Harper Adams University

A Thesis Submitted for the Degree of PhD at Harper Adams University

HARPER ADAMS UNIVERSITY

The Influence of *Syngamus trachea* on pheasant populations.

Owen John Gethings BSc (Hons), MSc

PhD

A dissertation submitted to Harper Adams University in accordance with the requirements for award of the degree of Doctor of Philosophy in the Department of Crop Sciences.

Author’s declaration

I declare that the work in this dissertation was carried out in accordance with the requirements of the University's Regulations and Code of Practice for Research Degree Programmes and that it has not been submitted for any other academic award. Except where indicated by specific reference in the text, the work is the candidate's own work. Work done in collaboration with, or with the assistance of, others, is indicated as such. Any views expressed in the dissertation are those of the author.

SIGNED:..... DATE:.....

Abstract

Syngamus trachea is a highly pathogenic parasite affecting managed pheasant populatons within the U.K. Morbidity and mortality rates are high, particularly when birds are managed at high densities, however very little is known about its disease dynamics and direct effects on pheasants. This study sought to determine the spatio-temporal factors influencing disease occurrence on pheasant estates, and to determine what direct effect, if any, *S. trachea* is having on pheasant populations.

Several factors influenced within and between-year disease transmission; the most important being pen age, average stocking density and soil moisture. Both stocking density and pen age were positively correlated with the number of eggs per gram of soil, whereas soil moisture was best modelled by means of polynomial regression. Larval recovery was both moisture and temperature dependent, with increased movement and abundance of hatched larvae on herbage, which in turn, facilitated transmission. Infection status, i.e. positive faecal egg counts, was associated with larval abundance.

The infectious stages of *S. trachea* were highly spatially aggregated, with feeders acting as a spatial focus for disease transmission in release pens. This relationship was highly linear, with a steep decline in the number of eggs per gram of soil within the first 2 m's from the feeder.

Syngamus trachea was previously thought to be relatively benign, however it was demonstrated that even sub-clinical infections were sufficient to reduce body condition in managed pheasants. It is clear that *S. trachea* is pervasive among managed pheasant populations, and the implications for survival and reproduction are likely to be significant in the absence of anthelmintic treatment. The finding of spatial *foci* upon estates provides a simple, low-cost solution to managing environmental contamination that should be incorporated into gamekeeper's disease management plans.

Table of contents ***Page number***

List of tables

List of figures

List of appendices

Chapter 1. The influence of *Syngamus trachea* on pheasant populations

1.0 Introduction

The common pheasant, *Phasianus colchicus*, is arguably the most common and important game bird species within Europe (Santilli and Bagliacca, 2012) and the industry is estimated to be worth around £2 billion to the UK economy (PACEC, 2014). Its importance as a game species means that in order to maintain adequate population levels, up to 35 million pen-reared birds are released every summer within the UK (Tapper, 1999; Draycott *et al.,* 2000). Despite the supplementation of natural populations with pen-reared birds, the bag size of shot birds has only increased by 2.5% since 1960 (Aebischer *et al.,* 2011) with a disproportional loss of birds between August and December in the year of release. Reproductive success of released ring-necked pheasants is generally poor compared with their 'wild' counterparts (Leif, 1994), but it is currently unclear as to why this is the case (Leif, 1994; Draycott *et al.,* 2000; Millan *et al.,* 2002; Draycott *et al.,* 2006; Villanua *et al.,* 2006). A number of factors such as increased parasitic worm infections and reduced food availability/quality are believed to be major components governing life-history traits in game birds (Hudson *et al.,* 1992). It is commonly known that excessive helminth infection leads to reduced body condition (Molina, 1999; Shutler *et al.,* 2012) and poor reproductive status (Woodburn, 1995), however many studies have also shown that increased parasite burden in released pheasants is directly correlated with increased predation (Van Dobben, 1952; Holmes & Bethel, 1972; Vaughan & Coble, 1975; Temple, 1987). The unnaturally high stocking densities, high levels of stress and poor sanitary conditions lead to the high incidence of parasitic infections in pre-released penned populations (Draycott *et al.,* 2006; Gortazar *et al.,* 2006; Villanua *et al.,* 2008).

Of the parasites to affect pheasants, *Syngamus trachea* (Montagu 1811), Chapin 1925, is among the most pathogenic and economically important (Atkinson *et al.,* 2008). S*yngamus trachea* is a parasitic nematode belonging to Family Syngamidae and the Superfamily Strongylidae, and has been recovered from a number of gallinaceous and passerine species (Morgan & Clapham, 1934; Campbell, 1935; Goble & Kutz, 1945; Bandelj *et al.,* 2015) with successful experimental transfer between species (Clapham, 1938). As its name suggests, *S. trachea* is a tracheal nematode, and male and female adults are found in permanent *copulo* within the trachea (Krone *et al.,* 2007). Infection with *S. trachea* can occur either directly by the ingestion of eggs or third stage larvae, or indirectly, by the ingestion of an infected invertebrate paratenic host, most commonly the earthworm (Morgan & Clapham, 1934; Campbell, 1935). The life cycle of *S. trachea* is

direct, and no intermediate host is required for development. Egg and larval survival rates on pasture are estimated to be ~6 months (Barus, 1966b,c), however once *S. trachea* larvae have been ingested by a paratenic host, survival rates increase to around 4 years (Nevarez *et al.,* 2002). Although no intermediate host is required for development, the ability to persist in invertebrate hosts may provide *S. trachea* larvae with the opportunity to over-winter in colder climates (Barus 1966b).

Clinical signs of syngamosis vary depending on the size and age of the animal and the extent of the parasitic infection (Atkinson *et al.,* 2008). The migration of larvae from the stomach to the lungs leads to severe pathological disturbances within infected pheasants, often leading to secondary pneumonia infections (Clapham, 1939a; Guildford & Herrick, 1954), especially in young pheasants (Atkinson *et al.,* 2008). Heavy parasite burden can lead to partial obstruction of the trachea, giving way to the classic symptom of "gaping" or gasping for air (Atkinson *et al.,* 2008); and/or protein loss via exsanguination. Particularly in young birds, this can lead to decreased food and fluid intake leading to decreased body condition and even death. Young birds are particularly susceptible to infection and the confinement of young birds within release pens prior to release leads to heavy pasture contamination and high occurrence of disease (Goldova *et al.,* 2006; Villanua *et al.,* 2006; Atkinson, *et al.,* 2008).

Economical costs associated with parasitism are a result of increased mortality, poor growth rates and egg production, and increased use of anthelmintics (Ruff, 1999). There has been little work conducted to evaluate the cost of parasitism to the worldwide poultry and game industries, but it is estimated that *Eimeria* spp. alone is responsible for global loses of £1.8 billion per annum (Reid *et al.,* 2014). Parasites are a major problem within confined pheasant populations and one of the most economically important parasites to affect game and poultry is the tracheal nematode, *Syngamus trachea,* which has high morbidity and mortality rates associated with clinical, and potentially even sub-clinical disease.

The distribution of parasite species is determined by a complex interaction between many environmental, biological and ecological factors (Pullan *et al.,* 2012). Arguably the most important factor is the overlap between parasites and their host species (Gethings *et al.,* 2015c*)*, which will determine not only the extent but also the timing of infections (Pullan *et al.,* 2012). Understanding of the biological factors behind disease occurrence will lead to

better disease management and potentially enable the spatial and/or temporal manipulation of disease by means of management factors such as pasture resting and rotating. The use of pasture rotation and resting is a common feature within ruminant farming (Morley and Donald, 1980; Morgan and van Dijk, 2012), but little work has been conducted in regards to disease manipulation within game species. Our current epidemiological understanding of syngamosis is based on laboratory hatching experiments conducted over 50 years ago (Barus, 1966a). Parasites that spend a significant proportion of their life cycle outside of their host are extremely efficient, in part due to their short generation interval, at adapting to local climatic conditions. In addition, the current temporal trends in regards to the advancement of spring (Gethings *et al.,* 2015c) and increasing temperatures due to climate change will likely also affect disease dynamics.

In order to better understand the epidemiology of *S. trachea* we must first determine the spatial distribution of the infectious stages, in both time and space, and determine what factors affect larval availability. The ability of larvae to survive in paratenic hosts makes the modelling of the spatial distribution of disease extremely difficult and will influence management decisions regarding the rearing of pheasants. If the contribution by paratenic hosts is minimal, then it may be possible to introduce control methods currently adopted by livestock producers i.e. manipulate disease risk by the subsequent resting and rotating of release pens to ensure adequate time for the desiccation of larval stages, and provide a 'fresh' pasture for the following season.

1) The spatio-temporal factors that influence disease dynamics,

2) The relationship between ambient temperature, rainfall, humidity, soil moisture content and larval development, hatching and mortality,

3) The relative contribution by earthworms and eggs/larvae in soil to disease occurrence,

4) The effect of significant *S. trachea* infections on body condition, weight and fecundity

2.0 Literature review

2.1.1 Nematodes

Nematodes are among the most abundant and ubiquitous group of organisms on the planet with the majority being parasitic in nature with the exception of a few free-living species (Nikolaou & Gasser, 2006). Typically, nematodes are elongated, bilaterally symmetrical and tapered to a point at both ends. They possess a complete digestive tract with a mouth at the most anterior aspect and an anus in the posterior region (Bird & Bird, 2012). They are pseudocoelamates as they possess a pseudocoel, a cavity derived from the embryonic blastocoel that functions to maintain shape and facilitate movement by acting as a hydrostatic skeleton (Harris & Crofton, 1959; Sutherland & Scott, 2009). Nematodes are arguably the most important parasite species to affect game birds in terms of their number, diversity and the pathological disturbances associated with infection (Ruff, 1999).

2.1.2 Common parasites of poultry and game

Endoparasites are relatively common among wild bird populations, and rarely lead to the death of the host species (Woog *et al.,* 2013), possibly due to the wider spatial distribution of parasites among wild populations. Common pheasants are highly susceptible to parasitic infections (Draycott *et al.,* 2006) and the most common parasites to affect pheasants within the UK are *Heterakis gallinarum*, *Capillaria* spp., *Ascaridia* spp. and *Syngamus trachea* (Ruff, 1999; Draycott *et al.,* 2006). *Capilaria* spp. and *S. trachea* in particular can cause significant production losses, poor weight gain and even mortality, especially when birds are managed at high densities (Ruff, 1999). There is marked pathology associated with infection with *S. trachea,* (Fernando *et al.,* 1971; Nevarez *et al.,* 2002; Atkinson *et al.,* 2008) and mortality rates of affected birds can be as high as 80 % (Wojcik *et al.,* 1999). It is estimated that the average mortality rate of birds infected with *S. trachea* is ~25% (Andreopoulou *et al.,* 2012). Wojcik *et al.* (1999) also observed an increase in the proportion of deaths associated with clinical syngamosis over time, which could have implications for current management practices, i.e. the continued use of discrete areas for releasing of birds.

Intestinal and tracheal parasites are commonplace within pheasant-rearing systems and shooting estates, but no accurate data are available concerning prevalence rates within the UK. Kotrla *et al.* (1984) found the presence of endoparasites on 82.5% of pheasant

farms in the Czech Republic and Golodova *et al.* (2006) found 497 samples positive for endoparasites out of 1030 in Slovakia. The most prevalent parasite was the *Eimeria* spp. with prevalence rates of 64% and 73% in pheasant chicks up to 2 weeks and 2 to 8 weeks respectively. The most prevalent nematode was *S. trachea* with 45.8% of birds infected. It is probable, given the similarities in pheasant rearing practices, that similar levels of infection are found within pheasant populations within the UK.

It is believed that the subsequent releasing of pheasants on shooting estates maintains or even exacerbates the levels of disease in the environment (Wojcik *et al.,* 1999; Santilli & Bagliacca, 2012). For instance, the releasing of birds annually within the UK is believed to maintain and/or increase the levels of *Heterakis gallinarum* burdens in wild pheasants (Draycott & Sage, 2005), which is implicated as a significant factor in the decline of the Grey Partridge (Tompkins *et al.,* 2001). Furthermore, Santilli and Bagliacca (2012) compared parasite egg and oocyst prevalence between 'restocking areas' and 'wild areas' in Tuscany. 'Restocking areas' consisted of 7 sites where the releasing of birds took place every year. The 'wild' areas consisted of 5 estates where the population of pheasants was maintained only by the breeding of wild pheasants i.e. no releasing took place. Significant differences were identified in egg and oocyst prevalence between sites. *Eimeria* was identified in 25.6% of samples from wild compared with 51.3% on restocked sites and nematode eggs were identified in 16.3% and 49.6% of samples for wild and restocked sites respectively. Significant differences were identified between sites for *Capillaria* spp. which was found in 31.9% of samples from restocked estates compared with 3.1% from wild sites and *S. trachea* was found in 10.1% and 3.1% of samples from restocked and wild sites respectively. These data suggest that the annual release of pheasants maintains or even increases the levels of disease in the environment, potentially contributing to the reduced breeding success and survival of pen-reared birds post-release (Woodburn, 1995; Draycott *et al.,* 2000; Millan *et al.,* (2002) and potentially contributing to the decline of native species.

2.1.3 Releasing of pen-reared pheasants

In order to supplement wild populations of pheasants and maintain a large enough population to keep up with demand for shooting, it is common practice within many parts of Europe to rear pheasants in confined systems (Draycott et al., 2006; Goldova et al., 2006). It is estimated that approximately 12 million pheasants are harvested each year within the UK (Tapper, 1999), though given recent trends, it is likely this number is considerably higher. In order to maintain this increased demand for game shooting, around 35 million, 6-8 week old pen-reared pheasants are released into the countryside

every year (Tapper, 1999; Draycott et al., 2006). Although the total biomass of British Birds declined between 1968 and 1988, the number of pheasants has risen substantially and now accounts for 30% of the total biomass (Dolton & Brooke, 1999; Lees *et al.,* 2013).

Within release pens, pheasants are commonly kept at stocking densities of ~1800 birds per hectare (Sage et al., 2005) and are commonly released at densities of 250 birds/km2 (Aebischer, 2003). This high concentration of birds within release pens, combined with the increased availability of density-dependent parasitic and bacterial pathogens can lead to significant losses (Goldova et al., 2006). Not only are significant losses seen within release pens, but the increased likelihood of disease significantly effects pheasants' spring body condition, making them more susceptible to predation once released (Draycott *et al*., 2002); as seen in red grouse (Hudson *et al., 1992a*). It is believed that heavily-parasitised females emit more scent than uninfected females, and are therefore more likely to be detected by mammalian predators (Hudson *et al.,* 1992a). The effect of parasitism on host morbidity may also benefit predators, as the reduced energy requirements needed to predate infected prey would be significantly lower than for uninfected birds, meaning it would be advantageous to select weaker individuals of a given population; as evidence in red grouse (Hudson *et al.,* 1992a). Several studies have been conducted concerning the effect of parasitism on body condition in birds, which will be discussed in a separate section.

2.1.4 The effect of parasitism on survival and body condition

A number of studies have been conducted to determine the survival of pen-reared pheasants post-release (Leif, 1994; Draycott *et al.,* 2000; Millan *et al.,* 2002; Sage *et al.,* 2002; Woodburn *et al.,* 2002; Villanua *et al.,* 2006; Draycott *et al.,* 2006) and the understanding is that the survival of pen reared birds is significantly lower than that of wild pheasants. Currently however, it is unclear as to why this is the case. For instance, several investigators have assessed the effect of parasitism on pheasant body condition. Draycott *et al.* (2002) showed that infection with *Heterakis gallinarum, Capillaria* spp. and *S. trachea* did not significantly influence spring body condition of pheasants on the estates monitored. In contrast, Delahay *et al.* (1995) found that red grouse infected with *Trichostrongylus tenuis* had increased metabolic rates at 12 days post-infection (P.I), 38% less energy consumption 16 days P.I and significantly reduced body condition from 16 days P.I compared with control birds. These significant differences coincided with the time

that L3 and L4 larvae were becoming adult worms (Delahay *et al.,* 1995), and differences in body condition and energy reserves after adult worm establishment diminished. The study by Delahay *et al.* (1995) however only used 4 pairs of cocks, and 3 pairs of hens so the differences could just be accounted for by natural variation, and the birds were treated with a single high dose of larvae (6000 in 1ml), and it is unclear as to whether wild birds would encounter similar infective doses in such a short space of time. Despite the limitations, it suggests that body condition could be affected early during infection when immature larval stages are migrating to their predilection site, potentially reducing fecundity and survival during this period, whilst returning to pre-infection levels following acquired immunity (Shaw & Moss, 1990; Delahay *et al.,* 1995). This is certainly the case for *S. trachea* as the majority of pathological changes are associated with the migration of immature larvae to their predilection site and not adult worms present in the trachea (Clapham, 1939a). This could explain how the study by Delahey *et al.* (1995) and Draycot *et al.* (2002) differ so significantly. The study by Draycott, *et al.* (2002) conducted sampling in spring, when most of the pheasants released the following year would have developed immunity to infection and would have been subjected to a lower infection pressure prior to the hatching of worms in that year, which generally occurs towards the end of July. It is also likely that the highly infected birds would have been predated (Hudson *et al.,* 1992a; Millan *et al.,* 2002) or died as a result of infection with large amounts of immature larvae. The study by Draycott, *et al.* (2002) also only quantified bird survival as the number of experimental birds surviving. A more appropriate method of calculating survival would have been to look at the proportion of birds surviving from the original number of birds released in the previous year. Further, Sage *et al.* (2002) noted that even relatively low *H. gallinarum* burdens (mean 118 ± 14 worms) negatively affected pheasant body condition. Without 'sex' as factor, infection with *H. gallinarum* accounted for the variation in observed body and breast muscle mass for both males and females (Sage *et al.,* 2002). With 'sex' as factor, the abundance of cloacal fat was negatively correlated with *H. gallinarum* burdens in females but not males.

Hwang (1964) experimentally infected turkey poults with varying numbers of *Syngamus trachea*-infected earthworms to observe their effect on weight gain and packed cell volume (PCV). Turkey poults were split into three groups of twenty and each fed 1, 3 and 10 infected earthworms, resulting in mean post-mortem *S. trachea* counts of 0.2, 4 and 56 respectively. Interestingly, significant differences were identified in weight gain between treatment and control birds in all groups. Birds given 1 earthworm had an average weight gain of 1482g compared with 1619g for control birds. Birds given 3 earthworms had an average weight gain of 694g compared with 757g for control and birds given 10

earthworms had an average weight gain of 51g compared with 341g for controls. The results of this study suggest a relationship between the levels of parasite burden and weight gain, which could have implications for birds managed at high densities and could be a causal factor of poor breeding and survival of birds post-release. A similar finding was observed by Loman (1980), who found that crows (*Corvus cornix*) infected with *S. trachea* showed a tendency to be lighter than non-infected individuals and were less likely to be observed later in year. All birds found dead during the study had an average of 12-20 worms within the trachea (Loman, 1980). These results suggest a link between weight and condition and susceptibility to infection, which could have implications for breeding success, as heavily parasitized individuals were less likely to survive long enough to breed. Although many other factors could be implicated as the cause of poor weight gain, e.g. differences in mean pre-trial body weight could be accounted for by normal variation among individuals, and the relatively small sample size, the results are still worth noting in the context of parasite-host relationships.

