for yearling tups was 870 ± 1.1 epg (mean ± s.e.), decreasing to 630 ± 1.1 epg for two-year olds, and tups that were three years and older had the lowest FEC of 310 ± 1.1 epg. Tup body weight also appeared to influence parasite burden, with the heaviest tups having the lowest FEC ($\chi^2_1 = 15.4$, $p = 0.0001$).

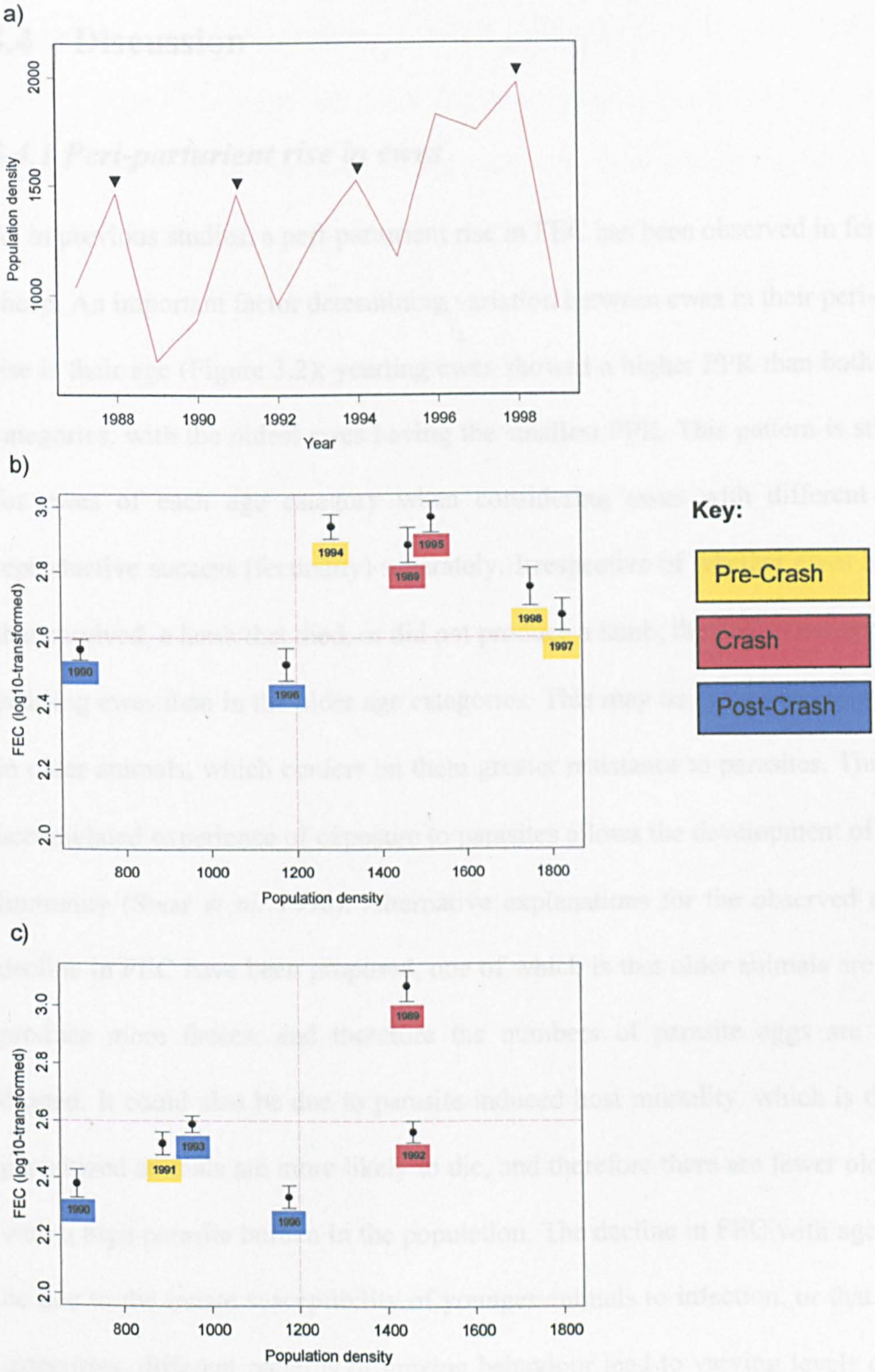


Figure 3.7: a) Yearly fluctuations in population (major crashes marked with arrow). Population density is that counted in August, and deemed to be the population entering the following winter. Mean Spring FEC (log10-transformed) in tups (b) and in ewes (c) at different population density/years. Population density corresponds to that entering the previous winter (i.e. population density from the previous August). The red dashed vertical line represents the estimated crash threshold (Clutton-Brock *et al.* 2004) at population size 1200 animals. The red horizontal dashed line represents the median FEC (~ 400 epg).

3.4 Discussion

3.4.1 Peri-parturient rise in ewes

As in previous studies, a peri-parturient rise in FEC has been observed in female Soay sheep. An important factor determining variation between ewes in their peri-parturient rise is their age (Figure 3.2); yearling ewes showed a higher PPR than both older age categories, with the oldest ewes having the smallest PPR. This pattern is still evident for ewes of each age category when considering ewes with different levels of reproductive success (fecundity) separately. Irrespective of whether ewes had a lamb that survived, a lamb that died, or did not produce a lamb; the FEC remained higher in yearling ewes than in the older age categories. This may be due to acquired immunity in older animals, which confers on them greater resistance to parasites. This is where accumulated experience of exposure to parasites allows the development of protective immunity (Stear *et al.* 1996). Alternative explanations for the observed age-related decline in FEC have been proposed, one of which is that older animals are larger and produce more faeces, and therefore the numbers of parasite eggs are effectively diluted. It could also be due to parasite-induced host mortality, which is that heavily parasitized animals are more likely to die, and therefore there are fewer older animals with a high parasite burden in the population. The decline in FEC with age could also be due to the innate susceptibility of younger animals to infection, or that within age categories, different patterns of grazing behaviour lead to varying levels of exposure to parasite larvae. These alternative suggestions have all been considered and rejected in favour of the accepted explanation of the observed age-related decline in FEC, which is that older animals acquire immunity to parasites (Wilson *et al.* 2002, Wilson *et al.* 2004).

The PPR was earlier in yearling ewes that successfully reared a lamb, than in older lambing ewes. This trend may occur simply as a result of age-related differences in immunity to parasites. Superior immune defence in older animals may delay the onset of the PPR, but not prevent it.

Of considerable interest is the importance that body weight had on spring parasitism in both ewe and tup faecal egg counts. Although a weight-related decline in FEC can normally be explained by age (and acquired immunity) in ewes (Wilson *et al.* 2004), and previous work has shown that male parasite burden measured in the summer (August FEC) is indistinguishable between animals over one year old (Wilson *et al.* 2004); these observations have revealed both an age- and a weight-related decline in spring parasitism in both sexes. These findings highlight the importance of the general body condition that the animals are in when they emerge from the harsh winter months, and how this can be reflected in their spring parasite burden.

In ewes that successfully reared a lamb, FEC increased prior to parturition and reached a maximum between ten and twenty days after lambing had occurred, before starting to drop (Figure 3.3a). In contrast, ewes with lambs that died showed a rise in FEC that increased to a maximum level at the time of lambing, and then dropped rapidly (Figure 3.2b). In the majority of these cases, lambs died at or shortly after birth; therefore these ewes would have minimal costs of lactation. These results lend support to the notion that the costs of lactation are important in maintaining the PPR (Gibbs 1967, Connan 1968a,b, Salisbury & Arundel 1970). However, as ewes that lost their lamb (and therefore had minimal lactation costs) still had the initial rise in FEC, this also suggests that the peri-parturient rise is caused by a combination of factors and events associated with lambing (Connan 1976, Fleming & Conrad 1989, Jeffcoate *et al.* 1992).

Ewes that had no lamb had the most variable pattern in FEC (Figure 3.3c). Yearling ewes had high FEC and showed the characteristic peak in FEC around the mean lambing date (for lambing ewes). However, in contrast to previous findings, FEC was not higher in yearling ewes that did not lamb, in comparison to yearling ewes that lambed (Wilson *et al.* 2004). Two-year old, non-lambing ewes also had a small peak in FEC. Crucially, however, ewes in the oldest age category (three years and above) had no rise in FEC, as levels of parasitism remained low and relatively constant. The absence of a PPR in non-lambing adult ewes (3 years and older) conforms to patterns seen in domestic sheep, indicating that the PPR does not occur in adult ewes that do not breed (Crofton 1958, Field *et al.* 1960, Brunsdon 1964, Procter & Gibbs 1968, Gibbs & Barger 1986). In further support of this, the model predictions suggest that lambing ewes have a significantly higher rise in FEC than the ewes that did not rear a lamb, when other factors such as age are accounted for (Figure 3.4). Ewes not subject to the costs of reproduction, particularly lactation, would not become immunocompromised at this time, and therefore parasitism would not be able to increase via either of the methods proposed by various authors: (a) establishment of newly acquired larvae (Morgan *et al.* 1951, Brunsdon 1970b, Jeffcoat *et al.* 1992); (b) maturation of dormant larvae (Naerland 1949, Spedding and Brown 1956, Field *et al.* 1960, Dunsmore 1965, Gibbs 1967, Connan 1968a, b, Herd *et al.* 1983, Gibbs & Barger 1986, Fleming *et al.* 1988, Jeffcoat *et al.* 1992); (c) reduced expulsion of existing worms (Connan 1976, Gibbs & Barger 1986); (d) increased fecundity of existing worms (Cushnie & White 1948, Naerland 1949).

As non-lambing ewes in the youngest age categories demonstrated an increase in FEC similar to a peri-parturient rise, it is likely that stresses other than the costs of reproduction may contribute to a rise in FEC at this time. During the winter months, body condition is very poor and mortality is often high due to food shortage (Gulland 1992, Clutton-Brock *et al.* 1997a). In May and June there is a peak in infective larvae on the pasture, largely due to peri-parturient ewes depositing parasite eggs in faeces, which then develop through the larval stages and are ingested by a new host (see Chapter 2). The combination of poor body condition and an increase in availability of infective larvae may explain why ewes, in particular the younger ewes, that have lower immunity, were more susceptible to parasitism and showed a rise in FEC despite not producing a lamb. Older ewes had the advantage of acquired immunity, and without the costs of reproduction, were less susceptible to parasitism, hence the absence of a rise in FEC during the spring.

3.4.2 PPR in ewes treated with anthelmintic bolus

In adult Soay ewes that have successfully reared a lamb, there is a peri-parturient rise in FEC, which peaks approximately 10-15 days after parturition. As all the ewes in the bolussing experiment were adults (4 - 7 years old) with well developed acquired immunity, and nearly all ewes (apart from 2 out of 43) reared a lamb successfully, much of the age-related variation that was seen in the previous analysis of ewe PPR was avoided. This enabled consideration of how other factors affecting over-winter body condition may influence the spring PPR.

Application of the anthelmintic bolus successfully removed the parasite burden in treated ewes for several months (see Chapter 5). Furthermore, it is likely that treated ewes had reduced costs of fighting parasitic infections going into, and probably throughout, much of the winter, since densities of infective larvae on the pasture are very low during the winter months (Wilson *et al.* 2004). Evidently, it appears that the reduced stress afforded by the anthelmintic treatment throughout the winter months, may have allowed the treated ewes to emerge from the winter in a superior overall body condition to the control ewes, which was manifested in a smaller peri-parturient rise (Figure 3.5).

3.4.3 Spring FEC in tups and ewes

During the spring, ewes become immunocompromised due to the costs of reproduction, which leads to a characteristic rise in FEC (peri-parturient rise). This effect may be exacerbated by high population density. As population size increases, the density of infective larvae on the pasture increases, and exposure to infection is higher (Gulland and Fox 1992). High population density also increases competition for food, keeping animals on a lower plane of nutrition, and making them more susceptible to infection. Tups, despite not having similar costs of reproduction, also showed an increase in FEC during the spring (Figure 3.6), which was higher when population density was high (Figure 3.7b). In a previous study, ewe and tup spring FEC was observed to be higher after a population crash in 1989, and much lower in the following spring when population density was low (Gulland and Fox 1992). In support of this, it has been found that during crash years, and in pre-crash years where population density was high, FEC also tended to be higher in both tups and ewes (Figures 3.7b and 3.7c). In contrast, during post-crash years where the population was low and food availability was high, spring FEC were low. This pattern is more apparent in tups, perhaps emphasizing the relative simplicity of the spring rise in tup

FEC in comparison to ewes. In ewes, FEC is low for all post-crash years, and high during a crash year, however there are two notable exceptions (Figure 3.7c). In contrast to tups, where FEC was high in pre-crash years, ewe FEC was notably low in spring 1991 which preceded a crash in the population. This may be due the fact that population was low in this year. The second anomaly occurs in the following year during the population crash: whereas in other crash years ewe and tup FEC was very high, a relatively low FEC was observed in spring 1992 in ewes (Figure 3.7c).

These results serve to emphasize the complexity of the situation on St Kilda. In tups, which appear to lack an effective acquired immune response (Wilson *et al.* 2004), the pressures of winter food shortage, combined with increased numbers of infective larvae on the pasture, causes an increase in their spring parasite burden (as measured by faecal egg counts); an effect which is exacerbated in years of high population density. It is unlikely that tups suffer any costs of reproduction in the spring, as these occur during, and after the rutting season (Stevenson *et al.* 2004). In contrast, in ewes, which develop effective, age-related acquired immunity, spring parasitism is affected similarly by population density, food shortage and infective larvae, but the effect is compounded by the costs of reproduction. More data are required to fully investigate the effects of ewe fecundity, body condition and year on the peri-parturient rise of ewes. Nevertheless, a crucial finding here is that both ewe and tup spring FEC varies across years and is associated with annual changes in population density and food availability.

3.5 Summary

Soay ewes, like their domestic cousins, suffer an increase in their parasite burden during the spring coinciding with lambing, called the peri-parturient rise (PPR). The PPR occurred in all ewes that successfully reared a lamb, with the peak in faecal egg count (FEC) occurring between ten and twenty days after parturition. In contrast, in ewes that lost their lamb, an initial increase in FEC was followed by a rapid decrease shortly after parturition. In all ewes that lambed, the rise in FEC was higher in yearling ewes than in two year old ewes, and was lower still in ewes that were three years and older.

Non-lambing adult ewes (3 years and older), did not show the characteristic rise in FEC, which conforms to patterns observed in domestic sheep. In contrast, yearling non-lambing ewes showed a large increase in FEC during the spring, although this was not higher or lower than lambing yearling ewes.

The reason for the increase in FEC in non-lambing yearling and two-year old ewes was probably due to increased susceptibility to parasitism at this time of year. Sheep are in poor condition after the food shortage that occurs during the winter (Gulland 1992, Clutton-Brock *et al.* 1997a), and ewes that failed to lamb are probably in the poorest condition. As younger animals do not have the acquired immunity that older ewes do, they will be more susceptible to the increase in infective larvae during the spring. Hence, younger non-lambing ewes, despite not incurring costs of parturition and lactation, had an increase in FEC during the spring.

Interestingly, lambing adult ewes with improved winter body condition afforded by anthelmintic treatment (see Chapter 5), had a lower PPR than control ewes. This emphasizes the importance of body condition for the ability of even the strongest animals to cope with the costs of lambing. However, the pressures of poor body condition and increased infective larvae on the pasture during the spring do not only affect ewes. These costs are exacerbated when population density throughout the winter is high, and causes an increase in parasitism in both ewes and tups. In Soay sheep, the peri-parturient rise that occurs as a cost of reproduction, is confounded and exacerbated by their population dynamics.

Chapter 4

Factors affecting lambing success in Soay ewes

4.1 Introduction

The impact of parasites on host survival, growth and fecundity has been the subject of many observational, and some experimental, studies, especially over the past three decades (reviewed in Gulland 1995, Grenfell & Gulland 1995, Tompkins & Begon 1999). Part of this recent growth in studies is a growing appreciation of the role that parasites might play in regulating their host's population dynamics (Anderson & May 1978, May & Anderson 1978, Tompkins *et al.* 2002). In particular, theoretical models highlight the importance of parasite-induced changes in host fecundity as a destabilising impact on host population dynamics (Dobson & Hudson 1992). Thus, population cycles are more likely when parasites reduce host fecundity more than they increase host mortality. Several recent studies have examined the impact of parasites on host reproduction. For example, in red grouse populations, there is evidence to suggest that the parasite *Trichostrongylus tenuis* impacts negatively upon grouse survival and fecundity, and may drive population cycles (Hudson 1986, Hudson & Dobson 1989, Dobson & Hudson 1992, Hudson *et al.* 1998). In an experimental study on Svalbard reindeer, *Rangifer tarandus*, removal of gastro-intestinal parasites caused an average increase of 11% in the rate of pregnancy through positive effects on female body condition (Stien *et al.* 2002, Albon *et al.* 2002). In bighorn sheep (*Ovis canadensis*), lower survival was observed in the lambs of ewes with higher lungworm burdens (*Protostrongylus* spp.) (Festa-Bianchet 1988a). However, in an experimental study to remove parasite burdens of Fulbe ewes in Cameroon, no differences in lamb survival were observed between treated and control groups (Njoya *et al.* 2005).

In Soay sheep (*Ovis aries L.*) on St Kilda, the probability of lambing is typically high: approximately 80% of adult ewes produce lambs, irrespective of population density.

In contrast, the incidence of lambing in younger females varies with population density, ranging from around 10% when the population size is high, to over 80% when the population is low (Clutton-Brock *et al.* 1996, Clutton-Brock *et al.* 2004). There is evidence that ewe weight also affects fecundity: yearling ewes are less likely to produce a lamb if they were light in weight during the previous summer (Clutton-Brock *et al.* 1996). However, in contrast, variation in body weight appears to have no effect on fecundity in adult ewes (Clutton-Brock *et al.* 1996). The incidence of twinning is weight related in ewes however; varying from <8% in ewes of low weight (in the previous summer), to 23-38% in heavier ewes (>25kg) (Clutton-Brock *et al.* 1996).

Observational studies indicate that certain traits of the ewe can have negative effects on the survival and development of their lambs. Older ewes tend to give birth earlier in the season (Clutton-Brock *et al.* 1992), which can affect the survival and growth rates of their lambs (Clutton-Brock *et al.* 1992, Milner *et al.* 1999a). Early-born lambs are heavier at four months old (Clutton-Brock *et al.* 1992), which positively affects their survival and reproductive success (Clutton-Brock *et al.* 1996, Coltman *et al.* 1999c). However, being born early also increases the likelihood of dying within the first few weeks of life (Clutton-Brock *et al.* 1992, Clutton-Brock *et al.* 1996). The age and weight of the ewe has an effect on the birth weight of the lamb: middle-aged ewes produce heavier lambs than young or old ewes, and heavier ewes tend to produce heavier lambs (Clutton-Brock *et al.* 1996). High birth weight increases lamb survival rates, and also correlates with the weight of the individual throughout much of their lifetime (Albon & Clutton-Brock 1988, Albon *et al.* 1991), which increases individual fitness (Clutton-Brock *et al.* 1996, Coltman *et al.* 1999c).

Several experimental and observational studies on St Kilda, have shown that gastrointestinal parasites cause increased mortality in Soay sheep (Gulland 1992, Illius *et al.* 1995, Coltman *et al.* 1999, Milner *et al.* 1999). However, the impact on fecundity is less clear. In a previous experiment to remove strongyle parasite burdens with an anthelmintic bolus, conducted in a year of high mortality, it was observed that there were no differences in fecundity between surviving treated and control ewes (Gulland *et al.* 1993).

In an attempt to clarify the effects of parasites on ewe fecundity, a further experiment has been carried out, in which adult ewes were treated with anthelmintic boluses. The results of this experiment are detailed in Chapters 5 and 6. As a prelude to this, the aim of this chapter is to use observational data collected on St Kilda from 1985 to 2004 to examine the factors affecting fecundity in ewes. In particular, the aim here is to determine if there are any observable effects of parasite burden (measured in the previous summer), on the probability of successfully raising a lamb to weaning age.

4.2 Methodology

Data were analysed using generalized linear mixed models with partial quasi-likelihood (GLMMpql) using the MASS library (Venables and Ripley 2002) in S-PLUS 2000 release 1 (Mathsoft, Inc). This approach was used to account for repeated measures of binary data, as data were available on the same ewe across different years. The statistical significance of a term in a GLMM is assessed by the change in deviance of the model when the term is dropped from it. This is distributed asymptotically as χ^2, on the number of degrees of freedom associated with the term.

Minimal models were obtained by step-wise deletion of non-significant terms, using an α-value of 0.05.

Data were collated from the Soay sheep database spanning the years 1985-2004. The response variable was binary, reflecting whether or not ewe successfully reared a lamb to weaning age (i.e. if the lamb was still alive in the August of that year, when the lamb would be approximately four months old). A small number of ewes in any given year produced twins where one lamb died and the other survived (n = 50 / 2503 ewe-years); for simplicity, these ewes were excluded from the analyses. The random effects structure specified ewe identity:

$$y \sim a + b + c \ldots, \text{random} = \sim 1 \mid \text{ewe ID}$$

Explanatory terms tested in the model were: the age of the ewe (categorised into 1 year old (yearlings), 2 years old, and 3+ years old; these age classes were chosen in light of previous statistical analyses (Wilson *et al.* 2004)); weight in the previous summer (kg); hind-leg length (mm); coat type (DW- Dark Wild, DS - Dark Self, LW - Light Wild, LS - Light Self); horn type (scurred, polled, or normal-horned); population size entering the previous winter and parasite burden. Individual ewes' August strongyle faecal egg count (FEC) data were highly skewed (Figure 4.1) and fitted the negative binomial distribution, as is typical of data of this type (see Chapter 2 and Wilson *et al.* 2004). FEC was therefore log 10 transformed prior to inclusion in the model as an explanatory term (log10 (FEC+100)).

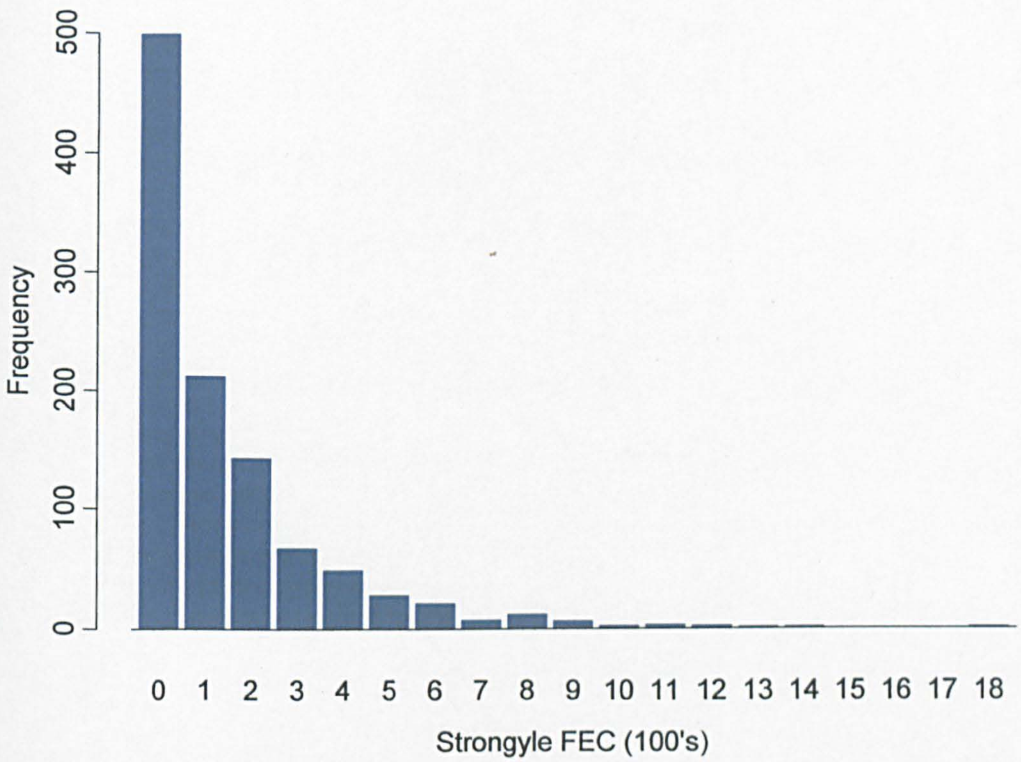


Figure 4.1: Histogram of ewe August strongyle faecal egg counts (FEC), measured as eggs per gram (epg) of faeces (1985 -2004).

4.3 Results

The probability of a ewe successfully rearing a lamb to weaning age (four months old) was affected by her age, her weight in the previous summer, her coat type, her parasite burden and the population size she experienced upon entering the previous winter (Table 4.1). The variance explained by the random effects structure, which accounted for ewe identity as a repeated measure, was large, explaining 80% of the total variance ($\chi^2_1 = 8.05$, p=0.005). This clearly indicates that ewe August FEC was highly repeatable within individual females.

The probability of rearing a lamb successfully was affected by the age of the sheep, with ewes in the oldest age category being more likely to rear a lamb that survived to weaning (χ^2_2= 13.55, p<0.001). In ewes that were aged three years and older, 89% of their lambs survived to weaning age (four months old), compared to 68% in two-year old ewes, and only 49% in yearling ewes (Table 4.2).

Although reproductive success varied among age categories, the weight of the ewe also influenced the probability of rearing a surviving lamb (Interaction: age category: weight (χ^2_2 = 7.74, p<0.001). The probability of a yearling, or a two year old ewe successfully rearing a lamb, increased with body weight in the previous August (Figure 4.2). In contrast, the probability of successfully rearing a lamb in the oldest age category (three years and older), increased only slightly as ewe weight increases, though remained high (>90% success) for all of these ewes.

The probability of successfully rearing a lamb to weaning decreased with increasing population size entering the previous winter (χ^2_1 = 19.8, p<0.001). The age of the ewe was also important: ewes aged three years and older had greater success in rearing lambs compared to yearling and two year old ewes (Interaction: age category: population size χ^2_2 = 5.85, p=0.003, Figure 4.3). There was no difference in the decline of reproductive success with increasing population size, between the age categories (Interaction: age category: population size, χ^2_2 = 0.80, p=0.45).

Table 4.1: Generalized linear mixed model (GLMM) of ewe reproductive success. The response is binary, indicating whether or not a ewe successfully reared a lamb to weaning age (four months old). All other interactions were excluded at p>0.05.

Terms	**Value**	**Std. Error**	**d.f.**	χ^2	**p**
Intercept	7.789	4.866		-	-
Ewe weight	**0.304**	**0.065**	**1**	**6.20**	**0.013**
Ewe age (2yo)	**1.999**	**1.346**			
Ewe age (3yo+)	**1.849**	**0.598**	**2**	**13.55**	**<0.001**
Population size	**-0.002**	**<0.001**	**1**	**19.80**	**<0.001**
Ewe coat (DS)	**10.138**	**3.767**			
Ewe coat (LW)	**-1.449**	**1.349**			
Ewe coat (LS)	**0.827**	**4.424**	**3**	**4.32**	**0.005**
FEC (log10-transformed)	-3.515	1.895	1	2.14	0.144
Ewe weight : age (2yo)	**-0.085**	**0.085**			
Ewe weight : age (3yo+)	**-0.131**	**0.033**	**2**	**7.74**	**<0.001**
Ewe age (2yo) : population size	**-0.001**	**<0.001**			
Ewe age (3yo+) : population size	**0.001**	**<0.001**	**2**	**5.85**	**0.003**
Ewe coat (DS): FEC (log10)	**-3.972**	**1.485**			
Ewe coat (LW): FEC (log10)	**0.634**	**0.536**			
Ewe coat (LS): FEC (log10)	**-0.328**	**1.761**	**3**	**4.99**	**0.002**
Excluded terms					
Population size^2			2	0.27	0.60
Ewe hindleg length			3	3.29	0.07
Ewe horn			2	0.07	0.94

Table 4.2: The number and percentage of ewes that successfully reared a lamb to weaning age, in different age categories: yearlings, two years old, and three years and older.

	Number of ewes lambing successfully (total number of ewes)	*Percentage of ewes lambing successfully (%)*
Yearling	152 (312)	48%
Two year olds	243 (356)	68%
Three years and older	1585 (1785)	89%

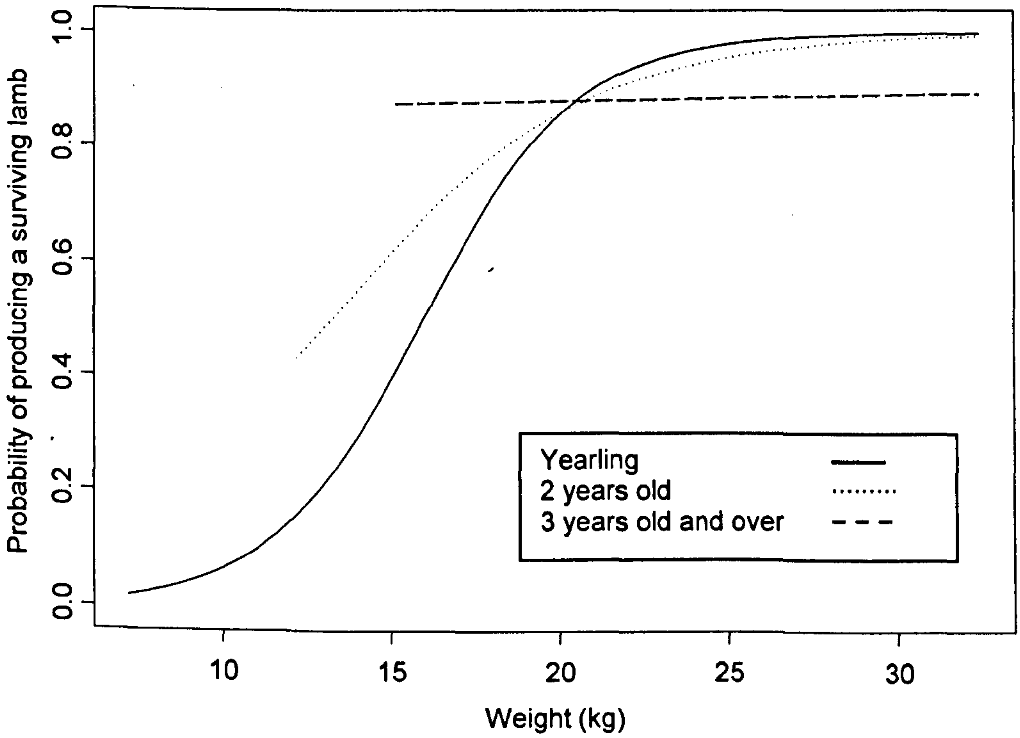


Figure 4.2: Effect of ewe weight (kg), in the previous summer, on the probability of producing a lamb that survives to weaning age (four months old), in three ewe age groups (yearlings, two years old, and three years old and over). Lines are drawn from model predictions.

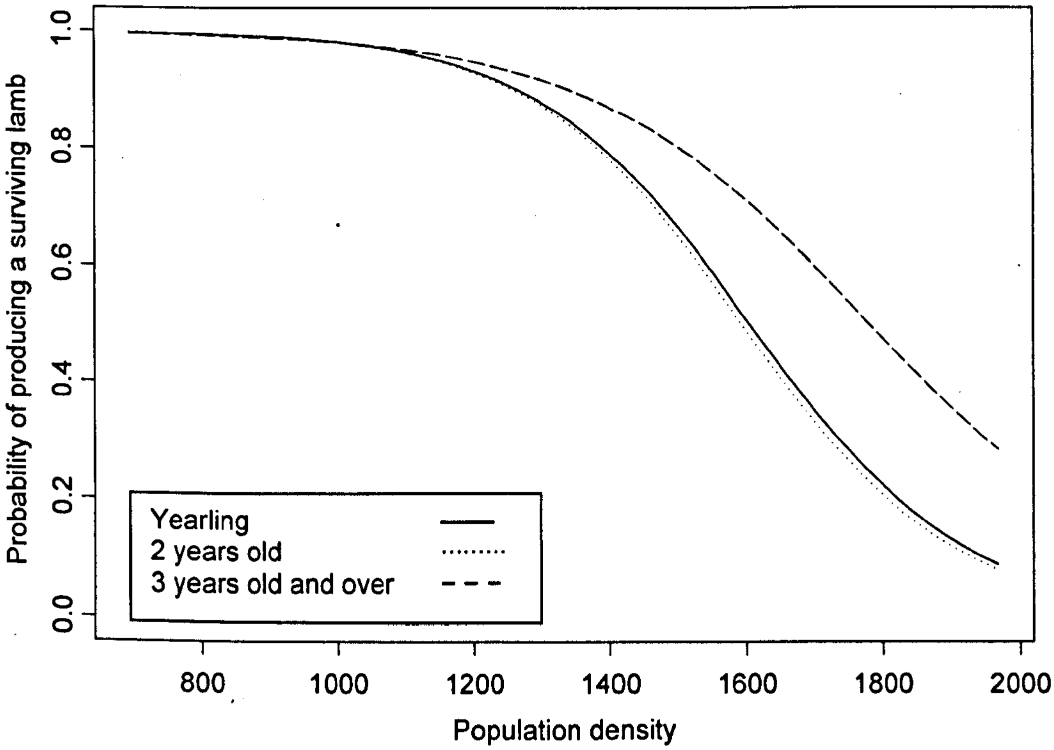


Figure 4.3: Effect of population size entering the previous winter on the probability of producing a lamb that survives to weaning age (four months old), in three ewe age groups (Yearlings, two years old, and three years old and over). Lines are drawn from model predictions

The main purpose of this chapter was to determine whether there was any evidence from observational data for a negative impact of parasites on ewe reproduction. Some support for this idea was found: parasite burden was associated with the probability of rearing a lamb to weaning age, in an interaction with ewe coat type ($\chi^2_3 = 4.99$, p=0.002, Figure 4.4). Specifically, the probability of rearing a lamb to weaning decreased as parasite burden increased in ewes with 'Dark Self' and 'Light Self' coats. In contrast, ewes of 'Dark Wild' and 'Light Wild' coat types had a high probability of rearing a lamb (>80% success) regardless of parasite burden (Figure 4.4). The factor coat can be collapsed into just two levels: 'Self', which includes

'Dark Self' and 'Light Self' coats; and 'Wild', which includes both 'Dark Wild' and 'Light Wild' coat types. In ewes of 'Self' coat type, the probability of rearing a lamb to weaning decreased as parasite burden increased, as described above, however the probability of rearing a lamb remained high in ewes of coat type 'Wild', with increasing parasite burden (Interaction: FEC (log10-transformed): Ewe coat (2 levels) ($\chi^2_1 = 6.70$, p=0.01).

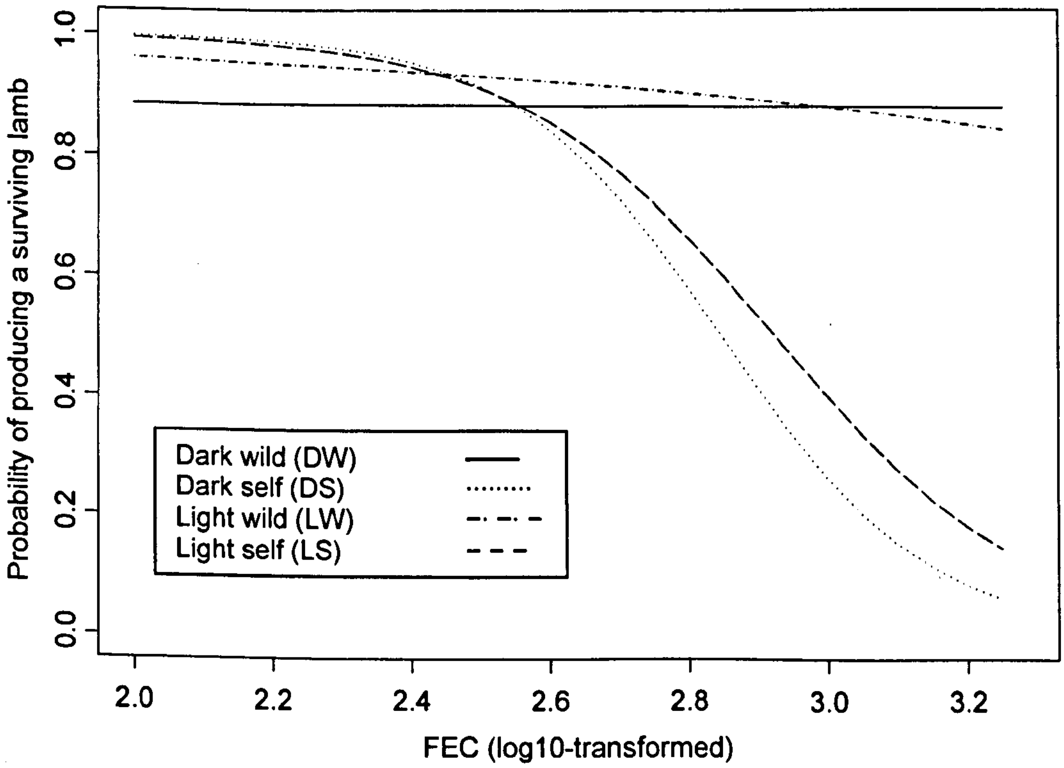


Figure 4.4: Effect of strongyle faecal egg count (FEC) on the probability of producing a lamb that survives to weaning age (four months old) in the four ewe coat types (Dark wild, Dark self, Light wild, Light self). FEC has been log-10 transformed. Lines are drawn from model predictions

4.4 Discussion

In accordance with previous studies, it was found that the probability of a ewe successfully rearing a lamb to weaning age (four months) was affected by her age, her weight in the previous summer, and the population density in the previous winter (Clutton-Brock *et al.* 1996, Clutton-Brock *et al.* 2004, Tavecchia *et al.* 2005). Adult ewes (three years and older) were most successful, with 89% rearing their lamb to weaning age, compared to only 49% in yearling ewes. However, body weight was also important, with heavier ewes (in the previous summer) having an increased chance of successfully rearing a lamb in yearling and two year old ewes. In contrast, the reproductive success of ewes aged three years and older was largely unaffected by their body weight in the previous summer. Their lambing success remained high (~ 90%) and was improved only marginally in the heaviest ewes. Since fecundity is high in Soay ewes (Clutton-Brock *et al.* 2004), it is very likely that ewes that are three years and older have previous breeding experience and that this is important in successfully rearing a lamb.

When the population on Hirta was high entering the winter, stresses caused by food shortage, and increased numbers of infective larvae on the pasture, reduced the ability of surviving ewes to successfully rear a lamb. This effect was exacerbated by age, as older ewes were able to cope with the pressures of increasing population density better than younger ewes (yearlings and two-year olds). This is probably due to the relative inexperience of younger ewes in rearing lambs, in comparison to ewes that are three years and older, and to their relatively poorer overall condition.

The main focus of this chapter was in trying to determine was there was any evidence from observational data for a negative impact of parasites on ewe reproduction. Surprisingly, a negative impact of parasite burden on ewe lambing success was observed only in ewes with Dark Self or Light Self coat types (see Chapter 2 for definitions); with lambing success dropping from >80% to approximately 20% when strongyle faecal egg count was high (1800 epg). In contrast, 'wild-type' sheep (i.e. Light wild and Dark wild) had consistently high lambing success regardless of parasite load. The reason that parasites impact on ewes of different coat types is unclear. Previous studies found no evidence of an effect of coat type on ewe lambing success, however the selection on coat colour and phenotypic responses are, as yet, not fully understood in Soays (Milner *et al.* 2004). Nevertheless, there is some evidence here that high parasite burden (measured in the previous August) may reduce the probability of successfully rearing a lamb to weaning age (four months old). This effect may be brought about by a trade-off between current reproductive effort and immunity to parasites. Resources must be directed towards fighting parasitic infection, in order to limit their pathogenic consequences (Gulland 1992); however it appears that this may have been costly in terms of limiting the availability of resources for successfully rearing a lamb to weaning.

Whilst the results of this analysis are suggestive of a possible trade-off between parasite resistance and reproductive effort, they are derived from purely observational data. As discussed in Chapter 1, it is notoriously difficult to establish the nature of life history trade-offs based on phenotypic correlations alone, due to variation between individuals in resource levels (van Noordwijk & de Jong 1986). Only by conducting appropriate manipulative experiments are the true underlying trade-offs likely to be revealed. For example, in *Callosobruchus* beetles, there is a positive phenotypic correlation between lifespan and fecundity, and it is only when egg production rates are experimentally manipulated that the inherent trade-off between egg production and residual lifespan is revealed (Wilson 1989, Lessells 1991). Similarly, it was only after experimental manipulation of gastro-intestinal parasites in Svalbard reindeer that it became clear that parasites can depress pregnancy rates (Stien *et al.* 2002). Thus, the next two chapters report the results of an experiment designed to manipulate parasite burdens in adult female Soay sheep, with to the aim of determining the effects of this on ewe body condition and reproductive success.

4.5 Summary

Ewes aged three years and older were more likely to rear a lamb successfully than younger ewes. Yearlings and two year olds were much less likely to rear a lamb to weaning age (four months old), however the success rate was improved substantially in heavier ewes. High population density reduced the probability of rearing a lamb successfully in all age groups, however the effect was minimised in ewes aged three years and older, probably due the benefits of previous breeding experience.

The only observed negative effect of parasite burden on lambing success was on ewes with 'Self' coat types. Ewes with Dark Self and Light Self coats had low success in rearing lambs when parasite burden was high, in contrast to the high lambing success observed in ewes with Dark Wild and Light Wild coat types.

Chapter 5

The impact of nematode parasites on body condition and fecundity in female Soay sheep: an experimental study

5.1 Introduction

There is growing evidence to suggest that parasites can have significant detrimental effects upon host survival, growth and fecundity (reviewed in Gulland 1995, Grenfell & Gulland 1995, Tompkins & Begon 1999). Comprehensive studies on red grouse have suggested that its main parasite (*Trichostrongylus tenuis*) may drive population cycles; through direct effects on mortality and by limiting reproductive success (Hudson 1986, Hudson & Dobson 1989, Dobson & Hudson 1992, Hudson *et al.* 1998). An experimental study on St Kilda has shown that removal of parasites by anthelmintic treatment causes an increase in daily survival in Soay sheep (Gulland 1992), and several other studies have confirmed that high levels of parasitism are correlated with lower survival in Soays (Illius *et al.* 1995, Coltman *et al.* 1999, Milner *et al.* 1999). However, there are few studies on wild ruminants that can clearly demonstrate an impact of parasites upon fecundity. One such study on reindeer (*Rangifer tarandus*) clearly demonstrates a significant effect of parasites on female reproductive success (Stien *et al.* 2002, Albon *et al.* 2002). Experimental removal of gastro-intestinal nematodes from a group of female reindeer caused an increase in pregnancy rate due to positive effects on female body condition.

The pathogenic consequences of gastrointestinal nematode infection in ruminants include reduction in food intake, weight loss, diarrhoea and endogenous protein losses (reviewed in vanHoutert & Sykes 1996, Holmes 1985, Symons 1985). Studies on domestic sheep have shown that protein is essential to mounting an immune-response to gastrointestinal parasites, which deflect protein resources away from growth, pregnancy, lactation and wool production (Adams & Liu 2003, Liu *et al.* 2003). Parasitic infection can cause reduction in weight gain; experimental infection of

Teladorsagia circumcincta in domestic sheep resulted in a 37% reduction in weight in grazing lambs (Coop *et al.* 1985). Domestic sheep in North-West Syria (Awassi ewes) treated for nematode infections were heavier and in a better condition than untreated ewes, leading to increased birth weight and weaning weight of their lambs (Thomson *et al.* 2000). In a recent study in Mexico, an experiment was set up that investigated the effect of natural and experimental infection of parasites on phenotypic variation in domestic sheep (*Ovis aries*, Pelibuey sheep) (Morteo-Gomez *et al.* 2004). Sheep were classified according to whether they were resistant or susceptible to nematode infection (*Haemonchus contortus*). Those that were classified as resistant had greater body weight and body condition scores, in comparison to susceptible sheep (Morteo-Gomez *et al.* 2004).

The Soay sheep population on the archipelago of St Kilda is an ideal system to examine the impact of parasites on host reproductive success. These primitive sheep have existed on St Kilda for at least 1000 years, and remain unmanaged with no predators or competitors. Soay sheep population dynamics on Hirta are unstable. There are typically periods of 2-3 years of rapid population growth with low levels of mortality; followed by a year in which there is a crash in population numbers due to high over-winter mortality (Clutton-Brock *et al.* 1997a, Clutton-Brock *et al.* 2004). This is primarily due to food shortage, and can be exacerbated by parasites and adverse weather conditions (Clutton-Brock *et al.* 2004). Parasitic prevalence in Soays is high. The gastrointestinal nematodes are the most important, in particular *Teladorsagia circumcincta,* as it is the most pathogenic and dominant species (Gulland and Fox 1992, Craig 2005. See Wilson *et al.* 2004, for a full description of the parasite community in Soay sheep).

By experimentally manipulating parasite burden in female Soays, it is possible to attempt to quantify the impact of parasites on fecundity. In August 2003, parasites were temporarily removed from a group of females. This should have allowed them to enter the harsh winter months with reduced costs of fighting parasitic infection, and therefore in a better general body condition. Does reduced cost of fighting parasitic infection throughout the winter months allow resources to be utilised elsewhere? If so, will these resources be directed towards improved survival and body condition in the ewe, and/or investment in future offspring? There are three main questions which will be addressed in turn in this chapter:

1) After the direct effects of the treatment (when the active ingredient has run its course), are there any longer term effects on parasite burden in the following months?

2) Can any effect of treatment be seen in terms of ewe reproductive success the following spring? Are there any quantifiable effects on the probability of lambing, or on the rate of twinning?

3) After accounting for inevitable differences between females pre-treatment, does treatment have any effect on body condition in the following summer?

5.2 Background & methodology

5.2.1 Parasitism in Soay ewes

During the spring there is a characteristic rise in parasite burden, that coincides with lambing (April to June), which is termed the Peri-Parturient Rise (PPR) (Connan 1968, O'Sullivan & Donald 1970,1973). PPR is usually measured in nematode strongyle egg counts, and the most important gastro-intestinal nematode contributing to the PPR in domestic sheep is widely regarded as *Teladorsagia spp.* (Morgan *et al.* 1951, Parnell *et al.* 1954, Crofton 1954, Dunsmore 1965, Connan 1968b, Herd *et al.* 1983, Bishop & Stear 2001). The PPR appears to occur as a cost of reproduction; in domestic sheep it has been shown that it does not occur in ewes that fail to breed or have lost their lamb (Crofton 1958, Field *et al.* 1960, Brunsdon 1964, Procter & Gibbs 1968, Shubber *et al.* 1981, Gibbs & Barger 1986).

In Soay sheep, a similar rise occurs, however the effect appears to be more complex and we are still striving to understand their spring rise in parasite burden. Parasite burden was measured by counting the number of strongyle parasite eggs per gram (epg) in faeces, the Faecal Egg Count (FEC) (see Chapter 2 for more information). For simplicity, in this analysis, a single *average* FEC (see section 5.3), was used as a measure of the peri-parturient rise in females. Chapter 3 considers patterns and trends in PPR among Soay sheep. After the spring rise from April to June, the parasite burden falls, and is lowest during late summer when the ewes are in peak body condition.

5.2.2 Bolussing experiment

During the annual August catch-up of 2003, fifty-one female Soay sheep aged four to seven years old, were caught and processed as normal, and assigned to either the Bolus (treated) or Control (not treated) group. Demographically the largest age group in the population, 24/51 ewes were four years old; the remainder were made up of ewes aged five (n=14), six (n=6) and seven (n=7) years old. Within each age group, ewes were assigned randomly to either the Bolus (n=25) or the Control (n=26) group using a predetermined sequence, which had been created using a random number generator (MS Access). The ewes in the Bolus group were administered with an anthelmintic bolus (Captec slow-release bolus; active ingredient Albendazole) which removes the parasite burden for at least 100 days. The boluses were administered orally with an applicator by Mrs Jill Pilkington, under a Home Office License (HO Project License no. PPL 60/2543). During the following seven days, a faecal sample was obtained from nearly all experimental ewes and faecal egg counts (FEC) were measured: some animals fled the study area for a few days after the summer catch-up, making it difficult to obtain faecal samples for all the ewes.

5.2.3 Data collection (Spring 2004)

During the lambing period of 2004, experimental ewes were monitored from April 1st until May 31st, and data were collected on lambing success and parasite burden. Regular censuses of the study area allowed accurate estimates of birth date, still births, neonatal mortality, ewe mortality, etc. All new lambs were easily identifiable either from their ear tag or, if untagged, through distinguishing morphological features, heft, and physical association (suckling) with their known mother. 55 out of

59 (93%) of the lambs from the study group were caught and tagged. Information about untagged lambs, such as sex and morph, was ascertained from direct observation.

Multiple faecal samples were collected throughout April and May for each ewe, with an average of eight samples per ewe (ranging from 2-14 samples). Parasite burden was measured by obtaining faecal egg counts.

5.2.4 Data collection (August 2004)

During the annual August catch-up in 2004, one year after bolussing, 25/49 experimental ewes were re-caught (2 of the initial 51 ewes had died since the spring). Many of their lambs were also caught (26/51, 8 of the initial 59 had died since the spring). As is usual, morphometric measurements were recorded and faecal egg counts obtained.

5.3 Data analysis

Data were analysed using general linear models (GLM's) in S-PLUS 2000 release 1 (*Mathsoft, Inc*). Minimal models were obtained by step-wise deletion of non-significant terms, using an α-value of 0.05. As only 25 of the 51 ewes were re-caught in August 2004, the sample size for analysis of these data was reduced quite considerably. Due to the small number of animals in this dataset, and the lack of residual degrees of freedom if too many terms were included in the model, interactions between terms were not tested in the maximal model, although interactions between final terms left in the minimal model were checked using an α-value of 0.025 to account for the greater number of tests carried out (Crawley 2002).

The response variable in all analyses was the trait in 2004 (e.g. body weight), or change in the trait from 2003 to 2004. Measures of ewe body condition were weight, skeletal growth and Body Condition Index (BCI). Hind-leg length is used as an indicator of skeletal size (Clutton-Brock & Pemberton 2004), and can be used to monitor growth. BCI was estimated using the following calculation (Stevenson 1994):

$$\text{Body Condition Index (BCI)} = \text{weight}/\,(\text{hind-leg length})^3$$

Parasite count data (post-treatment FEC, and early spring FEC) were analysed using a log-linear model in S-PLUS (GLM with Poisson errors and log link function). As explained above (section 5.2.1), the measure of the PPR used in this analysis was a single average of the spring FECs obtained for each ewe. However, spring FEC data was skewed with a negative binomial distribution, and therefore it was necessary to log10-transform the counts before taking an average value for each ewe. These data were then analysed using a GLM with normal errors. Parasite burden in the summer was analysed as the difference in FEC between August 2003 (before treatment) and August 2004 (after treatment). This approach was implemented for two reasons: including data from 2003 allowed comparisons with 'typical' August FEC, and using the *difference* as the response variable dealt with problems with the distribution of the data. August FEC values in 2003 and 2004 were not normally distributed, nor did they approximate the negative binomial distribution as expected, and attempts to transform the data were unsuccessful. However the difference between 2003 and 2004 did approximate a normal distribution and so these data were analysed using a GLM with normal errors.

The response variables, parasite burden in the spring and summer, and body weight, Body Condition Index (BCI), hind-leg length in the summer, were analysed in a two-stage analysis. In the first stage analysis, only terms relating to the ewe were included, e.g. age, weight, etc. In the second stage, the analysis was repeated and lambing traits such as lamb birth weight were added to the maximal model. This approach was taken to attempt to extract potentially important seasonal effects, as explained below.

Consider a trait that relates to the ewe, for example her body weight in August (2003). August weight may impact upon a measure of ewe general health during lambing in the following spring (e.g. spring 2004 parasite burden), and may also impact upon a measure of ewe health in the following summer (e.g. summer 2004 parasite burden). This approximates the first stage of the analysis: how does the trait (e.g. weight) of the ewe in summer 2003, impact upon traits (e.g. parasite burden) in the following spring (2004) and in the following summer (2004)?

Now consider additional traits or events that occur at lambing (2004), for example lamb birth weight. These lambing traits may also affect ewe health/condition during lambing (e.g. spring 2004 parasite burden) and in the following summer (e.g. summer 2004 parasite burden). However, as the lambing traits (e.g. lamb birth weight in spring 2004) may also be affected by the ewe traits (e.g. ewe weight in summer 2003), they must only be included in the second stage of analysis. In this chapter, measures of general health/condition in the ewe are considered as the response variables (e.g.

spring and summer parasite burden). Lambing traits such as lamb birth weight will be considered as the response in the next chapter.

Terms considered in all models (ewe traits) were the age of the ewe, coat type, horn type and treatment group. Ewe coat and horn type were included as explanatory variables, since there is evidence to suggest that horn type in particular influences ewe breeding success (Clutton-Brock *et al.* 1997). When analysing a particular response in 2004 (e.g. body weight), the value of that trait in 2003 was also included in the model. Terms considered to be lambing traits were as follows: lamb survival (alive or dead in August), lamb birth weight (corrected for day of capture, see below), Julian birth date (days after January 1st), litter size (twin/single) and lamb sex (male, female or both in the case of twin lambs)

As the date of first capture for newborn lambs varied, and weight increases rapidly over the first few days of life, birth weight was corrected for age at capture. Most lambs were caught within 5 days of birth, although several were just over a week old when first caught. To account for this, as in previous studies (e.g. Forchhammer *et al.* 2001, Wilson *et al.* 2005), relative birth weights were estimated by using the residuals from a linear regression of weight at first capture with age at first capture. This model provided a very good prediction of birth weight ($F_{1,39} = 46.44$, $p<0.0001$, $r^2=0.55$).

5.4 Results

5.4.1 Parasite burden

a) Post-treatment FEC (August 2003)

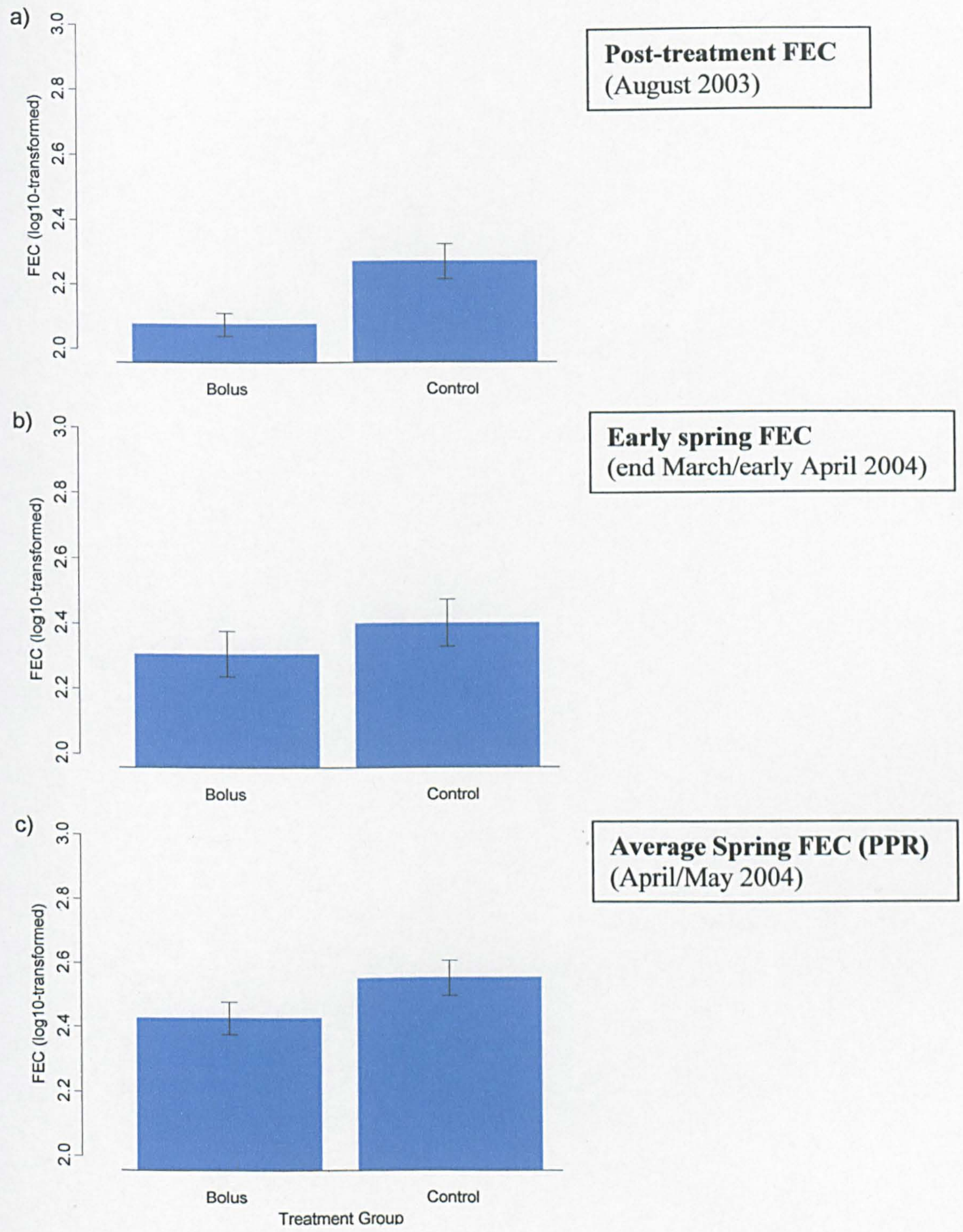
Measurement of FEC of ewes within a few days of starting the experiment in August 2003, confirmed that the administration of the anthelmintic had significantly reduced the parasite burdens of bolussed ewes (Figure 5.1a). Bolussed ewes had significantly lower FEC than the control animals ($F_{1,38} = 8.45$, $p=0.006$), with only four bolussed animals having a FEC higher than zero. In contrast, the control ewes averaged 100-200 epg (mean = 120 epg).

b) Spring FEC prior to lambing

Faecal egg counts before lambing (end of March, beginning of April 2004), and before the onset of the peri-parturient rise (PPR) were similar for all experiment ewes (100-200 epg). Of particular interest, there was no difference in FEC between treatment group ($F_{1,50} = 0.64$, $p=0.43$, Figure 5.1b). Thus, 6-7 months after bolussing, FEC of treated ewes were similar to those of control ewes.

c) Peri-parturient rise (average FEC)

The average spring faecal egg count in 2004 was not affected by the age of the ewe, horn type, coat type or treatment (Table 5.1). However, there was a trend for bolussed ewes to have a lower average FEC over the spring period (PPR), than control ewes ($F_{1,49} = 2.91$, $p=0.09$).



Post-treatment FEC
(August 2003)

Early spring FEC
(end March/early April 2004)

Average Spring FEC (PPR)
(April/May 2004)

Figure 5.1: Faecal egg counts (FEC) in bolus and control ewes a) post-treatment, b) early spring/pre-lambing and c) average spring FEC (PPR) (mean log10-transformed FEC). Error bars are standard errors. Data are log10-transformed for graphs. Analysis results (non-transformed data GLM with Poisson errors): a) post treatment, $F_{1,38} = 8.45$, p=0.006, b) prior to lambing, $F_{1,50} = 0.64$, p=0.43. Analysis results (log10-transformed GLM with normal errors): c) $F_{1,46} = 6.15$, p=0.017.

Adding lambing traits

After including lambing traits to the model, average spring faecal egg count was affected by ewe weight in 2003, treatment group and litter size (Full model: $F_{3,46}$ = 4.33, p=0.009, r^2 = 0.22; Table 5.2). The peri-parturient rise in Soay sheep is discussed in more detail in Chapter 3, however ewes treated with an anthelmintic bolus in 2003, had a lower parasite burden than untreated control ewes ($F_{1,46}$ = 6.15, p=0.017, Figure. 5.1c). The heaviest ewes in 2003 had the lowest average faecal egg count during lambing the following spring ($F_{1,46}$ = 4.87, p=0.032, Figure 5.2), and ewes that produced twins had a higher average parasite burden than ewes with single lambs ($F_{1,46}$ = 8.40, p=0.006, Figure 5.3).

Table 5.1: Analysis of variance table for GLM of average spring FEC (PPR). Stage 1 analysis (ewe traits only). Excluded terms were calculated by retesting them against the minimal model.

Terms	**d.f.**	**Sums of squares**	**Mean Squares**	**F**	**P**
Residual	50	3.59	0.07	-	-
Excluded terms					
Treatment group	**1**	**0.20**	**0.20**	**2.91**	**0.09**
Ewe age	1	0.05	0.05	0.69	0.41
Ewe coat	2	0.02	0.02	0.12	0.89
Ewe horn	2	0.05	0.05	0.37	0.69

Table 5.2: Analysis of variance table for GLM of average spring FEC (PPR). Stage 2 analysis (ewe and lambing traits). Excluded terms were retested against the minimal model.