Syngamus trachea for example, has been shown to affect both survival and fecundity of sparrows (*Passer domesticus*) in Helgeland, northern Norway (Holand *et al.,* 2014, 2015). Holand *et al.* (2014) collected faecal samples from 603 sparrows and found that birds infected with *Syngamus trachea* demonstrated significantly reduced survival probability compared with asymptomatic individuals. Similarly, the proportion of eggs hatching from mothers with high faecal egg counts was considerably reduced compared with uninfected individuals (Holand *et al.,* 2015).

2.1.5 The use of anthelmintics

Several experimental parasite reduction experiments have demonstrated increased survival and reproductive success in a number of avian species (Hudson, 1986; Draycott *et al.,* 2006; Reed *et al.,* 2008). Within pre-release penned populations, parasitic infections, and their deleterious effects, are well documented and frequently controlled through the use of anthelmintic products administered to feed or drinking water (Villanua *et al.,* 2006). Routine de-worming practices appeal to gamekeepers due to convenience, and as such may be the only control method practiced in lieu of more sustainable alternatives such as pen and feeder rotation. Birds are generally treated within the release pens, and as anthelmintic treatment is usually suppressive in nature, due to the high levels of environmental contamination (Gethings *et al.,* 2015a,b), birds are rapidly re-infected (Pennycott, 2000; Woodburn *et al.,* 2002).

It has been demonstrated that released pheasants generally disperse <1km from release sites (Leif, 1994), with birds often returning to nest and roost in the release pens overnight. As anthelmintic supplementation must cease as soon as birds are released, the continued use of heavily infected pens will result in the rapid reinfection of pheasants, facilitating the dissemination of infectious stages. This lends itself to the possible transmission of drug-resistant generalist-parasites between species, enabling the dissemination of resistant genes across species home ranges. Indeed, Villanua *et al.* (2007) showed that the commonly used anthelmintic in Portugal, albendazole, demonstrated limited efficacy at removing *Aonchotheca caudinflata* and *Heterakis gallinarum* worms in pre-released red-legged partridges. Similarly, many passerine species have been shown to harbour drug-resistant bacteria, and are believed to be a significant factor in the spread of drug-resistance alleles between farmed and wild bird populations (Benskin *et al.,* 2009). This inter-species transfer not only poses a potential threat to wild bird species, but also pheasants themselves and, through the shift to free-range systems with increased accessibility to wild birds, poultry. This demonstrates that blanket anthelmintic treatment alone will be ineffective at controlling disease incidence long term due to the continued use of heavily infected ground.

2.1.6 The effect of parasitism on reproduction

Not only is survival of pen-reared pheasants significantly lower than wild pheasants but reproductive success is also considerably reduced. The timing of breeding is one of the most important factors governing breeding success and chick survival (Verhulst & Tindergen, 1991), and it is believed that birds that breed later have a comparatively lower reproductive success (Drent & Daan, 1980) owing perhaps to reduced food availability and/or quality at this time (Allander & Bennett, 1995). Indeed, Davies and Lunderg (1985) demonstrated that birds supplemented with feed began breeding earlier than un-supplemented birds, while Draycot *et al.* (2002) demonstrated that birds supplemented with feed during spring fledged twice as many chicks as un-supplemented birds. In addition, Draycott *et al.* (2002) demonstrated that hen pheasants collected from sites where supplementary feeding continued into spring had comparatively higher fat reserves than sites where feeding ceased after shooting, which would coincide with the time when pheasants should be accumulating fat reserves for the onset of breeding. It is therefore likely that the nutritional status of the female directly influences breeding onset and reproductive success (Perrins, 1970; Price *et al.,* 1988).

It has been demonstrated that birds with reduced body condition spend considerably less time on the nest (Persson & Goransson, 1999), with Sage *et al.* (2003) noting that five birds that left the nest during the study were found dead shortly after and severely emaciated. Furthermore, Villanua *et al.* (2006) found that parasite burdens were significantly higher in female compared with male birds which, combined with increased energy input into breeding and egg production and the low abundance of food, could significantly affect host fecundity and chick survival by delaying the onset of breeding (Allander & Bennett, 1995) and egg laying (Linden *et al.,* 1992) by increasing pressure on the incubating hen. It has been suggested that in order to initiate egg laying, birds must reach a body condition threshold, and that individual host body condition can delay or advance threshold attainment (Drent & Daan, 1980). For example, parasites that undergo hepato-pulmonary migration and/or cause anaemia via exsanguination compete with the host for protein during the time when energy input is concentrated on egg production (Allander & Bennett, 1995). Proteins required for yolk production are synthesised within the liver, and the migration of *S. trachea* larvae across the liver parenchyma could impair the production of these proteins (Allander & Bennett, 1995) thus potentially affecting the onset of laying. Indeed, Jones and Ward (1976) demonstrated that reduced yolk proteins delayed the onset of breeding in Red-Billed Quaeleas.

It has been shown in red grouse populations, that the number of eggs laid is directly related to host body condition and energy intake in the preceding weeks (Delahey *et al.,* 1995). Delahey *et al.* (1995) showed that infection with *Trichostrongylus tenuis* reduced host body condition and could explain poor breeding performance of wild birds. During incubation, birds consume less and lose a considerable amount of body condition (Delahey *et al.,* 1995). Birds with underlying *T. tenuis* infections or the development of hypobiotic larvae in the spring could reduce body condition further. Hen pheasants spend approximately 25 days incubating eggs after laying the clutch (Robertson, 1991) and it has been demonstrated that birds can lose up to 80% of their body fat during this period (Britenbach & Mayer, 1959) with pen-reared birds losing up to twice as much body weight as wild birds (Robertson, 1991). This may therefore be the reason for nest abandonment and emaciation during disease outbreaks (Hudson, 1986), and the differences in body weight may be attributed to the high density of parasites within release pens, meaning birds are released with already high worm burdens (Villanua *et al.,* 2006), with significant pathological effects occurring during incubation (Delahay *et al.,* 1995). In male birds for instance, the onset of breeding sees a significant increase in the levels of testosterone,

which is known to have a suppressive effect on host immunity (Hillgarth & Wingfield, 1997). The onset of breeding and egg laying could reduce host immunity, making them much more susceptible to infection. This has been demonstrated in other species, principally sheep with the periparturient relaxation of immunity (PPRI) around lambing, making them much more susceptible to infection with already infected sheep beginning to shed more parasite eggs, contaminating pasture for immunological naïve lambs (Abbott *et al.,* 2012). In addition to sheep, Molina *et al.* (1999) found significant differences between burdens of *Andrya cuniculi* between pregnant/lactating female and non-pregnant female rabbits (*Oryctolagus cuniculus*). It was identified that pregnant and lactating female rabbits had significantly higher worm burdens than non-pregnant or lactating females (Molina *et al.,* 1999). Although no quantification of the effect of higher worm burdens on breeding success was undertaken during this study, the results suggest that the onset of breeding combined with significant parasitic challenge is a significant factor driving population dynamics (Watson, 2013). A meta-analysis conducted by Watson (2013) on 38 datasets encompassing 26 animal species, found that clutch size, hatching success and the number of young produced were all significantly reduced in parasite-infected animals. The direct effect of parasites on animal hosts is therefore predicted to be negligible, as the balance between virulence and transmission success approaches equilibrium (Watson, 2013), with the indirect effects being apparent in reduced breeding success and survivability of offspring. It has been demonstrated in a further meta-analysis that parasitism is at least as important as predation in managing populations (Cote & Sutherland, 1997; Watson, 2013).

Furthermore, Newborn and Foster (2002) identified, unsurprisingly, that birds with access to grit medicated with fenbendazole had lower *T. tenuis* burdens and higher body condition scores than control birds. Interestingly, birds from the treated plots demonstrated significantly higher breeding success and reared twice as many chicks as birds from control plots. Chick survival was also significantly greater in treated plots compared with control (Newborn & Foster, 2002). It appears that breeding success may be a combination of host body condition, food abundance and quality, the intensity of the parasitic infection and host susceptibility. The increased loss of condition in reared pheasants may however be attributed to physiological differences due to the feeding of grain *ad libitum,* making them less able to digest seeds and shoots, as seen in the grey partridge (Putaala & Hissa, 1995; Sage *et al.,* 2003), or altered feeding behaviour and reduced predator avoidance due to being reared in the absence of adult birds (Dowell, 1990). It could potentially be a combination of all of these factors.

Woodburn *et al.* (2002) demonstrated that birds dosed with anthelmintics, reared twice as many chicks as un-dosed controls. It is currently unknown however whether the anthelmintic had a direct effect on breeding success by reducing parasite challenge, or because the treatment was associated with greater bird survival due to reduced predation (Hudson, *et al.,* 1992a; Millan, *et al.,* 2002; Woodburn *et al.,* 2002).

2.2.1 Syngamus trachea

Syngamus trachea is a parasitic nematode belonging to the family Strongylida and the superfamily Syngamadiae that causes syngamosis in poultry, game and wild birds (Andreopoulou *et al.,* 2012). *Syngamus trachea* has a monoxenous lifecycle, with development restricted to avian-host tissues (Atkinson *et al.,* 2008), and although eggs can be ingested by an earthworm, no further development takes place and it acts merely as a mechanical vector (Taylor, 1935). Adult worms are sexually dioecious, and show marked sexual dimorphism from 7-9 days post-infection (PI) with female worms being considerably larger than males; female length = ~> 13mm, male length = > 4mm at 14 days PI (Fernando *et al.,* 1971).

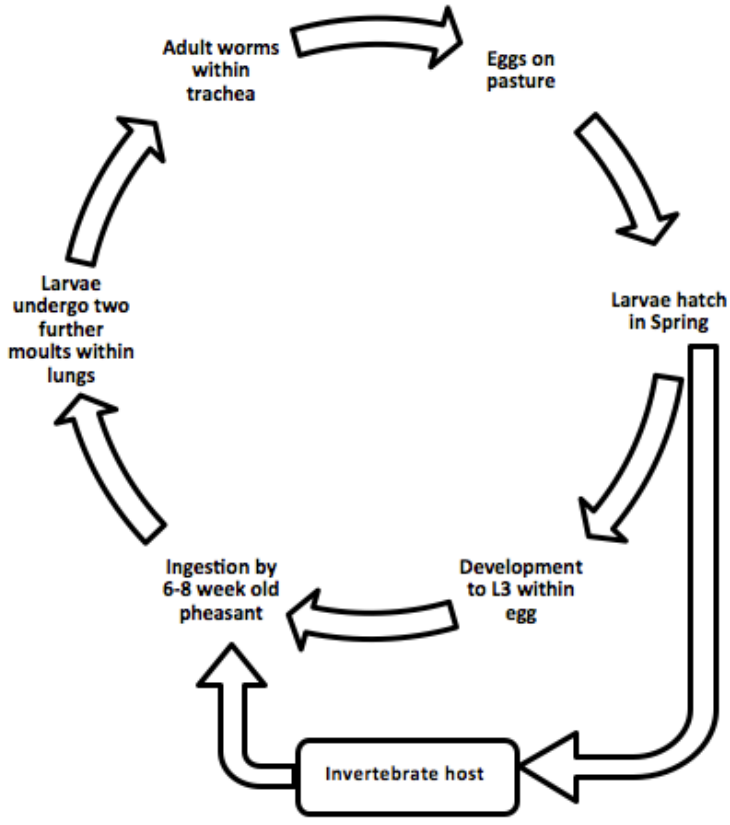

Figure 1 Life cycle of *Syngamus trachea*.

2.2.2 Host species

Syngamus trachea has been recovered from a number of avian genera, however it is believed that the turkey is the original host (Campbell, 1935).

Table 2 Non-exhaustive list of host species for *Syngamus trachea*.

Host common name	Host species	Country reported	Reference
Ring-necked Pheasant	*Phasianus colchicus*	Serbia	Pavlovic *et al.* (2012)
		Canada	Moynihan and Musfeldt (1950)
		Britain	Campbell (1935)
Willow Grouse	*Lagopus lagopus*	Norway	Wissler and Halvorsen (1975)
Carrion Crow	*Corvus carone*	Britain	Campbell (1935)
Rook	*Corvus frugilegus*		
Eurasian Jackdaw	*Corvus monedula*		
Eurasian Magpie	*Pica pica*		
European Starling	*Sturnis vulgaris*		
House Sparrow	*Passer domesticus*		
Purple Sandpiper	*Calidris maritima*		
Greater Rhea	*Rhea americana*	USA	De Wit (1995)
Wild Turkey	*Meleagris gallopavo*	Eastern Europe	Barus (1966a)
Red-and-yellow Barbet	*Trachyphonus erythrocephalus*	USA	Nevarez *et al.* (2002)
Song Thrush	*Turdus philomelos*	Britain	Campbell (1935)
Redwing	*Turdus iliacus*		

Eurasian Blackbird	*Turdus merula*		
American Robin	*Turdus migratorius*	USA	Welte and Kirkpatrick (1986)
Chough	*Pyrrhocorax pyrrhocorax*	Britain	Bignal *et al.* (1987)
Red-headed Woodpecker	*Melanerpes erythrocephalus*	USA	Cooper (1974)
Japanese Dwarf Quail	*Coturnix coturnix japonica*		Enigk & Dey-Hazra (1970)
Domestic Goose	*Anser anser domesticus*		
Jay	*Garrulus glandarius*	Britain	Lewis (1928)
Chicken	*Gallus domesticus*	Britain	Morgan & Clapham (1934)
Gray Partridge	*Perdix perdix*	Britain	
Red-legged Partridge	*Alectoris rufa*	Britain	Tompkins *et al.* (2002)
Barn Owl	*Tyto alba*	Nigeria	Egbetade et al. (2014)
Greylag Goose	*Anser anser*	Germany	Woog et al. (2013)

2.2.3 The life cycle of Syngamus trachea

The 'free living' portion of the life cycle of *S. trachea* is similar to that of other stronglyid nematodes, the major difference being the temperature threshold for development and the facultative paratenic host. There is however, a marked departure from the typical strongylid development once inside the host (Wehr, 1937). The development and hatching requirements of *S. trachea* at various temperatures have been widely studied in the literature (Ortlepp, 1923; Wehr, 1937; Barus, 1966a). The temperature threshold for the development of *S. trachea* eggs is 16 – 35 °C, and development does not take place above or below this threshold (Barus, 1966a; Atkinson *et al.,* 2008). The time it takes to reach the L3 stage generally decreases with increasing temperatures, however as with other nematode species, there is a trade-off between the time taken to reach development

and the numbers of L3 surviving towards the upper end of the threshold (Van dijk and Morgan, 2010; 2012). Barus (1966a) found that larvae developing within the egg reached L3 by 42 days at 17 °C, 25 - 28 days at 19 °C, 13 -14 days at 25 °C and 9 days at 27 °C. Once developed, it was noticed that eggs hatched 'spontaneously' (Atkinson *et al.,* 2008) via one of the two *opercula* (Wehr, 1937), however it is highly unlikely that egg hatching was not driven by some exogenous factor, i.e. temperature. Indeed, there may exist a hatching threshold in which eggs do not hatch above or below in order to prevent larvae hatching in adverse conditions, i.e. extreme heat potentially causing desiccation (Van Dijk and Morgan, 2012). Indeed, Ortlepp (1923), found that eggs kept below 20 °C did not hatch; however those eggs did hatch when exposed to temperatures of 28 °C. Further hatching data could potentially allow for the development of predictive mathematical models in order to forecast larval emergence in regards to temperature and climate change, i.e. temporal trends.

Wehr (1937) observed that eggs incubated at between 24 and 30 °C had usually hatched at around 12 days, however it must be noted that these experiments were conducted in constant temperature chambers and although they provide useful data for theoretical modelling of disease, are not indicative of natural conditions. In contrast to previous published literature, Wehr (1937) found that *S. trachea* larvae hatched as infective third stage larvae (L3), and not 2nd stage larvae (L2) as previously thought (Ortlepp, 1923).

Infection occurs either by the ingestion of eggs or hatched infective larvae, or by the ingestion of an infected paratenic host (Atkinson *et al.,* 2008). Although no intermediate host is required for development, *S. trachea* larvae are able to remain viable and infective encysted inside invertebrate hosts (Taylor, 1935). Once ingested by a definitive host, such as the pheasant, larvae hatch and migrate from the intestines to the liver via the hepatic portal vein within as little as 6 hours post-infection (Clapham, 1939a; Fernando *et al.,* 1971). Early work (Clapham 1935; Wehr 1937) established the cardiovascular system as the main route of larval migration from the intestines to the lungs. From the liver, the larvae migrate to the heart via the posterior *vena cava* to the right atrium before travelling via the pulmonary artery to the lungs (Clapham, 1939a). Larvae can be detected in the interlobular connective tissue as early as 4 hours post-infection and within the atria of the lungs 24 hours post-infection (Clapham, 1939a; Fernando *et al.,* 1971). The larvae then undergo a third moult to L4 ~2 days post-infection and can be sexually differentiated (Fernando *et al.,* 1971; Atkinson *et al.,* 2008). Larvae can be recovered from the lungs up to 7 days post-infection in the chicken, and adult worms can be recovered from the

trachea from as early as 7 days post-infection (Fernando *et al.,* 1971). In the pheasant, adult worms are present in the trachea ~9 days post-infection (Guildford & Herrick, 1952). Adults are sexually reproductive by 12-14 days and eggs can be found in the faeces 17-20 days post infection (Fernando *et al.,* 1971; Atkinson *et al.,* 2008), although this can vary between species.

Guildford and Herrick (1954) conducted a vast amount of preliminary work on the development and length of infections in the pheasant. One pheasant examined on the 27th day post-infection and all pheasants examined after 36 days post-infection had considerably more granulomatous nodules than adult worms present in the trachea. This suggests that pheasants begin to develop immunity to gapeworms fairly rapidly following the initial infection, which results in the expulsion of adult worms already established and prevents new worms from establishing (Guildford & Herrick, 1954).

2.2.4 Epidemiology

Although not necessarily considered a seasonal disease, syngamosis is most commonly seen in young birds, thus disease patterns tend to follow breeding and rearing cycles of pen-reared populations. Currently, very little is known about the longevity and dynamics of the infectious stages in the environment. Barus, (1966a), found an increase in disease occurrence in response to increased precipitation, which they attributed to an increase in the abundance of paratenic hosts. Maximal earthworm abundance however, occured in March and April, whereas disease occurrence did not peak until July, August and September. Given the relatively short pre-patent period between the ingestion of infectious stages and the ability to detect embryonated eggs in faeces, it is unlikely that these infections came from the ingestion of infected earthworms. Alternatively, the relationship between disease occurrence and rainfall could be explained by increased larval movement from faeces in the presence of excess environmental moisture (O'Connor *et al.,* 2006). The finding of distinct peaks in disease occurrence in July, August and September coincides with the period that immunologically-naïve pheasants are being placed into the pens for release, and the time when anthelmintic provision must cease prior to release. There is however currently very little information on the hatching and development of eggs in the environment outside of pheasant rearing conditions. In contrast, disease occurrence in wild bird populations appears to be earlier in the year, and again, driven by breeding cycles. Rooks, (*Corvus frugigelus*), particularly juveniles, tend to be highly infected in April and May (Elton & Buckland, 1928).

2.2.5 Pathogenesis

The gross pathology associated with *Syngamus trachea* infections is mainly a result of the migration of larval stages from the stomach to the lungs (Clapham, 1939). From the blood capillaries, the larvae migrate into the air capillaries causing significant haemorrhage and inflammation to surrounding tissues (Clapham, 1939). Early pulmonary lesions include a significant increase in the number of lymphocytes within connective tissue and parabronchi, resulting in the disruption of the normal capillary architecture (Guildford & Herrick, 1954; Fernando *et al.,* 1971; Atkinson, 2008). Giant cells and granulocytes infiltrate the lumina and atria of the parabronchi 4-7 days post infection. The majority of the pathology associated with infection with *S. trachea* is a result of the host's immune response (Atkinson *et al.,* 2008). In severely infected birds, the lungs can become ecchymotic and oedematous with increased erythrocytes and leucocytes leading to impaired respiration and gaseous exchange (Clapham, 1939). Secondary bronchitis is a common feature of syngamosis. Many investigators have noted that the migration of larvae through the lung parenchyma can often lead to pneumonia (Clapham, 1939; Andreopoulou *et al.,* 2012), particularly in young birds (Atkinson *et al.,* 2008). Secondary pneumonia is not only unique to syngamosis, but also occurs from the migration of larvae through the lungs of cattle and pigs infected with *Ascaris suum* (McCraw and Lautenslager, 1971).

Once established in the trachea, adult *S. trachea* worms give rise to haemorrhagic catarrhal tracheitis by the direct mechanical disruption of tracheal mucosa (Fernando *et al.,* 1971; Nevarez *et al.,* 2002; Atkinson *et al.,* 2008). The presence of worms within the trachea, coupled with the host-mediated immune response leads to a significant increase in mucus, potentially leading to asphyxiation in young birds or birds with a heavy parasitic load (Clapham, 1939). Although a number of bird species are affected by syngamosis, there appears to be a vast difference in pathogenicity among species. The turkey for instance, which is previously believed to be the natural host of *S. trachea,* rarely shows signs of infection (Clapham, 1935). In comparison, the parasite-host relationship in the chicken is such that many young birds die during the early stages of infection (Clapham, 1939). In the pheasant and partridge for instance, there is a significant mortality rate among young chicks, however once infected, chicks develop immunity rapidly and many adult birds are found harbouring *S. trachea* with little or no clinical symptoms (Clapham, 1935). In the pheasant, unlike other bird species, many investigators have noted that haemorrhagic tracheal nodules form at the point of attachment (Clapham, 1935; Atkinson *et al.,* 2008). These nodules consist of hyperplastic peritracheal connective tissue

(Clapham, 1935; Fernando *et al.,* 1971; Atkinson *et al.,* 2008). In some cases these nodules can become large enough to be visible externally and have been described as "resembling a small pea" upon pathological examination (Clapham, 1935; Atkinson *et al.,* 2008). Within these nodules, male worms are often found deeply embedded within the tracheal cartilage and can occasionally cause proliferation of the perichondrium (Fernando *et al.,* 1971; Atkinson *et al.,* 2008). The tissue directly adjacent to the point of attachment is often necrotic and infiltrated by granulocytes and giant cells (Fernando *et al.,* 1971). In contrast, *S. trachea* larvae within the chicken only cause small petechial haemorrhages at the point of attachment. Although Clapham (1935) first noticed the large tracheal nodules within the pheasant, it was concluded that these nodules were caused by the length of time the adult worms were present in the trachea, which was upwards of one-year post infection. In contrast, Fernando *et al* (1971) observed these nodules as early as 6-weeks post infection. It is currently unclear as to why the pathology varies so considerably between species.

Anaemia and hypoproteinaemia are common features of parasitic infection, however Varga (1971) was unable to substantiate this in regards to syngamosis. This however can be explained by the fact that the larvae used within this study were administered via the brachial vein, whereas the resultant anaemia would most probably be caused by the migration of larvae through the liver parenchyma. Andreopoulou *et al* (2012) however, indicates that anaemia is a common feature of syngamosis and can be identified by examination of the wattles.

2.2.6 Clinical signs and diagnosis

Clinical signs of syngamosis are generally associated with the presence of adult worms in the trachea. The characteristic sign, 'gaping', has become synonymous with syngamosis, hence the common name, 'gapeworm', to which the disease is more commonly referred. General signs of respiratory distress, i.e. dyspnoea, coughing, sneezing and head shaking are the most common signs of infection and are usually a result of infected birds trying to expel attached worms from the trachea (Davenport & Cairns, 1962). Birds may appear emaciated, anaemic and show a marked depression. Young birds may develop pneumonia due to the mechanical disruption of host tissues and show a general inappetence and dehydration (Clapham, 1934; Atkinson *et al.,* 2008). Diagnosis based on clinical signs alone is unreliable, as there are a number of conditions, which may lead to respiratory distress (Welchman *et al.,* 2013). Diagnosis is generally confirmed by faecal

egg count or *post-mortem* examination, however, a number of birds may have died during the early stages of infection as the majority of the pathology associated with syngamosis is a result of immature larvae migrating across the liver and lung parenchyma.

2.2.7 Control

2.2.7.1 Anthelmintics

The incidence of syngamosis can be effectively controlled by the use of several anthelmintic compounds, the majority of which are benzimadozole based. The benzimadazole group of anthelmintics are part of a large chemical family used to effectively control nematode infections in domestic animals, and are frequently chosen because of their broad-spectrum of activity, relative safety margins and ease of administration, most of which are soluble in water. Flubendazole (Flubenvet™) and fenbendazole (Panacur™) for example, are available as oral suspensions; readily facilitating their administration through drinking water in poultry and pigs. Demonstrated efficacies at controlling the number of adult worms and inhibiting immature larval establishment in experimental infections have been demonstrated for thiabendazole™ in turkeys (Wehr & Hwang, 1967) and pheasants (McGregor, 1963; Sharpe, 1964), mebendazole in turkeys (Thienpont *et al.,* 1972) and chickens (Devada & Sathianesan, 1989), albendazole in turkeys (Varga *et al.,* 1998), and flubendazole in the goose (Vanparijs, 1984) and pheasant (Draycott *et al.,* 2006), and fenbendazole (Panacur™) showing high levels of efficacy in pheasants and partridges (Kirsch, 1984; Griffith *et al.,* 2014), and red grouse (Newborn & Foster, 2002), whilst being available under the cascade system for Veterinary Medicines as prescribed by a Veterinary Surgeon. Flubendazole (Flubenvet™) is currently the only anthelmintic licensed for use in Gamebirds.

Generally, pheasants are treated for seven days every three weeks to follow the pre-patent period for *S. trachea* (Atkinson *et al.,* 2008), usually around week 3, 7 and 11 following placement into the release pens. However, anthelmintic supplementation must cease prior to release and therefore birds are generally released with high parasite burdens due to rapid reinfection and continued use of the release pens (Villanua *et al.,* 2007). Reports of reduced efficacies and/or complete resistance have been indicated for virtually every livestock host and every anthelmintic class currently available (Kaplan, 2004), and there is presently no suitable alternative to chemical control in intensive systems (Wolstenholme *et al.,* 2004). For poultry and game birds, the benzimadazole

class of anthelmintics is currently the only licensed compound for controlling parasitic worm infections. Their use has however, been decreasing in ruminants due to widespread drug resistance. In birds for example, Villanua *et al.* (2007) showed that albendazole, the most commonly used anthelmintic in Spain, demonstrated limited efficacy in removing *Aonchotheca caudinflata* and *Heterakis gallinarum* worms in pre-released red-legged partridges. Similarly, Yazwinski *et al.* (2013), found efficacies of <90% for both albendazole and fenbendazole for chickens and turkeys in the United States. Though currently there is no evidence of BZ resistance in poultry or gamebirds in the UK, given the widespread use of anthelmintics in pheasant systems, it is highly likely to have gone undetected.

2.2.7.2 Resting and rotating release pens

An alternative method of reducing disease pressure is by limiting host contact with the infectious stages through resting and rotating strategies. These methods are widely employed within other livestock producing systems, i.e. sheep and cattle (Abbott *et al.,* 2012) and generally involve regulating land use to allow for the natural mortality of infectious stages. Indeed, Simon *et al.* (2011) assessed the effectiveness of utilising 'fresh' pens to reduce infection pressure in broiler chickens. Five cohorts of broilers were utilised and they found significant reductions in parasite prevalence in each of the new runs, with zero worms being recovered in one pen. Each of the other pens had a prevalence ranging between 0.8 and 16%; interestingly, no *Syngamus trachea* worms were recovered in any of the pens, only *Ascaridia galli* and *Heterakis gallinaurm*. Simon *et al.* (2001), attributed the absence or low prevalence of disease with the seeding of the ground by wild birds, which were apparently highly infected with parasites particularly *S. trachea* and *Capillaria* spp. Neither of these nematode species were however, recovered from any of the chickens and interestingly there was a higher prevalence of disease in open air runs than in those in forests, so it is unlikely that spill-over from wild birds was the cause of the reinfection. Environmental contamination prior to the study was apparently zero, however none of the birds received anthelmintic treatment before being placed in the pens so could have been the source of the initial infection. Although the moving of pens may be a viable method of controlling environmental contamination, it is clear that further research into its effectiveness is needed, as well as assessing the viability of such drastic methods on pheasant estates with permanent release pens. Anecdotal evidence suggests that moving release pens can control *Syngamus* spp. infections, and a number of respondents interviewed during the study claimed to have no cases of *Syngamiasis* on

estates where release pens are completely dismantled and relocated annually (*Pers. Comms* – VLA Game Fair).

Similarly, the resting of pens to allow for the natural mortality of parasites could be a viable alternative to moving pens, however a greater knowledge of the longevity of the infectious stages of avian parasites is required in order to be effective. Long-lived species such as *Ascarida* spp can remain viable in the environment for upwards of four years and it may not be financially viable to avoid using pens on small estates.

2.2.7.2 Stocking densities

The risk of disease for pen-reared birds is significantly increased when birds are managed at high densities. It may be possible to reduce disease risk by reducing population size and stocking density, either by reducing the numbers of birds placed into the pens or by increasing the overall size of the release pen. Several studies suggest that population size and stocking densities are major components in contributing to observed disease patterns, and there is a direct relationship between host abundance and the health status of a population (Kellogg & Prestwood, 1968; Permin *et al.,* 1998; Kjaer, 2004; Gortazar *et al.,* 2006; Heckendorn *et al.,* 2009; Sherwin *et al.,* 2013). For infectious diseases that rely on ingestion of larval stages, the increased density and aggregation of a population will facilitate disease transmission i.e. there will be a greater contact time between host and parasite, thus increasing the likelihood of infection (Sherwin *et al.,* 2013). The confinement of a large population of birds prior to release will increase the levels of stress within the population, reducing the host's immune system making them more susceptible to reinfection. Relationships between high stocking densities and parasite burden have been identified in a wide range of species under varying management techniques; species including but not limited to Quail (Kellogg & Prestwood, 1968), Chickens (Onbasilar *et al.,* 2008; Permin *et al.,* 1998; Sherwin *et al.,* 2013) and Pheasants (Pennycott, 2000).

Heckendorn *et al.* (2009) conducted a study in order to compare infection pressure and disease transmission in outdoor pens managed under different stocking densities. It was demonstrated that the abundance of *Ascaridia galli* and *Heterakis gallinium* eggs within the soil decreased with increasing distance from the hen house and communal areas, however it was concluded that increased stocking density did not contribute to increased levels of disease. This can however, be attributed to the very low sample sizes across

pens (*N*=25 per treatment), with only 3 replicates of each density. This stocking density does not accurately represent densities of birds managed in the commercial setting. In contrast, Sherwin *et al.* (2013) conducted a similar study with a much larger sample size consisting of 19 flocks from a range of management settings with average stocking density of 4373 (±3887) birds per flock. It was concluded that both indoor and outdoor stocking density directly influenced nematode faecal egg counts (FEC), perhaps due to the increased risk of transmission. It was also noted that the age of the system, i.e. number of years the pen was in use, was positively correlated with the numbers of *H. gallinium* eggs within faecal samples (Sherwin *et al.,* 2013), possibly due to accumulation of infected soil and/or paratenic hosts over time increasing risk of infection for subsequent flocks. A significant association was also identified between feeders and the numbers of both *H. gallinium* and *Syngamus trachea* eggs.

In many parts of Europe it is common practice to supplement the natural diet of pheasants through the employed use of feed hoppers (Draycott *et al.,* 2002), and to implement predator control in order to minimise losses during the rearing and release phase (Gortazar *et al.,* 2006). These feed hoppers may however, contribute to disease transmission, as many species of birds will congregate around them, continually shedding eggs on to the ground and allowing for a wider spatial distribution among natural bird populations (Hofle *et al.,* 2004) e.g. the apparent decline in grey partridge as a result of host-mediated parasite competition among pheasants (Tompkins *et al.,* 1999; Tompkins *et al.,* 2000).

2.2.8 Relative contribution to disease by larvae/eggs

As previously discussed, pheasants can become infected with *S. trachea* either by the direct ingestion of eggs or hatched larvae, or by the ingestion of an infected invertebrate host (Clapham, 1934; Atkinson *et al.,* 2008). The ability of *S. trachea* larvae to survive inside a paratenic host enables the increased longevity of infective stages and allows for a greater spatial distribution among wild and penned pheasants. The longevity of hatched *S. trachea* larvae on pasture is approximately 4-6 months, depending on climatic factors, although it should be noted that no work has been conducted regarding the longevity of larvae within the UK. In contrast, the longevity of larvae within a paratenic host can be up to 4 years (Taylor, 1935; Atkinson *et al.,* 2008), which could enable *S. trachea* to over-winter in colder climates (Atkinson *et al.,* 2008). Free-living larval stages of *S. trachea* are highly susceptible to desiccation at low temperatures (Barus, 1966a). Although Barus

(1966c) found that the eggs of *S. trachea are* capable of surviving for 4.5 months at -15 °C and 3 months at -25 °C, they concluded that eggs on pasture were unable to survive during winter months, despite temperatures during the study not decreasing below -11 °C. It seems unlikely that within the UK, where winter temperatures rarely decrease below -5 °C for sustained periods, that eggs on pasture would desiccate by the following spring, especially when taking into account diurnal fluctuations in temperature. It is worth noting that Barus (1966a), maintained their cultures in water, i.e. 100% humidity. As humidity has been shown to be a significant limiting factor for larval development, these results provide useful information on larval survival in vitro but not in the environment. The results of Barus (1966c) suggests that the role of paratenic hosts in contributing to disease epidemiology may be of greater importance in colder climates, whereas in the UK, where the winter temperatures rarely drop below -5 °C, the primary mode of transmission may be bird-to-bird transmission or eggs on pasture. Furthermore, Guilford and Herrick (1952) noted that the numbers of *S. trachea* eggs were higher in soils with higher moisture content. They noted that the numbers of infected eggs within moist soil was upwards of 40 eggs per cm^3, compared with their sporadic occurrence in dry soil. This suggests that larval longevity may be moisture dependant; meaning geographical variation could be a significant factor in disease epidemiology. For instance, the incidence of fascioliasis in sheep and cattle is greatly increased in years with significantly higher rainfall, with distinct geographic foci in many parts of Wales and Scotland as a result (McCann *et al.,* 2010; Ducheyne *et al.,* 2015). If it follows that *S. trachea* is significantly more abundant in soil with higher moisture content and/or disease incidence is greatly increased following above average rainfall, then more targeted treatment strategies could be conducted around periods of increased rainfall to reduce drug resistance pressure. If however, eggs and larvae do not survive for prolonged periods in the soil, it may be that increased rainfall leads to a greater abundance of infected paratenic hosts.

Furthermore, Guilford and Herrick (1952) undertook soil sampling of pheasant pens over a 2-year period to quantify the numbers of *Heterakis* spp.*, Capilara* spp.*, Coccidia* and *Syngamus trachea* eggs remaining viable in the soil. They noted that small numbers of *Heterakis* spp.*, Coccidia, and Capilara* spp. remain viable over winter to infect pheasants in the following spring. *Syngamus trachea* eggs were also found in small numbers throughout the sampling period, and a large number of 'disintegrated' eggs identified in August resulted in a high incidence of disease (Guilford and Herrick, 1952). The presence of disintegrated eggs indicates that these cases of syngamosis occurred by direct ingestion of large amounts of infective larvae.

It is naïve to think that disease epidemiology observed by Barrus (1966a,b,c) in the Czech Republic is comparable to that of *S. trachea* populations within the UK. For instance, McGregor *et al* (1961) reported that syngamosis can be effectively controlled by adequate sanitation and segregation of infected birds. If earthworms were of high significance in contributing to the epidemiology of syngamosis, adequate sanitation and segregation of infected birds would have little or no influence on the number of cases. As no real experimental work has been conducted regarding the longevity of larvae in the UK, we do not know the relative contribution of either larvae or paratenic hosts to disease epidemiology.