Terms	d.f.	Sums of Squares	Mean Squares	F	P
Ewe weight 2003	**1**	**0.28**	**0.28**	**4.87**	**0.032**
Litter size	**1**	**0.49**	**0.49**	**8.40**	**0.006**
Treatment group	**1**	**0.36**	**0.36**	**6.15**	**0.017**
Residuals	46	2.67	0.06	-	-
Excluded terms					
Ewe age	1	0.01	0.01	0.09	0.77
Ewe coat	2	0.03	0.03	0.22	0.80
Ewe horn	2	0.03	0.03	0.22	0.80
Lamb birth weight	1	0.04	0.04	0.70	0.41
Lamb birth date	1	0.05	0.05	1.00	0.32
Lamb sex	2	0.03	0.03	0.52	0.60
Lamb survival	1	0.01	0.01	0.11	0.74

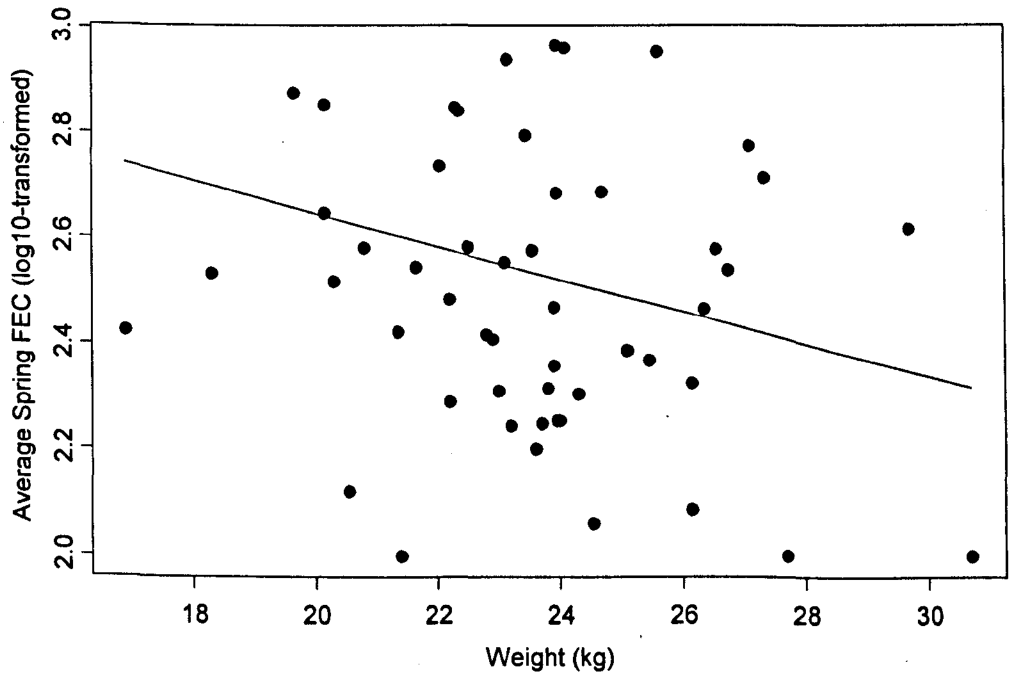


Figure 5.2: Relationship between ewe weight (kg) in August 2003 and the average spring FEC (mean log10-transformed FEC) in 2004 ($F_{1,46} = 4.87$, p=0.032). The fitted line is drawn from model predictions.

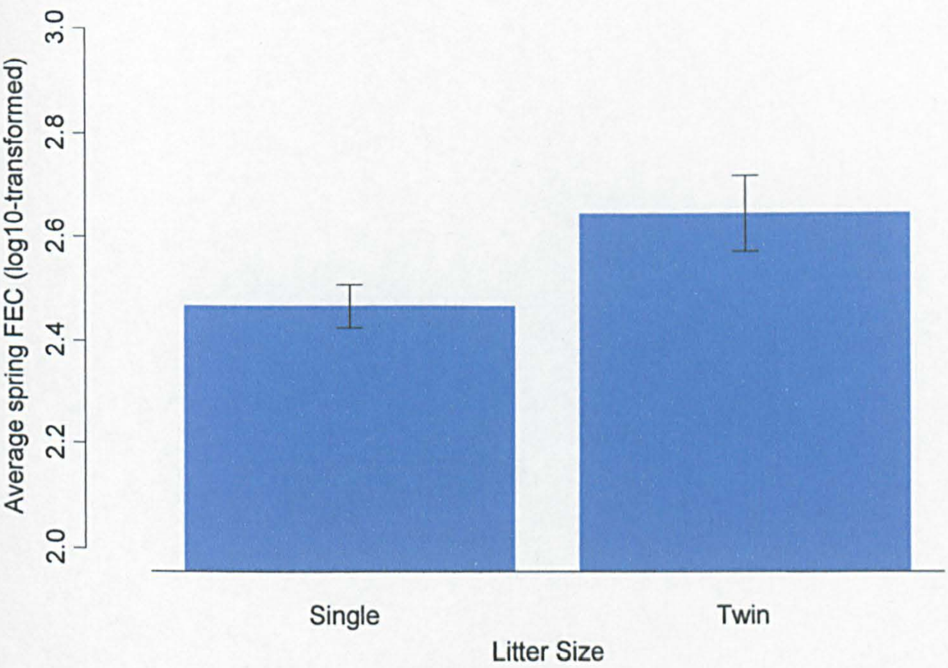


Figure 5.3: The average spring FEC (mean log10-transformed) of ewes giving birth to single and twin lambs ($F_{1,46} = 8.40$, p=0.006). Error bars are standard errors.

d) August FEC (2004)

The faecal egg count of ewes in August 2004, accounting for FEC in August 2003 (pre-treatment) was analysed, and results show that the bolussed ewes showed a drop in FEC, with an average difference in FEC of -200 epg, compared to the previous year (Figure 5.4). In contrast, control ewes had similar FEC to the previous year, on average there being zero difference in FEC. This change in FEC was significantly different between treatment group ($F_{1,18} = 4.6$, p=0.046, $r^2= 0.20$; Table 5.3).

Adding lambing traits

None of the lambing traits had any effect on August FEC in 2004 (after accounting for August FEC 2003) (see Table 5.3). The only factor effecting a change in August FEC was treatment group (see above).

Table 5.3: Analysis of variance table for GLM of August FEC. Stage 1 & 2 analysis (ewe and lambing traits). Excluded terms were retested against the minimal model.

Terms	**d.f.**	**Sums of Squares**	**Mean Squares**	**F**	**P**
Treatment group	1	**144500**	**144500**	**4.60**	**0.046**
Residuals	18	565000	31388	-	-
Excluded terms					
Ewe weight	1	27259		0.86	0.37
Ewe age	1	18270		0.57	0.46
Ewe coat	1	16000		0.49	0.49
Ewe horn	2	8728		0.13	0.88
Litter size	1	44696		1.38	0.26
Lamb birth weight	1	9918		0.26	0.62
Lamb birth date	1	66164		2.12	0.16
Lamb sex	2	94444		1.51	0.25
Lamb survival	1	4000		0.11	0.74

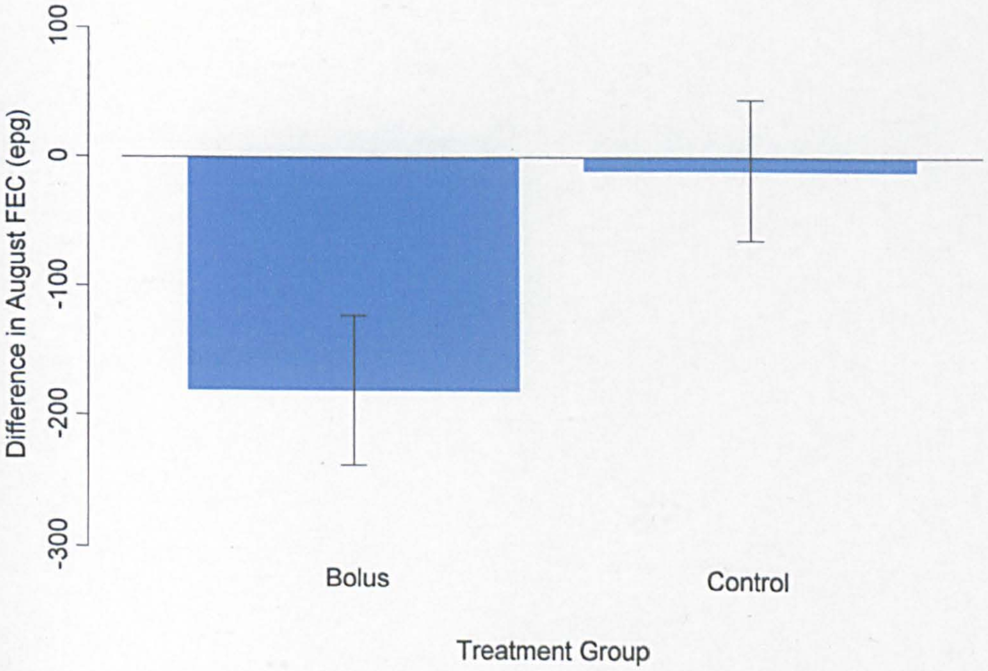


Figure 5.4: Faecal egg counts (FEC) in bolus and control ewes as the difference in August FEC from 2003 to 2004. Error bars are standard errors. Analysis results (GLM with normal errors): $F_{1,18} = 4.6$, p=0.046.

e) Average spring FEC (PPR) and August FEC (2004)

Since the peri-parturient rise (PPR) in FEC that occurs during the spring is linked to lambing events (see section 5.2.1 and Chapter 3) it is not feasible to include average PPR as an explanatory term in the analysis of August FEC in 2004. Nonetheless it is interesting to investigate possible associations between the FEC measured in spring 2004, and in August 2004. Therefore, PPR as the average spring FEC (mean log10-transformed FEC) was included as an explanatory term in a repeat of the stage two analysis (adding lambing traits) of August FEC (part d). This did not change the minimal model (see part d results above). Average spring FEC was not retained in the model as a non-significant term ($F_{1,17} = 3.67$, $p=0.072$).

5.4.2 Ewe survival & reproductive success

a) Ewe survival

Over-winter survival (2003/2004) in the study population was high (94%), with only 32 of the 494 sheep entering the winter recorded dead between January and May 2004. None of the ewes in the experiment died during the winter of 2003-2004. Only 2 ewes died (one Bolus, one Control) between lambing (parturition) in spring 2004 and August 2004; neither of these deaths occurred at parturition. As survival was very high among ewes, little could be attributable to the few cases where mortality did occur.

b) Probability of lambing

Only 1 out of 51 experimental ewes failed to lamb in 2004. Therefore there was no effect of treatment on the probability of lambing.

c) Probability of having twins

The incidence of twinning amongst the experimental ewes was 18% (9/50 ewes). The probability of twinning amongst the experimental ewes was not significantly affected by any term in the model. However, there was a non-significant positive association with ewe weight (in August 2003); in that the heaviest ewes were more likely to produce twins ($F_{1,48} = 3.29$, $p=0.075$). Across treatment groups, bolussed ewes were *twice* as likely to twin as control ewes: 25% (6/24) of bolus ewes had twins, while only 12% (3/26) of control ewes produced twins. However, this trend was not statistically significant, ($F_{1,48} = 1.40$, $p>0.1$), possibly because of low statistical power caused by small sample size.

5.4.3 Ewe body weight and condition (August 2004)

a) Skeletal growth (hind-leg length)

Hind-leg length in 2004 was correlated with hind-leg length in 2003, and the age of the ewe ($F_{2,22} = 222$, $p<0.0001$, $r^2 = 0.95$). Hind-leg length 2004 was strongly dependent on length in 2003 ($F_{1,22} = 430.06$, $p<0.0001$), and change in hind-leg length was reduced in older ewes ($F_{1,22} = 10.90$, $p=0.0032$).

Adding lambing traits

Including lambing traits does not change the minimal model; they do not affect hind-leg length.

Table 5.4: Analysis of variance table for GLM of ewe hind-leg length (mm) in 2004. Stage 1 & 2 analysis (ewe and lambing traits). Excluded terms were calculated by retesting them against the minimal model.

Terms	d.f.	Sums of Squares	Mean Squares	F	p
Ewe hind-leg 2003	**1**	**1209.64**	**1209.64**	**430.06**	**<0.0001**
Ewe age	**1**	**30.68**	**30.68**	**10.91**	**0.003**
Residuals	22	61.88	2.81	-	-
Excluded terms					
Treatment group	1	1.18		0.41	0.53
Ewe coat	1	0.02		0.01	0.93
Ewe horn	2	2.14		0.36	0.70
Litter size	1	0.85		0.28	0.60
Lamb birth weight	1	1.09		0.38	0.55
Lamb birth date	1	0.27		0.09	0.77
Lamb sex	2	6.91		1.21	0.32
Lamb survival	1	2.98		0.51	0.61

b) Body weight

Body weight in 2004 was related to body weight in 2003, treatment group and horn type ($F_{3,21} = 58.34$, $p<0.0001$, $r^2= 0.89$; Table 5.5). The largest effect on body weight in 2004, was weight in 2003 ($F_{1,21} = 131.95$, $p<0.0001$) explaining 63% of the variance in the data (Figure 5.5). Nearly all ewes lost weight between 2003 and 2004, on average 0.9 kg. Ewes of different horn types had different weight changes from 2003 to 2004: scurred ewes had less weight loss than polled and normal horned ewes (these two levels of the factor "horn" were collapsed into one level) ($F_{1,21} = 5.70$, $p=0.026$, Figure 5.6a). Treatment was also related to weight loss (Figure 5.6b), with the treated ewes showing less weight loss (0.43kg), than the control ewes (1.24 kg) ($F_{1,21} = 5.50$, $p=0.029$).

Adding lambing traits

After including lambing traits to the full model, the minimal model changed considerably (Table 5.6). Ewe weight in 2004 was affected by weight in 2003, and lamb survival ($F_{2,21} = 114.4$, $p<0.0001$, $r^2 = 0.92$). Once again, the most important predictor of weight in 2004 was weight in 2003 ($F_{1,21} = 153.2$, $p<0.0001$). After accounting for this, ewes that lost their lamb (within 8 weeks of parturition) lost less weight than ewes whose lambs survived until August 2004 ($F_{1,21} = 11.69$, $p=0.0025$, Fig 5.6c).

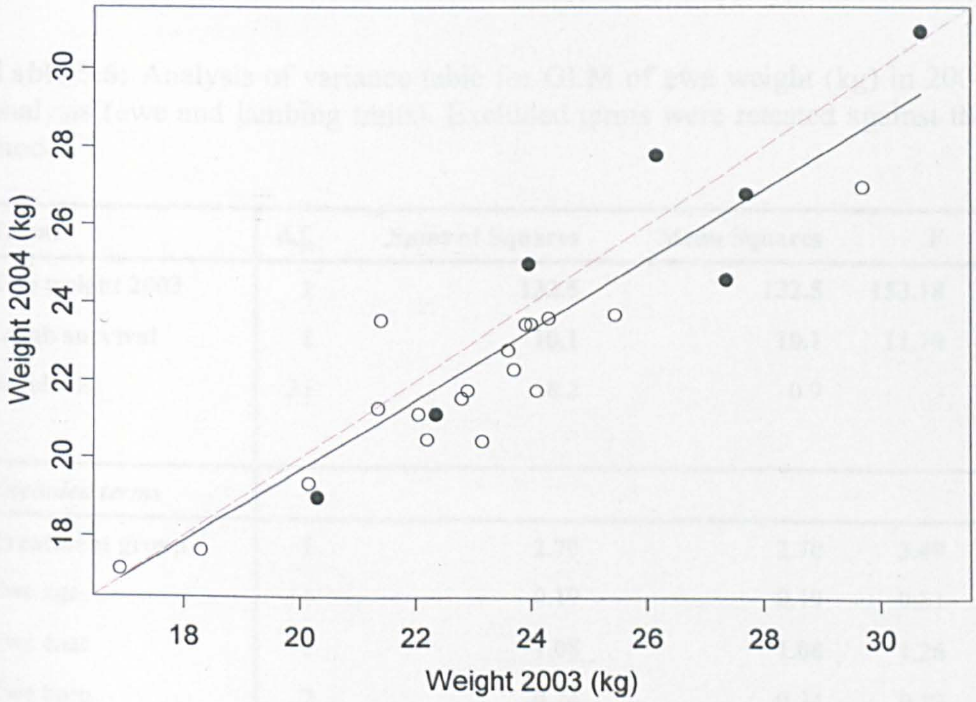


Figure 5.5: Correlation of ewe weight in August 2003 with weight in August 2004 (kg) ($F_{1,21} = 131.95$, $p<0.0001$). Fitted line is drawn from model predictions. The red dashed line is 1:1. Scurred ewes are represented with closed circles (•), normal horned and polled ewes are represented with open circles (◦).

Table 5.5: Analysis of variance table for GLM of ewe weight (kg) in 2004. Stage 1 analysis (ewe traits only). Excluded terms were calculated by retesting them against the minimal model.

Terms	**d.f.**	**Sums of Squares**	**Mean Squares**	**F**	**p**
Ewe weight 2003	**1**	**163.65**	**163.65**	**131.95**	**<0.0001**
Ewe horn	**1**	**7.07**	**7.07**	**5.70**	**0.026**
Treatment group	**1**	**6.82**	**6.82**	**5.50**	**0.029**
Residuals	21	26.04	1.24	-	-
Excluded terms					
Ewe age	1	1.00	1.00	0.80	0.38
Ewe coat	1	0.16	0.16	0.13	0.73

Table 5.6: Analysis of variance table for GLM of ewe weight (kg) in 2004. Stage 2 analysis (ewe and lambing traits). Excluded terms were retested against the minimal model.

Terms	**d.f.**	**Sums of Squares**	**Mean Squares**	**F**	**p**
Ewe weight 2003	**1**	**132.5**	**132.5**	**153.18**	**<0.0001**
Lamb survival	**1**	**10.1**	**10.1**	**11.70**	**0.0025**
Residuals	21	18.2	0.9	-	-
Excluded terms					
Treatment group	**1**	**2.70**	**2.70**	**3.49**	**0.08**
Ewe age	1	0.19	0.19	0.21	0.65
Ewe coat	1	1.08	1.08	1.26	0.28
Ewe horn	2	0.24	0.24	0.12	0.88
Litter size	1	0.11	0.11	0.12	0.73
Lamb birth weight	1	0.88	0.88	2.20	0.16
Lamb birth date	1	0.49	0.49	1.21	0.28
Lamb sex	2	1.08	1.08	0.60	0.56

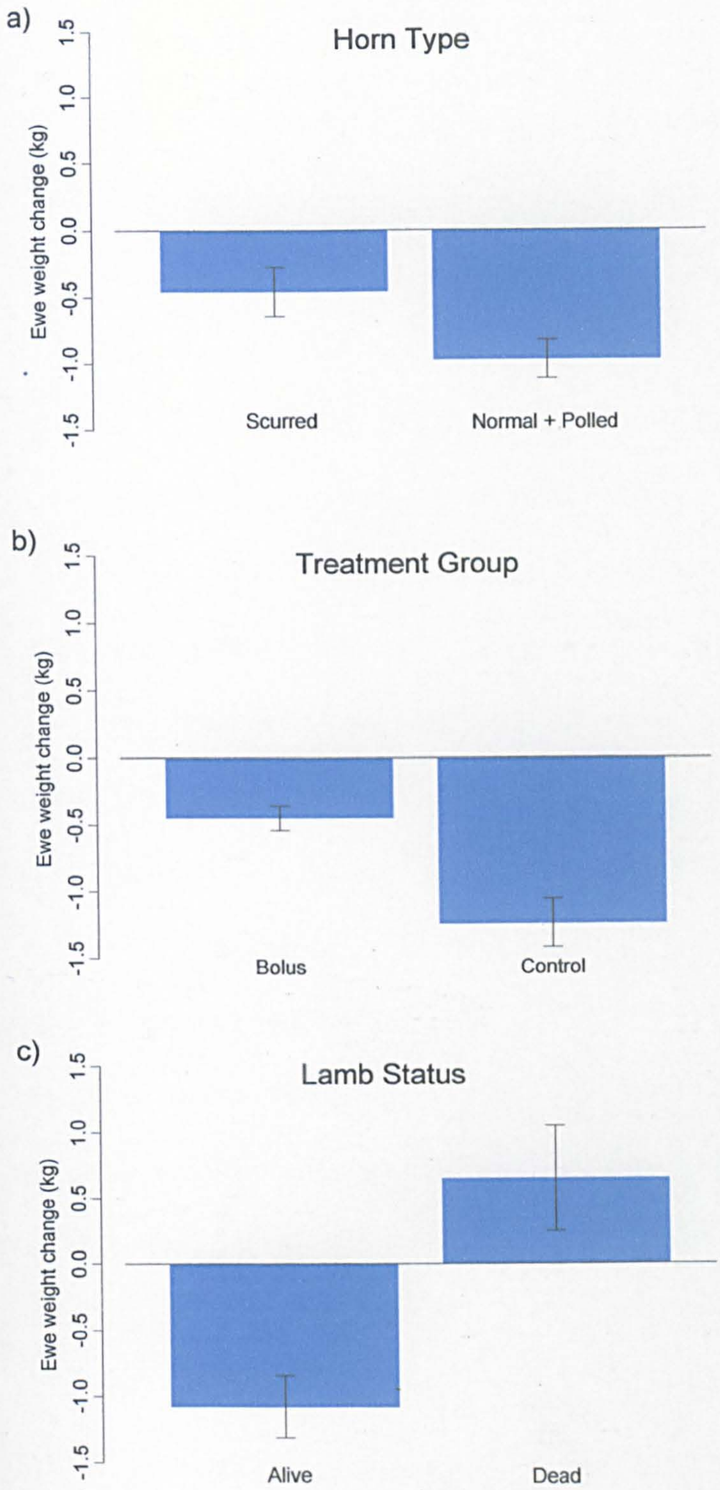


Figure 5.6: Weight change (kg) in ewes from August 2003 to August 2004, a) with different horn-types ($F_{1,21}$ = 5.70, p=0.026), b) in different treatment groups ($F_{1,21}$ = 5.50, p=0.029), and c) whose lambs were either alive, or had died by August 2004 ($F_{1,21}$ = 11.69, p=0.0025). Error bars are standard errors.

c) Body Condition Index (BCI)

As the BCI is a function of body weight, it is not surprising that the minimal model for BCI in 2004 is very similar to that of weight in 2004. The minimal model retains BCI in 2003, horn type and treatment group ($F_{3,20} = 53.81$, $p<0.00001$, $r^2= 0.89$; Table 5.7). BCI in 2003 correlates strongly with BCI in 2004, explaining 60% of the variance ($F_{1,20} = 133.0$, $p<0.00001$). As with weight, there was a general trend for ewes to lose body condition over this period, with an average drop in the BCI of 6%, from 2003 to 2004. Polled ewes lost more BCI between 2003 and 2004, than normal and scurred horn types ($F_{1,20} = 5.29$, $p=0.032$). However, the most pertinent result was that the loss in BCI in control ewes was significantly greater than that in bolussed ewes ($F_{1,20} = 4.69$, $p=0.043$); with bolussed ewes losing 4.6% in BCI from 2003 to 2004, compared to a loss of 7.5% in control ewes.

Adding lambing traits

Including lambing traits changed the model, such that BCI in 2004 was related to BCI in 2003, lamb survival, litter size and treatment group ($F_{4,18} = 52.84$, $p<0.0001$, $r^2 = 0.92$; Table 5.8). Body Condition Index in 2004 was strongly dependent on BCI in 2003 ($F_{1,18} = 187.44$, $p<0.0001$). The positive effect of the anthelmintic on body condition was still evident after including lambing traits; control ewes lost more in their BCI than bolussed ewes between 2003 and 2004 ($F_{1,18} = 8.10$, $p=0.011$, Figure 5.7a). Ewes with lambs that died did not lose as much in BCI as ewes with lambs that were still alive in August 2004 ($F_{1,18} = 4.99$, $p=0.038$, Figure 5.7b). Litter size was also associated with the ewe body condition index; ewes that bore twin lambs lost more of their BCI than those with single lambs ($F_{1,18} = 5.58$, $p=0.03$, Figure 5.7c).

Table 5.7: Analysis of variance table for GLM of ewe Body Condition Index (BCI) in 2004. Stage 1 analysis (ewe traits only). Excluded terms were calculated by retesting them against the minimal model.

Terms	d.f.	Sums of Squares	Mean Squares	F	p
Ewe condition 2003	**1**	**4.08e-012**	**4.08e-012**	**133.01**	**<0.0001**
Ewe horn	**1**	**1.62e-013**	**1.62e-013**	**5.30**	**0.032**
Treatment group	**1**	**1.43e-013**	**1.43e-013**	**4.67**	**0.043**
Residuals	20	6.13e-013	3.07e-014	-	-
Excluded terms					
Ewe age	1	6.75e-015		0.21	0.65
Ewe coat	1	2.52e-015		0.08	0.78

Table 5.8: Analysis of variance table for GLM of ewe Body Condition Index (BCI) in 2004. Stage 2 analysis (ewe and lambing traits). Excluded terms were calculated by retesting them against the minimal model.

Terms	d.f.	Sums of Squares	Mean Squares	F	p
Ewe condition 2003	**1**	**4.48e-012**	**4.48e-012**	**187.44**	**<0.0001**
Litter size	**1**	**1.33e-013**	**1.33e-013**	**5.58**	**0.029**
Treatment group	**1**	**1.94e-013**	**1.94e-013**	**8.10**	**0.011**
Lamb survival	**1**	**1.19e-013**	**1.19e-013**	**4.99**	**0.038**
Residuals	18	4.30e-013	2.39e-014	-	-
Excluded terms					
Ewe age	1	3.59e-014		1.55	0.23
Ewe coat	2	5.85e-014		2.68	0.12
Ewe horn	2	6.47e-014		1.42	0.27
Lamb birth weight	1	2.62e-014		1.86	0.19
Lamb birth date	1	1.91e-016		0.01	0.92
Lamb sex	2	6.70e-014		1.47	0.26

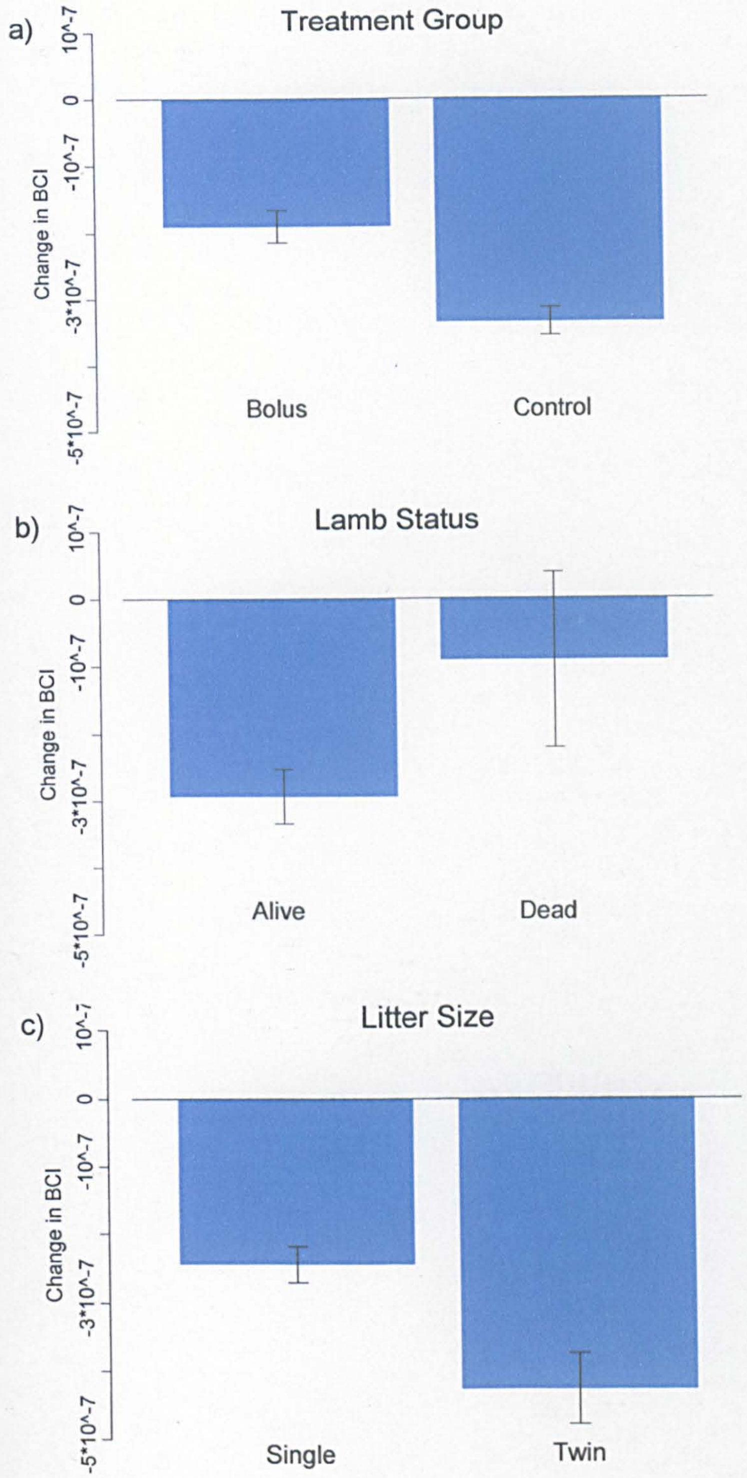


Figure 5.7: Change in Body Condition Index (BCI) in ewes from August 2003 to August 2004, a) in different treatment groups ($F_{1,18} = 8.10$, $p=0.011$), b) whose lambs were either alive, or had died by August 2004 ($F_{1,18} = 4.99$, $p=0.038$), and c) in ewes with different litter size ($F_{1,18} = 5.58$, $p=0.029$). Error bars are standard errors.

d) Average spring FEC (PPR) and ewe body condition (weight and BCI).