Currently, it is not known definitively how many invertebrate species are able to carry and transmit *S. trachea* larvae, but much work has been conducted on earthworms, slugs and snails (Taylor, 1935). Although Clapham (1939) believed that earthworms acted as intermediate hosts for *S. trachea*, no development occurs within these paratenic hosts and they act merely as mechanical vectors. Taylor (1935) heavily infected experimental plots with *S. trachea* eggs and periodically recovered the earthworms from the soil to experimentally infect pheasants. Although *S. trachea* is able to encyst within earthworms and transmit syngamosis, which could increase the longevity of eggs, the number of earthworms used during the study was extremely high compared with the number of adult worms found in the trachea at post-mortem (Taylor, 1935). In one experiment, 200 infected earthworms were fed to each of 21 pheasant chicks, but the largest number of adult worms found during post-mortem was 8. Even more intriguing is the fact that 12 of the 21 birds did not develop any infection and no adult worms were found in the trachea (Taylor, 1935). This method was repeated by Clapham (1934), who obtained *S. trachea* eggs from pheasants, partridges chickens and rooks and experimentally infected chickens. Although a larger number of adult worms were found upon post-mortem compared with Taylor (1935), it is known that *S. trachea* is more pathogenic in chickens than in pheasants and could explain the differences in adult worm establishment (Clapham, 1935). Similarly, Campbell, (1935) obtained earthworms (*Lumbricus terrestris)* from pheasant pens where heavy mortalities had occurred the previous year from clinical syngamosis. Campbell, (1935) fed each of 8 pheasant chicks 11 earthworms however only two birds were found to be harbouring 1 pair of worms each on post-mortem. Two hundred and ninety three earthworms recovered from the same pheasant pen were then fed to each of 40 pheasant chicks, however again only two birds were found to be harbouring 1 and 6 pairs of adult worms upon post-mortem. The results of Campbell's (1935) experiments demonstrate that disproportionately large numbers of earthworms would need to be consumed in order to result in clinical syngamosis among pen-reared

birds. Barus (1966b), however, considered earthworms as the most important factor in the transmission and longevity of *S. trachea* and claimed that eggs and larvae within the pens are of little epizootological importance. This, however, is a surprising conclusion, as Barus (1966b) only observed 6.3% of 338 earthworms (*Lumbricus terrestris*) to contain encysted *S. trachea* larvae. Of these earthworms, the number of encysted larvae within the infected worms only varied between 1 and 12 per worm.

With the exception of Campbell, (1935), all of these previous studies have either used discrete plots of land or used Petri dishes in order to infect earthworms, increasing the likelihood that a given earthworm will come into contact with an egg. In the natural setting however, it is unclear as to what proportion of earthworms within a release pen are indeed infected, and even what proportion of those infected earthworms get ingested by pheasants. It could be that a small proportion of infected earthworms are responsible for the initial infection pressure among penned birds, and due to the high biotic potential of *S. trachea,* the subsequent infections are a result of seeding of the ground with contaminated faeces. It is perhaps more probable that disease transmission and propagation are a result of direct contact with the infectious stages around communal areas due to poor sanitary conditions within release pens, and that earthworms play very little, if any role in the epidemiology of *S. trachea.* It is important to mention that a number of nematode species may be ingested by earthworms, but are simply passed out along with other waste products. The fact that the eggs hatch inside the earthworm, and that the hatched larvae migrates through the intestinal mucosa and encysts in the muscle tissue is clearly noteworthy. If the earthworm were not an important factor then there would be no further hatching inside a non-avian host. It may be that either in the past, or in the future, that natural selection may favour earthworms as the primary method of transmission.

Several authors are of the opinion that there are host strains, and sub-species among *S. trachea* populations, and that direct transmission of eggs between species is not a viable method of disease transmission (Taylor, 1928; Clapham, 1938). Clapham, (1938) was of the opinion that the passage of eggs from different bird species through an earthworm removed any physiological constraints determining host specificity. This hypothesis was borne from the work of Taylor, (1928) who found ‘difficulty’ in producing clinical syngamosis in chicks with eggs cultured from starlings. This, however, is unlikely to be the case as there is no evidence of any parasite species changing host following passage through a non-essential transport host, and in Taylor’s, (1928) work, 5 out 8 chicks died following exposure to eggs obtained from starlings. The earthworms that were used in the

Clapham, (1938) study were infected with large numbers of eggs in small containers that would increase their likelihood of becoming infected. Indeed, Lewis (1928), undertook detailed morphological examination of *Syngamus* spp. worms taken from a variety of wild and farmed avian hosts. It was concluded that different worms obtained from chickens, pheasants, turkeys, rooks, starlings and blackbirds were indeed *S. trachea* and that there is much phenotypic plasticity determining the size and shape of structures used to distinguish between species among individuals within and between hosts (lewis, 1928). Recently however,

2.2.8.1 Eggs

Many researchers have focused on the apparent importance of earthworms in the transmission of syngamosis, however very few have focused on the epizootological importance of embryonated and over-wintered eggs and larvae within release pens. Guildford and Herrick (1954) conducted artificial infection experiments using pheasant chicks to observe the host pathological response to embryonated eggs. Each of twenty six, 22-day-old pheasants, were dosed with approximately 200 *S. trachea* eggs directly into the crop. Each of the 26 pheasants developed syngamosis, with the number of paired adult worms in the trachea ranging from 1 to 28. Similarly, Devada and Sathianesan (1988) infected day-old Leghorn chicks with ~3000 infective eggs. Again, each of the birds developed syngamosis and the number of adult worms ranged from 1 – 94 pairs. Despite this high parasitic load, the largest number of adult worms found on post-mortem examination was 94 pairs. Although this would certainly be enough worms to lead to the mortality of the host, this suggests that infections with *Syngamus trachea* may be dose dependant. In contrast, Clapham (1939b) infected each of 36 chickens with ~60 *S. trachea* larvae. Each bird developed respiratory distress and died within two weeks of the initial infective dose, with parasite intensity ranging from 6-21 pairs of worms per bird. It is not clear however where these eggs came from, as Clapham has previously infected chickens with eggs of corvid origin with little success.

Chapter 3. Spatio-temporal factors influencing the occurrence of *Syngamus trachea* within release pens in the South West of England.[1]

3.1 Introduction

An understanding of the factors influencing infectious disease dynamics is fundamental to the control and prevention of disease (Pullan *et al.,* 2012). Knowledge of these factors can potentially influence management decisions, leading to the development of alternative practices to promote sustainable disease control (Abbott *et al.,* 2012; Morgan & Van Dijk, 2012). With the development of drug resistance to anthelmintics in many animal species, sustainable disease control is a growing concept in agriculture (Morgan & Wall, 2009). It must be noted however, that drug resistance has not yet been identified within game birds, perhaps due to the lack of studies within this area. As only one anthelmintic, Flubenvet™, has been licensed for use in pheasants in the UK (Pennycott, 2000; NOAH, 2014), the need for alternative control methods could become a significant issue in the coming years. Other anthelmintics such as Fenbendazole (Panacur™) are available under the cascade, however must be prescribed by a Veterinary Surgeon (NADIS, 2014).

In order to supplement wild populations of pheasants and maintain a large enough population to keep up with demand for shooting, it is common practice within many parts of Europe to rear pheasants in confined systems (Draycott et al., 2006; Goldova et al., 2006). It is estimated that approximately 12 million pheasants are harvested each year within the UK (Tapper, 1999). In order to maintain this increased demand for game shooting, around 25 million 6-8 week old pen-reared pheasants are released into the countryside every year (Tapper, 1999; Sage *et al.,* 2003; Draycott et al., 2006). Within release pens, pheasants are commonly kept at stocking densities of ~1800 birds per hectare (Sage et al., 2005) and are commonly released at densities of 250 birds/km2 (Aebischer, 2003). This high concentration of birds within release pens, combined with the increased density of parasitic and bacterial pathogens can lead to significant losses (Ruff, 1999; Goldova et al., 2006).

Pheasants *(Phasianus colchicus)* are susceptible to a number of parasitic nematodes (Ruff*,* 1999); *Syngamus trachea* in particular can cause significant production losses, poor weight gain and even mortality in heavily infected birds (Ruff, 1999; Krone *et al.,* 2007; Atkinson, *et al.,* 2008). *S. trachea* is a parasitic strongylid nematode that causes syngamiasis in poultry and game birds (Krone *et al.,* 2007; Atkinson, *et al.,* 2008), Generally, unembryonated eggs

[1] Published in *Veterinary Parasitology,* 207 (2015), 64-71.

are deposited in the faeces and develop to the L3 stage within the egg, with development times generally decreasing at increasing temperatures (Barus, 1966a). The confinement of pheasants within 'release pens' prior to release is believed to be a major component in the epidemiology of syngamiasis, and as the same pens are frequently used between years, could facilitate the maintenance and propagation of disease within the environment (Ruff, 1999; Goldova et al., 2002; Draycott *et al.,* 2006; Goldova *et al.,* 2006). The added complication is syngamiasis can be either direct or in-direct, and many invertebrate species can ingest *S. trachea* eggs, thus serving as paretenic hosts for disease, increasing its spatial distribution and increasing the longevity of infective stages (Clapham, 1934; Taylor, 1935; Nevarez *et al.,* 2002; Atkinson *et al.,* 2008; Holand *et al.* 2013).

Although the potential for disease is high within release pens, currently however, it is unclear how long eggs and larval stages of poultry parasites are able to remain viable in the environment, and thus contribute to disease between years (Clapham, 1934). It is suggested that the between and within-year variation in the abundance of free-living stages of parasites of veterinary importance is primarily weather dependent (Moss *et al.,* 1993; O' Connor *et al.,* 2006; Morgan & van Dijk, 2012), with temperature, humidity and rainfall being the most significant factors (Pullan *et al.,* 2012: Dybing *et al.,* 2013; Morgan & van Dijk, 2012; Formenti *et al.,* 2013). It has also been suggested that soil moisture is important in governing egg longevity in parasites that have relatively high temperature thresholds for development (Guildford & Herrick, 1952; Khadijah *et al.,* 2013a; Khadijah *et al.,* 2013b). As *S. trachea* larvae are extremely susceptible to desiccation ('drying out') (Barus, 1966a), it is predicted that only low numbers of hatched larvae are able to survive the winter, although eggs are able to survive prolonged exposure to low temperatures when kept in water. In the natural environment, however, other factors such as humidity and soil moisture may be important for disease persistence (Guildford & Herrick, 1952), with the potential for discrete disease foci on estates (Kocan, 1969; Draycott *et al.,* 2000; Goldova *et al.,* 2006). In conjunction with environmental factors, the aggregated distribution of birds within estates, and the aggregated nature of faeces within release pens (Saunders *et al.,* 2000b) could explain the variation in infection pressure of all nematodes, not just *S. trachea*, which may provide an opportunity to manipulate disease risk spatially.

It is predicted that the potential for disease in release pens is high, with significant disease transmission occurring around communal sites such as feeders and water baths, with the potential transfer between species and the wider spatial distribution of disease among wild and penned birds. It is hypothesised that levels of disease of *S. trachea* are higher in pens

that have not been relocated, and higher in pens with greater annual stocking densities. Here we report the results of a study investigating the influence of climatic variables on both egg survival and larval abundance, and speculate on how this could be used to reduce disease incidence in pheasant flocks.

3.2 Materials and Methods

3.2.1 Selection of field sites

The two field sites were recruited subject to certain criteria. Field sites had to have a history of releasing pheasants continuously for a number of years (10) and have had some previous history of *S. trachea* infections. Due to anonymity requests, sites are only being referred to by approximate grid references. Site 1 was situated approximately at grid reference - SU 17769 30326 and consisted of 7 release pens. Site 1 undertook Corvid control via the use of Larsen. Site 2 was situated approximately at grid reference - SU 67340 48539, and consisted of 13 release pens. Unlike site 1, site 2 did not undertake any Corvid control using Larsen traps. Both sites provided Flubendazole (Flubenvet™) in the feed as a prophylactic. One disused pen (> 5 years without use) per site served as a control. No *S. trachea* eggs or larvae were found at any point during the study within control pens.

3.3.1 Sample collection

To ascertain the parasite distribution within and surrounding release pens, the spatio-temporal modelling of disease was undertaken from April 1st 2014 to August 2014.

3.3.1.1 Collection of soil samples

Soil samples were collected at the beginning of the study (01/04/2014) in order to quantify the number of eggs and larvae remaining in the pens over winter. Fifteen soil samples were collected haphazardly from within the pens. Soil samples were collected using a 20 cm wide shovel. A 20 x 20 cm surface area was marked out and excavated to a depth of 1.5 cm totalling 600 cm^3 before collecting in a sealable plastic bag. Herbage and vegetation was removed from the area prior to collection of soil. Analysis of soil samples was conducted within two hours of collection. When this was not possible, samples were stored at 4 °C in a refrigerator for no longer than 24 hours.

3.3.2 Soil egg counts

The number of eggs within the soil in each release pen was calculated to determine disease risk between pens. The method of egg recovery was a slightly modified version of one devised by Guildford & Herrick (1952). Samples were collected into 30g containers to ensure uniformity. After removing large rocks and debris, the 30g sample of soil was mixed with 100ml of water and 1 drop of anionic surfactant (Fairy Liquid^{TM}) in order to ensure egg and soil separation. The sample was thoroughly mixed and left to stand for 24 hours in order to ensure maximum egg recovery. The sample was then passed through sieves of decreasing apertures (500μm, 250μm, 125μm and 40μm) to filter out large soil particles and retain eggs. The eggs retained on the 40μm sieve were recovered using a fine spray of water and transferred to a 500ml-measuring beaker. The egg-water mixture was then transferred to a 100ml-measuring cylinder in order to facilitate sedimentation. The sample was left to settle for 2 hours before removing the excess water with a 100ml syringe until a 15ml water-soil mixture remained. This was then transferred to a 15ml conical centrifuge tube before being centrifuged at 1500 rpm (554rcf (G)) for 3 minutes (SciSpin One Compact Centrifuge). The supernatant was poured away and the pellet was re-suspended in Saturated Sodium Chloride (NaCl; Specific gravity – 1.20) to 15ml volume. The sample was then spun again at 1500rpm for 3 minutes to allow eggs and larvae to float to the top of the test tube. A 1ml aliquot was then transferred to a Nematode Counting Slide (Chalex corp) before examination under a microscope at 100x magnification.

3.3.3 Determination of soil moisture content

Initial volumetric soil moisture content was determined for each release pen in order to evaluate the effect of moisture content on the numbers of viable nematode eggs. Sampling took place at the beginning of the study (01/04/2014). Moisture content was measured using a Time-domain Reflectometer (TDR) (Fieldscout® TDR 100 Soil Moisture Meter) at 15 haphazardly selected points across all release pens.

3.3.4 Size of pen and previous stocking densities

Data on previous stocking densities and the age of pen were collected from detailed records kept by the gamekeepers. The size of the pen was calculated by walking around the perimeter of the release pen with a Garmin E-Trex GPS whilst using the 'Calculate Area' function to determine the area of the pen in acres before converting to m^2.

3.3.5 Collection and analysis of faecal material

Faecal samples were collected weekly from the date when pheasants were placed into their respective release pens (From 16/07/2014 – 31/07/2014). In order to account for within-group variation in parasite burdens, 10 faecal samples were collected per week per release pen (Yazwinski *et al.,* 2003). The selection of faecal samples to determine overall numbers of disease per pen (eggs per gram of faeces) was conducted haphazardly. Faecal egg counts were performed with a Modified McMaster Technique, conducted according to WAAVP standard guidelines as outlined by Coles *et al.* (1992).

3.3.6 Larval sampling

Larval sampling was conducted weekly from the 18/04/2014 from one pen per study site in order to quantify the numbers of infective larvae (L3) over time. Each week, the pen was traversed in the typical 'W' sampling method as described by (Taylor, 1939) and 3-4 herbage plucks were collected every 2 feet. Black plastic bags were filled with enough grass to total 1kg of dry weight, which was determined by trial and error before conducting sampling. Following collection, the herbage was transferred to buckets and filled up with water. Several drops of anionic surfactant (Fairy Liquid™) were added to each bucket and mixed by hand for several minutes. Each bucket was then allowed to stand for 2 hours to ensure optimum larval separation. After 2 hours, larger plant material was removed and the remaining liquid was poured through sieves of decreasing apertures (250, 125 and 40μm). The larvae-containing residue on the 40μm sieve was washed and collected into a 15ml conical test tube and shaken for 10 seconds. After several seconds, a 1ml sample was collected from the middle of the test tube and transferred to a Nematode Counting Chamber (Chalex Corp), before being stained with Lugol's Iodine. The total numbers of larvae within the chamber were counted before multiplying by the original sample volume to give an accurate representation of the total number of larvae per kg/dm.

3.3.7 Parasite identification

Eggs within the soil and faeces, and larvae in herbage samples were identified using the Veterinary Parasitology Reference Manual (Foreyt, 2001) and online photographs from the RVC/FAO Guide to Veterinary Diagnostic Parasitology available on the Royal Veterinary College website. Due to the scarcity of images of the larval stages of *S. trachea,* personal photographs were taken from cultures obtained by dissecting gravid female worms taken from pheasants which were maintained in the laboratory at 24° C (Wehr, 1937). Eggs were cultured to the infective stage (L3) and manually hatched by applying light pressure between two cover slips.

3.3.8 Climatological factors

Air and soil temperature were recorded at both sites using Tinytag™ Plus 2 (TGP-4020) Data Loggers measuring at 15-minute intervals throughout the duration of the study. Soil temperature was measured at a depth of 2-inches using a Tinytag™ (PB-5002-1M5) Thermistor Probe. Relative humidity was also measured at 15-minute intervals using a Tinytag™ Plus 2 (TGP-4500) Internal Temperature and Relative Humidity logger. Rainfall was measured using a standard rain gauge at both field sites.

3.3.9 Statistical analysis

All data were analysed using SPSS (20) for Macintosh. Data were tested for normality using the standard One-Sample Kolomogrov-Smirnof test before analysis. The number of eggs per gram of soil and the number of eggs per gram of faeces were not normally distributed so were subjected to Log (Base-E) transformation. Residual values had a mean of 0 and equal variance after transformation so the assumption of normality was confirmed. To assess between-site variation in the numbers of eggs per gram of soil, differences in the log transformed mean numbers of eggs per gram of soil per pen were compared between sites using an Independent Samples T-Test. The numbers of larvae recovered per week were compared for significant difference using an Independent Samples T-Test with site as factor. Within-site variation in the number of eggs per gram of soil per pen was assessed using a One-Way Analysis of Variance (ANOVA) with *Post-hoc* Least Significant Difference (LSD). In order to establish differences in infection risk between pens, manual multiple regression analysis was conducted with 'number of eggs per gram of soil' as the dependant variable and all possible combinations of 'pen number', 'years in use', 'average faecal eggs per gram', 'stocking density' and 'Soil moisture content' as independent variables. Again, manual multiple regression analysis was conducted with the log(E) transformed mean number of eggs in faeces per pen, and larval abundance as dependant variables, and all possible combinations of air temperature, soil temperature, rainfall, humidity and soil moisture to assess the importance of various climate and environmental variables on observed disease patterns. Goodness of fit for all model combinations was determined by adjusted R-squared, with a higher adjusted R-squared indicating greater model fit.

3.4 Results

3.4.1 Numbers of eggs per release pen

The mean number of eggs per gram of soil differed significantly between pens at site 1 ($F_{4,70}$=9.738, p=0.001) and site 2 ($F_{6,99}$=15.276, p=0.001), but did not differ significantly between sites ($F_{1,10}$=12.113, p=0.854). Site 1 had, on average, 54.6 (Std=±23.98) eggs per gram of soil across all pens compared to 54.1 (Std=±17.93) eggs per gram of soil at site 2 (Individual means and standard deviations given in figure 2).