Since the peri-parturient rise (PPR) in FEC that occurs during the spring is linked to lambing events (see section 5.2.2 and Chapter 3) it is not feasible to include average PPR as an explanatory term in the analysis of ewe August weight and Body Condition Index (BCI) in 2004. However, as a post-hoc analysis to examine any possible relationship, PPR as the average spring FEC (mean log10-transformed FEC) was included as an explanatory term in a repeat of the stage two analysis (adding lambing traits) of weight and BCI (part b and c).

Including PPR in the analysis changed the minimal model considerably for both ewe weight and BCI in August 2004. Weight in 2004 was related to weight in 2003, lamb survival and average spring FEC ($F_{3,20} = 168.4$, $p<0.0001$, $r^2 = 0.96$). High average spring FEC was associated with greater weight loss by August 2004 ($F_{1,20} = 24.15$, $p<0.0001$, Figure 5.8a). BCI in August 2004 was related to BCI in 2003, and average spring FEC ($F_{2,21} = 190.1$, $p<0.0001$, $r^2 = 0.95$). Similarly, high average spring FEC was associated with greater loss in Body Condition Index (BCI) by August 2004 ($F_{1,21} = 41.66$, $p<0.0001$, Figure 5.8b).

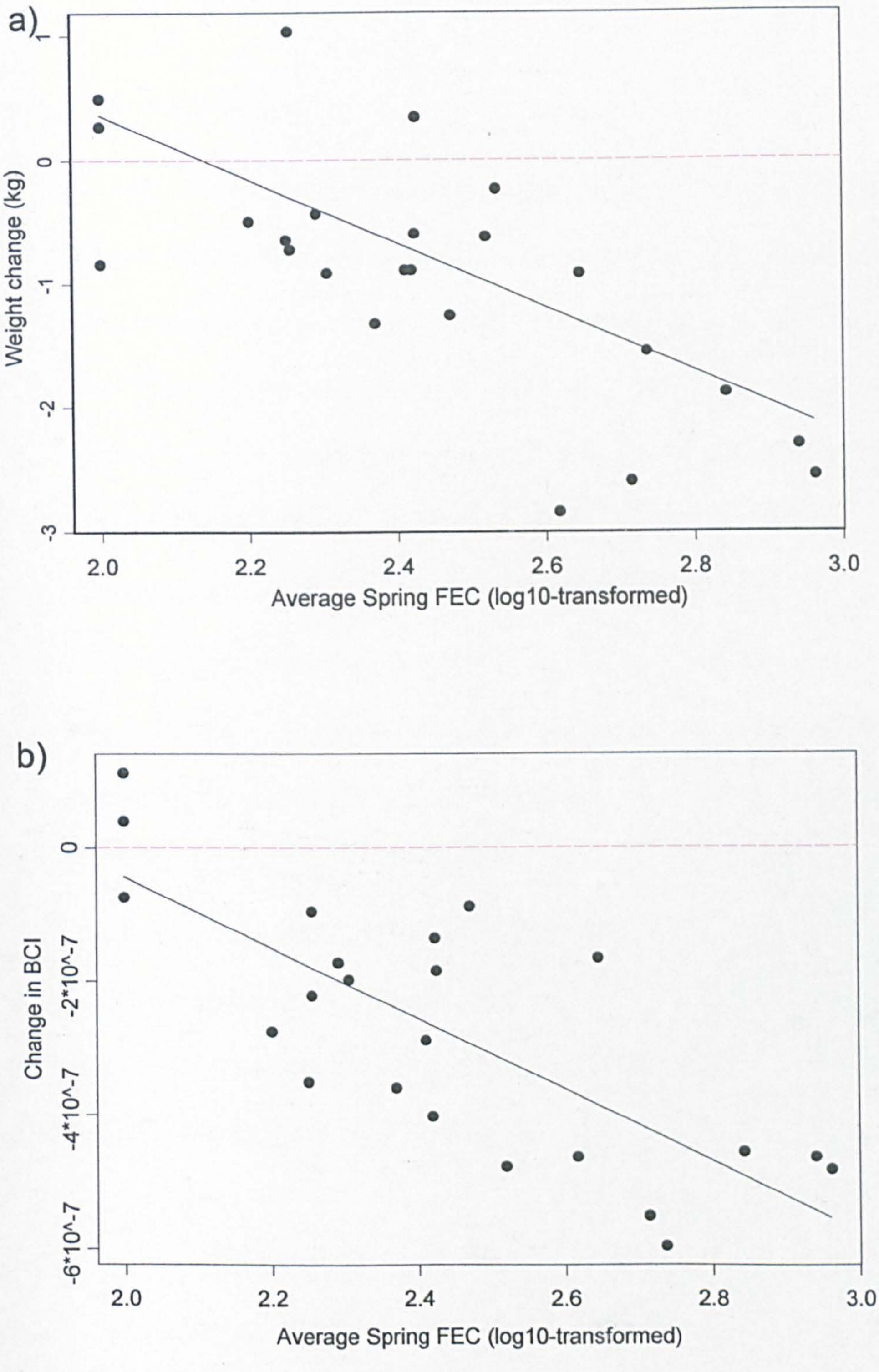


Figure 5.8: Relationship between average spring FEC (2004) (mean log10-transformed FEC), and a) weight change (kg) in ewes from 2003-2004 ($F_{1,20} = 24.15$, $p<0.0001$), and b) change in BCI in ewes from 2003-2004 ($F_{1,21} = 41.66$, $p<0.0001$). Fitted lines are drawn from model predictions.

5.5 Discussion

5.5.1 Parasite burden

Pivotal to this experiment, administration of anthelmintic bolus successfully removed the parasite burden from the treated ewes, as the low FEC measured in the days following treatment clearly demonstrated. The manufacturer's state that the bolus is effective at preventing establishment of parasites for approximately 100 days in domestic sheep, after which time, parasites may become re-established. This was shown to be the case, as by early April, approximately 7 months after bolussing, all ewes had a similar faecal egg count, with an average of 100-200 eggs per gram. We can therefore be confident that the treated ewes had reduced costs of fighting parasite infection going into, and probably throughout much of the winter, since densities of infective larvae on the pasture are very low during the winter months (Wilson *et al.* 2004). However, just before lambing (towards the end of March, or beginning of April) and prior to the onset of the characteristic spring rise in parasite burden (PPR), there were no detectable differences between the parasite burdens of treated and control sheep. So, did this temporary relief of parasite burden manifest re-direction of resources into other aspects of the ewe's life?

The peri-parturient rise occurs as the ewes become immuno-compromised, due to the costs of gestation, parturition, and particularly lactation. Although the direct effect of the treatment had worn off by the start of spring, the putative benefits of having reduced costs of fighting parasitic infection during the winter may have allowed treated ewes to enter the spring in a better condition and to deal with these reproductive costs more successfully. This may have been a reason for a reduced peri-parturient rise in parasite burden amongst bolussed ewes. Alternatively, the lower PPR in treated ewes could be due to the direct effect of the bolus on the larval stages of parasites. In the life cycle of *Teladorsagia circumcincta*, arrested development of fourth stage larvae can occur in the abomasum in late winter (Armour *et al.* 1969). De-arrestment occurs approximately three months later, at around the time of lambing and the PPR. Therefore the lower PPR in treated ewes could be because putative arresting larvae have been killed by the anthelmintic bolus prior to arrest.

Apart from the benefit of treatment, the other main effects on PPR were litter size and weight. Ewes that produced twins (normally the heaviest, see below) suffered a higher peri-parturient rise, as this is presumably more costly than producing a single lamb. However, even after accounting for this, heavy ewes were in better condition and had a lower PPR than lighter ewes.

August faecal egg counts, twelve months after bolussing, were affected only by treatment group. Bolussed ewes had lower FEC than control ewes, which may suggest that the treated ewes coped more effectively with the costs of reproduction during the spring, resulting in a lower parasite burden in the summer. However, was the lower August FEC in bolussed ewes' merely a result of the lower PPR in the spring, since high PPR was associated with high summer FEC? Including the average spring FEC (PPR) in the analysis, treatment group was retained in the model, and PPR was non-significant in describing August FEC. Therefore in terms of parasite burden, there were long-term effects of the bolus treatment on spring and summer FEC.

Treatment was successful in relieving ewes from their parasite burden temporarily, however by early spring the direct effects of bolussing had worn off and all ewes had similar parasite burdens. Longer term effects of having reduced parasitic infection throughout the winter were manifested in suffering lower costs of reproduction in terms of a lower PPR. It also appears that this was transferred through to the summer months, where lower FEC were also observed in bolussed ewes. Is this advantage of improved condition throughout the lambing period transferred to the offspring? Are ewes in a better condition able to invest more to their offspring during gestation leading to higher birth weights; or provide an increase in milk production/quality which could increase lamb growth rates? These are questions that will be addressed in the next chapter. In the next section, the possible consequences that the improved condition of the ewe may have upon the likelihood of lambing and prevalence of twinning, are considered.

5.5.2 Survival and reproductive success

Survival

There was no effect of treatment on survival in the experiment ewes. None of the ewes died over winter, and only 2 died in the period between lambing and August 2004. In 1988, Gulland (1992) treated a group of male lambs, yearlings and two-year olds, and female yearlings with anthelmintic boluses. During the winter of 1988/1989, there was a population crash in which over 70% of all individuals died. There were no effects of treatment on absolute survival. However, the rate of daily survival in treated animals was significantly higher than in control animals. In a similar experiment during a year of *low* mortality (1991/1992), survival of female lambs and yearlings, and male yearlings, was significantly higher in treated animals compared to controls (Gulland *et al.* 1993). These experiments successfully showed direct effects of parasites on survival in younger age-classes of Soay sheep, in years of both high and low mortality. In a further experiment, involving anthelmintic treatment of young adult male and female Soays (two-year old) prior to the crash of 2001/2002, there was no difference in the survival of treated and control animals, however statistical power was very low due to very high mortality (Jones 2004). Until now, experimental evidence for the negative effects of parasites on survival in older animals has not been available. This experiment suggests that in a year of low mortality, experimental removal of parasites may not have a discernable impact upon survival in adult female Soay sheep.

To summarise, it appears that in years in which overall mortality is *low*, parasite burden can affect survival in lambs and juveniles (Gulland *et al.* 1993). In adults, survival may not be affected in females; however the effect on males is as yet untested. This could suggest that only juvenile animals are affected by parasites to such an extent as to cause death, rather than just a loss in condition. This could be because adults have stronger immune systems or there is a genetic advantage to adults since they have already undergone selection. However, this can only be speculation until further experimental work can be done in adult males in a year of low mortality.

In years of *high* mortality, removal of parasites improved daily survival in lambs and yearlings (Gulland 1992); however it does not appear to affect the survival of young adults (Jones, unpublished data). It has yet to be determined experimentally, the effect of parasites on survival in older animals during a year of high mortality.

Probability of lambing

The probability of lambing is typically high in female Soay sheep: approximately 80% of adult ewes produce lambs, irrespective of population density. In contrast, the incidence of lambing in younger females varies with population density, ranging from around 10% when the population size is high, to 80% when the population is low (Clutton-Brock *et al.* 1996, Clutton-Brock *et al.* 2004). In this experiment, all ewes were adults (see section 5.2.3), and fecundity was high, with all but one of the ewes producing lambs in spring 2004. These figures also confirm that neither the condition of adult ewes, nor treatment to remove parasites, had any effect on fecundity (in 2004). In support of this, Clutton-Brock *et al.* (1996) found that variation in body weight had no effect on fecundity in adult ewes. A previous bolussing experiment, performed during a year of high mortality found no difference in fecundity between surviving treated and control ewes (Gulland *et al.* 1993). It is interesting to compare those results with this experiment where, during a year of *low* mortality, there was also no evidence of a positive effect of treatment on fecundity.

These fecundity trends within Soay females are in stark contrast to those in another wild ruminant. In a similar bolussing experiment on Svalbard reindeer, removal of gastro-intestinal parasites caused an average increase of 11% in the rate of pregnancy (Stien *et al.* 2002, Albon *et al.* 2002). The positive effect on fecundity was channelled through effects on condition, with treated hinds showing an increase in body mass and back fat depth.

Probability of twinning

It has previously been shown that females that bear lambs at 12 months old do not produce twins and that among adult females the rate of twinning ranges from 2-23% across years, with the highest incidence of twinning occurring in years of low population density (Clutton-Brock *et al.* 1991). In this (2003-2004) experimental group, all females were mature adults, therefore variation in twinning rates is more likely to be dependent on weight. Clutton-Brock *et al.* (1996) showed that among adult ewes of weight category 15-20kg, 0-8% of ewes bore twins, while twinning in adult ewes over 25kg ranged from 29-38%. In this experiment (2003-2004), weight in August did not significantly affect rates of twinning, however there was a trend for the heaviest ewes to bear twins. Within different weight categories, rates of twinning fell within the ranges previously described: no ewes (0% - 0/3) between 15-20kg bore twins; 14% (5/35) in the weight range 20-25kg bore twins; and 33% (4/12) of ewes over 25kg bore twins.

Although statistically there was no effect of treatment on rates of twinning, probably due to low statistical power (small sample size), there was a positive trend for bolussed ewes to bear twins: 25% (6/24) of bolussed ewes produced twins, while the incidence in control ewes was only 12% (3/26). In support of this, there is some evidence to suggest that ewes bearing twins have a lower FEC in the previous August than ewes with single lambs (L.J.Tempest unpublished data). This suggests that parasites may impact upon reproductive success in Soay females.

Previous work has shown that, as a strategy, twinning is best adopted by females in the best condition (Clutton-Brock *et al.* 1996). This is primarily a function of body weight, and it has been shown that heavy ewes have greater reproductive success via heavier lambs, higher conception rates and higher rates of twinning (Clutton-Brock *et al.* 1997b). However, in this experiment there is some evidence to suggest that ewes in superior condition, through lower costs of parasitism, are also able to transfer this into production of more twins.

5.5.3 Ewe body condition (August 2004)

Ewe body weight, Body Condition Index (BCI) and hind-leg length in 2004 were highly correlated with the value in the previous year and, in general, ewes lost weight and body condition between 2003 and 2004. This is probably due to food shortages resulting from high population density. Over the winter months, food stresses are high, and the vegetation intake during this time is below the level necessary to maintain body condition (Milner & Gywnne 1974, Clutton-Brock *et al.* 1997a). Therefore, all animals lose weight during winter, and only start to regain lost weight when plant productivity increases in late spring. As mortality was low throughout the winter of 2003-2004, the population density remained high, increasing from 1568 animals in August 2003 to 1996 in 2004. This inevitably led to increased competition for food and may have delayed weight gain into the summer of 2004.

Selection for horn type is not fully understood in Soays. However there appears to be a fitness advantage in scurred females: scurred ewes had lowest weight and condition losses between 2003 and 2004, with polled ewes suffering the greatest losses. Once the reproductive traits had been added to the models, any effect of horn type on ewe body condition and weight in August 2004 disappeared. This would suggest that benefits of being a scur may be prominent during the lambing season. Scurred females tend to be heavier than other females, and previous studies have shown that this leads to higher breeding success due to higher conception rates, and higher weaning rates (Clutton-Brock *et al.* 1997b). In this analysis, horn type did not have any effect on parasite burden during the spring or on conception rates.

Not surprisingly, ewes with greater costs of reproduction suffered a greater toll on their body weight and condition. Raising twins proved costly, as these ewes lost more body condition than ewes raising single lambs. In the few cases where lambs died, ewes had reduced condition and weight losses. These differences are probably due to the costs of lactation, with mothers of twins having increased lactation efforts, while lactation ceases with the death of a lamb. The main cost of reproduction for females is lactation, as it is energetically more expensive than gestation and parturition (Clutton-Brock *et al.* 1989).

Initially, without considering effects of reproductive effort, and after accounting for the trait in 2003, it was found that removal of parasite load by anthelmintic treatment had a positive effect on ewe body weight and BCI, but no observable effect on hindleg length. Ewes that were bolussed to remove their parasites had lost significantly less weight and condition, than those ewes in the control group (bolussed ewes lost only 0.43 kg in weight, and 4.6% in BCI, compared to 1.24 kg weight loss, and 7.5% loss in BCI, in control ewes). The positive effect of treatment on ewe weight disappeared when reproductive traits were included in the analysis. However treatment was retained in the model describing ewe Body Condition Index, with the positive effects of treatment still evident in bolussed ewes. These results would suggest that treatment afforded the bolussed ewes the advantage of improved body weight and higher BCI, but that this advantage may have been expressed during lambing. Indeed this does appear to be the case: if average spring FEC (PPR) is included in the model, it explains much of the variation in summer weight and condition, and is highly significant. Those ewes with the highest average FEC during lambing time, suffered the greatest losses in body weight and BCI.

Previous work on summer FEC has shown negative correlations with weight and hind leg length in both sexes (Wilson *et al.* 2004). In contrast, there was no effect of FEC on growth (change in weight and hindleg length), except in male lambs where FEC was found to significantly reduce growth in hindleg length. During the bolus experiment of 1991/1992 (Gulland *et al.* 1993), the removal of parasites had no effect on hindleg growth or weight gain in female lambs or yearlings, but positively affected weight gain in yearling males. Perhaps it is possible that the benefit of treatment on the condition of female yearlings was instead expressed and utilised during the spring, as has been suggested in the adult females in this study.

The positive effect of treatment on BCI and weight in the August of 2004 appears to be channelled through factors related to ewe condition around the time of lambing. In this experiment it seems possible that positive effects of treatment are transferred and utilised at lambing, so that treated ewes may be less immuno-compromised and suffer a lower PPR. This is then, at least in part, transferred to the summer when these ewes are in a better condition. There is also a tendency for treated ewes to have higher twinning rates. It seems quite likely, therefore, that positive effects of treatment should be transferred to compensate other reproductive costs, or provide direct benefits to offspring. It is expected that a treated ewe should be able to invest more in offspring; leading to higher birth weights, faster growth rates, and greater survival. This is clearly a very complex relationship, and further work is needed to tease apart the effects of the costs of parasitism, and how resources are partitioned into reproductive effort.

5.6 Summary

The anthelmintic treatment of adult ewes to temporarily remove parasite burden, does have an effect on reproductive success and body condition in Soay ewes. In terms of long term effects on parasite burden in the year following treatment, treated ewes appeared to be less immuno-compromised around the time of lambing, and consequently had a lower peri-parturient rise. This was partly translated into lower August parasite burdens. During a winter of low mortality, survival and the probability of having a lamb were not affected by treatment. Nonetheless treated ewes did show a tendency to produce twins more commonly than the control ewes. Body Condition Index (BCI) and weight in treated ewes in August (2004) was significantly higher than in control animals. However this effect is likely to have been expressed during the spring, as August BCI and weight appear to be correlated with a measure of condition in the spring, the peri-parturient rise in FEC. It is unfortunate, although ethically necessary, that data cannot be obtained on the weights of ewes during lambing time. This is because the sheep are in their poorest condition during the spring, and attempting to capture them at this time puts unnecessary stresses upon them. Despite this, measures of parasite burden in the spring and summer, and morphometric measurements obtained in the summer, all indicate that treatment had a positive effect on body condition in the ewes during the lambing season.

Positive effects of treatment in Svalbard reindeer are on pregnancy rates, whereas in Soays this is not the case. In such a highly fecund population where pretty much everything that can lamb, does lamb, perhaps this is hardly surprising. Parasites may not affect fecundity *per se*, but it does affect the body condition of the ewe during lambing time which impacts on reproductive success in terms of litter size. Whether treatment influences the ewes' ability to invest in their lambs, is the next subject to be addressed.

Chapter 6

The impact of nematode parasites in female Soay sheep on the survival, early development and suckling behaviour of their offspring: an experimental study

6.1 Introduction

The aim of the previous chapter was to assess if the chemical removal of parasites in female Soay sheep had any effects on ewe body condition and fecundity. It was found that the removal of parasites from a group of ewes improved their body condition compared to ewes in a control group. Treatment resulted in a marginally non-significant increase in average litter size, with the incidence of twinning being twice as high in treated ewes as control ewes. The next obvious question to ask is if any positive effects of treating the *ewe* are transferred to their *lambs*; in terms of enhanced survival, growth and/or development.

In animal populations, the condition of the mother is very important and can impact strongly upon offspring birth weights, growth rates and survival. Offspring birth weight is positively related to maternal body weight in several mammalian species, including: mountain goats (*Oreamnos americanus,* Cote & Festa-Bianchet 2001b), grey seals (*Halichoerus grypus* F., Pomeroy *et al.* 1999), Alaskan moose (*Alces alces gigas,* Keech *et al.* 2000) and domestic sheep (Kenyon *et al.* 2004). Birth weight is also related to mother's age in reindeer (*Rangifer tarandus,* Weladji *et al.* 2002), and the age and condition of the mother impacts on offspring survival in moose, *Alces alces gigas* (Keech *et al.* 2000).

However, there are few cases in wild ungulate populations where the burden of maternal parasitic infection has been shown to negatively impact on the early development of their offspring. There is evidence from bighorn sheep (*Ovis canadensis*) that lamb survival is lower for highly parasitized ewes. Ewes with higher lungworm burdens (*Protostrongylus spp.*) nursed their lambs less, and provisioned lower quantities of milk, having a negative impact on lamb survival (Festa-Bianchet 1988a).

In domestic sheep, maternal parasites have been shown to depress lamb growth rates. Experimentally infecting ewes with nematode parasites (40% *Teladorsagia circumcincta*, 40% *Trichostrongylus colubriformis*, 20% *Haemonchus contortus*) before and after parturition caused a drop in the growth rate of their lambs compared to the lambs of uninfected control ewes (Valderrabano & Uriarte 2003). However, this depressive effect of parasitic infection was ameliorated by improving the diet quality of the ewe during early pregnancy. In another study on domestic sheep, treatment of sarcoptic mange (*Sarcoptes scabiei*) increased milk production in dairy ewes by over 22% compared to untreated controls (Fthenakis *et al.* 2000). In contrast, treatment of Fulbe ewes in Cameroon to remove parasitic infection, had no effect on growth or survival of lambs; however, maternal dietary supplementation increased lamb survival, weight at birth and weight at weaning (Njoya *et al.* 2005).

There are also some examples of the negative effect of maternal parasites on offspring traits in non-ungulate populations. For example, in house mice (*Mus musculus*), experimental infection with naturally-occurring nematode parasites (*Heligmosomoides polygyrus*) in the mother caused a depression of offspring growth rates (Kristan 2002b). In the red-backed shrike (*Lanius collurio*), females infected with blood parasites delayed egg-laying compared to uninfected females: hatching late in the season is costly, as this misses the super-abundant food sources that occur earlier in the season (Votypka *et al.* 2003).

In Soay sheep (*Ovis aries L.*), observational studies indicate that certain traits of the ewe can have negative effects on the survival and development of their lambs. Older ewes tend to give birth earlier in the season (Clutton-Brock *et al.* 1992), and birth date can affect the survival and growth rates of lambs (Clutton-Brock *et al.* 1992, Milner *et al.* 1999a). Early-born lambs tend to grow at a slower rate than those born late in the season (Clutton-Brock *et al.* 1992). In spite of this, early-born lambs are heavier at four months old, although by 16 months there are no discernable weight differences relating to birth date (Clutton-Brock *et al.* 1992). Birth date also affects survival; early-born lambs are more likely to die shortly after birth than lambs born later in the season (Clutton-Brock *et al.* 1992, Clutton-Brock *et al.* 1996). The age and weight of the ewe has an effect on the birth weight of the lamb: middle-aged ewes produce heavier lambs than young or old ewes, and heavier ewes tend to produce heavier lambs (Clutton-Brock *et al.* 1996). Birth weight has immediate and long lasting repercussions; high birth weight increases lamb survival rates, and is thought to correlate with the weight of the individual throughout much of their lifetime (Albon & Clutton-Brock 1988, Albon *et al.* 1991). This is important because heavier individuals have improved survival and reproductive success in comparison to lighter individuals (Clutton-Brock *et al.* 1996, Coltman *et al.* 1999c).

The aim of this study was to determine experimentally the effects of parasite removal from mature Soay ewes on the survival and early development of their lambs. In order to do this, it is necessary to determine whether the factors that had previously been reported as influencing the survival and development of Soay lambs, also impacted on the fitness of the lambs of the study animals. The ultimate aim however, was to determine whether temporarily removing parasites from the mother had any further effects on the survival and development of her offspring. It has already been observed that the reduced costs of fighting parasitic infection over winter, has allowed the treated ewes to invest more resources into improving their own body condition (Chapter 5). Do treated ewes also invest more into improving the fitness of their lambs?

The second part of this chapter addresses an important issue affecting offspring development, which is the behavioural time budget of the lamb, in particular suckling behaviour. The main cost of reproduction for females is lactation, as it is the most energetically expensive (Clutton-Brock *et al.* 1989). Measuring lactation efforts, i.e. milk intake by the infant, provides an estimate of maternal investment in current offspring. Milk quality and rate of milk transfer is known to be strongly associated with offspring growth and survival (Loudon *et al.* 1983, Oftedal 1984). It is not possible to measure milk quality in Soay sheep due to logistical and ethical reasons, but it is possible to attempt to gain a measure of milk intake rate. This is most easily measured by recording suckling behaviour, and is widely used to measure milk intake rates in ungulates (e.g. red deer, *Cervus elaphus*, Loudon *et al.* 1983, bighorn sheep, *Ovis canadensis*, Festa-Bianchet 1988, mouflon, *Ovis gmelini m.*, Reale *et al.* 1999, domestic horse, *Equus caballus*, Cameron *et al.* 1999, feral horse, *Equus caballus,* Cameron & Linklater 2000). However, there are conflicting opinions as to whether measures of suckling behaviour can accurately predict milk transfer (reviewed in Cameron 1998). Despite these issues, assessment of suckling behaviour is used as a proximate measure of lactation effort and maternal investment. In bighorn sheep, nursing behaviour was lower in highly parasitized ewes (Festa-Bianchet 1988a). Are the treated ewes in this study, with their improved body condition, able to devote more time into nursing and lactation effort?

6.2 Methodology

6.2.1 Bolussing experiment (ewes)

In August 2003, fifty-one female Soay sheep aged 4-7 years old, were caught and randomly assigned to either the Bolus (treated) or Control (not treated) group (see chapter 5 for more details on how this was done). The ewes in the bolus group were administered with an anthelmintic bolus which removes the parasite burden for at least 100 days. The ewes in the control group were not manipulated. Parasite burden was measured by counting the number of strongyle parasite eggs per gram (epg) in faeces, the faecal egg count. (FEC) (see Chapter 2 for more information). Analysis of the FEC of the ewes subsequent to starting the experiment confirmed that bolus ewes had significantly reduced parasite burden going into the winter months, in comparison to control ewes (see Chapter 5 for results).

6.2.2 Morphometric data collection

During the lambing period of 2004, lambs born to experimental ewes (both those treated with anthelmintic and those in the control group) were monitored from April 1st until May 31st. All new-born lambs were easily identifiable either from their ear tag or, if untagged, through distinguishing morphological features, heft, and physical association (suckling) with their known mother. Regular censuses of the study area allowed accurate estimates of birth date, still births and neonatal mortality.

Birth weight was obtained for 55 out of 59 (93%) of the lambs from the experimental group and corrected for day of capture (see section 6.3.1 for how this was done). 17 of the lambs with known birth weight were re-weighed within the first 8 weeks, in order to obtain an estimate of early growth rate; they were stalked while asleep, caught and weighed at least once between the ages of 24-53 days old. See section 6.3.2 for details on how growth rate was calculated.

During August 2004, when the lambs were approximately 4 months old, 26 of the surviving 51 lambs (8 had died since the spring) were caught during the annual catch-up, and their weight and other morphometric measurements were recorded.

6.2.3 Behavioural data collection

During April and May 2004, a team of 3 observers recorded the behaviour of lambs born to the experiment ewes (Bolus and Control groups). Telescopes were used to facilitate identification of lambs from their plastic ear tags, and they were monitored from a suitable distance (> 10 metres). This method has been successfully used on numerous occasions in previous studies on St Kilda (e.g. Preston *et al.* 2003a, 2003b).

Lamb behaviour was recorded during the first four to five weeks after birth using a hand-held computer (Psion II Organiser, software written by I. R. Stevenson, *Sunadal Data Solutions*, Edinburgh). Each lamb focal watch lasted for 1 hour, during which time all behaviours and their durations were recorded (see Table 6.1 for definitions of behaviour).

Table 6.1: Definitions of lamb behaviour categories used to record lamb behaviour.

Behaviour	***Definition***
Feed	Behaviour similar to adult *Feed- Searching for, and processing, food. Includes short periods of head-down movement between bites.* Food processing by young lambs is inefficient until the rumen is fully developed at 8 weeks (Lyford 1988).
Doze	Lamb is lying down and appears to be asleep.
Suckle	A suckling bout occurs when the lamb makes contact with the teat, and sucks from it. The head is usually tilted upwards slightly, and some movement of the head can occur.
Nuzzle	Particularly evident in the first few days after birth, nuzzling is when the lamb appears to be searching for the teat under the ewe's belly, but fails to make contact.
Play	Either with other lambs or individually (includes running in a playful manner)
Move	Any prolonged movement not resulting from interaction with other sheep. Note that this does not include head-down movement during feeding bouts.
Other	Periods of no observable activity covered by above categories, grooming, and periods of general alertness. Also used to describe the periods in between suckling bouts.

Each lamb was watched on 7-9 occasions (mean = 8.04 ± 0.87 (st.dev.), n = 48 lambs), which approximates to at least twice a week during the whole watch period. For each lamb, focal watches were distributed as evenly as possible throughout the day, and between the observers recording their behaviour.

For the duration of each watch, behaviour was recorded continuously, and all behaviours were, by definition, mutually exclusive. The categories include 'sleep', 'feed', 'move', 'suckle', 'nuzzle', 'play' and 'other' (Table 6.1).

6.2.4 Litter size

In Chapter 5, it was shown that removing parasites from ewes had a positive (but marginally non-significant) effect on litter size. Across treatment groups, twin lambs from bolussed ewes were *twice* as common as twins from control ewes: 25% (6/24) of bolus ewes had twins, while only 12% (3/26) of control ewes produced twins. This is important, because being a twin has potentially huge repercussions for the early development of lambs, and this must be taken into account when interpreting the results.