3.4.2 Background levels of disease: influence of soil moisture, years in use and stocking density

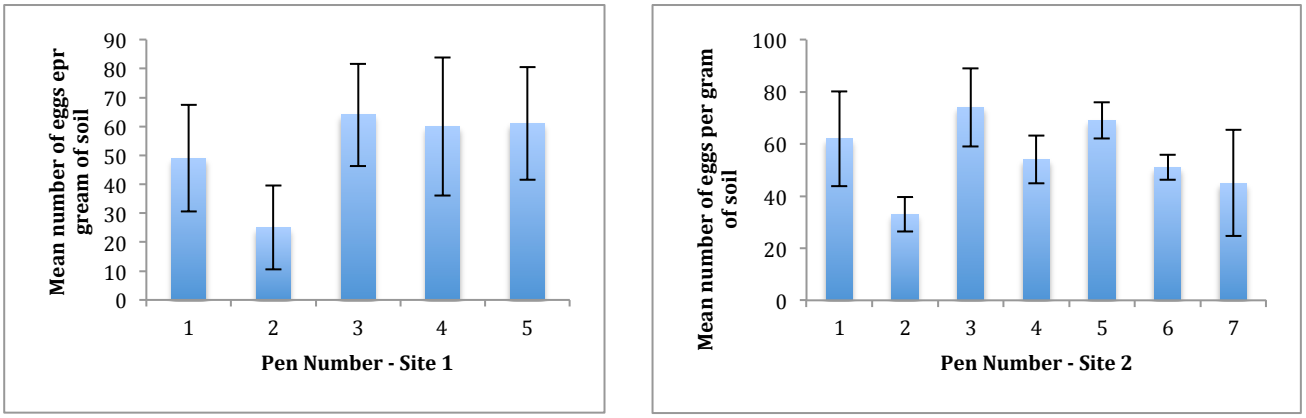

Figure 2 Average number of eggs per gram of soil within release pens between sites.

3.4.2.1 Soil moisture

The relationship between soil moisture content and the number of eggs per gram of soil per pen was not linear; fitting a quadratic model accounted for much more of the variation in the numbers of eggs per gram of soil per pen than the linear model (R-squared change = 0.191, p=0.003). The linear model explained 49.2 % of the variation ($F_{1,\ 10}$=11.115, R=0.726, adjusted R-squared=0.479, p=0.008), whereas the quadratic model accounted for 71.8% of the variation in the numbers of eggs per gram of soil per pen ($F_{2,9}$=11.435, R=0.847, adjusted R-squared=0.655, p=0.036). The model captures the relatively steep increase in egg abundance at 30-35%, and the relative stability at 40% and above.

3.4.2.2 *Pen age and stocking density*

Stocking density (See appendix A), expressed as birds/m^2, accounted for 47.2% of the variation in the numbers of eggs per gram of soil between pens ($F_{1,10}$=10.828, R=0.721, adjusted R^2=0.472, p=0.008), with more eggs being found in pens with higher annual stocking densities. Finally age of the release pen accounted for 38.4% of the variation in the numbers of eggs per gram of soil ($F_{1,10}$=7.852, R=0.663, adjusted R^2=0.384, p=0.019), indicating a higher number of eggs in pens that have been in place longer.

3.4.2.3 The effect of multivariate comparisons

When models were combined, in terms of adjusted R^2, the best predictor of the numbers of eggs per gram of soil was a combination of pen age, average stocking density and volumetric soil moisture content which explained 84.7% of the variation in the numbers of eggs per gram of soil ($F_{3,8}$=21.267, R=0.943, adjusted R^2=0.847, p=0.001). The combination of moisture and average stocking density explained 84.9% of the variation in the numbers of eggs per gram of soil ($F_{2,9}$=31.878, R=0.936, adjusted R^2=0.849, p=0.001), whereas pen age and its respective stocking density accounted for 55.5% of the variation in the numbers of eggs per gram of soil across sites ($F_{2,9}$=7.851, R=0.797, adjusted R^2=0.555, p=0.011), indicating that soil moisture content is an important factor determining egg longevity, more so than pen age.

3.4.3 Numbers of larvae over time

The numbers of recoverable larvae differed significantly between sites (t^{12}=4.213, p=0.001) with an average of 1730±195.57 L3/Kg/DM recovered at site 1, and 3211±759.02 L3/Kg/DM at site 2. In general, the numbers of recoverable larvae increased with time at both site 1 (Fig. 3) (R=0.79, p=0.001) and site 2 (Fig.4) (R=0.78, p=0.001), though in some weeks higher numbers of larvae were recovered. Maximal larval recovery rates occurred at the beginning of July at site 1 (04/07/2014) and site 2 (07/07/2014).

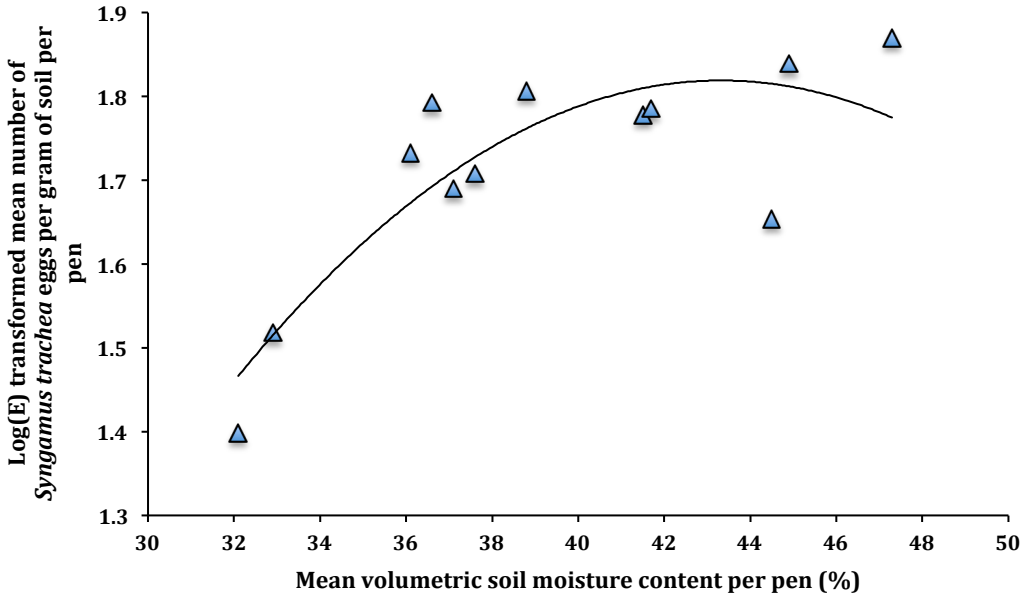

Figure 2 Relationship between volumetric soil moisture content and the abundance of Log(Base-E) transformed number of eggs per gram of soil per pen.

3.4.4 Climate variables and larval development

Temperature and humidity were the greatest factors determining larval development at site 1, with greater numbers of larvae being recovered at higher temperatures (R=0.698, p=0.004) and higher relative humidity (R=0.636, p=0.017) (appendix B). Similarly, temperature (R=0.751, p=0.001) and humidity (R=0.625, p=0.006) influenced larval development and recovery at site 2 (appendix C). Rainfall did not influence larval recovery at either site 1 (R=0.012, p=0.966) or site 2 (R=-0.125, p=0.620). When climate variables were combined the best predictor in terms of adjusted R^2 was the combination of temperature, humidity and rainfall which accounted for 62.1% of the variation in the numbers of larvae recovered per week at site 1 ($F^{3,11}$=6.020, R=0.788, p=0.011), and 68.5% of the variation at site 2 ($F^{3,14}$=10.161, R=0.828, p=0.001), The exclusion of rainfall also explained much of the variation in the number of larvae recovered per week at site 1 ($F^{2,12}$=9.466, R=0.782, p=0.003) and site 2 ($F^{2,15}$=12.133, R=0.786, p=0.001), demonstrating rainfall added very little to the model in terms of predictive ability.

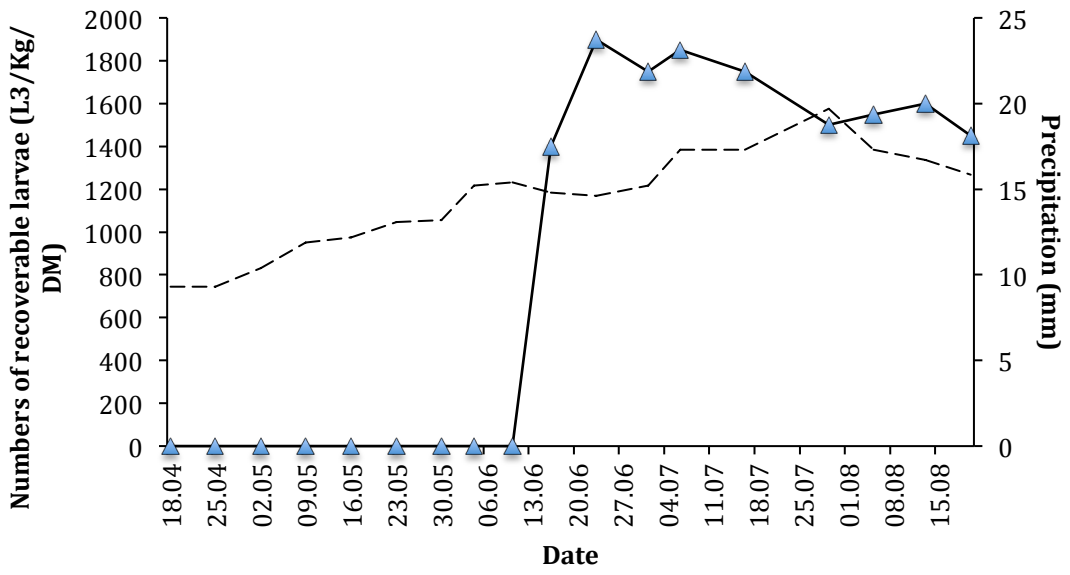

Figure 3 Numbers of *S. trachea* larvae (Solid line) recovered per Kg of Dry Matter (L3/Kg/DM) and weekly precipitation (Dashed line) in mm at site 1.

3.4.5 Climate variables and infection status

The overall incidence of clinical syngamiasis remained low on both estates throughout the study period. The numbers of recoverable larvae and rainfall (Site 1) were the greatest factors influencing infection status in pheasants with higher faecal egg counts (FEC) being identified in weeks with higher numbers of infective larvae at site 1 (R=0.642, p=0.013) (appendix D) and site 2 (R=0.951, p=0.001) (appendix E), and increased rainfall at site 1 (R=0.946, p=0.001) but not at site 2 (R=-0.020, p=0.936). Neither temperature (R=0.453, p=0.307) nor humidity had any effect on infection status at site 1, although there was a trend for higher FECs at higher relative humidity (R=0.603, P=0.152), although this was not considered statistically significant. In contrast, both temperature (R=0.688, p=0.002) and humidity (R=0.547, p=0.019) influenced infection status at site 2 with higher FECs in weeks with higher temperature and higher relative humidity.

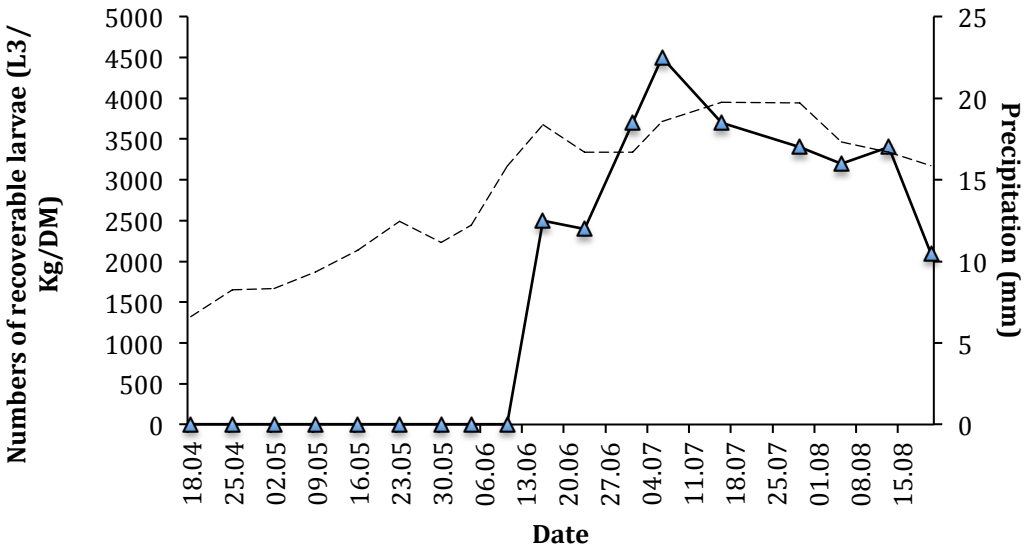

Figure 4 Numbers of *S. trachea* larvae (Solid line) recovered per Kg of Dry Matter (L3/Kg/DM) and weekly precipitation (Dashed line) in mm at site 2.

3.4.6 Multivariate comparisons

Predictive ability was again increased with a combination of variables, with the greatest predictor being a combination of rainfall, temperature and humidity at both site 1 ($F^{3,3}$=26.837, R=0.982, p=0.011) and site 2 ($F^{3,14}$=7.660, R=0.788, p=0.003). When humidity was removed from the model, rainfall and temperature still explained much of the variation in the numbers of *S. trachea* eggs identified in faecal samples per week at site 1 ($F^{2,4}$=47.020, R=0.979, p=0.002).

3.5 Discussion

Parasite development, and therefore transmission and infection success is dependent on many variables (Moss *et al.,* 1993). For parasites whose development is reliant on certain exogenous variables, such as temperature and humidity (Dybing *et al.,* 2013), disease occurrence and therefore risk is going to vary in both time and space (Pullan *et al.,* 2012: Dybing *et al.,* 2013; Formenti *et al.,* 2013). The effect of increased rainfall, and invariably moisture and ground saturation, on moisture dependent species is therefore predicted to determine disease incidence between years (Moss *et al.,* 1993; Magwisha *et al.,* 2002; O' Connor *et al.,* 2006; Dybing *et al.,* 2013; Holand *et al.,* 2013). For a parasite whose temperature developmental range is fairly high, it is not surprising that soil moisture content was the most important factor governing initial infection status and longevity of eggs within

pens in this system, in agreement with Guildford & Herrick (1952). It is, however, the first mention of soil moisture dependence in regards to *S. trachea* and the first suggestion of such profound intra-site variation in infection pressure, which may suggest the presence of discrete disease foci within estates (Draycott *et al.,* 2000). The importance of soil moisture content in disease propagation has been identified in other nematode species, most notably *Haemonchus contortus* (Silangwa & Todd, 1964; Rossanigo and Gruner, 1994; Khadijah *et al.,* 2013a; Khadijah *et al.,* 2013b), with significantly more L3 being recovered from soil and herbage with higher moisture content. As *S. trachea* larvae are extremely susceptible to desiccation (Barus, 1966a), it is likely that moisture is important for perpetuating the disease within the environment by increasing larval survival, aiding larval dispersal within the soil profile and permitting their vertical migration up available herbage (Silangwa & Todd, 1964; Moss *et al.,* 1993; Saunders *et al.,* 2000b; Dybing *et al.,* 2013). The quadratic model suggests a sharp increase in egg abundance and survival at 35-40% moisture content, with recovery rates showing relative stability at 40% and above. It is possible, that with a larger sample size and a greater selection of variable moisture content, that egg recovery and abundance could decrease when soil becomes waterlogged, similarly to *Ancylostoma caninium* (Dwight and Bowman, 2013).

Despite previous studies suggesting that *S. trachea* eggs do not survive long enough to contribute to disease incidence between years (Barus, 1966b) the present study found a higher abundance of viable *S. trachea* eggs in pens that had been in use longer. The nature of pheasant releasing is not conducive to the frequent movement and/or resting of pens, so generally the same pens are used for release between years. The frequent use of discrete areas for rearing and release over time would lead to highly infective ground (Goldova *et al.,* 2002), especially when disease can be transmitted via wild birds, eggs and larvae in the soil and paratenic hosts. Sherwin, *et al* (2013) found a similar relationship between the number of consecutive years a pen had been in use and the numbers of *Heterakis gallinarum* eggs within faecal counts in chickens, although no quantification of the number of eggs in the soil was undertaken. Although a higher number of viable eggs were recovered in older pens, egg recovery at the beginning of the study was low, indicating a relatively low carry-over within these two systems from previous years. It has been demonstrated that eggs are capable of surviving prolonged exposure to temperatures of between 0 - +3 °C degrees, however eggs kept at the lower end of the development threshold did not survive for prolonged periods when exposed to fluctuating temperatures of +12-+24 °C and humidity ranging from 36-97% (Barus, 1966a). It is clear, that eggs are capable of remaining viable in the soil, however only when soil moisture, humidity and rainfall conditions are optimal. This could explain the within-site and within-year variation in infection pressure, as soil moisture was highly correlated with egg

longevity, and the reason larval recovery was greater during high rainfall periods (Formenti, *et al.,* 2013). As these conditions cannot always be guaranteed, paratenic hosts may play a greater role in disease maintenance when climatic conditions are unfavourable for larval survival, suggesting a potential evolutionary strategy to avoid local extirpation.

In addition, annual stocking density influenced the abundance of viable *S. trachea* eggs within the pens, with a higher number of eggs being found in pens with greater stocking densities. This is in agreement with Sherwin *et al* (2013), who found that annual stocking densities influenced the abundance of *T. tenuis, H. gallinarum* and *Ascaridia* spp within faecal samples, and Permin *et al* (1998), who found that increased stocking densities increased *Ascardia galli* establishment in chickens, whilst negatively effecting body weight gain. The increased density of birds would most likely increase the likelihood of subsequent infections within flocks, as the contact time between host and parasite increases at greater densities (Permin *et al.,* 1998; Abbott *et al.,* 2012; Borovkov *et al.,* 2013; Sherwin *et al.,* 2013).

Results from the present study are in line with previous studies concerning the influence of temperature and humidity on the numbers of *S. trachea* eggs within FEC (Barus, 1966b). To our knowledge however, the present study is the first to quantify larval abundance over the course of the season and relate larval abundance to climatic variables. Temperature, humidity and rainfall were the greatest factors determining larval abundance during the study period, and higher numbers of larvae were recovered during weeks with higher average temperature, relative humidity and rainfall at both sites. High humidity levels have been shown to influence survival and distribution of free-living stages of parasitic species, as it invariably determines moisture content within microenvironments (Callinan & Westcott, 1985; Dybing *et al.,* 2013), aiding larval development and preventing desiccation. It has been demonstrated that in the absence of suitable levels of moisture, larvae remain within the faecal pats and/or migrate into the soil beneath the pats in order to prevent desiccation (Uriarte & Gruner, 1994; Stromberg, 1997). Although temperature and humidity influenced larval development, this was only true for weeks in which the average weekly temperature exceeded 16 °C. Once this development threshold had been attained, the numbers of larvae increased rapidly, shortly after pheasant placement. In agreement with Barus (1966b), the numbers of larvae recovered from herbage reached their peak towards the end of July/August, presumably when conditions were optimal for larval development. Interestingly, high numbers of unembryonated *S. trachea* eggs were found per week at site 2, in comparison to negligible numbers at site 1. An examination of the Corvids at site 1 identified that ~75% (n=55) were infected with at least 1 pair of gapes (Mean 6.4±9.6 gapes per bird), whereas the small number of pheasants that were recovered, were

found to be uninfected (Personal unpublished data). The rapid fluctuations in the numbers of recoverable L3 per week, and the presence of unembryonated eggs, may be attributed to the seeding of the ground with fresh *S. trachea* eggs by Crows, Rooks and Jackdaws (Simon *et al.,* 2011). As site 1 employed heavy Corvid control throughout the season via the use of Larsen Trapping, and site 2 did not, could explain the differences in numbers of recovered eggs and larvae between sites. It is a possibility that these eggs may have come from Corvids, which in turn may have picked up the initial infection by consuming infected invertebrate hosts (Taylor, 1935).

3.6 Conclusions

Despite previous ecological studies aiming to determine egg and larval abundance and survival over time, these studies have been conducted under controlled conditions that do not accurately represent natural conditions, especially in regards to moisture availability (O' Connor *et al.,* 2006). The results of the present study clearly demonstrate that the continued use of discrete releasing areas upon estates is maintaining and even exacerbating the levels of disease within pheasant populations within these systems. In contrast to previous studies (Barus, 1966a,b), results presented here clearly demonstrate the increased longevity of *S. trachea* eggs and their ability to contribute to disease in subsequent bird populations, however only when conditions are optimal. It is recommended that pens are 'rested' or where possible, moved, between releases to ensure sufficient larval and egg mortality, as is common practice within livestock farming (Abbott *et al.,* 2012). Indeed, Simon *et al.* (2011) showed that the movement of pens to uninfected ground significantly reduced the occurrence of all nematodes within chickens. It was also concluded that the absence or very low worm burdens were probably the result of seeding by wild birds (Simon, *et al.,* 2011). Similarly to the present study, Simon *et al.* (2011) found a high proportion of infected corvids, with 61% being infected with *S. trachea*. Although there are differences in stocking density and target species, the results are still noteworthy and suggest a potential strategy to reduce disease occurrence. Although variation in parasite prevalence has previously been suggested in a metapopulation of house sparrows (Holand *et al.,* 2013), the previous study was conducted over a much greater spatial scale, whereas the present study suggests possible disease variation within relatively small pheasant estates. This presents the possibility of managing disease risk by the regulation of releasing areas to potentially allow the natural mortality of parasitic nematodes whilst reducing the reliance on anthelmintics, although further work is needed to accurately determine egg and larval longevity, and the speed of re-seeding following pheasant placement.

Chapter 4. Spatial distribution of infectious stages of the nematode S*yngamus trachea* within pheasant (*Phasianus colchicus*) release pens on estates in the South West of England: potential density dependence? [2]

4.1. Introduction

The spatial distribution of infectious stages of parasitic nematodes is one of the most important factors influencing disease transmission. The pattern and distribution of infectious stages of parasites in most animal species is best described as a truncated form of the negative binomial distribution (Crofton, 1971), with few hosts harbouring a large number of parasites with the vast majority of hosts harbouring few to zero parasites (Crofton, 1971). Because of this observed aggregation among discrete host populations, there is often a certain degree of aggregation in the infectious stages of parasites in the environment (Shaw & Dobson, 1995; Brockhurst *et al.,* 2006). Although the distribution of infectious stages of free-living species is indeed aggregated in space and time, the non-random congregation and social interaction of birds around feeders and drinkers within confined systems is likely to lead to a greater degree of spatial aggregation (Gortazar *et al.,* 2006; Real & Biek, 2007; Villanua *et al.,* 2008), with the 'tail' of the binomial distribution potentially occurring around communal sites. This could, in theory, have implications for the distribution of parasites within the host species, and could potentially account for the differences in pathogenicity of *Syngamus trachea* between wild and penned bird species due to differences in encounter rates.

Heterogeneity in exposure and susceptibility to parasitism is said to be the most significant factor determining parasite aggregation within host populations. In terms of variability in parasite exposure rates, it is generally believed that the uneven distribution of infectious stages of parasitic species in both space and time is predominantly responsible for this observed aggregation (Poulin, 2013). In general, host-parasite interactions occur at specific points, both spatially and temporally and the frequency of overlap between a susceptible host and parasite will determine the extent, intensity and establishment rate of subsequent infections (Real & Biek, 2007). In pheasant release pens however, the provision of designated feeding and drinking sites is likely to exacerbate parasite transmission due to the aggregated nature of immunologically naïve hosts (Hofle *et al.,* 2004; Villanua *et al.,* 2008).

Acquisition rates of new infections will generally increase as the contact time between host and parasite increases, and thus if feeders are representing distinct disease foci within release

[2] Published in *Veterinary Parasitology,* 212 (2015), 267-274.

pens, then it is likely that new infections are being acquired from the communal gathering of infected and susceptible hosts. As it has been demonstrated that many bacterial and mycotic agents can be transferred between hosts attached to grain (Hofle *et al.,* 2004), it is likely that feeders, feeder placement and feeder type, represent a major component in disease transmission (Villanua *et al.,* 2008). Much work has been conducted on the importance of faecal avoidance behaviour in grazing ruminants (Hutchings *et al.,* 2001), with both cattle and sheep generally avoiding grazing around faeces where possible. No such research however has been conducted on commercially managed poultry and game birds, and as birds are generally fed in specified locations, faecal contamination could be contributing substantially to the observed disease patterns. The importance of feeders in the transmission dynamics of *S. trachea* has been suggested elsewhere (Draycott *et al.,* 2006; Villanua *et al.,* 2008; Santilli & Bagliacca, 2012; Gethings *et al.,* 2015b), but no data currently exist to support or refute this hypothesis. In other animal species, the aggregation of animal populations at artificial feeding and watering sites has been shown to greatly increase the incidence of infectious diseases (Vincente *et al.,* 2007), in particular those with direct lifecycles.

Syngamus trachea is a parasitic tracheal nematode of many avian species, and can lead to morbidity and mortality in poultry and game birds, especially when birds are managed at high densities (Villanua *et al.,* 2008). Generally, *S. trachea* has a direct life cycle, with infection occurring following the ingestion of infective eggs or hatched infective larvae (L3), however transmission can involve the use of a paratenic host, usually an earthworm (Clapham, 1934). Despite this however, the epidemiological importance of paratenic hosts in the maintenance and propagation of syngamosis among game birds is disputed (Gethings *et al.,* 2015a,b) and it is believed that the majority of disease transmission is via ingestion of infectious stages with faecal-contaminated feed (Villanua *et al.,* 2008).

If feeders represent major disease foci within pens, then it may be possible to manipulate disease risk, either by the frequent movement of feeders, or the use of feeders that limit the amount of faecal-grain contamination. The aim of the present study is to identify to what extent, if any, infectious stages of avian parasites are aggregated within the release pen, and to evaluate what effect, if any, this aggregation has on the distribution of the adult stages within the host species. Relationships between varying management practices, i.e. moving or not moving feeders and feeder type will be also be compared between and within sites.

4.2. Materials and method

4.2.1 Sampling locations

The two estates chosen for the study were the same as per Gethings *et al.* (2015a). Both sites provided Flubendazole (Flubenvet™) in the feed as a prophylactic treatment against intestinal and other parasites in the release birds. One disused pen per site served as a control. No *S. trachea* eggs or larvae were found at any point during the study within control pens. Each of n = 10 (5 per site) pens were divided into equal quadrants of 5 m^2. Quadrants were constructed using parallel transects spaced at 5m intervals with perpendicular intersecting transects completing the quadrant. Each quadrant was then numbered and entered into a random number generator for selection of sampling locations.

4.2.2 Sampling regime

4.2.2.1 Random sampling

The first part of the analysis was conducted at random to determine the general distribution of *S. trachea* eggs across pens. Fifteen quadrants were sampled at random per pen (5 pens per site) and one soil sample was taken per quadrant. No bias towards areas with or without feeders or drinkers occurred. Sampling of quadrants took place on 1/10/2014.

4.2.2.2 Purposive comparisons

As the initial sampling method indicated a trend for greater abundance of *S. trachea* eggs around feeders in all pens (p = <0.001), purposive sampling between quadrants with and without feeders was undertaken on the 16/10/2014 for site 1, and 21/10/2014 for site 2, shortly after pheasant release, to quantify the observed difference. The pen was again split into quadrants, however quadrants with feeders were treated as separate variables and numbered consecutively. Fifteen samples from each of the two groups (i.e. feeder or random) totalling n = 30 were collected as per section 1 per pen. Samples from quadrants with feeders were collected from underneath the feed hopper, whereas samples from random quadrants were determined using a thrown quadrat. Ten pens were utilised for analysis with n = 30 soil samples per pen (n = 300 in total).

4.2.2.3 Distance from feeder

To determine whether pheasant feeders represent distinct disease foci within release pens, soil samples were taken at increasing distances at right angles from the feeder. Feeders were placed towards the centre of the pen, or at woodland edges, in order to hold the pheasants within the pens for as long as possible. On account of this, the distance sampling did not extend beyond the perimeter fence and sampling transects were maintained, unobstructed, for 15 metres in all directions. The first sample was collected from directly beneath the feeder and subsequent samples were collected at 2, 5, 10 and 15 metres away at right angles in each direction totalling 25 samples per feeder (*n* = 250 in total).

4.2.2.4 Abundance of eggs within soil samples

The number of eggs within the soil in each release pen was calculated to determine disease risk between pens. The method of egg recovery from soil samples was identical to the one used in Gethings *et al.* 2015a, which was a slightly modified version of one devised by Guildford & Herrick (1952). Eggs within the soil and faeces, were identified using the Veterinary Parasitology Reference Manual (Foreyt, 2001) and online photographs from the RVC/FAO Guide to Veterinary Diagnostic Parasitology available on the Royal Veterinary College website.

4.2.3 Collection and analysis of faecal material

Faecal samples were collected weekly from the date when pheasants were placed into their respective release pens (from 16/07/2014 to 31/07/2014). In order to account for within-group variation in parasite burdens, 10 faecal samples were collected per week per release pen (Yazwinski et al., 2003). The selection of faecal samples to determine overall infection levels of syngamosis per pen (eggs per gram of faeces) was conducted haphazardly. Faecal egg counts were performed with a Modified McMaster Technique, conducted according to WAAVP standard guidelines as outlined by Coles et al. (1992).

4.2.4 Determination of soil moisture content, pen size and stocking density

Initial volumetric soil moisture content was determined for each release pen on 1/10/2014 to evaluate the effect of moisture content on the numbers of viable nematode eggs. Moisture content was measured using a Time-domain Reflectometer (TDR) (Fieldscout® TDR 100 Soil Moisture Meter) at 15 randomly selected points across all release pens. The size of pen and stocking density were obtained from detailed records kept by the gamekeepers.

4.2.5 Egg deposition

As the same pens were used as per Gethings *et al.* (2015a), the abundance of eggs before and after pheasant release could be evaluated for difference overtime, as well as potential relationships between egg deposition and volumetric soil moisture content.

4.2.6 Post-mortem examinations

As site 2 was undertaking corvid control, crows were opportunistically sampled throughout the study to asses the levels of infection and determine the distribution of parasites within wild animal hosts. The number of adult worms per trachea for both crows and pheasants was determined by dissection.

4.2.7 Statistical analysis

All data were analysed using R for Macintosh. Data were assessed for normality, first by visual inspection of normal probability and Q-Q plots, then by statistical analysis using a Sharpio-Wilks test of normality and exploration of the skewness. The number of eggs per gram of soil and the number of eggs per gram of faeces were not normally distributed so were subjected to Log (Base-E) transformation prior to analysis. Differences in the number of eggs in quadrants were compared within and between sites using General Linear Modelling with 'Site' or 'Pen' as factors. The effect of soil moisture content on explaining the variation in the abundance of eggs per pen was assessed by Linear Regression Analysis between soil moisture content and the variance to mean ratio. Egg deposition before and after pheasant placement was assessed using a paired sample T-test. The 'distance from feeder' data were not normally distributed and visual inspection of the residuals indicated a non-random U-shaped distribution, so a quadratic model was fitted to the data.

4.2.7.1 Measures of parasite aggregation

Raw data concerning the spatial distribution of eggs per gram of soil within the release pens were assessed for aggregation by calculating a variance/ to mean (VMR) ratio (Barbour, & Pugliese, 2000), index of dispersion and estimating the negative binomial, the exponent, k. A value greater than 1 for the v/m ratio implies more variation than would be expected if the eggs were randomly distributed, and suggests the data are clumped or over-dispersed, i.e. aggregated. A value less than 1 indicates a regular or under-dispersed distribution (Elston *et al.,* 2001). The extent of aggregation was assessed by means of linear regression analysis

(Taylor's Power Law) between log variance and log mean and estimating the slope of the regression equation (Shaw & Dobson, 1995). Values of *k,* VMR and the index of dispersion were calculated for each pen.

4.2.7.2 Variance to mean ratio was calculated by;

(a) σ^2/μ

Where μ is the sample mean and σ^2 is the sample variance.

4.2.7.3 The corrected moment estimate of k

The corrected moment estimate (*k*) of the negative binomial distribution quantifies the degree of aggregation within a population, with an increase in the degree of aggregation for a constant known mean resulting in a diminishing value of *k.* Values for *k* range from a theoretical value of 0 to infinity, with a lower value of *k,* commonly less than 1, indicating a higher degree of aggregation. Alternatively, as the value of *k* increases for the same given mean then the degree of aggregation decreases with the distribution tending towards a Poisson (random) distribution and then a positive binomial as the value of *k* increases to infinity (Shaw & Dobson, 1995; Sherrard-Smith, *et al.,* 2015). (For reference, *k* < 1 = highly aggregated, *k* = 5 = aggregated, *k* > 20 = random or Poisson).

(b) $$k = \frac{\mu^2 - \sigma^2/n}{\sigma^2 - \mu}$$

Where *k* is the degree of clumping, μ is the sample mean, *n* is the sample size and σ^2 is the sample variance. Values for *k* range from 0 to infinity, with a lower value (commonly less than 1) indicating greater aggregation.

4.2.7.4 Index of dispersion (I_D) was calculated by;

(c) $I_D = \sigma^2(n-1)/\mu$

Where μ is the sample mean, σ^2 is the sample variance, *n* is the sample size – 1 degree of freedom.

4.2.7.5 Chi Square Goodness of fit

Raw counts of eggs per gram of soil per pen were compared to an estimated Poisson distribution using the chisq.test function in R. The aggregation parameters I_D and the VMR of the number of eggs per gram of soil, number of eggs per gram of faeces and the number of worms per bird were also compared to a Poisson distribution ($\mu = \sigma^2$) with n-1 d.f. to confirm the assumption of a negative binomial distribution.

4.3. Results

4.3.1 Egg abundance and distribution within pen – between site analyses

The variance to mean ratio for egg abundance for the randomly collected samples was significantly greater than 1, with values ranging from 27.31 to 914.90 indicating a highly aggregated parasite distribution within pens for both sites that differed significantly from the Poisson distribution (x^2_9 = 4437.96, p = < 0.001) with k being less than 1 in each case (Table 2). Similarly, the I_D differed significantly from the Poisson distribution (x^2_9 = 128701.1, p = <0.001). The estimated slope of the regression of log mean on log variance for both sites combined (b = 2.11 ± 0.037, p = < 0.001) (Fig 7) was significantly greater than 1, indicating eggs are highly aggregated within release pens. Results from the general linear model revealed a significant main effect of 'Site' on the abundance of *S. trachea* eggs in quadrants with a feeder ($F_{1,140}$ = 162.847, p = < 0.001), with site 2 displaying a mean difference of +211.693 eggs per gram of soil compared to site 1. The effect of 'Site' was also significant for quadrants without a feeder ($F_{1,140}$ = 4.104, p = 0.045), with site 2 again having a mean difference of +2.493 eggs per gram of soil across all pens compared to site 1.

4.3.2 Intra-site analysis – Site 1

The regression of log mean on log variance revealed a very good fit ($F_{1,8}$ =280.58, R^2 = 0.972, p = < 0.001) with the estimated slope, b = 1.79 (± 0.035 SE). Both the VMR (x^2_4 = 390.59, p = < 0.001) and the I_D (x^2_4 = 11357.90, p = < 0.001) differed significantly from the estimated Poisson distribution. The mean number of eggs per gram of soil differed significantly between release pens for quadrants with ($F_{4,70}$ = 56.461, p = < 0.001) and without a feeder ($F_{4,70}$ = 4.518, p = 0.003) at site 1. Mean egg abundance was significantly higher in quadrants that had a feeder (Mean = 87.1 ± 126.76 epg) when compared with empty quadrants (Mean = 7.64 ± 7.54 epg) (t^{148} = 12.761, p = < 0.001).

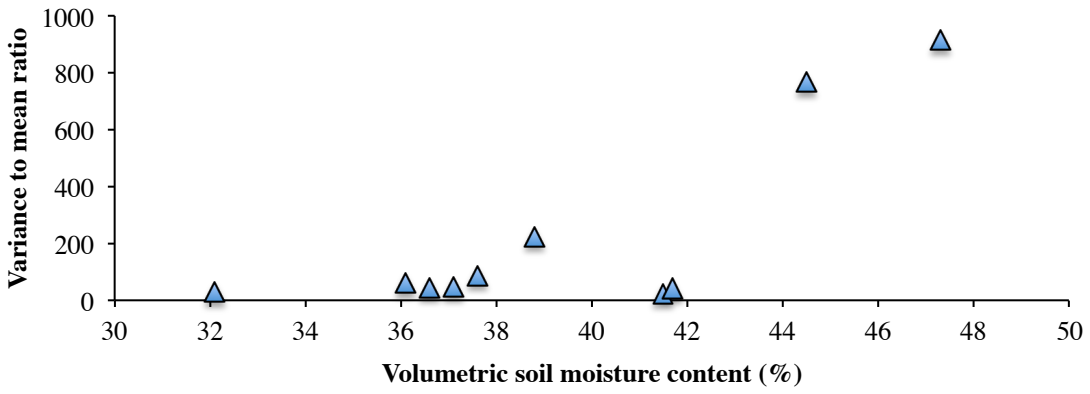

Figure 6 The relationship between average volumetric soil moisture content and the variance to mean ratio for all pens at both study sites. n = 10 pens, n = 30 samples per pen.