6.3 Data analysis

6.3.1 General

Data were analysed using the statistical package S-PLUS 2000 release 1 (*Mathsoft, Inc.*). Terms tested in the maximal models were ewe treatment group (Bolus or Control), ewe age, ewe weight (in August 2003), ewe coat type, ewe horn type, litter size (single or twin), lamb sex, Julian date of birth (days after January 1st) and corrected lamb birth weight. Ewe coat and horn type were included as explanatory terms because there is evidence to suggest that they may influence ewe breeding success (Clutton-Brock *et al.* 1997). Minimal models were obtained by step-wise deletion of non-significant terms, using an α-value of 0.05. Due to small sample sizes, interactions between main effects were not tested in the maximal model (unless stated). However, interactions between final terms in the minimal model were checked. Interactions were tested using an α-value of 0.025 to account for the greater number of tests carried out (Crawley 2002).

As the date of first capture for newborn lambs varies, and weight increases rapidly over the first few days of life, birth weight was corrected for age at first capture. Most lambs were caught within 5 days of birth, although several were just over a week old when first caught. To account for this, as in previous studies (e.g. Forchhammer *et al.* 2001, Wilson *et al.* 2005), relative birth weights were estimated by using the residuals from a linear regression of weight at first capture with age at capture. This model provided a very good prediction of birth weight ($F_{1,47} = 49.01$, $p<0.0001$; $r^2 = 0.51$).

6.3.2 Survival and development

Lamb birth weights, August weights and Julian dates of birth were analysed with general linear models (GLMs), using analysis of covariance (ANCOVA) methods. The maximal models tested all of the terms mentioned above, except where that term was the response variable. Lamb growth rates were analysed using general linear mixed models (GLMMs) to account for repeat weighings of each lamb. The fixed effects tested in the model were the age of the lamb interacting with each of the terms described above (except for birth weight). The best model had individual intercepts and slopes for each lamb, and so the random effects in the model were age within lamb ID.

$$\text{Weight} \sim \text{Age} * (a + b + c\ldots),\ \text{random} = \sim \text{Age} \mid \text{Lamb ID}$$

Early growth rate was defined as the change in weight during the first 8 weeks of the lambs' life. Growth to weaning was defined as the change in weight from birth to when the lambs were approximately 4 months old (August 2004).

Lamb survival analysis was performed using Cox's proportional hazards model (a non-parametric technique) in S-PLUS, testing all of the terms mentioned above in the maximal model. Terms were tested by the change in deviance resulting from its removal, which was tested against the Chi-square distribution to obtain the p value. Excluded terms did not cause a significant change in deviance ($p>0.05$), when added to the minimal model.

6.3.3 Behavioural data

The proportion of time spent in any behaviour was often correlated with time spent in another behaviour category (see Table 6.2). For example, the proportion of time spent sleeping was strongly negatively correlated with the proportion of time spent feeding. Due to the correlations between behaviours, a multivariate analysis of variance (MANOVA) was carried out, in which multiple response variables were analysed as a group to allow for covariance between them and minimise Type I errors (e.g. Wilson *et al.* 2001). Terms retained in the minimal model of the MANOVA, were then tested in separate univariate ANOVAs for each response variable, after correction for other terms in the model. Where necessary, proportion of time data were arc-sine transformed prior to analysis, as is often recommended for data of these type (Crawley 2002). The response variable was the average proportion of time spent in each behaviour, calculated over all focal watches for each lamb. During the course of the fieldwork, it was noticed that i) the weather was very changeable, but generally improved as the season progressed, and ii) the weather appeared to have a strong effect on lamb behaviour. It was therefore deemed appropriate to include weather in the analysis.

Table 6.2: Correlation coefficients (Pearson's product-moment correlation) between behaviour categories. Significant correlations are highlighted in bold (significance indicated by asterisks: extremely significant - $p<0.001$ ****, highly significant - $p<0.005$ ***, significant - $p<0.02$ **, marginal significance - $p<0.06$ *).

	Feed	**Doze**	**Suckle**	**Nuzzle**	**Play**	**Move**	**Other**
Feed		****__0.74__	-0.23	-0.01	*__0.27__	0.24	-0.18
Doze			-0.06	-0.19	-0.23	****__0.53__	****__0.51__
Suckle				***__0.41__	-0.13	-0.01	0.21
Nuzzle					-0.03	-0.01	0.24
Play						-0.02	-0.15
Move							**__0.36__
Other							

Daily weather data (average of rainfall (mm), wind speed (ms^{-1}). temperature (°C) and hours of sunshine, in 24 hours) was obtained for each focal watch on each lamb (see Chapter 2 for more information). An average of these weather variables was taken relating only to the dates that each individual lamb was monitored.

The explanatory variables used in the MANOVAs included traits relating to the lamb and the ewe (as described in section 6.3.1), in addition to the weather variables. To avoid problems with masking effects of one explanatory trait over another, separate analyses was performed with each set of explanatory variables, before combining them as one, and comparing the results. Initial analysis suggested that there were no masking effects between ewe and lamb traits, as the same traits were retained in the minimal models with both separate and combined analyses. However the weather variables were being compromised in the combined analysis, due to two reasons; correlations between weather variables, and correlations between all weather variables and Julian date of birth.

As expected, weather variables were highly correlated (see Table 6.3). For example, average daily temperature was strongly negatively correlated with average daily wind speed. Due to the close association of the weather variables, and that there were obvious weather changes as the lambing season progressed, they were analysed together in a MANOVA with Julian date of birth. All weather variables were strongly associated with Julian date of birth ($F_{1,46} = 41.02$, $p<0.0001$).

Table 6.3: Correlation coefficients (Pearson's product-moment correlation) between behaviour categories. All correlations are significant (significance indicated by asterisks: extremely significant – $p<0.001$****, highly significant - $p<0.005$ ***, significant - $p<0.02$ **, marginal significance - $p<0.06$ *).

	Temperature	**Sunshine**	**Rainfall**	**Wind Speed**
Temperature		****0.65	***-0.41	****-0.57
Sunshine			**-0.34	****-0.49
Rainfall				****0.58
Wind speed				

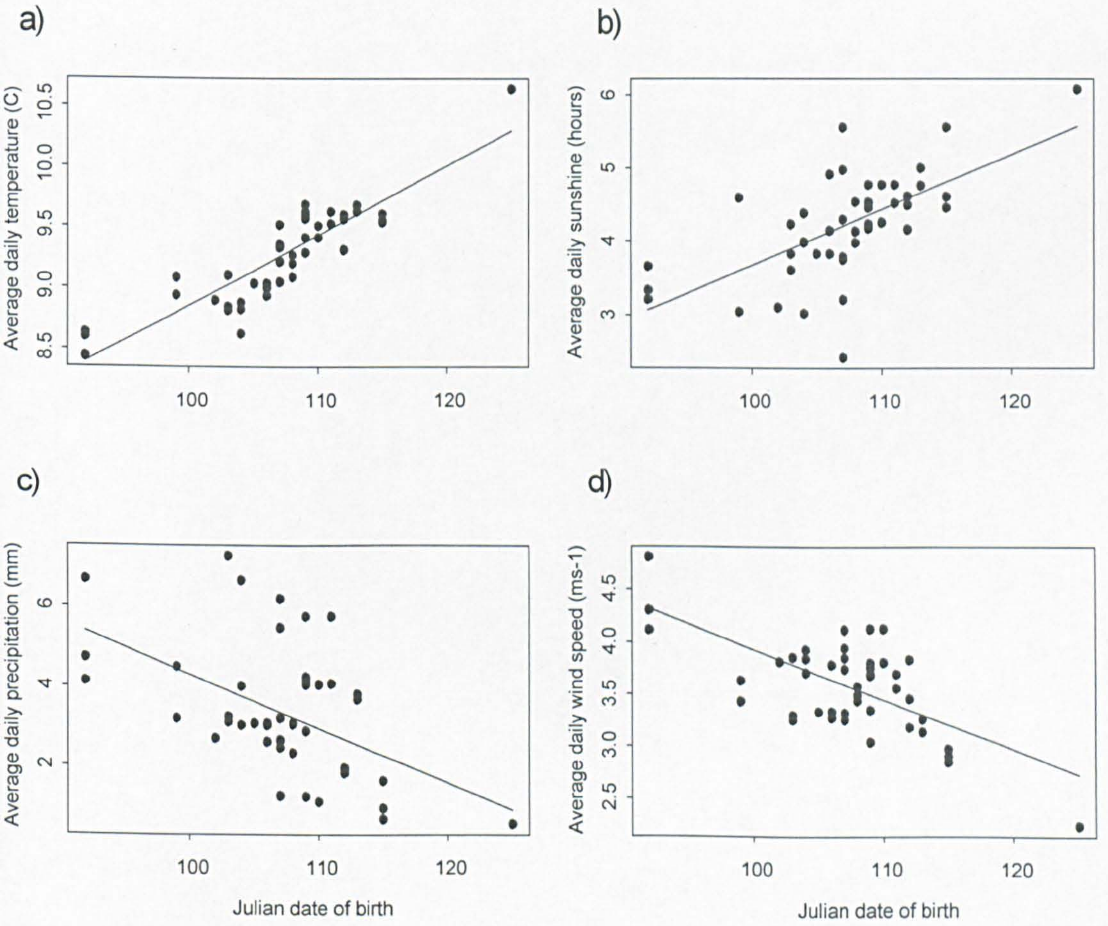


Figure 6.1: The relationships between weather variables (average over all the focal watches for each lamb) and a) temperature, b) sunshine hours, c) precipitation, and d) wind speed) and lamb date of birth (Julian day). Each data point represents one individual. Fitted lines are drawn from model predictions.

Generally, the weather improved for lambs born later in the season as the average daily temperature and sunshine hours increased, and average daily wind speed and precipitation decreased (Figure 6.1). As weather experienced by the lambs was related to their birth date, and Julian date of birth was clearly sufficient to encompass these effects, weather terms were excluded from the full MANOVA of lamb behaviour. In the cases where Julian date of birth was retained in a model explaining lamb behaviour, the model was re-tested using the weather variables (separately) as a replacement.

As the amount of suckling decreases as lambs get older, this behaviour category was examined in more detail. Specifically, the frequency of suckling bouts per hour (rate of suckling) and the average duration of individual suckling bouts were recorded in each focal watch. Bout lengths and rates were log10-transformed before a mean value was taken for each focal watch.

These data were analysed using general linear mixed models (GLMMs) to account for repeated measures of suckling as the lambs become older. The fixed effects tested in the model were the age of the lamb plus the explanatory terms described above, tested up to second order interactions. The random effects structure used in the model was as follows:

Suckling bout length ~ Age * (a + b + c...), random = ~ 1 | Lamb ID
Suckling rate ~ Age * (a + b + c...), random = ~ Lamb age | Lamb ID

During this analysis, the behaviour of the lamb whose mother was of coat type 'Dark Self' (DS) did not differ significantly from the behaviour of the lambs of the 'Dark Wild' (DW) ewes, and so these 2 levels of the factor 'coat' were collapsed into one level.

6.4 Results

6.4.1 Survival and development

a) Survival

Survival of lambs was high: 51 out of 59 lambs (86%) survived to weaning. Lamb survival was affected by litter size (Table 6.4). The rate of lamb mortality during the first few months was significantly higher in twins than in single lambs ($\chi^2_1 = 9.51$, p=0.002; Figure 6.2). Only 7% (3/41) of single lambs died, whereas 22% (5/18) of twins died before weaning (at four months old). Although there was no overall difference in the birth weight of lambs that lived or died; there was a significant interaction between treatment group and lamb birth weight, on the survival of lambs ($\chi^2_1 = 5.66$, p=0.017, Table 6.4). It appears that there may have been a threshold birth weight for survival; the critical birth weight for lamb survival was lower for bolussed ewes, than for control ewes. Thus, the mean birth weight of lambs from bolussed ewes that died was 1.40 kg; lambs that died from control ewes had a mean birth weight of 2.23 kg.

For the purposes of graphical representation, the lamb birth weights have been converted into a two-level weight category (heavy or light). The cut-off point was

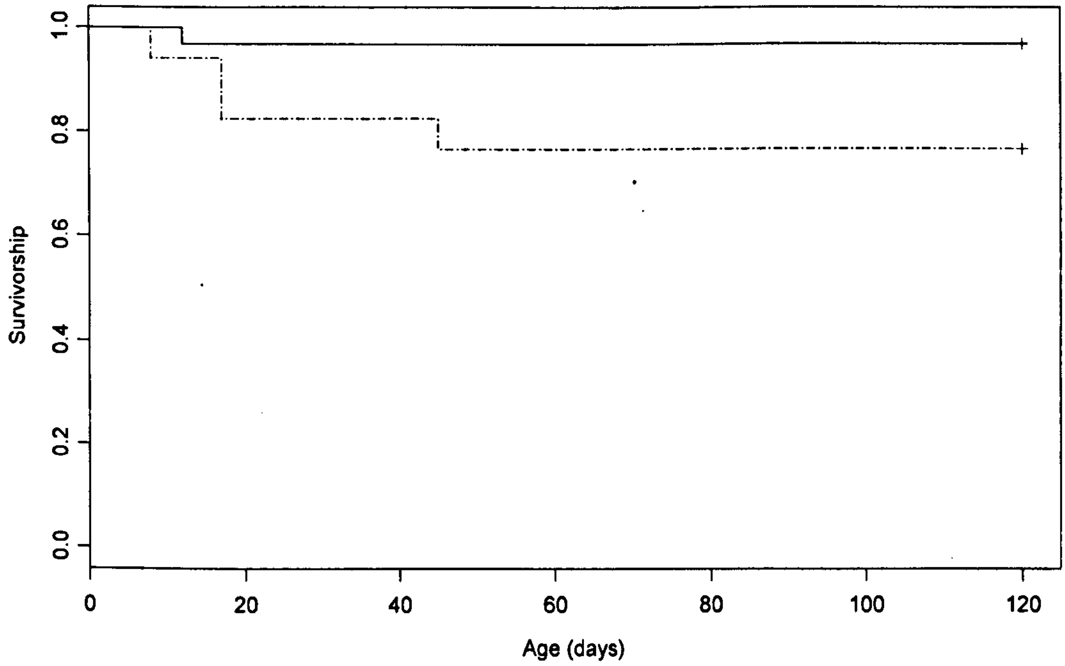


Figure 6.2: Survivorship (Kaplan-Meier plot) in single lambs (solid line) and twin lambs (dashed line). Survival to weaning (four months old) was significantly higher in single lambs than in twin lambs ($\chi^2_1 = 9.51$, $p<0.002$).

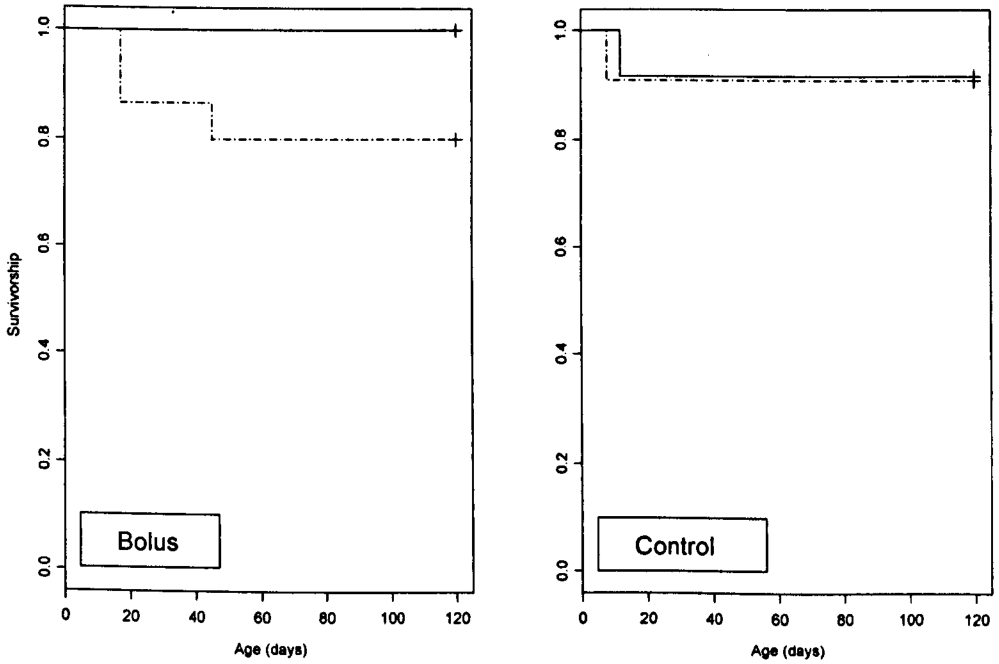


Figure 6.3: Survivorship (Kaplan-Meier plot) of heavy lambs (solid line), and light lambs (dashed line) from Bolus ewes (left) and Control ewes (right). Lambs are separated into heavy and light weight categories using a median birth weight of 1.9 kg. There is a significant interaction between treatment group and lamb birth weight, on the survival of lambs to weaning (four months old) ($\chi^2_1 = 5.66$, $p<0.017$).

Table 6.4: Lamb survival to weaning (four months old). Data were analysed using Cox's proportional hazards model. The table reports the change in deviance resulting from removal of the explanatory term, which was tested against the Chi-square distribution to obtain the p value. Excluded terms did not cause a significant change in deviance (p>0.05), when added to the minimal model.

Terms	**Coefficient**	**Std. Error**	**Δ Dev**	**d.f.**	**p (χ^2)**
Birth weight	0.261	1.38	1.09	1	0.30
Treatment group (C)	-24.473	22.81	0.01	1	0.92
Litter size (twin)	-13.862	11.24	**9.51**	**1**	**0.002**
Birth weight: Treatment	14.124	12.74	**5.66**	**1**	**0.017**
Excluded terms					
Ewe weight			0.4	1	0.53
Ewe age			0.0	1	0.99
Ewe coat			0.2	2	0.90
Ewe horn			1.2	2	0.55
Lamb sex			2.1	1	0.15
Julian birth date			1.3	1	0.25

based on the median birth weight of 1.9 kg. Figure 6.3 shows that for bolussed ewes only light lambs died, whereas all heavy lambs survived. In contrast, the lambs that died from control mothers belonged to both weight categories. Ewe treatment group, *per se* (as a main effect in the minimal model), had no effect on lamb survival (χ^2_1 = 0.01, p=0.92); even after the exclusion of litter size from the model (χ^2_1 = 0.02, p=0.89).

b) Julian date of birth

The julian date of birth of lambs was unaffected by any of the explanatory terms. All terms tested dropped out of the maximal model, including treatment group ($F_{1,56}$ = 0.11, p=0.75; Table 6.5).

c) Birth weight

Litter size significantly affected birth weight, with single lambs being born heavier than twins ($F_{1,47} = 31.3$, $p<0.0001$). The average birth weight for single lambs was 2.2 kg (n = 32), while twins were lighter at an average of 1.4 kg (n = 17) (Figure 6.4). To maximise sample size, both members of each twin was included in the analysis, but randomly excluding one member of each twin-pair produced qualitatively similar results. After accounting for litter size, there was no effect of treatment of ewes on the birth weight of their lambs ($F_{1,46} = 0.54$, p=0.47, Table 6.6); however treated ewes were twice as likely to have twins (see section 6.2.6 above). Accounting for litter size, there was also a non-significant trend for heavy ewes to bear the heaviest lambs ($F_{1,46} = 3.02$, p=0.09).

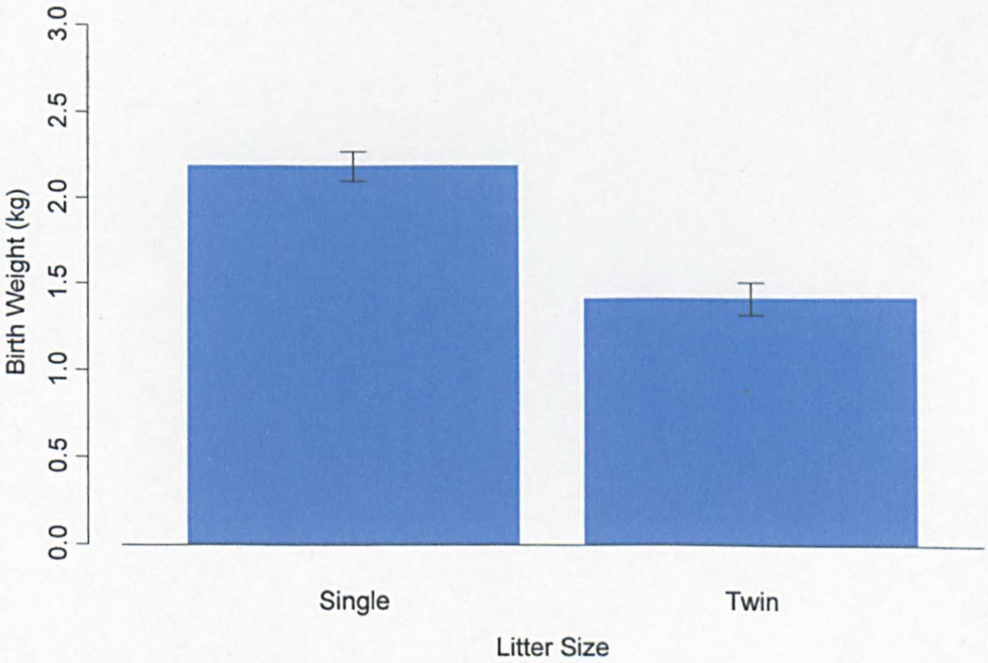


Figure 6.4: Birth weight (kg) in single and twin lambs. Birth weight is corrected for day of capture. Error bars are standard errors from the mean. ($F_{1,47} = 31.27$, $p<0.0001$, n=49).

Table 6.5: Analysis of variance table for GLM of lamb Julian date of birth. No explanatory terms affected lamb birth date.

Terms	**d.f.**	**Sums of Squares**	**Mean Squares**	**F**	**p**
Residual	57	4797.88	84.17	-	-
Excluded terms					
Treatment group	1	9.12		0.11	0.75
Ewe weight	1	1.79		0.02	0.89
Ewe age	1	57.91		0.68	0.41
Ewe coat	2	144.75		0.86	0.43
Ewe horn	2	279.59		1.70	0.19
Lamb sex	1	83.11		0.97	0.33
Litter size	1	67.10		0.79	0.38

Table 6.6: Analysis of variance table for GLM of lamb birth weight (kg).

Terms	**d.f.**	**Sums of Squares**	**Mean Squares**	**F**	**p**
Litter size	1	6.57	6.57	**31.27**	**<0.0001**
Residuals	47	9.88	0.21	-	-
Excluded terms					
Treatment group	1	0.11		0.54	0.47
Ewe weight	1	0.61		3.02	0.09
Ewe age	1	0.15		0.73	0.40
Ewe coat	2	0.24		0.57	0.57
Ewe horn	2	0.66		1.60	0.21
Lamb sex	1	0.52		2.54	0.18
Julian birth date	1	0.29		1.51	0.22

d) August weight

Weight of lambs in August, when they were approximately 4 months old, was dependent on their birth weight (Figure 6.5). Lambs that were born heavy, were also heavier in their first August ($F_{1,22} = 13.92$, p=0.001). Twins were lighter in August than single lambs; however this was due to differences in their birth weight (see Figure 6.4). After excluding birth weight from the model, twins were significantly lighter ($F_{1,24} = 6.34$, p=0.019). Treatment of ewes did not affect lamb August weight ($F_{1,21} = 0.76$, p=0.39, Table 6.7).

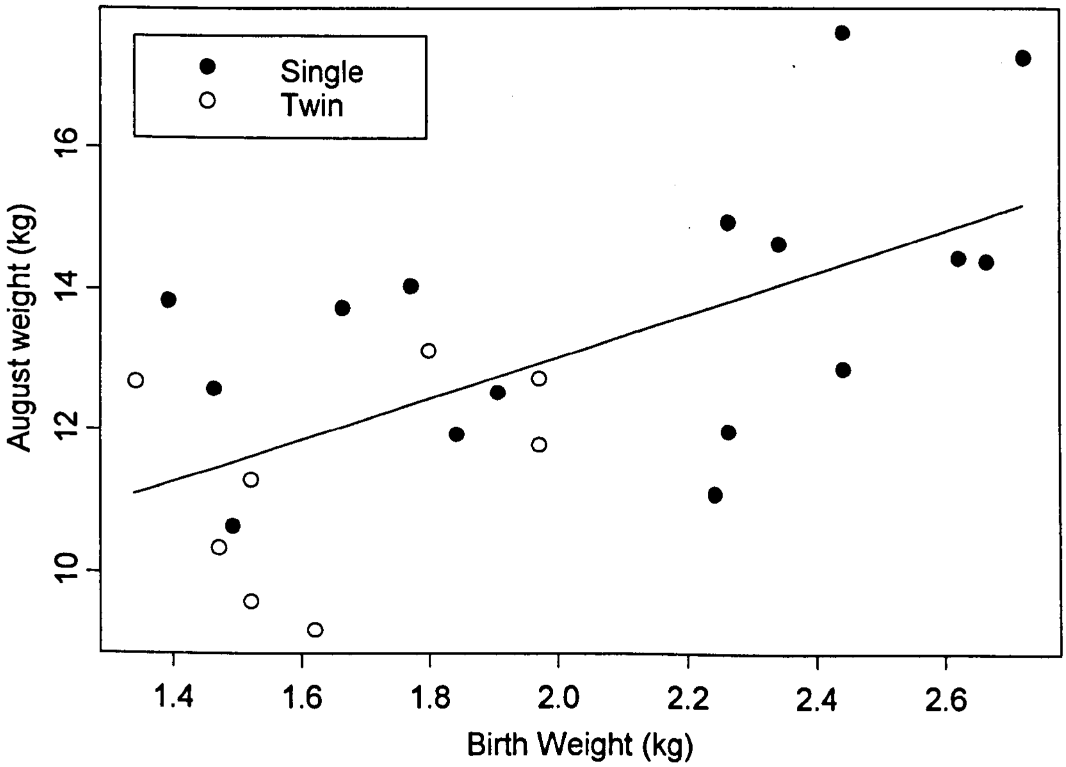


Figure 6.5: The relationship between lamb birth weight (corrected for day of capture) and their August weight (kg), four months later. ($F_{1,22} = 13.92$, p=0.001). The fitted line is drawn from model predictions. August weight is also affected by litter size ($F_{1,24} = 6.34$, p=0.019), although this is due to difference in birth weight (see Figure 6.4). Closed circles are single lambs, open circles are twins.

There was a trend for lamb August body weight to decrease with birth date; i.e., late-born lambs tended to be lighter in their first summer ($F_{1,21} = 3.66$, $p=0.069$). Removal of an outlier data point (Julian Day = 125, open red circles in Figure 6.6), caused this relationship to become statistically significant ($F_{1,20} = 5.03$, $p=0.036$), and removal of a second outlier (Julian Day = 99, open red circles in Figure 6.6) caused the negative relationship between date of birth and August weight to become highly significant ($F_{1,19} = 10.1$, $p<0.005$).

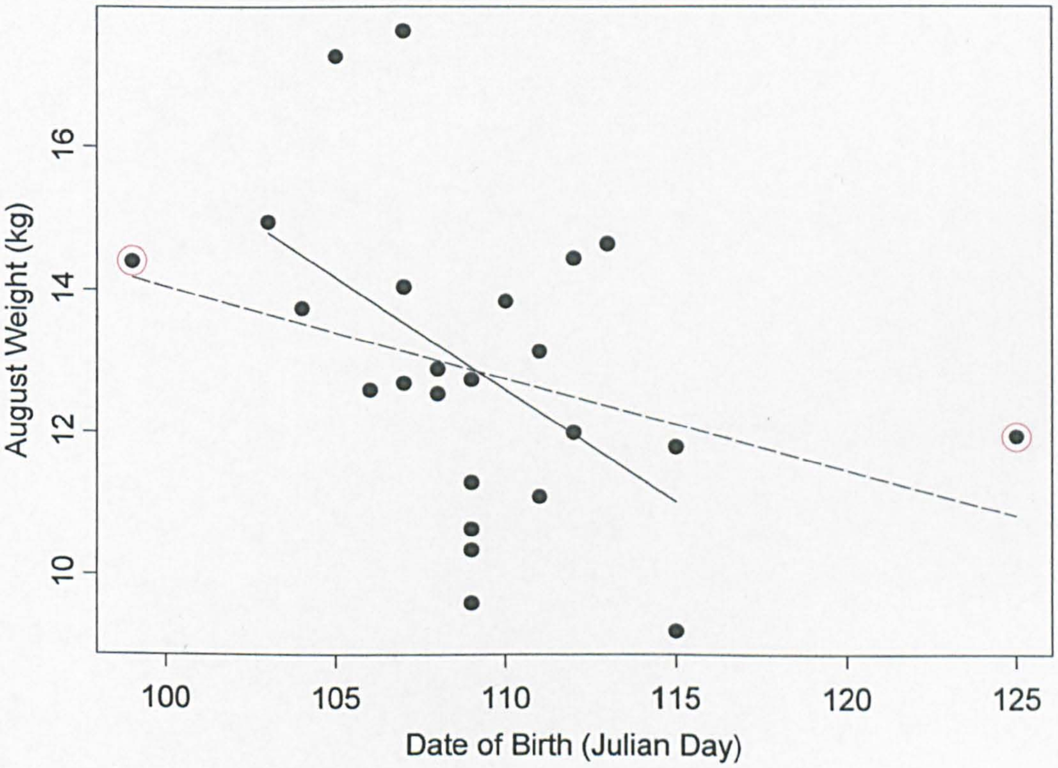


Figure 6.6: The relationship between lamb date of birth (Julian Day) and their August weight (kg), four months later. The fitted lines are drawn from model predictions. Date of birth does not significantly affect August weight ($F_{1,21} = 3.66$, $p=0.069$; fitted line is dashed). However after removal of outliers (open red circles), the negative effect of birth date on August weight becomes significant ($F_{1,19} = 10.1$, $p<0.005$; fitted line is solid).

Table 6.7: Analysis of variance table for GLM of lamb weight (kg) in August 2004 (when four months old).

Terms	d.f.	Sums of Squares	Mean Squares	F	p
Birth weight	1	39.17	39.17	**13.92**	**0.0012**
Residual	22	61.91	2.81	-	-
Excluded terms					
Treatment group	1	2.16		0.76	0.39
Ewe weight	1	0.47		0.16	0.69
Ewe age	1	6.52		2.47	0.13
Ewe coat	1	4.06		1.47	0.24
Ewe horn	2	8.94		1.69	0.21
Lamb sex	1	1.03		0.36	0.56
Litter size	1	6.93		2.65	0.12
Julian birth date	1	9.20		3.66	0.069

e) Growth rate

There were significant differences in the early growth rates of individual lambs, accounting for 13.1% of the variance ($\chi^2_2 = 12.83$, p=0.0016). This was still apparent up to four months old, as 13.6% of the variance in lamb growth up to weaning was explained by individual differences in growth rates ($\chi^2_2 = 62.94$, p<0.0001). However, despite the variation in individual growth, there were still some significant fixed (main) effects.

Weight gain during the first 8 weeks was best described by a linear relationship with age ($\chi^2_1 = 52.8$, p<0.0001). During this period, growth rate was affected by litter size (Figure 6.7); single lambs grew faster than twins ($\chi^2_1 = 7.69$, p=0.006, Table 6.8). The average growth rate for single lambs was 140 g per day, whereas twins grew at only 100 g per day.

The difference in growth rate between twin and single lambs was still apparent in August (Figure 6.8). Single lambs had a higher growth rate than twin lambs. (χ^2_1 = 14.05, p=0.0002, Table 6.9). Growth rate had slowed for all lambs by 4 months old, and the relationship was best described by a quadratic (age^2) relationship (χ^2_1 = 48.48, p<0.0001).

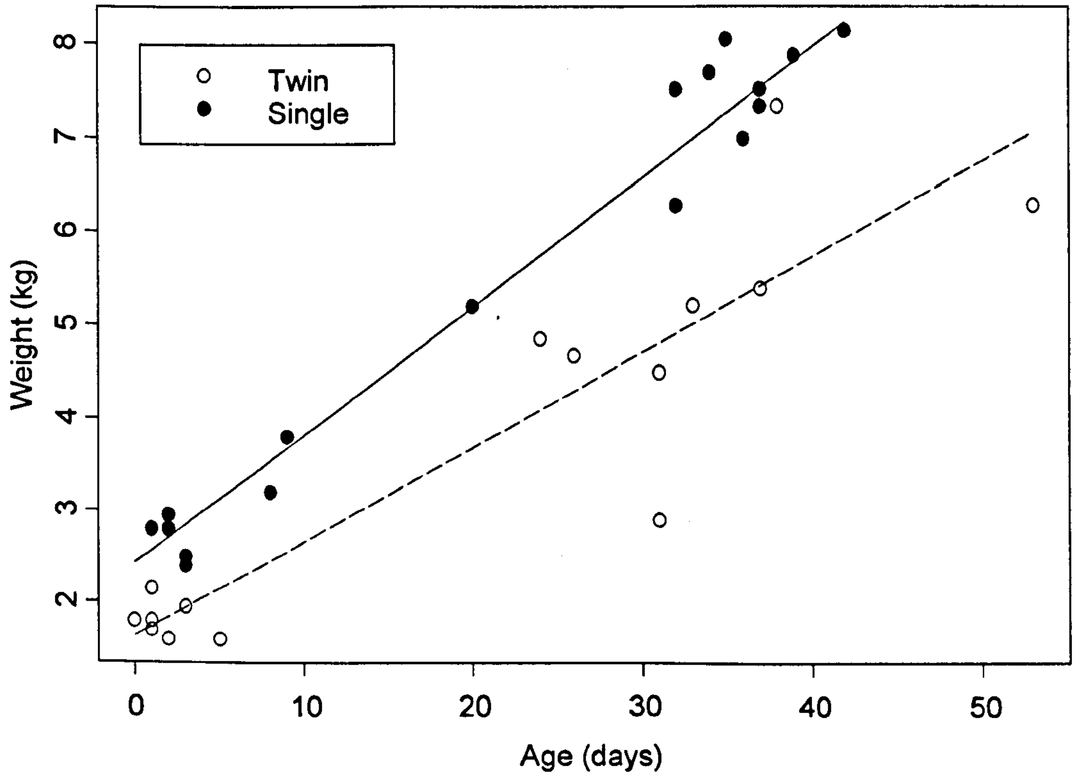


Figure 6.7: Early growth rate (weight gain during first 8 weeks of life) in twin and single lambs. Growth in single lambs is higher than in twin lambs (χ^2_1 = 7.69, p=0.006). The fitted lines are drawn from model predictions. Dashed line and open circles are twins; solid line and closed circles are single lambs.

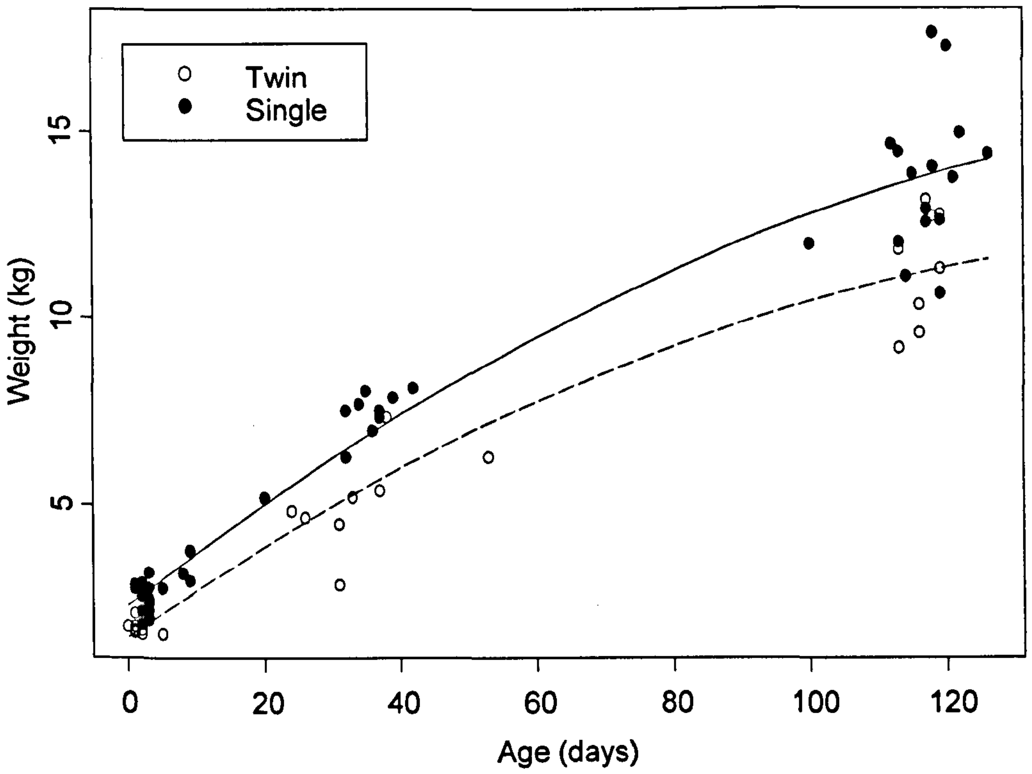


Figure 6.8: Growth rate in lambs up to 4 months old in single and twin lambs: growth in single lambs is higher than in twin lambs (χ^2_1 = 14.05, p=0.0002). The fitted lines are drawn from model predictions. Dashed line and open circles are twins; solid line and closed circles are single lambs.

The treatment group of the mother did not affect the growth rates of the lamb during their first eight weeks (χ^2_2= 1.44, p=0.49, Table 6.8), or growth to weaning (χ^2_2= 0.90, p=0.64, Table 6.9). Bearing in mind that treating the ewe influences litter size, it is possible that litter size could be masking an affect of treatment. With this in mind, litter size was excluded from the models, and the effects of treatment group on lamb growth were tested independently. However, ewe treatment group had no effect on either early growth rates (χ^2_2= 0.67, p=0.72), or growth of the lambs to weaning (χ^2_2= 2.0, p=0.37).

Table 6.8: Fixed effects of the general linear mixed model on early lamb growth rates (weight gain during the first 8 weeks of life (kg)).

Terms	**Coefficient**	**Std. Error**	**χ^2**	**p- value**
(Intercept)	2.420	0.111		
Age	0.137	0.007	**52.81**	**<0.0001**
Litter size (twin)	-0.793	0.165	**13.70**	**0.0002**
Age * Litter size	-0.826	0.176	**21.37**	**<0.0001**
Excluded terms				
Age^2			0.31	0.58
Age : Treatment group			0.90	0.64
Age : Ewe weight			1.54	0.46
Age : Ewe age			4.15	0.13
Age : Ewe coat			7.24	0.12
Age : Ewe horn			5.54	0.24
Age : Lamb sex			0.47	0.79
Age^2 : Litter size			0.53	0.46
Age : Julian birth date			0.49	0.48

The weight of the ewe appeared to have a marginally significant positive effect on lamb growth to weaning (χ^2_2= 7.37, p=0.026, Table 6.9). Based on the *a priori* assumptions, that interactions such as age*weight should be tested with an α-value of 0.025, this is not significant. In addition to this, closer inspection of the data suggests that this result is most likely due to 2 high leverage points. Inclusion of ewe weight in the model does not change the minimal model.

Table 6.9: Fixed effects of the general linear mixed model on lamb growth rates to weaning (weight gain during the first four months of life (kg)).

Terms	Coefficient	Std. Error	χ^2	p- value
(Intercept)	2.245	0.095		
Age	0.150	0.006	**102.64**	**<0.0001**
Age^2	-0.001	0.001	**48.48**	**<0.0001**
Litter size (twin)	-0.633	0.144	**9.22**	**0.0024**
Age * Litter size	-0.657	0.150	**23.27**	**<0.0001**
Excluded terms				
Age :Treatment group			1.45	0.48
Age : Ewe weight			7.37	0.026
Age : Ewe age			3.33	0.19
Age : Ewe coat			2.24	0.69
Age : Ewe horn			0.03	0.98
Age : Lamb sex			4.16	0.13
Age^2 : Litter size			<0.01	0.95
Age : Julian birth date			1.17	0.56

6.4.2 Behaviour

a) Proportion of time spent undertaking different behaviours

The average proportion of time (calculated as an average over the full watch period of 4-5 weeks) spent undertaking each behaviour category varied greatly (Figure 6.9). Sleeping was the most common behaviour, with lambs spending on average just under half of their time budget resting/sleeping (average 26 minutes per hour). Feeding and 'other' behaviours were the second and third most common, lambs spending on average 14.5 and 15.5 minutes per hour in these behaviours, respectively. Time spent moving was much less common, on average less than 2 minutes per hour. Suckling and nuzzling behaviour together comprised just over 1.5 minutes per hour, while play was the least common of all behaviours with less than a minute (average 42 seconds per hour).

The explanatory variables retained in the MANOVA explaining variation in lamb behaviour were Julian date of birth ($F_{1,38} = 4.85$, $p<0.001$), birth weight ($F_{1,38} = 2.25$, $p=0.056$) and ewe coat type ($F_{2,38} = 1.94$, $p=0.038$). Ewe treatment group did not affect lamb behaviour ($F_{1,37} = 1.78$, $p=0.13$, Table 6.10) (even if lamb birth weight is excluded from model: ($F_{1,43} = 1.16$, $p=0.35$).

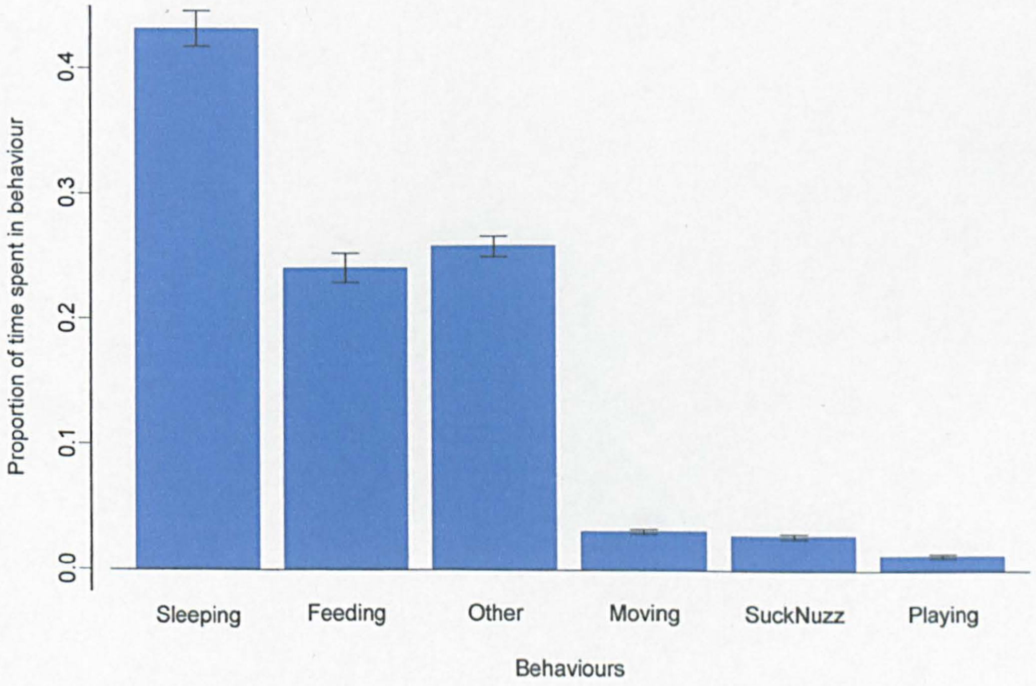


Figure 6.9: Proportion of time spent in each behaviour category averaged over the full watch period (four to five weeks for each lamb). Error bars are standard errors.

Table 6.10: Multivariate analysis of variance (MANOVA) table for all behaviour categories: the effect that traits had upon the proportion of time spent in each behaviour (sleeping, feeding, other, moving, suckling, nuzzling, playing) as a group.

Terms	DF	F	p
Ewe coat	**2**	**1.94**	**0.038**
Birth weight	**1**	**4.85**	**<0.001**
Julian birth date	**1**	**2.25**	**0.056**
Residuals	38	-	-
Excluded terms			
Treatment group		1.78	0.13
Ewe weight	□0.7	0.70□0.67	0.67□□E
Ewe age □□0.70□0.67□□Ewe		0.80	0.60
Ewe horn		0.51	0.92
Lamb sex		1.02	0.44
Litter size		1.69	0.15

Univariate analyses of the behaviours, based on the results from the MANOVA, showed that time spent sleeping was affected only by Julian date of birth ($F_{1,46} = 6.73$, p=0.013): lambs born later in the season spent more time sleeping (Figure 6.10). This was most likely associated with the weather conditions during the watch period. Replacing date of birth with average daily temperature (°C) in the model, shows that sleep was associated strongly with ambient temperature ($F_{1,46} = 5.09$, p=0.029). Lambs spend more time sleeping in the warmest weather. Ewe treatment group did not affect sleeping ($F_{1,45} = 0.71$, p=0.40).

The proportion of time spent feeding was negatively associated with lamb birth weight (Figure 6.11). Light-born lambs tended to spend more time feeding, although this relationship just failed statistical significance ($F_{1,41} = 3.69$, p=0.062).

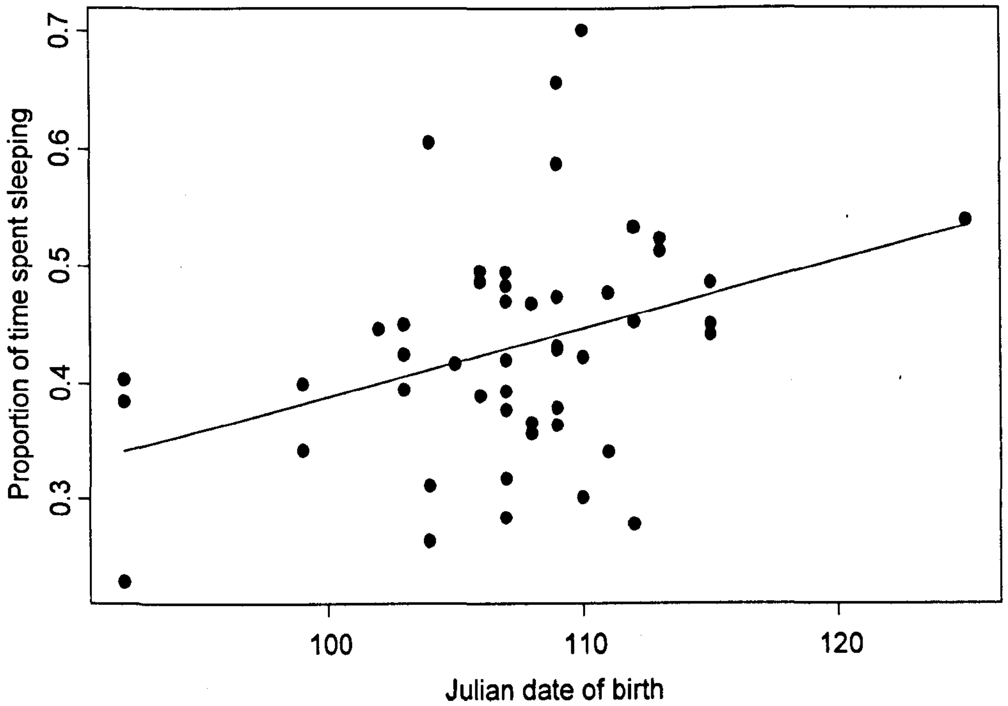


Figure 6.10: Relationship between lamb date of birth (Julian day) and the proportion of time spent sleeping ($F_{1,46} = 6.73$, $p=0.013$). The fitted lines are drawn from model predictions.

Ewe treatment group did not affect the feeding behaviour of the lambs ($F_{1,40} = 1.25$, $p=0.27$).

Proportion of time spent in 'other' behaviour (which included inactive standing and periods of general alertness) was affected by Julian date of birth and ewe coat type. Lambs born later in the season spent less time in 'other' behaviour than those born earlier ($F_{1,45} = 22.29$, $p<0.0001$, Figure 6.12a). This was largely due to weather conditions; time spent in 'other' behaviour was significantly positively related to average daily rainfall (replacing Julian date of birth with rainfall in minimal model: $F_{1,45} = 13.85$, $p<0.001$). The coat type of the ewe also affected the behaviour of the lamb, lambs of Dark Wild (DW) ewes spent more time in 'other' behaviour than lambs of Light Wild (LW) ewes ($F_{1,45} = 6.85$, $p=0.012$; Figure 6.12b).

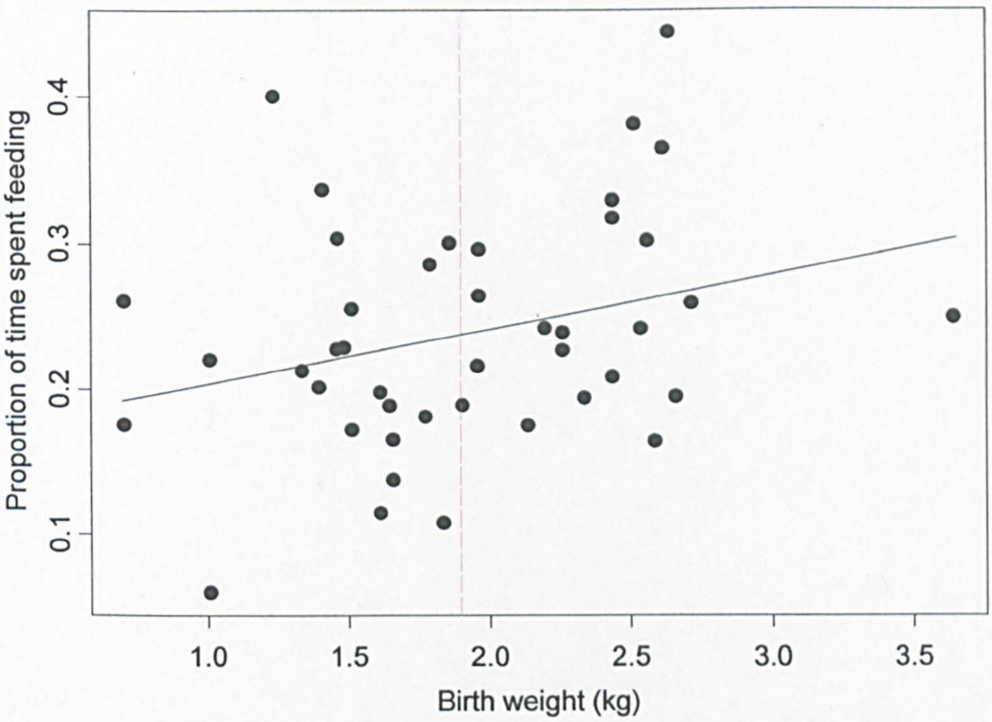


Figure 6.11: Relationship between lamb birth weight (kg) and the proportion of time spent in feeding behaviour ($F_{1,41}$ = 3.69, p=0.062). The fitted lines are drawn from model predictions. The red dashed vertical line indicates the median lamb birth weight (1.9kg).

Ewes of different coat type did not tend to produce their lambs at different stages in the season; as it has already been established Julian date of birth is unaffected by any of the explanatory variables (see section 6.4.1). Ewe treatment group did not affect the proportion of time spent in 'other' behaviour ($F_{1,43}$ = 0.84, p=0.36).

Proportion of time spent in play behaviour was affected only by lamb birth weight ($F_{1,41}$ = 5.52, p=0.023). Lambs with a higher birth weight spent more time in play than low birth weight lambs (see Figure 6.13). Ewe treatment group did not affect lamb play behaviour ($F_{1,40}$ = 0.87, p=0.36).

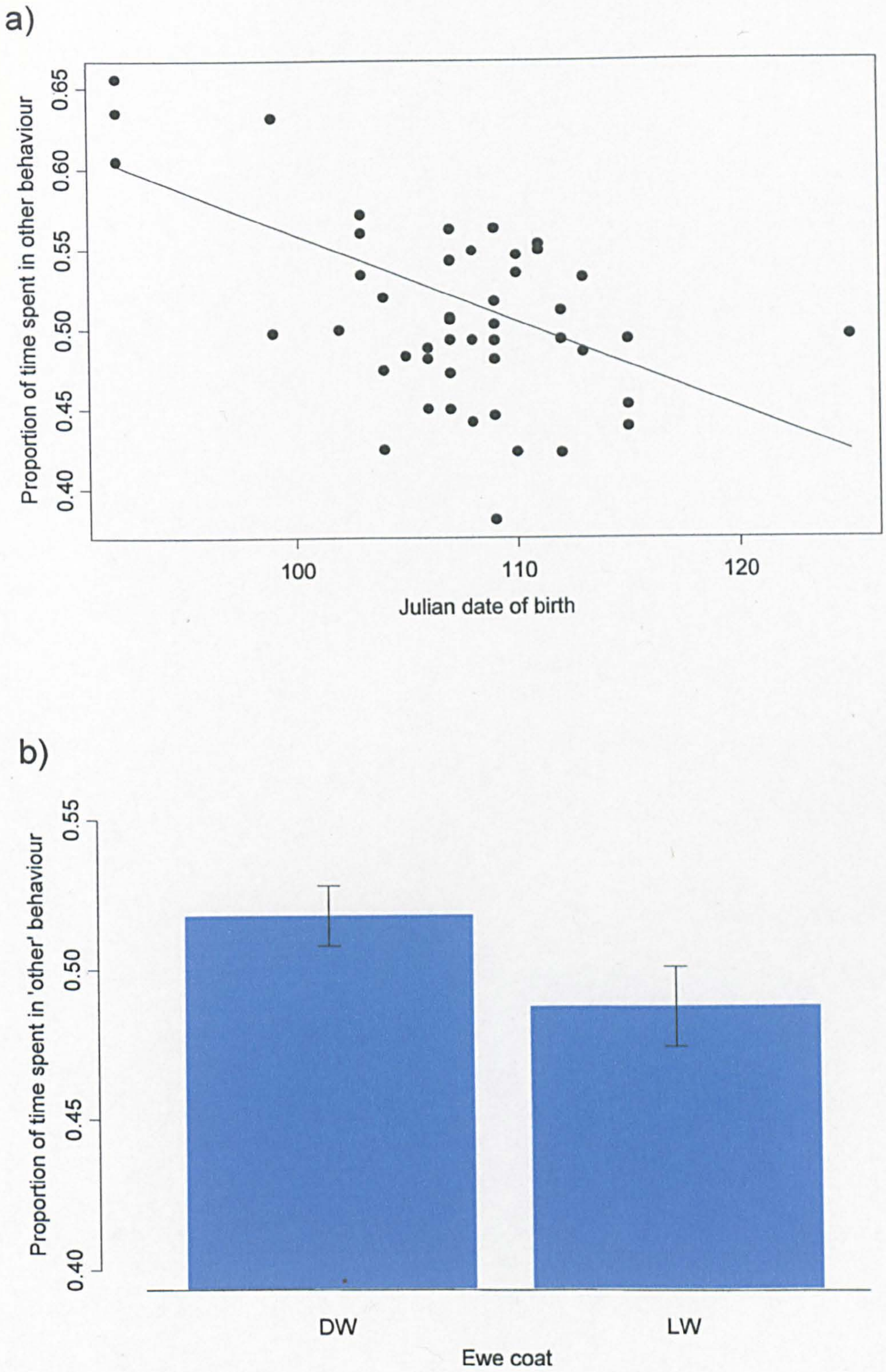


Figure 6.12: a) Relationship between birth date and the proportion of time spent in 'other' behaviour. Lambs born later spend less time in other behaviour ($F_{1,45} = 22.29$, $p<0.0001$). The fitted line is drawn from model predictions. b) The proportion of time spent in 'other' behaviour in lambs of ewes with different coat type. Lambs from ewes with dark wild (DW) coats spend more time in other behaviour ($F_{1,45} = 6.85$, $p=0.012$). Error bars are standard errors.

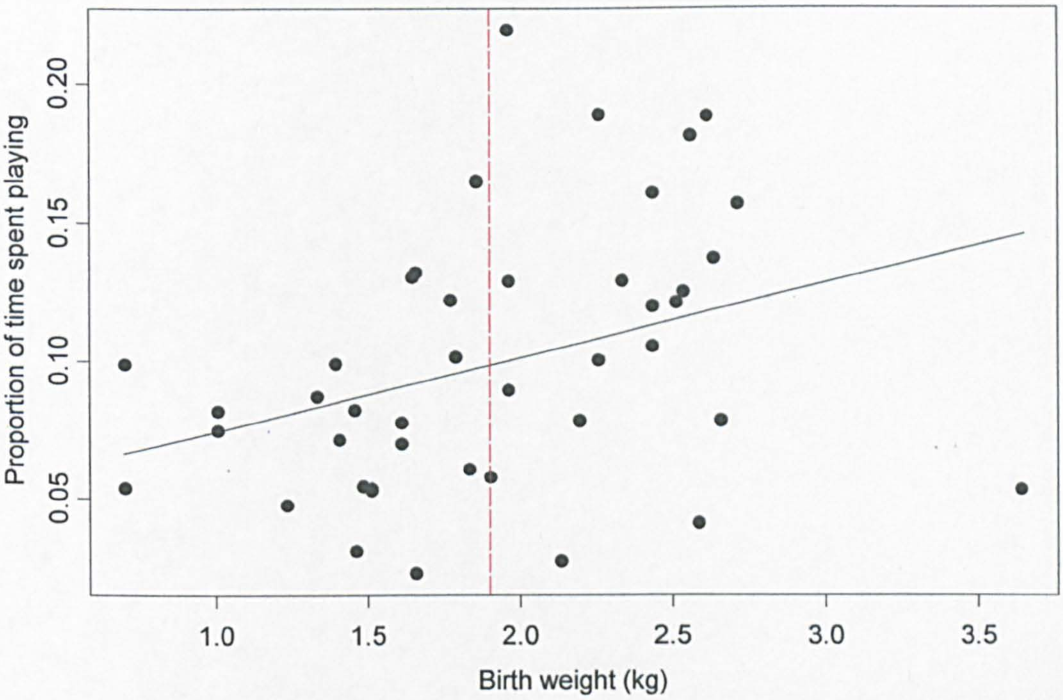


Figure 6.13: Relationship between lamb birth weight (kg) and proportion of time spent playing ($F_{1,41} = 5.52$, $p=0.023$). Fitted lines are drawn from model predictions. The red dashed vertical line indicates the median lamb birth weight (1.9kg).

Proportion of time spent moving was unaffected by any traits relating to the lamb or the ewe (including treatment group: $F_{1,46} = 1.46$, $p=0.23$), and was also unaffected by weather conditions. Similarly, there were no trends in the nuzzling behaviour of the lambs (including treatment group: $F_{1,46} = 2.68$, $p=0.11$).

However, the suckling behaviour of lambs was influenced by their birth weight and the coat type of their mother. The proportion of time spent suckling decreased as the birth weight of lambs increased ($F_{1,40} = 5.36$, $p=0.026$; Figure 6.14a). The lambs of DW ewes spent more time suckling than those of LW ewes ($F_{1,40} =21.62$, $p<0.0001$; Figure 6.14b). Ewe treatment group did not affect lamb suckling behaviour ($F_{1,39} = 0.37$, $p=0.54$).