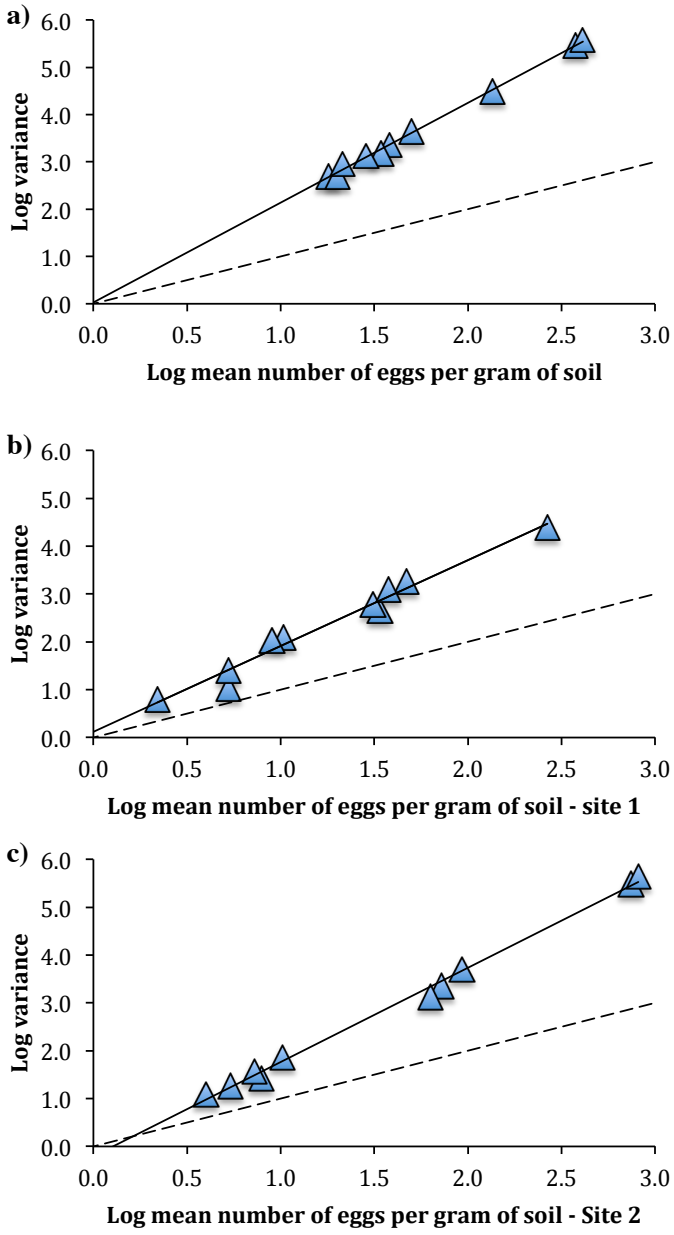

Figure 7 Relationship between log mean and log variance abundance of eggs per gram of soil. a) All pens across both sites, b = 2.11 ± 0.037, b) Site 1, b = 1.79 ± 0.035, c) Site 2, b = 1.97 ± 0.048. Dashed line represents a Poisson model where the log variance is equal to the log mean (log μ = log σ^2).

4.3.3 Intra-site analysis – Site 2

Again, both the VMR (x^2_4 = 1967.90, p = < 0.001) and the I_D differed significantly from the Poisson distribution (x^2_4 = 57067.45, p = < 0.001). The regression of log mean against log variance again revealed a very good fit ($F_{1,8}$ = 1694, R^2 = 0.995, p = < 0.001) with the estimated slope of b = 1.97 (± 0.048 SE). Similarly to site 1, significant differences were found between mean egg abundance between release pens for quadrants with ($F_{4,70}$ = 52.660, p = < 0.001) and without ($F_{4,70}$ = 5.058, p = 0.001) a feeder at site 2, which again can be accounted for by variation in volumetric moisture content across pens (R = 0.978, p = 0.004) (Gethings *et al.,* 2015a). Similarly, mean egg abundance was significantly higher in quadrants that had a feeder (Mean = 298.80 ± 416.46 epg) when compared with empty quadrants (Mean = 10.13 ± 9.39 epg) within each pen at site 2 (t^{148} = 17.370, p = < 0.001).

4.3.4 Egg deposition

The abundance of eggs within pens at both site 1 (t^4 = -3.298, p = 0.030) and site 2 (t^4 = -2.968, p = 0.041) was significantly higher after pheasant placement with more eggs being deposited in pens with higher average volumetric moisture content ($F_{1,8}$ = 11.154, R^2 = 0.582, p = 0.010).

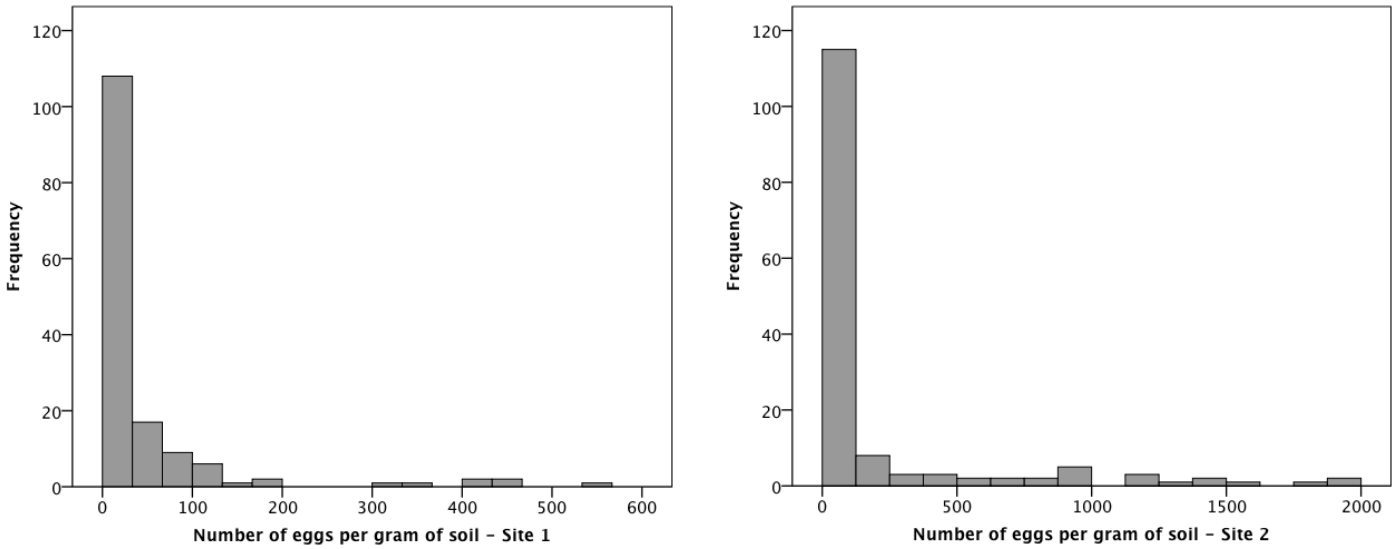

Figure 8 Frequency distribution of *Syngamus trachea* eggs across all pens for site 1 and 2.

4.3.5 Soil moisture, pen size, stocking density and aggregation

The fitting of a quadratic regression model between volumetric soil moisture content and the VMR explained 82.2% of the variation in spatial aggregation between release pens ($F_{2,7}$ =

16.135, R = 0.907, p = 0.002) (Figure 6). Similarly, pen size was significantly positively correlated with the VMR (n = 10, R = 0.834, p = 0.03), suggesting higher levels of aggregation in larger pens. Stocking density (birds/m^2) was significantly correlated with levels on environmental aggregation at site 2 (n = 5, R = -0.885, p = 0.045) and there was a trend for a relationship at site 1, however was not considered statistically significant (n = 5, R = 0.581, p = 0.304).

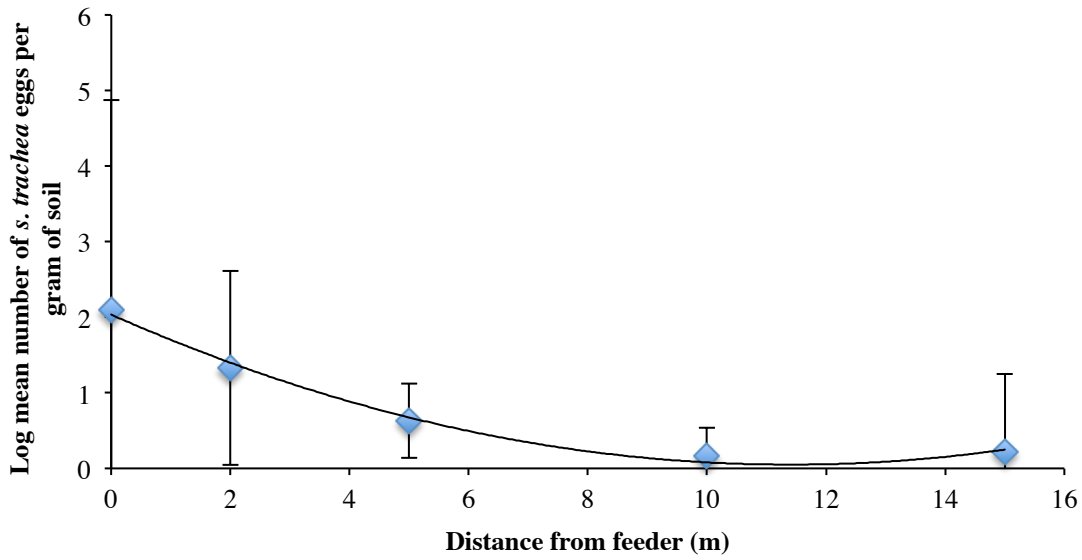

Figure 9 Distance from the feeder and the mean log-transformed abundance of *S. trachea* eggs for all pens at site 1.

4.3.6 Worm distribution within host species

The abundance and distribution of adult *S. trachea* worms within hosts were only obtained from site 1 due to varying management factors. The frequency distribution of the number of pairs of worms per bird differed significantly from the estimated Poisson distribution (x^2_5 = 16.240, p = 0.006). The levels of aggregation within culled crows were significantly aggregated (males (n = 19) - k = 0.91, females (n = 22) – k = 1.53) with the VMR differing significantly from a Poisson distribution (x^2_1 = 106.512, p = < 0.001). Female crows had on average 16.05 (variance = 178.62) worms per bird whereas males had an average of 2.68 (variance = 10.01) worms per bird, with an estimated slope (b) of the regression of log mean on log variance of 1.603 (±0.035).

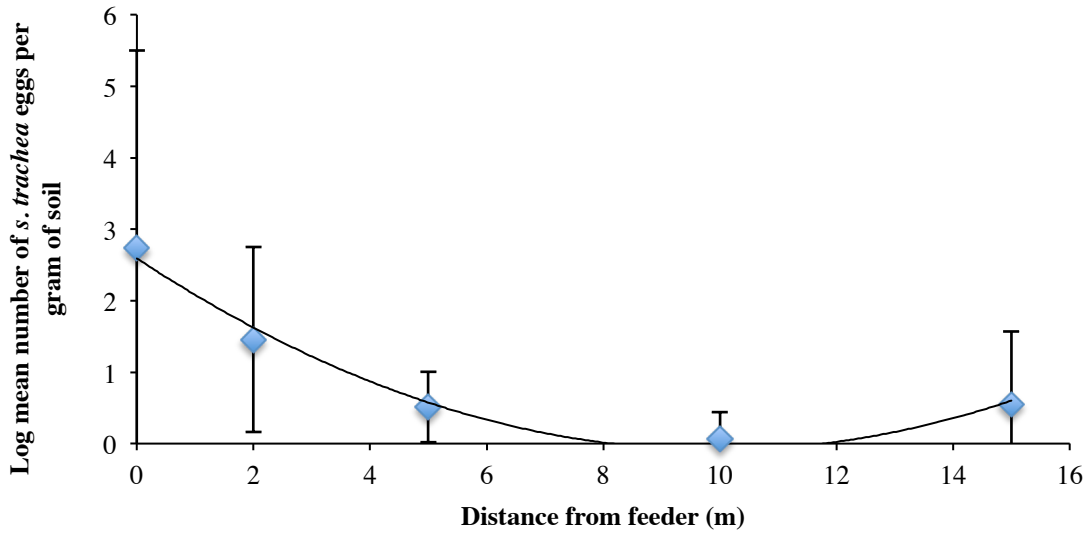

Figure 10 Distance from the feeder and the mean log-transformed abundance of *S. trachea* eggs for all pens at site 2.

4.3.7 Distance from feeder

The relationship between the distance from the feeder in metres and the abundance of *S. trachea* eggs per gram of soil was not linear, however the fitting of a quadratic model greatly improved model accuracy. The mean number of eggs per gram of soil decreased with increasing distance from the feeder within each pen on both estates (p = < 0.001). Tables 3 and 4 display individual Regression Coefficients and p values for each pen. Eggs were highly concentrated around feed hoppers, with the number of eggs decreasing, on average, by 73.7% (±12.1%) within the first 2 m (range = 58.9 - 91.2%) at site 1 (fig 9) (appendix G). Similarly, the eggs were also aggregated around feeders at site 2 (Fig 10) (appendix H), however the mean percentage loss within the first 2 m was higher at 93% (range = 71.9 – 95.1%).

Table 2 Mean, variance to mean ratio (VMR) and degree of aggregation (*k*) of the number of eggs per gram of soil per pen. *n* = 30 samples per pen (Site represented by number, pens within each site represented by letter). VMR values > 1 indicate aggregated data. A value of *k* < 1 indicates highly aggregated data. (* = *p* < 0.05, ** = *p* < 0.001). Chi square statistics for comparisons of frequencies of the number of eggs per pen compared with an estimated Poisson distribution. Degrees of freedom for Chi square analysis for all pens was 29.

Site and pen	Stocking density (birds/m^2)	Pen size (acres)	Mean number of eggs per gram of soil	VMR	Index of dispersio	Corrected *k*	Chi square
1(a)	0.18	1.972	28.6	47.14	1367.10	0.59	17.771*
1(b)	0.08	3.09	18.0	27.31	792.00	0.65	26.182**
1(c)	0.19	3.26	135.6	223.02	6467.68	0.58	29.897**
1(d)	0.15	4.07	20.1	24.47	709.69	0.82	38.503**
1(e)	0.15	2.96	21.4	42.50	1232.44	0.48	33.112**
2(a)	0.21	2.37	38.3	59.87	1736.23	0.62	15.200*
2(b)	0.18	2.15	34.3	44.04	1277.27	0.76	17.600*
2(c)	0.13	4.16	374.7	766.35	22224.05	0.46	39.706**
2(d)	0.17	2.94	50.1	86.57	2510.63	0.55	14.839*
2(e)	0.14	6.27	412.0	914.90	26532.04	0.42	31.000**

4.4 Discussion

The spatial distribution of eggs of *S. trachea* followed a highly aggregated distribution within the soil within each pen (Fig 8). Generally, the estimated slope (*b*) between log variance and log mean abundance of parasites within their host species is around 1.5 (Taylor *et al.,* 1978). The results of the present study however, suggest a much greater degree of environmental aggregation than one would expect if aggregation were driven by host-mediated heterogeneities in parasite abundance alone. Taylor's Power Law has frequently been applied to parasite models (Taylor *et al.,* 1978; Shaw & Dobson, 1995), however the extremely good fit of the slope (*b)* within these two systems suggests that spatial aggregation is driven by factors above and beyond those in the host, i.e. a combination of environmental and host factors. Gethings *et al.* (2015a) found that the abundance of eggs within release pens is generally higher in pens with higher average volumetric moisture content, and it could be that a combination of feeders and soil moisture are responsible for the high levels of aggregation observed within release pens (Moss *et al.,* 1993). Indeed, soil moisture explained 82.2% of the variation in the variance to mean ratio between release pens, which suggests much greater variance, and therefore a greater degree of aggregation in pens with higher moisture content. However, as this finding was only correlative, further research is required to definitely

determine the effect of environmental moisture on driving *S. trachea* spatial patterns. This is in line with the findings by Gethings *et al.* (2015a), that there is an optimum soil moisture threshold for egg survival. As the average pen moisture content increases, then the availability of dissolved oxygen will decrease meaning eggs will be confined to 'patches' of optimum moisture (Saunders *et al.,* 2000a; Nielsen *et al.,* 2010). Similarly, *Ancylostoma caninium* has a preferred moisture threshold, and it has been demonstrated that the abundance of viable eggs decreases in the presence of excess environmental moisture (Dwight & Bowman, 2013). The role of oxygen availability in the embryonation of eggs of poultry parasites has also been demonstrated in *Heterakis gallinarum* (Saunders *et al.,* 2000a). Saunders *et al.* (2000a) found that oxygen was essential for the development of *H. gallinarum* to the infectious stage, however further work is required to clarify that within these systems.

To the best of our knowledge, this is the first study to explicitly demonstrate the high spatial aggregation of eggs around feeding sites. A comparative examination of egg abundance between quadrants with and without a feeder, revealed that eggs of all parasites are heavily concentrated around feeding stations in all pens on both estates. Similarly, Hunter and Quenouille (1954) identified a greater degree of aggregation of parasites in animals that are constrained by feeding opportunities. They found that the distribution of infectious stages of parasites of ruminants was considerably more aggregated for populations of sheep grazing on heather hills compared with sheep grazing on pasture. Presumably because sheep grazing on pasture would be exposed to a more uniform infection pressure than those grazing in limited patches (Hunter & Quenouille, 1954; Donald, 1968). Although differing in magnitude, the abundance of eggs per gram of soil decreased with increasing distance from feeding stations within all pens on both estates (Figures 9 and 10, Table 2). On average, there was a 73.7% (±12.1%) reduction in the number of *S. trachea* eggs within the first 2 metres from the feed station at site 1, with a 91.2% reduction seen in one instance. In comparison, the mean reduction in the number of eggs per gram of soil was 93% at site 2, with a 95.1% reduction seen in one pen.

Table 3 Relationship between the distance from the feeder and the abundance of *Syngamus trachea* eggs per gram of soil at site 1.