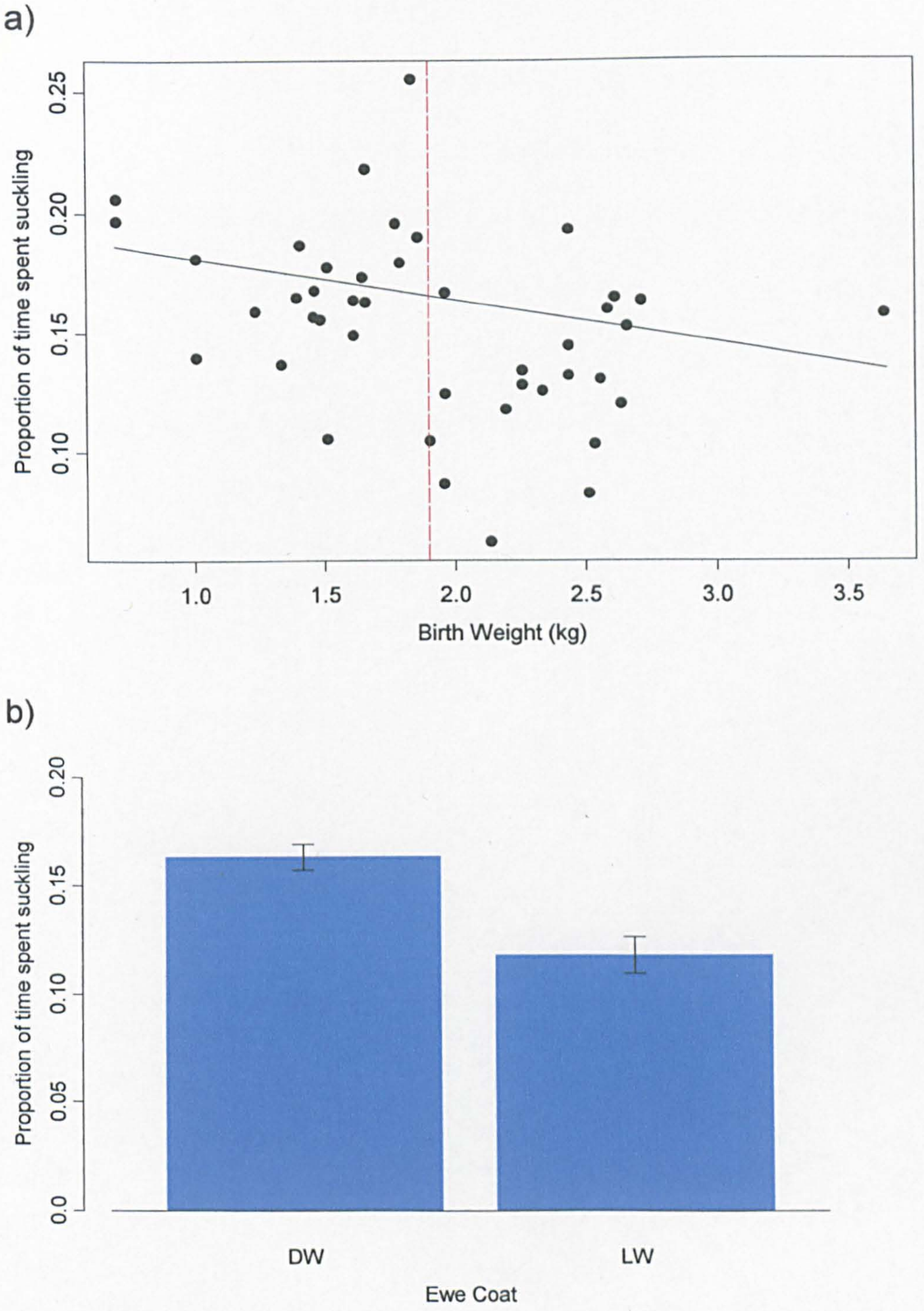


Figure 6.14: a) Relationship between lamb birth weight (kg) and the proportion of time spent in suckling behaviour. Lambs with a heavier birth weight spend less time in suckling behaviour ($F_{1,40}$ = 5.36, p=0.026). The fitted line is drawn from model predictions. The red dashed vertical line indicates the median lamb birth weight (1.9kg). b) The proportion of time spent in suckling behaviour in lambs of ewes with different coat type. Lambs from ewes with dark wild (DW) coats spend more time in suckling behaviour ($F_{1,40}$ =21.62, p<0.0001). Error bars are standard errors.

b) Suckling bout length & suckling rate

There was little variation in the length of suckling bouts between individual lambs, explaining less than 1% of the variance in the data. However the random effects structure was kept in the minimal model to account for repeated sampling for each lamb, as this did not affect the results of the fixed effects.

The length of suckling bouts became shorter as the lambs got older (Figure 6.15), with the rate of decline increasing with lamb age, i.e. in a quadratic relationship (χ^2_2 = 52.87, p<0.0001). The average bout length at 1 day old was 22 seconds, dropping to just 9 seconds by day 35.

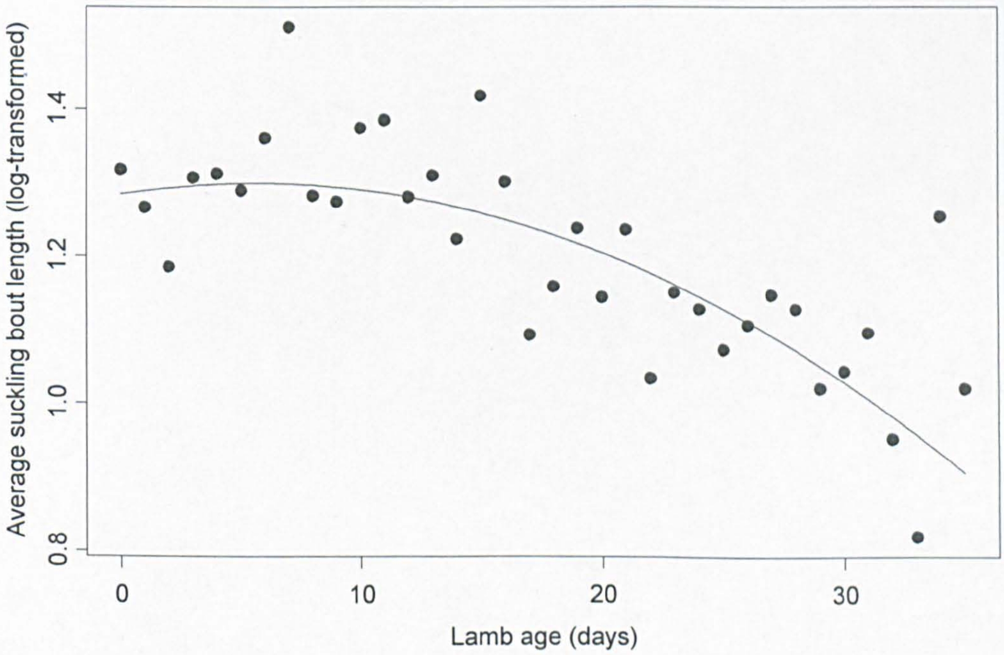


Figure 6.15: Change in suckling bout length with lamb age (χ^2_2 = 52.87, p<0.0001). The fitted lines are drawn from model predictions. Each data point represents grouped means for lamb age.

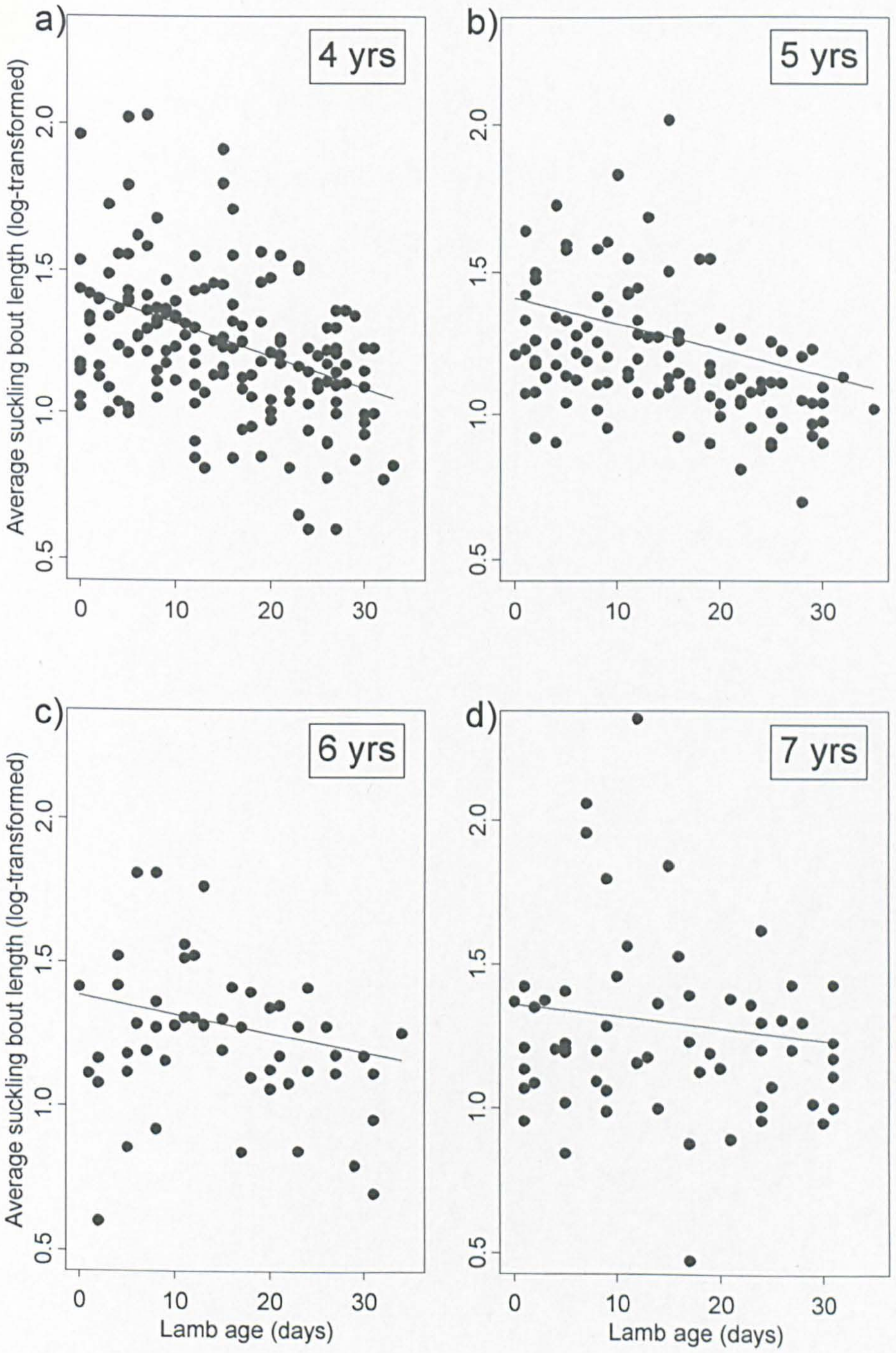


Figure 6.16: Change in suckling bout length with lamb age as ewe age increases from (a) 4 years, (b) 5 years, (c) 6 years, and (d) 7 years old. ($\chi^2_1 = 8.53$, p=0.004). The fitted lines are drawn from model predictions.

There was also an interaction between the age of the lamb and the age of the ewe in suckling bout length ($\chi^2_1 = 5.34$, p=0.02). The decrease in suckling bout length as the lambs aged was less apparent as the age of the ewe increased. In other words, whereas there is a large decline with lamb age in the length of bouts in the lambs of four-year old ewes, in the lambs of seven-year old ewes the change in length of suckling bouts is minimal (Figure 6.16)

Importantly, the average length of suckling bouts was affected by the treatment group of the ewe (Figure 6.17). Lambs from bolussed ewes had longer suckling bouts than lambs from control ewes ($\chi^2_1 = 4.66$, p=0.031). The average length of suckling bout for the Bolus group was 17.68 seconds, whereas the average for those in the Control group was slightly shorter at 16.24 seconds.

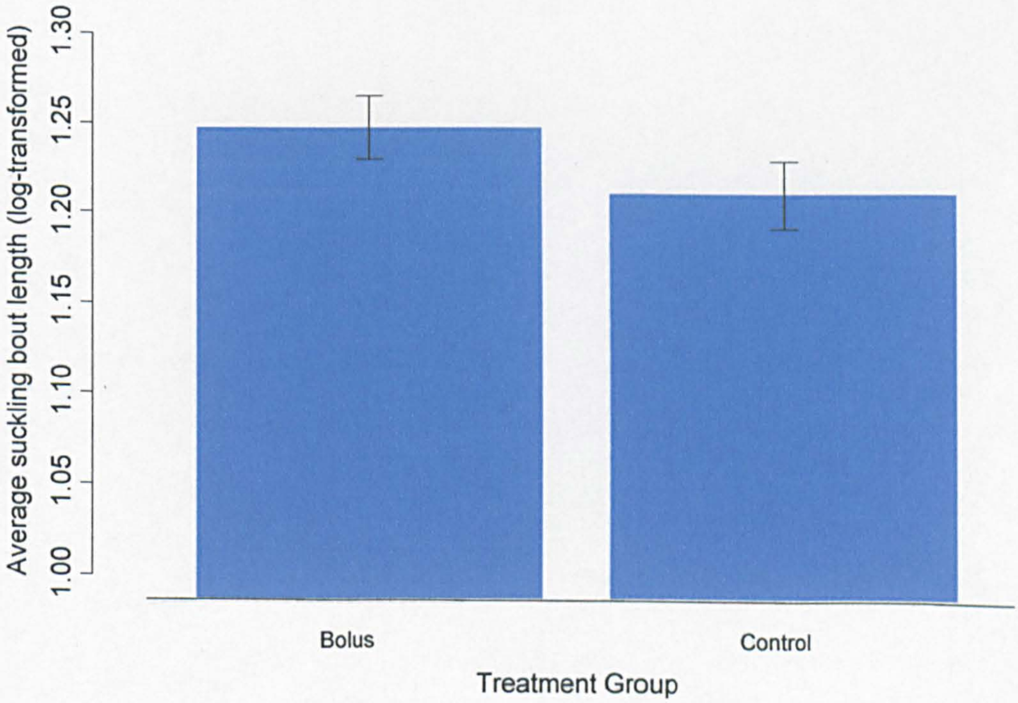


Figure 6.17: Average suckling bout length in lambs of ewes from each treatment group (Bolus or Control) ($\chi^2_1 = 4.66$, p=0.031). Error bars are standard errors.

The coat type of the ewe also affected suckling bout length ($\chi^2_1 = 5.28$, p=0.022; Figure 6.18). Lambs of DW ewes had longer suckling bouts (17.7 seconds) than lambs of LW ewes (15.35 seconds).

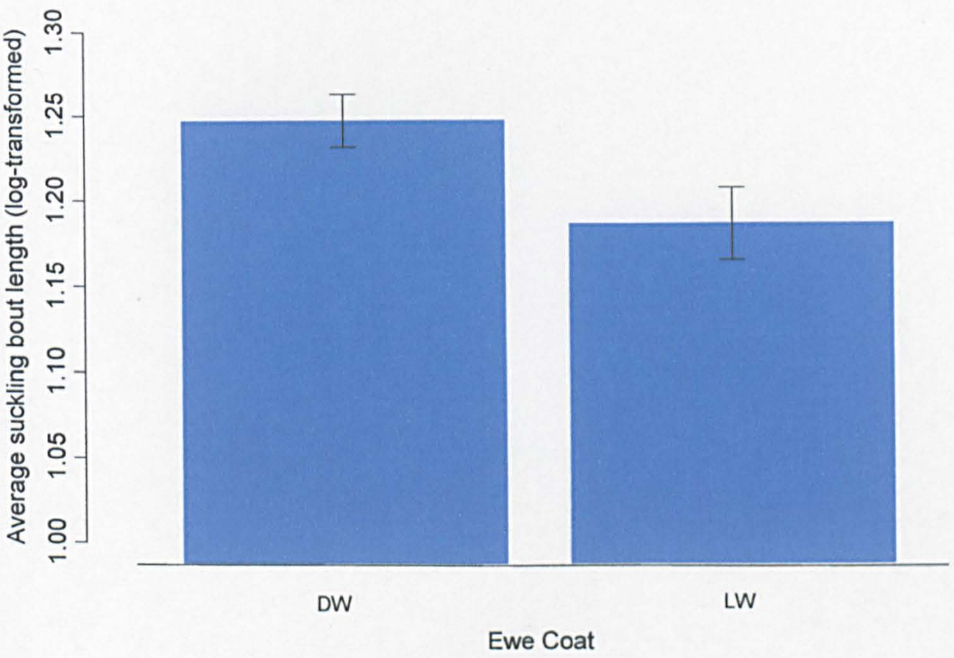


Figure 6.18: Average suckling bout length in lambs of ewes with different coat type (DW- Dark Wild, LW- Light Wild) ($\chi^2_1 = 5.28$, p=0.022). Error bars are standard errors.

In contrast to suckling bout length, there were large differences between lambs in their suckling rates (i.e. the number of suckling bouts per hour), explaining nearly 41% of the variance in the data. As the lambs became older, suckling rate decreased (see below), but there was also variation between lambs in slope of the decline ($\chi^2_2 = 20.19$, p<0.0001). However, it was the fixed effects that were of the most interest in this analysis, and these were still evident despite huge variation explained by individual lambs.

Rate of suckling decreased in a linear fashion as the lambs became older ($\chi^2_2 = 49.11$, $p<0.0001$, Figure 6.19), from 5.75 bouts per hour, at 1 day old, to just 1.32 bouts per minute by day 35. Even after accounting for this general decline, there were still other traits that affected the rate of suckling. A notable exception to this is treatment group; the rate of suckling was no different in lambs of bolussed or control ewes ($\chi^2_1 = 0.28$, $p=0.60$). However, suckling rate was higher in lambs of ewes with a DW coat, than in lambs of ewes with a LW coat ($\chi^2_1 = 3.99$, $p=0.046$; Figure 6.20). Lambs of DW ewes suckled at an average rate of 3.0 bouts per hour, whereas lambs of LW ewes suckled at a lower rate of 2.7 bouts per hour.

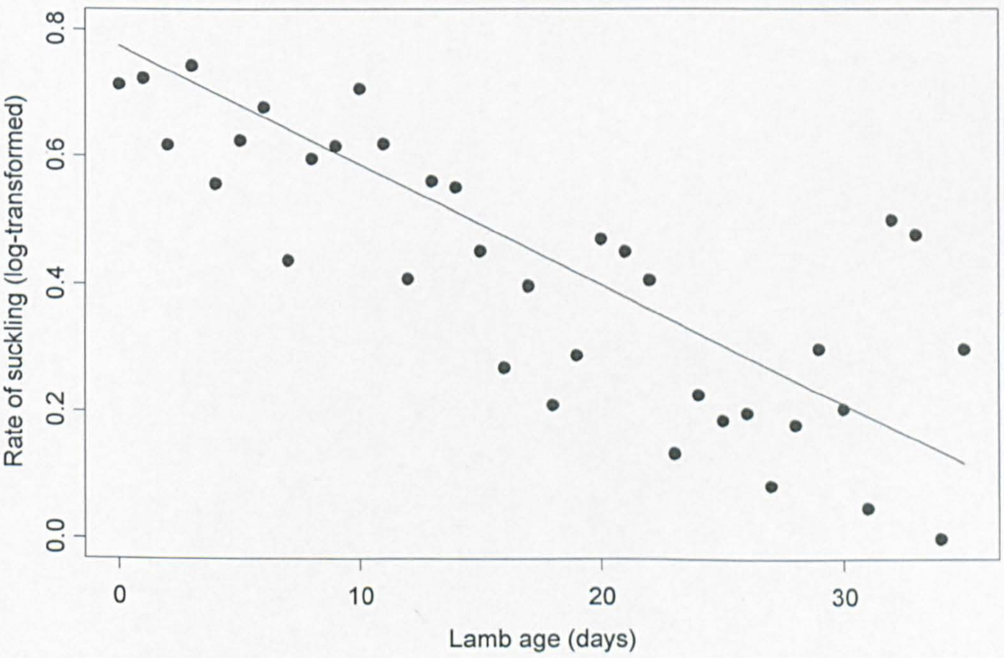


Figure 6.19: Change in suckling rate with lamb age ($\chi^2_2 = 49.11$, $p<0.0001$). The fitted lines are drawn from model predictions. Each data point represents grouped means for lamb age.

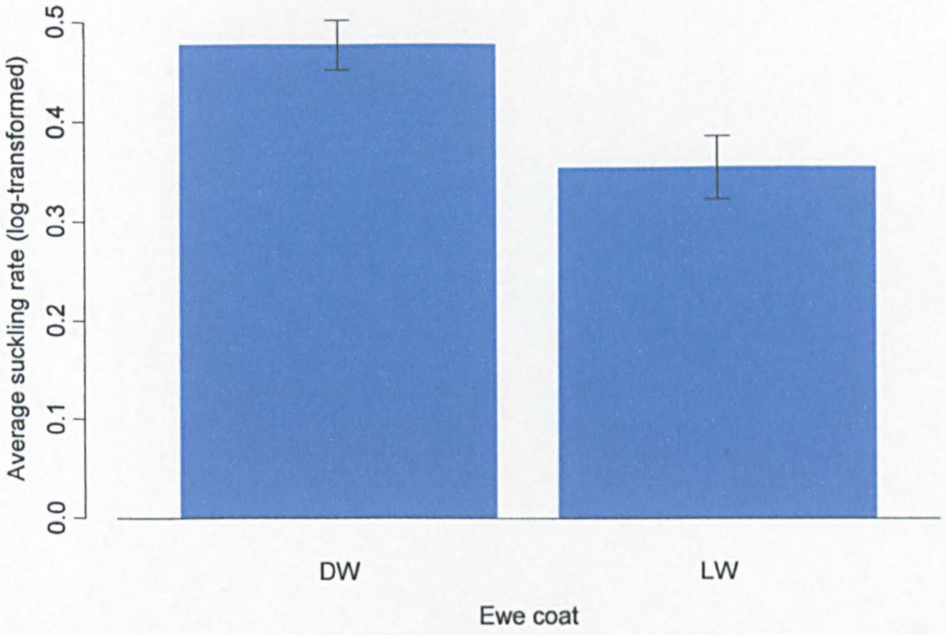


Figure 6.20: Average suckling rate in lambs of ewes with different coat type (DW- Dark Wild, LW- Light Wild) on suckling rate ($\chi^2_1 = 3.99$, p=0.046). Error bars are standard errors.

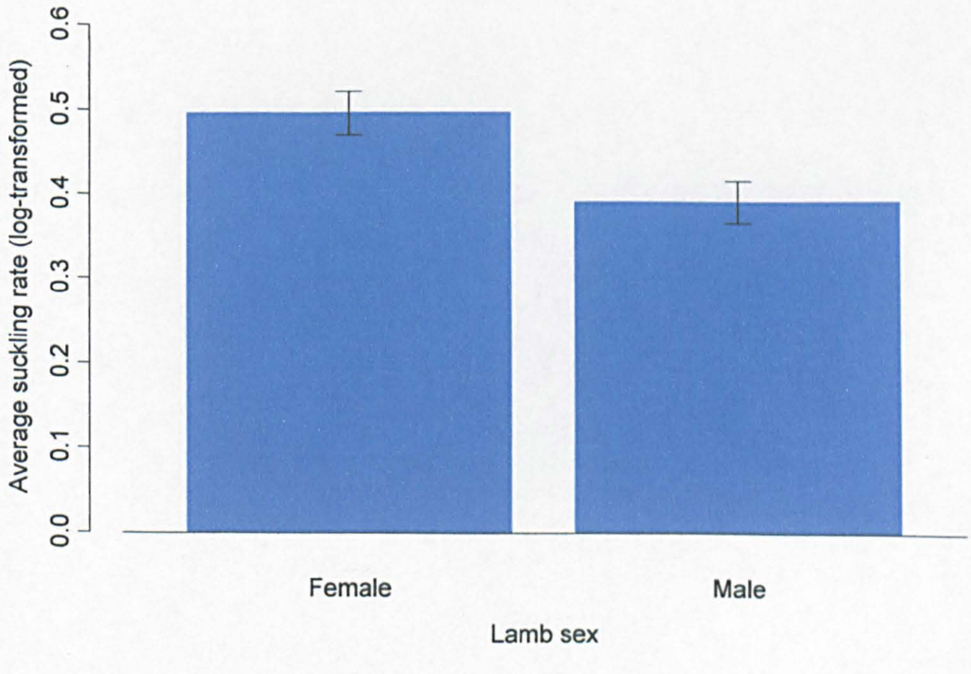


Figure 6.21: Average suckling rate in male and female lambs ($\chi^2_1 = 6.64$, p=0.01). Error bars are standard errors.

Rate of suckling varied with lamb sex, female lambs showing a higher average rate of suckling than male lambs ($\chi^2_1 = 6.64$, p=0.01; Figure 6.21). Female lambs had an average suckling rate of 3.2 bouts per hour; much higher than the male suckling rate of 2.5 bouts per hour. Suckling rate was also influenced by litter size interacting with the age of the ewe ($\chi^2_1 = 8.53$, p=0.004). The rate of suckling in singleton lambs decreased as ewe age increased; however among twin lambs the opposite was true, rate of suckling was higher in lambs with older mums (Figure 6.22).

6.5 Discussion

6.5.1 Lamb survival

Neonatal survival was predominantly affected by litter size, with twins suffering greater mortality than single lambs. This is probably due to the inability of the mother to provide sufficient resources to support both of her offspring, resulting in the death of one or both of them. It may also be an effect of the lower birth weight of twins, although birth weight was not retained in the model independently, even in the absence of the litter size term. This is quite surprising, as birth weight has been shown to be a factor affecting Soay lamb survival in previous studies (Clutton-Brock *et al.* 1992), and is also known to affect offspring survival in other populations (Cote & Fiesta-Bianchet 2001b, Keech *et al.* 2000, Bekele *et al.* 1992, Perez-Razo *et al.* 1998). However, Ylonen *et al.* (2004) found that in bank voles (*Clethrionomys glareolus*), birth weight had no impact on survival.

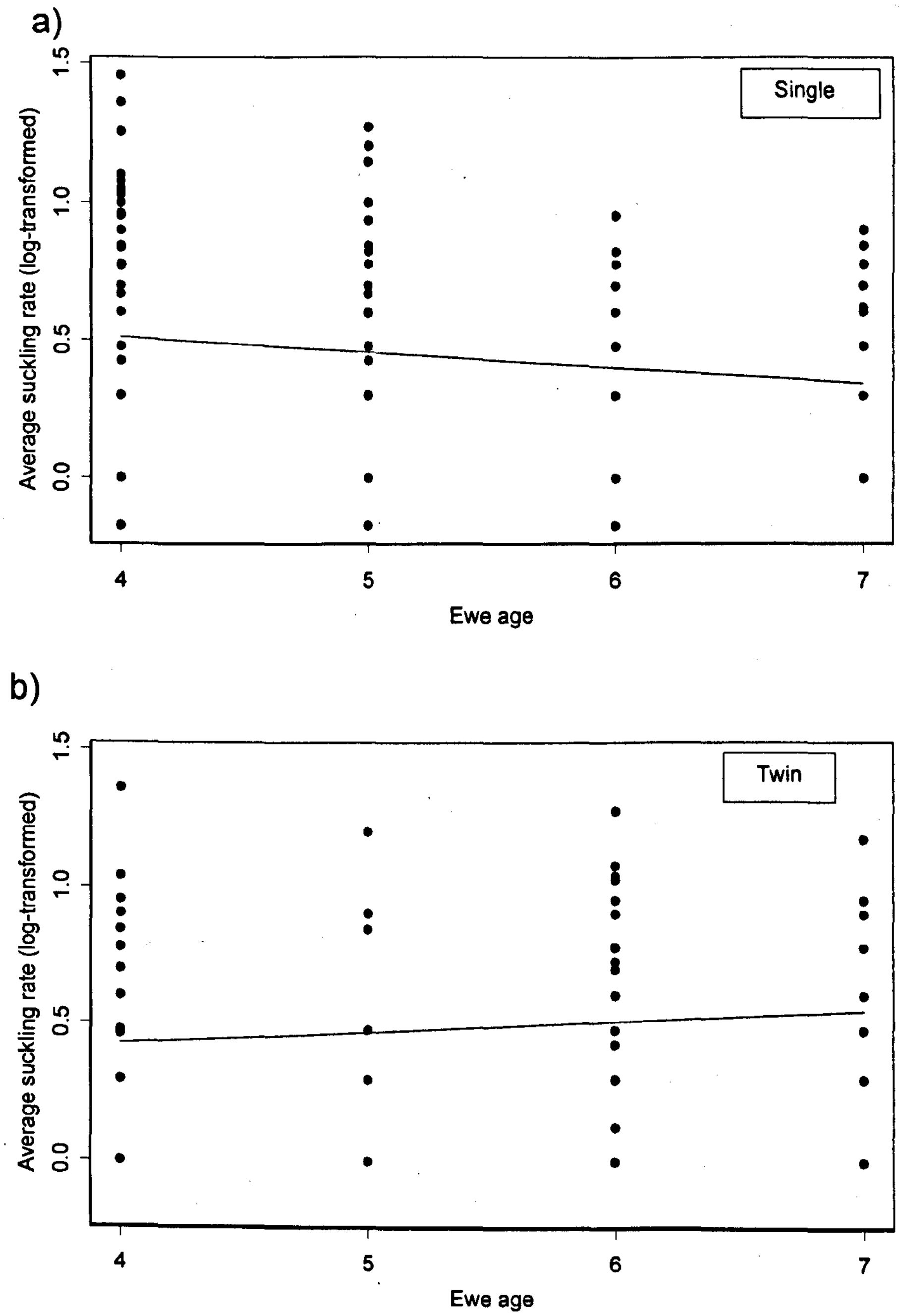


Figure 6.22: Relationship between ewe age (years) and the rate of suckling, in a) single lambs, and b) twin lambs ($\chi^2_1 = 8.53$, p=0.004). The fitted lines are drawn from model predictions.

The effect of ewe treatment on lamb survival is unclear. Although the interaction of treatment group and birth weight was statistically significant (as tested using both parametric and non-parametric survival analyses), the robustness of this result is undermined by the small number of deaths observed (n = 5). Nevertheless, the relationship is interesting, and so is worthy of further discussion. Essentially, although there were no differences in the birth weight of lambs born to treated and control ewes (see below), lambs that were born light in weight were more likely to survive if their mother had been bolussed the previous August. Thus, the threshold birth weight below which lambs died was lower for animals in the treated group (1.4kg), than for those in the control group (2.2kg). This is possibly because bolussed ewes were able to utilize their superior condition (see Chapter 5) to invest more resources into their lamb's survival.

6.5.2 Early development

The patterns of early development and growth in lambs in this study are broadly similar to those reported from earlier studies conducted on St Kilda. As in previous studies on Soay sheep (Clutton-Brock *et al.* 1992) and other species, including Alaskan moose (Schwartz & Hundertmark 1993) and South African goats (Lehloenya *et al.* 2005); litter size affected birth weight, with single lambs being heavier at birth than twin lambs. However, unlike previous studies on Soays (Clutton-Brock *et al.* 1992), there were no significant differences in the birth weights of male and female lambs. In Soay sheep, heavy ewes tend to produce heavy lambs; and middle-aged ewes tend to produce heavier lambs than young or old ewes (Clutton-Brock *et al.* 1996). In this study, there was no effect of ewe weight or age on lamb birth weight; although there was a statistically non-significant trend for heavy ewes to produce heavy lambs, after accounting for litter size. However, it is important to point out that, unlike most previous studies on St. Kilda, all of the ewes in this study were middle-aged (4-7 years old), and therefore some age or weight related issues may not be relevant. Birth date was unaffected by traits of the lamb or ewe in this study. However, previous work has shown that birth date gets earlier as the mother gets older, and twin lambs tend to be born 2-3 days earlier than single lambs on average (Clutton-Brock *et al.* 1992). Again, the absence of any effect on birth date in this study could be a reflection of the small sample size rather than a genuine null result.

Early growth rate (within the first 6-8 weeks) was dependent on litter size; single lambs growing faster than twins (140g per day versus 100g per day). This is slightly higher than earlier studies in which average growth rates for single lambs was 120g per day, and 75g per day in twins (Robertson *et al.* 1992). However, it is important to note that growth rates are typically higher in years of high population density; the population density during the present study was high (1996 sheep on Hirta), whereas the earlier figures were calculated as an average over two years (1988 to 1989) during which the population crashed from approximately 1500 to just under 700 animals. Robertson *et al.* (1992) also found an effect of birth date on early growth rates, with early born lambs growing at a slower rate than late born lambs. No such effect was detected in this study, probably due to small sample sizes and a small number of influential outlying birth dates. Although growth rates had slowed by the time lambs were 4 months old, the difference between single and twin lambs remained; single lambs showing a faster growth rate. Absolute weight at four months was highly correlated with birth weight; lambs born heavy were heavier in August. Previous studies have shown that the positive effect of birth weight on current body weight was still detectable in animals up to 2 years old, and is probably apparent throughout the life of the animal (Albon & Clutton-Brock 1988, Albon *et al.* 1991).

There were no discernable advantages to the lamb, in terms of their birth weight, growth, or weight at four months, in the treatment of their mother to relieve their parasite burden. A similar result was observed in Fulbe ewes, with the removal of parasites having no effect on the growth of their lambs (Njoya *et al.* 2005). In contrast, other studies have demonstrated negative effects of maternal parasites on offspring development in domestic sheep (Valderrabano & Uriarte 2003), and several examples in non-ungulate populations (Kristan 2002b, Votypka *et al.* 2003).

6.5.3 Behaviour

Not surprisingly, the weather affected lamb behaviour to quite a large extent. Lambs born early bore the brunt of the bad weather, whereas the later born lambs had the advantage of better weather in the first few weeks of their life. Lambs tended to stand apparently doing nothing (classed as 'other' behaviour) when it rained, and spent longer sleeping in the sun on hot days. Although these behaviours were influenced by the weather, the most important activities (feeding and suckling) were unaffected by the lamb's date of birth (or the associated weather).

It is expected that lambs in a poorer condition would have to devote more time to feeding on grass in an attempt to increase their nutrient intake. This was evident in the trend for light-born lambs to spend more time feeding than heavier lambs, to compensate for their poorer start in life. Light-born lambs also played less than heavy lambs, presumably because the weaker lambs did not have the energy resources to indulge in play.

Interpreting the significance of time spent in 'other' behaviour is not simple, as by definition 'other' is a catch-all behaviour category that was intended to include anything not described by the mutually exclusive behaviours. However in practice, the main behaviour included in the 'other' category was inactive standing. As has already been discussed, this was largely due to bad weather, but was also common in small lambs (pers. obs). It was noticed, especially in good weather, that while weaker lambs (i.e. those that appeared small and frail) would stand inactive, their stronger peers (healthy-looking lambs, that appeared bigger and well-nourished) were more likely to be involved in playing games within large group of lambs, feeding or asleep in the sun. Correlations between the proportion of time spent in various behaviours (Table 6.2) confirms that 'other' was quite strongly negatively correlated with sleeping (r=-0.51), feeding (r=-0.18), and play behaviour (r=-0.15). If personal observations were correct, and weaker lambs were more likely to remain inactive, then we would expect low birth weight to affect 'other' behaviour in the analyses. This was not the case, and apart from birth date, the main factor influencing this category of behaviour was the coat type of the ewe. This is an unexpected result. However, the analysis clearly shows that the lambs of ewes with dark wild (DW) coat type spent more time in 'other' behaviour than lambs of light wild (LW) ewes. Given that 'other' behaviour is generally not functional, and does not seem to serve a particular purpose, it is difficult to assign a reason as to why a genetic difference in the ewe should be related to this type of behaviour in the lamb. This is especially clear since no differences in early development and growth were detected in the lambs of ewes with different coat types. Perhaps when there is a greater knowledge of coat type in Soays, this type of question can be addressed in more detail.

Generally, suckling behaviour diminished (decrease in suckling rate and bout lengths) as lambs got older, due to the fact that they became more efficient during suckling and less reliant on milk. Feeding on grass increases as the rumen develops (Lyford 1988) and this becomes the ultimate source of nutrient intake.

Unexpectedly, the coat type of the ewe appears to have an effect on the suckling behaviour of the lamb. Lambs of ewes with dark wild coats suckled for a greater proportion of time, due to bouts of suckling being longer, and occurring at a higher rate. The reasons for this are unclear; however it would suggest that the coat type of the ewe has an affect on her mothering ability. As there were no differences in lamb growth or August weight relating to the coat type of the ewe (see Tables 6.7-6.9), it would seem that there may be a difference in the quality of the milk produced by ewes with different coats. As the lambs of dark wild ewes seem to receive more milk provisioning, with no add-on effect on their growth and development, then the milk they receive may be of a lower quality. Perhaps Light Wild ewes produce smaller quantities of higher quality milk than their Dark Wild counterparts. This is not necessarily problematic for the ewes or their lambs. For the ewes, the energetic costs of producing lots of dilute milk, or smaller quantities of more concentrated milk, may in fact be very similar. For the lambs, their nutrient intake is similar (as demonstrated by their growth) but their behaviour must alter accordingly.

The proportion of time spent in suckling behaviour was affected by lamb birth weight: lambs born lighter spent more time suckling than heavy born lambs. This was not surprising, as lambs would demand extra provisioning to compensate for their low birth weight and improve their chances of survival. Female lambs appeared to suckle at a higher rate than male lambs. This contrasts with the findings of an earlier study (Robertson *et al.* 1992) in which no sex differences were found in any aspect of suckling behaviour. However, there were no sex differences in the length of suckling bouts, or the overall proportion of time spent in suckling in the current study. As there were also no sex differences in August weights or growth rates, and therefore no energetic consequences, it would seem likely that during some of these suckling attempts, little or no milk was transferred to the lamb. This being the case, it may be possible that some of suckling behaviour in female lambs served a purpose other than nutritional gain.

Life- history studies of Soay sheep have shown that at around 6 months old, male sheep leave their home range (heft) and disperse to form bachelor groups (Grubb & Jewell 1974, Clutton-Brock *et al.* 2004). From this time on, they are nutritionally and socially independent from their mother. In contrast, female Soays tend to spend their lives in or near the place they were born, with their ranges frequently overlapping those of close female relatives (Coulson *et al.* 1999a). Up until they are about the 2 years old, when the bond between them has weakened, young ewes tend to associate with their mothers (Grubb 1974c, Jewell & Grubb 1974). If this behaviour can also be traced to their early life, in that female lambs may have spent more time nearby their mother than male lambs, this could explain why females have a higher rate of non-nutritional suckling. During the focal watches, it was noticed that as lambs became older and stronger they would move away from their mothers to interact with other lambs. Unfortunately, no information on the distance from lamb to ewe was recorded in this experiment, so it is not known if male lambs did in fact stray further away, or for longer periods of time. If it assumed that this may be a possibility, then the closer spatial association of female lambs to their mothers would provide the lamb with greater access to attempt suckling. Although this may not have any nutritional advantage, it could be important in developing a social bond, or be purely opportunistic.

The interaction of ewe age with litter size on suckling rate, and with lamb age on suckling bout length, gives us some insight into mother-offspring conflict, and the costs of reproduction in terms of the trade-off between current and future reproduction. The decline in suckling bout length with age that occurred in all lambs, varied according to the age of their mother. This decline was greatest in the lambs of the youngest ewes (4 years old), whereas the decline in the oldest ewes (7 years old) was minimal. It is likely that this is attributable to the nursing experience of the ewe, rather than any differences in their body condition. As shown in chapter 5, there were no detectable differences due to age in ewe body weight or condition. The oldest ewes are likely to have more experience in raising young, and will not permit the longer suckling bouts that the relatively inexperienced younger ewes allow. This pattern has been seen in other ungulates, including Mouflon (Reale & Bousses 1995, Reale *et al.* 1999).

In a previous study of maternal care on Soay sheep, Robertson reported that twin lambs suckled for longer than single lambs (Robertson *et al.* 1992). In contrast, in this study there were no differences in suckling bout lengths, and the rate of suckling was affected by litter size, dependent on the age of the ewe. Older ewes (7 years old) tended to let twin lambs suckle more than single lambs, whereas in the youngest ewes in the study (4 years old) the opposite was true. Generally, there is a trade-off between the amount of resources a mother can invest in her offspring, and the number of offspring in the litter (Clutton-Brock 1991). This is probably the explanation as to why single lambs suckled at a higher rate than twins in younger ewes. However, in the older ewes, the chances of breeding successfully the following year are diminished compared to the chances of a four year old. The cost of rearing twins would probably reduce this possibility further. It could be that older ewes are increasing investment in current reproductive effort, because the likelihood of future reproduction is low (Trivers 1974).

The only evidence for a difference in suckling behaviour due to ewe treatment group is in the length of the suckling bout. Lambs from bolussed ewes had longer suckling bouts than lambs of control ewes. However this difference was not apparent throughout the whole of the first four to five weeks of the lambs' life, as there were no differences in the overall proportion of time spent in suckling behaviour or the rate of suckling. An obvious explanation to these longer suckling bouts is that treated ewes produced lower quality milk than control ewes, which would necessitate an increase in bout length. However, treated ewes were in a better condition than control ewes (Chapter 5), so there is no biological reason to assume that this is the case (in fact, one would predict the opposite to be true). In addition to this, as there were no observable differences in growth or in August weight of lambs of treated ewes, there must have been no energetic consequence of the increased suckling bout length. In light of these observations, why then were longer suckling bouts reported in lambs of treated ewes?

The answer could lie with mother-offspring conflict. Frequently, offspring will demand more resources than the mother can, or wants to, provide, either because there is another sibling to invest in, or perhaps to maintain her own body condition for survival and/or future reproductive effort. Although the treated ewes are in a better condition, they do not appear to invest more resources in their lambs overall, as is evident in the growth and weights of their lambs. Day to day, with lower energetic stresses in place, treated ewes may occasionally allow longer bouts of suckling, being under less pressure to desist a persistent lamb. However, as this cannot have provided extra nutritional provision, it is possible that there may be variation in milk flow during suckling, with some bouts having reduced or no milk flow.

Generally then, there were no differences in lactation effort between treated and control ewes. There were no differences in time spent suckling or rate of suckling, and despite some variation in suckling bout length, there is no evidence for increased nutritional provisioning of offspring by the treated ewes, as ultimately there were no differences in the early development and growth of the lambs. In bighorn sheep, ewes highly parasitized with lungworm provided less maternal care (shorter nursing time and lower quantities of milk), which was correlated with low lamb survival (Festa-Bianchet 1988).

6.6 Conclusions

The results of this study indicate that some of the factors shown previously to influence the early development and growth of Soay sheep were also important in determining the fitness of the experimental sheep. However, the key question is, after accounting for these known effects (e.g. litter size on birth weight), was there any effect of removing parasites from ewes on the birth weight, growth rates, and August weight of the lambs? The simple answer to this is no. Despite the fact that treated ewes were in a superior condition to control ewes, they did not appear to invest more resources in their lambs to improve their early development. Perhaps the explanation for this lies in parent-offspring conflict. Mortality was generally low in this study; most lambs survived to weaning. Therefore, allocation of surplus resources towards pre-natal development (influencing birth weight) and post-natal development (lactation effort; influencing growth and August weight) may not have been necessary. If the amount of effort needed to successfully wean a lamb in a particular year is set at a certain level, is there any advantage to the mother in exceeding it? There certainly is an advantage to the lamb, and this scenario leads to mother-offspring conflict. A good start to life will improve the lamb's chances of survival and reproductive success (Clutton-Brock *et al.* 1996, Coltman *et al.* 1999c), but at what cost to the mother? Maternal investment ideally should be sufficient to give offspring a good start in life, without exceeding a limit over which the mother herself would suffer detrimental effects; through short-term energetic costs that could affect her survival and future reproduction (Festa-Bianchet *et al.* 1998, Pomeroy *et al.* 1999). Bighorn ewes are known to limit the resources they invest in lambs during years of increased stress, so that personal costs are not too high (Festa-Bianchet & Jorgenson 1998). A recent study of Harbour seals (*Phoca vitulina L.*) showed that females invest a constant proportion of body stores to pups rather than a constant amount, and females varied lactation effort according to their body mass (Bowen *et al.* 2001). In this way a balance is reached between the energetic costs to the mother, and the benefits of invested resources to the offspring (enhanced growth and survival).

In this study it appears that treated ewes do not exceed the maternal care necessary to wean their lambs successfully, as there were no differences in weight and growth of lambs from treated or control ewes, and no evidence for differences in milk provision in terms of suckling behaviour. Crucially, however, there may be some advantage to lambs born to a treated mother; lambs that were born particularly light in weight were more likely to survive if their mother had had their parasites removed the previous August. Thus, it appears that extra provision was may have been made by the Bolus ewes to ensure the *survival* of lambs, but perhaps not to enhance their development.

Chapter 7

Discussion

7.1 Aims and introduction

The aim of this thesis was to examine the interaction between the costs associated with parasitism and the costs associated with reproduction, and to identify any possible trade-offs between these traits, in a wild ungulate population of Soay sheep (*Ovis aries L.*) on St Kilda. Life history theory predicts that allocation of resources towards a trait, for example reproduction, occurs at the expense of allocation of resources to other traits, such as immunity to parasites or survival (Partridge and Harvey 1985, Stearns 1989, Lessells 1991, Partridge 1992). These trade-offs must occur since the resources available to an individual are limited, and therefore allocation decisions must be made (Levins 1968). How an individual allocates resources is dependent on their acquisition of resources, and the environment in which they live (van Noordwijk and de Jong 1986). Different circumstances caused by changes in their environment such as in predation risk, parasite burden, food availability and weather conditions, may alter the optimal life history strategy. Moreover, some individuals may have a genetic advantage in a certain environment, which affords them greater acquisition of resources, and allows them to allocate more resources to life history traits, than other individuals (van Noordwijk and de Jong 1986, Reznick *et al.* 2000). However, the ultimate goal in managing life history decisions is to maximise fitness in terms of survival and, ultimately, reproduction (Stearns 1989, Lessells 1991).

An important trade-off that appears to be prevalent in many individuals is that between immunity to parasites and reproduction. For example, when reproductive effort was increased in the collared flycatcher (*Ficedula albicollis*) by increasing brood size, parents suffered increased susceptibility to *Haemoproteus* parasites, and consequently lower survival (Nordling *et al.* 1998). In eider ducks, *Somateria mollissima*, antiparasitic treatment did not affect nest success, or the rate of return in successful females (those that were observed to hatch their eggs); but it did affect the subsequent return rate of females that were unsuccessful (those that lost or abandoned the nest before hatching) (Hanssen *et al.* 2003). In this case, the detrimental effects of parasites did not affect the host current reproductive success, but by removing them, birds in poorer condition were able to improve their chances of future reproductive success.

Parasites may have a two-fold effect upon their host: the pathogenic consequences of parasitism, which reduces body condition (e.g. Gulland & Fox 1992) and may directly affect survival or the ability to reproduce; and the costs associated with maintaining or mounting an effective immune response to parasites. To avoid the pathogenic effects of parasites, an individual may have to redirect resources and modify their life history strategy (Forbes 1993, Forbes 1996, Agnew *et al.* 2000). This can be achieved either by maximising current reproductive effort in a bid to minimise the impact of parasites, or by directing resources towards immunity, thereby lowering resources available for growth, body condition and investment into offspring (Minchella & Loverde 1981, Polak & Starmer 1998, Krist 2001, Chadwick & Little 2005). For example, high numbers of the blood-sucking larvae of blowflies (*Protocalliphora falcozi*) in the nests of marsh tits (*Parus palustris*), caused an increase in parental effort in terms of the food provisioning of their offspring. Consequently, nestling survival in highly parasitized nests was not compromised; however, parents suffered the cost of increased reproductive effort in reduced survival in the following year (Wesolowski 2001). The impact of parasites may not only cause alteration of life history strategy in hosts; but may also impact upon the fitness of their offspring. High ectoparasite loads of mites in the common lizard (*Lacerta vivipara*) were associated with decreased maternal survival (Sorci & Clobert 1995). However, the *offspring* of highly parasitized mothers subsequently showed higher fitness early in life; which was interpreted as a strategy to avoid the additive effects of parasites in the population (Sorci & Clobert 1995).

Since parasites can cause modification of life-history by creating an unfavourable environment, the question we need to ask is, how do parasites influence the life history strategy of Soay sheep on St Kilda? More specifically, how do they affect ewe current reproductive effort? When the parasite burden is high, do Soay sheep modify their life history strategy, to increase current reproductive effort in the anticipation of reduced future reproduction? Or do they divert valuable resources to resisting parasites, and suffer the consequences that this may have upon their current reproductive effort?

There is growing evidence that parasites affect host survival, growth and fecundity (reviewed in Gulland 1995; Grenfell & Gulland 1995; Tompkins & Begon 1999); and that they play a significant role in regulating their host's population dynamics (Anderson & May 1978, May & Anderson 1978, Tompkins *et al.* 2002). The effects of parasites on regulating host populations have been demonstrated in laboratory populations of mice (Scott 1987). Manipulating numbers of the parasite *Heligmosomoides polygyrus,* first by introducing parasites to the population, resulted in a drop in host population density by a factor of 20. Subsequent removal of the parasites by administration of anthelmintics, then allowed the population to increase, reaching original population density within twenty weeks (Scott 1987). Demonstrating parasite-mediated regulation of host densities is more difficult in wild populations, due to logistical and ethical considerations, and it may take many years to obtain sufficient data. However, a classic example of parasite-mediated regulation in natural populations is in the red grouse (*Lagopus lagopus*). Several studies have shown that parasites are a driving force behind the population dynamics of red grouse populations (Hudson 1986, Hudson & Dobson 1989, Dobson & Hudson 1992, Hudson *et al.* 1998). This was clearly demonstrated in an experiment to remove the parasite *Trichostrongylus tenuis*, by treatment with anthelmintic drugs. Parasite removal caused dampening of the cyclical fluctuations in population density in treated grouse populations, in comparison to the control populations (Hudson *et al.* 1998). The mechanism behind this was through negative effects of parasites on grouse fecundity, rather than on their survival (Dobson & Hudson 1992).

The effect of parasites on host fecundity has also been observed in ungulate populations. In bighorn sheep (*Ovis canadensis*), low survival was observed in the lambs of ewes highly parasitized with lungworm (*Protostrongylus spp.*) (Festa-Bianchet 1988a). Similarly, in Svalbard reindeer (*Rangifer tarandus*) pregnancy rate in hinds increased when they were treated to remove their burden of gastro-intestinal parasites (Stien *et al.* 2002; Albon *et al.* 2002).

On St. Kilda, the prevalence of parasitism by strongyle nematodes in Soay sheep is high (Gulland and Fox 1992, Wilson *et al.* 2004, Craig 2005) and several observational and experimental studies have demonstrated that parasites have negative effects on sheep survival (Gulland 1992, Illius *et al.* 1995, Coltman *et al.*

1999, Milner *et al.* 1999). In addition to this, there is evidence of age-related costs of reproduction on future survival (Gulland 1991, Clutton-Brock *et al.* 1996, Tavecchia *et al.* 2005). However, although the costs of reproduction and the costs of parasitism on survival are fairly well understood in Soay sheep, little is known about the trade-off between parasites and the costs of reproduction. This thesis attempted to explore the interaction between resistance to parasites and the costs of reproduction. In particular, it considered the impact of reproduction on the peri-parturient rise in parasite burden observed during the spring (Chapter 3), and the effects of parasites on ewe reproductive success (Chapters 4-6).

7.2 The impact of reproduction on parasite burden: the peri-parturient rise in Soay sheep

The peri-parturient rise, or PPR, is a well known phenomenon that has been documented widely in domestic sheep (Connan 1968, O'Sullivan & Donald 1970,1973). It is the temporary increase in parasite burden around the time of lambing from April to June. The most important gastro-intestinal nematode contributing to the PPR in domestic sheep is widely regarded as *Teladorsagia* spp. (Morgan *et al.* 1951, Parnell *et al.* 1954, Crofton 1954, Dunsmore 1965, Connan 1968b, Herd *et al* 1983, Bishop & Stear 2001).

In accord with studies on domestic sheep, the peri-parturient rise in parasite burden has been observed in lambing Soay ewes that incur the full costs of reproduction, which are the costs of gestation, parturition and lactation (Crofton 1958, Field *et al.* 1960, Brunsdon 1964, Procter & Gibbs 1968, Shubber *et al.* 1981, Gibbs & Barger

1986). This characteristic rise is likely to be associated specifically with lactation effort, since ewes whose lambs died shortly after birth showed a subsequent rapid decrease in parasite burden. Several studies in domestic sheep have observed that costs of lactation are most likely to be causative of the PPR, although the direct mechanisms are still unknown (Gibbs 1967, Connan 1968a, b, Salisbury & Arundel 1970, although see Jeffcoate *et al.* 1992).

In non-lambing adult ewes (three years and older), there was no evidence of a peri-parturient rise, which conforms to patterns observed in domestic sheep (Crofton 1958, Field *et al.* 1960, Brunsdon 1964, Procter & Gibbs 1968, Shubber *et al.* 1981, Gibbs & Barger 1986). In contrast, yearling non-lambing ewes showed a large increase in parasite burden (strongyle FEC) during the spring, and, to a lesser extent, so did the non-lambing two year old ewes. The reasons for this are probably due to increased susceptibility to parasitism at this time of year, combined with inferior acquired immunity: sheep are in poor condition after the food shortage that occurs during the winter (Gulland 1992, Clutton-Brock *et al.* 1997a), and ewes that failed to lamb are probably in the poorest condition. As younger animals do not have the acquired immunity of older ewes, they will be more susceptible to the increase in infective parasite larvae present on the pasture during the spring (Wilson *et al.* 2004). Hence, younger non-lambing ewes, despite not incurring costs of parturition and lactation, had an increased PPR during the spring.

In this study, in contrast to most studies of the PPR in domestic sheep, it has been feasible to examine the possible effects of factors not directly associated with reproduction. In particular, it has been shown that population density is important, and that males also suffer a spring rise in parasitism, which confirms what had previously been suggested in an earlier study (Gulland & Fox 1992). The importance of sheep body condition and environmental effects, were emphasized by the observations made on spring parasitism in tups, and in ewes experimentally treated to temporarily remove their parasite burden. Lambing adult ewes with improved winter body condition afforded by anthelmintic treatment (see Chapter 5), had a lower PPR than a control group of ewes. In addition to this, the pressures of poor body condition and increased infective larvae on the pasture during the spring (Wilson *et al.* 2004) caused an increase in parasite burden in ewes and tups, an effect that was exacerbated when population density throughout the winter was high. As population size increases, the density of infective larvae on the pasture increases, and exposure to infection is higher (Gulland & Fox 1992). High population density also increases competition for food, keeping animals on a lower plane of nutrition, and making them more susceptible to infection.

The importance of body condition was further confirmed by the observation that the weight of both ewes and tups made a significant difference to their ability to cope with spring parasite burden. The reason that this observation is important is because a weight-related decline in levels of parasite burden in ewes can normally be attributed to their age, and the acquired immunity that this affords (Wilson *et al.* 2004). Similarly in males, weight is not normally an issue; previous studies have shown that there is little variation in the parasite burden of tups over one year old (Wilson *et al.* 2004).

Supplementing Soay sheep diet is not logistically possible (since they are a free-living population). However, in an experimental study on West African goats, supplementation of basic diet requirements reduced the effect of the peri-parturient rise in parasite burden (measured by faecal egg count) in treated individuals (Faye *et al.* 2003).

In Soay sheep, it appears that there is a peri-parturient rise in parasite burden, which occurs as a cost of reproduction, as observed in domestic sheep. However, the trade-off that occurs between reproduction and immunity to parasites is confounded by their population dynamics. This has the effect of exacerbating the effect of the peri-parturient rise during periods of unfavourable weather conditions, and in animals that are in poor condition.

7.3 The effect of parasite burden on ewe reproductive success

Using observational data on the reproductive success of ewes, it was apparent that there was a correlation between high parasite burden in the summer, and reduced probability of successfully rearing a lamb to weaning age in the following spring. This suggests the *possible* existence of a trade-off between parasite resistance and reproductive effort. However, it is particularly difficult to establish the nature of life history trade-offs based on phenotypic correlations alone, due to variation between individuals in resource levels (van Noordwijk & de Jong 1986). Often it is possible to reveal the true nature of trade-offs between life history traits only by experimental manipulation, since positive correlations are often observed. Exposure to parasites can have a positive effect on current reproductive effort; however experimental manipulations have shown that this, known as 'fecundity compensation', is a strategy employed to reduce the impact of parasites on future reproduction, and/or survival (e.g. Minchella & Loverde 1981, Richner & Tripet 1999, Krist 2001, Tripet *et al.* 2002, Bouslama *et al.* 2002). For example, experimental manipulation of the microsporidian parasite (*Glucoides intestinalis*) in the crustacean *Daphnia magna*, revealed parasite-mediated fecundity compensation in *Daphnia* life history trade-offs, with exposure to parasites causing an increase of 39% in offspring numbers in early reproductive effort (Chadwick & Little 2005). Similarly, male *Drosophila* increased reproductive effort in response to being experimentally parasitized, but suffered the cost in having a shorter life span (Polak & Starmer 1998).

Given the limitations associated with analysis of observational data, an experiment was conducted to manipulate the parasite burden of ewes, and examined the consequences of this for the reproductive success and condition of ewes; and the survival, early development and growth of their lambs (Chapters 5 and 6).

Pivotal to this experiment, administration of anthelmintic bolus successfully removed the parasite burden from the treated ewes, affording them reduced costs of fighting parasite infection going into, and probably throughout, much of the winter (see Chapter 5). Did the reduction in the costs of immunity to parasites, afforded by the anthelmintic treatment, have any impact upon ewe condition and reproductive effort, and if so, was any benefit observed transferred to their lambs?

In terms of the effects of parasite removal on ewe body condition, the answer would appear to be yes. Longer term parasite burden in the following spring (the peri-parturient rise) and in the following summer (a year after treatment) were lower in bolussed ewes compared to control ewes. Bolussed ewes also appeared to be in a better condition than control ewes, having reduced loss in body weight and condition over the period from August 2003 to August 2004 (bolussed ewes lost 0.43 kg in weight, and 4.6% of body condition, compared to 1.24 kg weight loss, and 7.5% loss of condition in control ewes). By approaching the data analysis in two stages (the first determining the factors in 2003 that may affect ewe body condition in spring and summer 2004, and the second incorporating events that occurred in the spring of 2004) it was possible to discern that treatment afforded the bolussed ewes the advantage of improved body weight and condition, and that this advantage was expressed during the lambing period.

Given the superior condition of bolussed ewes, one might expect this to affect their reproductive success. However, the probability of lambing was not affected by treatment. Perhaps, in such a highly fecund population, this is hardly surprising. However, there was some evidence suggesting a trade-off between reproductive effort (in terms of the number of offspring produced), and parasite load: bolussed ewes had *twice* the incidence of twinning than control ewes (25% in bolussed ewes, compared to only 12% in control ewes).

It might also be expected that the improved body condition of bolussed ewes would be reflected in the survival and early development of lambs. The survival of lambs born to bolussed ewes was higher than lambs of control ewes, but this was dependent on their birth weight (Chapter 6). Although there were no differences in the birth weights of lambs born to bolussed and control ewes, lambs that were born light in weight were more likely to survive if their mother had been bolussed in the previous summer. Thus, the threshold birth weight below which lambs died was lower for animals in the bolussed group (1.4kg), than for those in the control group (2.2kg). This is possibly because bolussed ewes were able to utilize their superior condition (Chapter 5) to invest more resources into their lamb's survival. In contrast to this, there was no evidence that bolussed ewes increased lactation effort toward their offspring. This was apparent in both the suckling behaviour and the early growth of their lambs. There were no differences in time spent suckling or rate of suckling and, despite some variation in suckling bout length, no evidence for increased nutritional provisioning of offspring by the treated ewes, as ultimately there were no differences in the early development or growth of the lambs.

So, why did the bolussed ewes not increase their investment of resources into the early development of their lambs, despite being in a superior condition than control ewes? The most likely explanation for this is the trade-off between current reproductive effort and future reproductive effort; the cost of reproduction (Williams 1966). Hence, mothers should invest sufficient resources to ensure the survival of their offspring, while not investing so much that their own future fecundity and survival prospects are compromised. This has been demonstrated in Bighorn ewes, who, during periods of increased stress, limit the resources they invest in their lambs (Festa-Bianchet & Jorgenson 1998). Similarly, harbour seals vary their lactation effort depending on their body mass (Bowen *et al.* 2001). However in dung beetles, mothers were able to spend less on food provisioning, and still produce higher numbers of offspring, when given a choice to 'upgrade' to a higher quality food resources (Hunt & Simmons 2004). Although it is unlikely that the Soay ewes in this study were under extreme pressure, evidenced by the low mortality observed in ewes and lambs during this period, they may still apportion resources optimally to increase future survival and fecundity prospects.

In conclusion, there is some evidence for a trade-off between reproductive effort and parasite resistance, in terms of increased lamb survival and twinning rates in bolussed ewes. However, there is no evidence for increased investment into early development and growth of lambs, which would improve lamb survival and reproductive success later in life (Albon & Clutton-Brock 1988, Albon *et al.* 1991, Clutton-Brock *et al.* 1996, Coltman *et al.* 1999c). However, it should be remembered that this experiment focussed on prime aged ewes (four to seven years old), and it is possible that the main effects of parasitism on reproductive success are observed in younger females that have yet to fully develop acquired immunity to their nematode parasites. In addition, the current study has considered only the effects of parasites on *current* reproduction; but what about the effects on *future* reproduction? As the ewes in this study were prime aged individuals they are likely to have future breeding events before the effects of senescence takes hold (Tavecchia *et al.* 2005). It is likely that the bolussed ewes in this study did not exceed investment of resources into reproduction, despite having superior body condition; in anticipation of allocating resources to future reproductive events. Future work could include the continual monitoring of these study animals into their future reproductive effort and ultimately their survival. It would be very interesting to investigate lambing success, litter size, and ewe body condition in the next breeding year (at the minimum) and if possible, even further into future reproduction effort. The ultimate experiment (although logistics and permissions may be limiting), would be to repeat the bolussing experiment in both high and low density years; using animals in a wider age range and in both sexes, and then to follow the progress of those animals over the next two breeding seasons. This might help to identify trade-offs that exist, ascertain if they are important at different stages of life and under different environmental pressures, and aid understanding about the impact of parasites on both male and female reproductive strategies.

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