Pen	*F*	*df*	R^2	*p*
1	66.658	*2,17*	0.887	< 0.001
2	51.603	*2,17*	0.859	< 0.001
3	85.705	*2,17*	0.91	< 0.001
4	40.888	*2,17*	0.828	< 0.001
5	42.657	*2,17*	0.834	< 0.001

As parasite populations are spatially aggregated with many hosts/plots having few parasites and relatively few hosts/plots having many parasites, parasite-induced morbidity and mortality is greater for hosts in the 'tail' of the parasite distribution. The stability of this host-parasite relationship is heavily determined by the extent of the spatial aggregation of infectious stages, and it is generally agreed that the greater the degree of aggregation, the more stable the relationship between a parasite and its host, especially when host mortality is a function of parasite burden (Elston *et al.,* 2001; Brockhurst *et al.,* 2006; Real & Biek, 2007). Generally, heterogeneity in parasite load is determined by host exposure rates and intrinsic biological factors, i.e. age, immune status, sex, body condition and this individual level of variation is a key driver of environmental aggregation (Brunner & Ostfeld, 2008; Johnson & Hoverman, 2014). Seeing as the distribution is highly skewed within this system, with the tail of the distribution, i.e very high egg counts, occurring around feeding stations, could account for the high morbidity and mortality rates among pen-reared birds, however no data are currently available for this system. Further, when parasite populations are heavily spatially aggregated, hosts have rarer but more severe infections when they encounter infected 'clumps' (Cornell *et al.,* 2004; Brockhurst *et al.,* 2006; Real & Biek, 2007). Indeed, the VMR was highly correlated with pen size, suggesting a greater variance, and therefore a higher level of environmental aggregation in larger pens. Stocking density (birds/m^2), was a significant factor in determining the levels of aggregation at site 2, with a greater stocking density leading to a reduced VMR, indicating a more 'even' distribution of eggs at greater densities with eggs less confined to discrete clumps. There was a trend for a similar relationship at site 1, however due to differences in the number of birds released compared with site 2, i.e. the relatively low numbers of birds released and the fact that a larger pen did not necessarily mean a higher number of birds at site 1, was not considered statistically significant.

Table 4 Relationship between the distance from the feeder and the abundance of *Syngamus trachea* eggs per gram of soil at site 2.

Pen	*F*	*df*	R^2	*p*
1	95.778	*2,17*	0.918	< 0.001
2	61.368	*2,17*	0.878	< 0.001
3	63.811	*2,17*	0.882	< 0.001
4	68.08	*2,17*	0.889	< 0.001
5	60.814	*2,17*	0.877	< 0.001

Despite the high levels of environmental aggregation, the distribution of worms within their host species was in line with other studies examining the link between environmental factors and host aggregation (Taylor *et al.,* 1978; Shaw & Dobson, 1995). Although the placement of feeders and environmental moisture could be contributing to parasite aggregation, density-dependent processes seem to be ensuring the population does not become too over or under-dispersed, in order to maintain the transmission-virulence equilibrium. This could potentially explain the seemingly low burdens of *S. trachea* observed in most bird species, generally around 0 – 39 for crows (Loman, 1980; Gethings *et al.,* 2015a,b) and 0 – 31 for pheasants (Gethings *et al.,* unpublished data), even when administered a single large dose of ~3000 infective larvae (Devada and Sathianesan, 1988). Although burdens of up to 90 worms have been observed by Clapham (1934), this was a result of experimental infection. In comparison, it is not uncommon to see burdens of ~8000 for the caecal threadworm, *Trichostrongylus tenuis,* with low to moderate burdens of 1000 worms per host (Watson *et al.,* 1988; Moss *et al.,* 1993). As parasites become more pathogenic, mean worm burdens tend to decrease, especially when compared with parasites of lesser pathogenic importance (Shaw & Dobson, 1995). Further, density-dependent regulatory mechanisms are likely to be of greater importance in parasites that are large relative to their host (Burn, 1980; Luong *et al.,* 2011). This density-dependent regulation ensures that hosts in the 'tail' of the distribution do not die from increased parasite-induced mortality (Shaw & Dobson, 1995). Indeed, Churcher *et al.* (2005) found that the effect of density-dependence on regulating transmission dynamics is greater for parasites with greater levels of environmental and host aggregation, whilst Tompkins and Hudson (1999) found that density-dependent regulation of fecundity in *H. gallinarum* worms was maintaining population dynamics. Whether this regulation is a result of intraspecific parasite competition for predilection sites, or heterogeneities in host immuno-competence is currently unknown.

Interestingly, the degree of aggregation, i.e. the slope of the coefficient between log mean and log variance, was lower at site 1 than at site 2, indicating a lesser degree of spatial aggregation. This could be due to differing management practices, i.e. the occasional movement of feeders; so eggs and larvae would be distributed over a greater area, albeit in lower numbers, or the use of feeders that limit the amount of faecal-grain contamination at site 1, whereas the feeders at site 2 were frequently left in place for ease of access. The re-siting of feeders could, in theory, decrease overall infection pressure at hotspots, i.e. areas of high contamination, for transmission. Although the overall level of contamination will be greater, the reduced number of eggs could result in less-severe infections, thus potentially allowing birds to develop immunity whilst avoiding heavy parasite challenge.

Although 10 pens were used (*n* = 5 per estate), the fact that only 2 estates were utilised makes it difficult to draw conclusions about management factors at this stage. It would also be of epidemiological importance to assess any temporal changes in parasite aggregation, and how this relates to observed disease patterns. As there was no relationship between pen age and parasite aggregation, it is unlikely that aggregation will change dramatically within and between years.

4.5 Conclusion

The abundance of the eggs of *S. trachea* were highly aggregated within release pens, with very high levels of contamination apparently driven by a combination of feeder placement, available soil moisture and host-mediated heterogeneities in immuno-competence. The extremely good fit of log mean on log variance of the number of eggs per gram of soil suggests that host-mediated heterogeneities and environmental factors, i.e. feeder placement and soil moisture, are driving aggregation within this system. This could, in theory, have implications for disease management and could potentially account for the high virulence associated with syngamosis in game birds, however the present paper raises questions as to why such a virulent parasite, with increased opportunities for transmission only accounts for a mortality rate of around 10-20% (Andreopoulou *et al.,* 2011). It appears that faecal contamination of feed is the primary method of parasite transmission within this system, as a very high proportion of eggs were 'clumped' around feeding sites. The subsequent feeding and defecating within these discrete areas will inevitably influence disease dynamics, by the increase in contact time between parasite and host. The prophylactic use of anthelmintics on the two study sites appears to be effectively controlling disease incidence and severity for the time being. However, the more or less permanent provision of anthelmintic-treated grain in lieu of adequate environmental sanitation/removal of faeces or feeders that limit the amount of faecal-grain contamination could have serious implications for disease management in the coming years. The results of the present study also present evidence that density-dependent processes appear to be maintaining the equilibrium between virulence and transmission success despite the increased opportunities for transmission. However, whether this is due to host-mediated heterogeneities in immuno-competance, intraspecific parasite competition (for space or nutrients) or indeed a combination of both factors, is currently unknown and is a fascinating area of on-going research.

Chapter 5. Density dependent regulation of fecundity in *Syngamus trachea* infrapopulations in semi-naturally occurring ring-necked pheasants (*Phasianus colchicus*) and wild Carrion Crows (*Corvus corone*). [3]

5.1 Introduction

One recurring theme within parasite ecology is the relative stability of parasite populations in domestic and wild animal hosts (Anderson & May, 1978; Tompkins & Hudson, 1999), which suggests that some form of regulatory mechanism must be ensuring population stability. The majority of these mechanisms are driven by parasite density, i.e. are a function of parasite burden within individual hosts; thus acting on infra-populations as opposed to populations as a whole. Indeed, density-dependent regulatory mechanisms act on many aspects of the parasite lifecycle, such as parasite establishment, growth, fecundity, development and maturation times, and adult survival (Walker *et al.,* 2009). Growth and fecundity for instance, being the two most common aspects of the life cycle regulated by such mechanisms in helminth populations (Tompkins & Hudson, 1999), are particularly important at regulating the abundance of the 'free-living', infectious stages within the environment, and therefore determining the extent of future infections. These density-dependent mechanisms are important for regulating and stabilising transmission dynamics, and therefore the parasite-host relationship.

Despite knowledge of the existence of such regulation, the mechanisms underlying density-dependence are poorly understood, as it is difficult to disentangle host and parasite responses to increasing parasite challenge (Paterson & Viney, 2002). Host immune responses have been demonstrated to reduce establishment, survivability and fecundity of parasitic nematodes, and it is hypothesised that innate and adaptive immune responses, whose response to infection increases with increasing parasite density, are responsible for the manifestation of density dependence (Paterson & Viney, 2002). Similarly, intraspecific competition for space and resources once inside the host has also been implicated as a driver of density-dependent regulation. Indeed, Michael and Dunby (1989), hypothesised that parasite-mediated competition was responsible for *Trichuris muris* establishment in the mouse, owing to the finite carrying-capacity of the caecum.

[3] Published in *Parasitology*, 143 (2016), 716-722.

Syngamus trachea is a parasitic nematode occurring in a wide range of avian hosts (Lewis, 1928; Morgan & Clapham, 1934; Goble & Kutz, 1945). The non-specific nature of this parasite makes it possible to study differences in host-mediated responses to a natural challenge of *S. trachea.* In a previous paper by Gethings *et al.* (2015b), we highlighted the fact that despite increased opportunities for transmission of *S. trachea* within pheasant release pens, relatively low numbers of adult worms are consistently recovered upon post-mortem investigation. This is often the case in experimental infections using large numbers of infective larvae (Olivier, 1944; Guildford & Herrick, 1954). Despite these previous studies finding relationships between *S. trachea* establishment and host immunity, no such work has been conducted in semi-naturally occurring pheasant populations using natural infections of *S. trachea.* The aims of the present study were to determine firstly, whether worm length is a good indicator of fecundity within *S. trachea* populations, and secondly, to determine whether fecundity is impaired in response to increasing worm burden in two host species.

5.2 Materials and Method

5.2.1 Study Sites

The two study sites were selected due to their large size and annual occurrence of clinical syngamosis. Site 1 was located approximately at grid reference ST 97502 39837 and consisted of seven release pens. Site 2 was situated approximately at grid reference SU 17769 30326 and similarly consisted of seven release pens. Site 2 undertook rigorous corvid control as part of a game management program with the use of Larsen traps, whereas site 1 used traps sporadically.

5.2.2 Carcass recovery

Male and female ring-necked pheasants (*Phasianus colchicus*) were recovered from two pheasant estates in the South West of England from January 2015 to November 2015. All birds were either obtained during the shooting season or were found dead on the estates at various times of the year. Crows (*Corvus carone*) were opportunistically sampled throughout the season, as the sites were undertaking Corvid control via the use of Larsen traps. Crows and Rooks are known to be commonly infected with *S. trachea,* and any density-dependent effects would likely be more apparent as worm burdens tend to be larger than in pheasants. Crows were primarily recovered from site 2, as their corvid control program was more consistent. Age was roughly estimated by presence/absence and size of the bursa of fabricius,

which has usually atrophied by 6 months (Williams & Newton, 1969); however no formal analysis of the effects of age on either parasite burdens or length was undertaken during this study.

5.2.3 Adult worm recovery

Adult *Syngamus trachea* worms were recovered from the trachea of pheasants and crows. Adult worms are sexually dioecious, and show marked sexual dimorphism from 7-9 days post-infection (PI) with female worms being considerably larger than males; female length = ~> 13mm, male length = > 4mm at 14 days PI (Fernando *et al.,* 1971). The trachea was first resected from the underlying connective tissue and transected slightly above the proximal bifurcation of the bronchi. The trachea was then incised longitudinally through the tracheal cartilage and the worms recovered using fine-tipped forceps. Adult worms were distinguished from juvenile (L4) worms by observation under a microscope at varying magnifications in order to detect the presence of fertilised ova. Adult worms from both species were assessed according to Lewis (1928), which confirmed that these worms were indeed *S. trachea* and that the worms were identical between species justifying the between-species comparisons.

5.2.4 Worm length and fecundity

Fernando *et al.* (1971) conducted in-depth pathogenetic examinations detailing adult worm length at various stages of development, and determined the number of days post-infection (PI) to the production of fertilised ova. Female *S. trachea* worms are fertile by day 14 PI, with minimum female length at the adult stage generally averaging 10-15mm. Once fertile, Guildford and Herrick (1954) found no relationship between days PI and female worm length, so we concluded that the number of days PI was not a significant confounding factor within this study. As several authors have demonstrated that worm length is significantly correlated with worm fecundity (Michael & Dunby, 1989; Stear *et al.,* 1997; Stear & Bishop, 1999; Tompkins & Hudson, 1999; Walker *et al.,* 2009), the same principle was applied in this study. One hundred and fifty seven female worms were recovered using systematic sampling from ten randomly selected pheasants and crows in order to estimate the effect of length on the number of eggs per worm. Although adult worms were recovered from 130 birds, *in-utero* eggs were counted in female worms recovered from every seventh (130/20 = 6.5 rounded to 7) bird, providing it was infected, until 10 was reached for each species. Female worms were measured using a digital calliper (accuracy to 0.01mm) and the number of eggs were counted using a stereomicroscope. Genital tubes were liberated from female worms and each egg was counted *in-situ.* In order to ensure egg viability, eggs were recovered from each worm and

maintained in the laboratory at 24° C (Wehr, 1937). Eggs were cultured to the infective stage (L3) and manually hatched by applying light pressure between two cover slips. Male worms were not measured during this study.

5.2.5 Condition of the trachea

It has been demonstrated that prolonged infections with *S. trachea* result in the formation of hyperplastic tracheal cartilage in which the adult male worms are deeply embedded (Clapham, 1935). These nodules begin to form between 26 and 37 days PI and generally remain indefinitely; meaning previous exposure and duration of current infection can be determined. These nodules form at the point of attachment, so if no worms are found within these nodules, it can be concluded that the bird has been exposed to *S. trachea* previously, thus conferring some degree of immunity. To assess whether previous exposure influenced mean worm length or mean worm burden in subsequent infections, pheasant tracheas were examined for the present of nodules. These nodules do not form at the point of attachment in corvids so previous exposure cannot be determined. Therefore, crows were excluded from this part of analysis.

5.2.6 *Retrospective data analysis*

Guildford and Herrick (1954) experimentally infected pheasants with *S. trachea* larvae and counted the number of worms upon post-mortem examination, measured their length and noted the presence/absence of nodules. These data (n = 36) were combined with data recovered in this study (n = 21) to evaluate whether physiological evidence of previous exposure influenced parasite intensity and/or parasite length.

5.2.7 Statistical analysis

All data were analysed using the R statistical package for Macintosh. Differences in the mean number of worms and mean worm length between species were assessed using Welch's *t*-test for unequal samples. Unless stated otherwise, all regression analyses were conducted using generalised linear models with negative binomial error distributions and log link functions (*glm.nb* available in the MASS package). Data were assessed for negative-binomial model fit by comparing with models with Poisson error distributions by maximum likelihood using the *pchisq* function in R. Significance levels were calculated using x^2 tests from the deviance explained by each factor and pseudo R^2 values were calculated for each model (1 – residual

deviance/null deviance). The effect of parasite intensity on mean parasite length was analysed using log transformed mean counts for parasite intensity in the 37 pheasants and 92 crows sampled (Tompkins & Hudson, 1999; Ives, 2015). Non-constant error variance was assessed using the Breusch-Pagan test (B-P test) and "length" data were transformed to the appropriate power transformation (*y^-0.02)*. The B-P test was then conducted on the transformed data, which confirmed constant variance (x^2 = 0.30, df = 1, p = 0. 57). In order to ensure that worm length was a reliable indicator of fecundity, the effect of parasite length on the number of eggs per adult female was assessed using raw count data of 157 adult female worms recovered from 10 pheasants and 10 crows. In order to determine the minimum parasite density at which negative effects are observable, iterative backwards-stepwise deletion of the highest parasite densities was conducted until the regression was no longer significant at the P = < 0.05 level.

5.3 Results

The trachea of 37 pheasants (appendix K) and 92 crows (appendix L) were recovered and examined for the presence of adult *S. trachea* worms, of which 1307 pairs were recovered.

5.3.1 Parasite fecundity

Model comparisons suggested the negative binomial model best fit the data (x^2 = 3281.27, D.F. = 1, p = < 0.001) so data were analysed with negative binomial error distributions. *In-utero* egg counts were performed on 157 pooled adult female worms recovered from 10 crows (113 worms) and 10 pheasants (44 worms), with an average (±SEM) of 1066 (± 41.5) eggs per worm. Worm length (coef = 0.06, deviance = 590.47, D.F = 1, p = < 0.001), after accounting for within-host variation in worm burden (coef = -0.05, deviance = 7.77, D.F = 1, p = 0.006), was significantly correlated with the number of eggs per female worm; explaining 79% of the deviance (residual deviance = 157.05, D.F = 154, p = < 0.001) (fig 11)

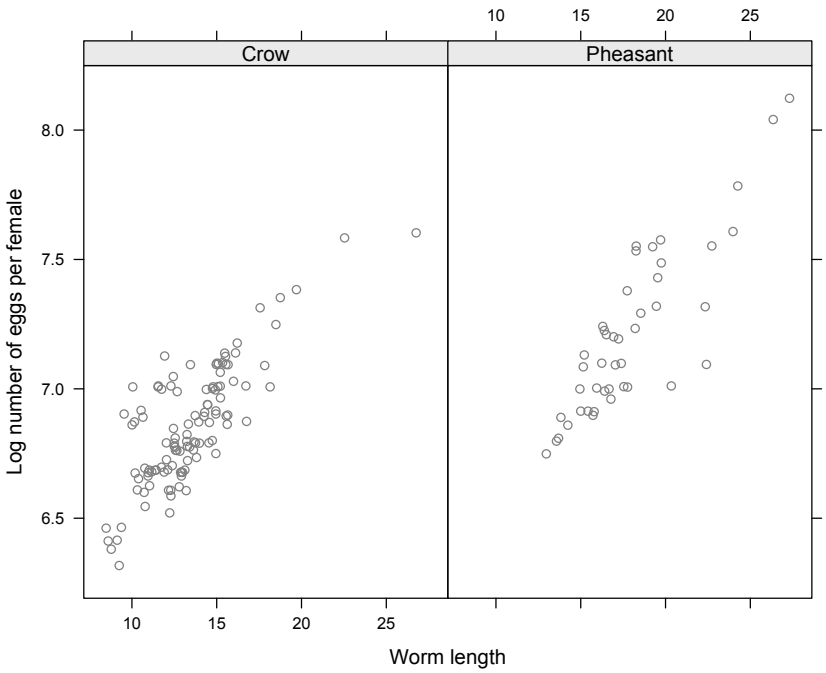

Figure 11 Relationship between worm length and the number of eggs per worm (Fecundity) for both species.

5.3.2 Worm length and parasite intensity

A total of 279 and 1028 adult *S. trachea* pairs were recovered from the trachea of 37 pheasants and 92 crows respectively. Mean worm length was significantly correlated with mean parasite density for pheasants and crows with a significant reduction in mean worm length at higher parasite densities ($F_{1,127}$ = 393.3, R^2 = 0.759, p = < 0.001) (Fig.12). For individual species, there was a stronger effect of mean worm burden on mean worm length for crows ($F_{1,90}$ = 340.2, R^2 = 0.79, p = < 0.001) than for pheasants ($F_{1,35}$ = 64.21, R^2 = 0.64, p = < 0.001). Stepwise data-point deletion of the highest parasite densities revealed that density-dependent effects begin to manifest above 4 worms per bird for pheasants, and 2 worms per bird for crows, with the regression model not reaching the significance level of p < 0.05 below these thresholds

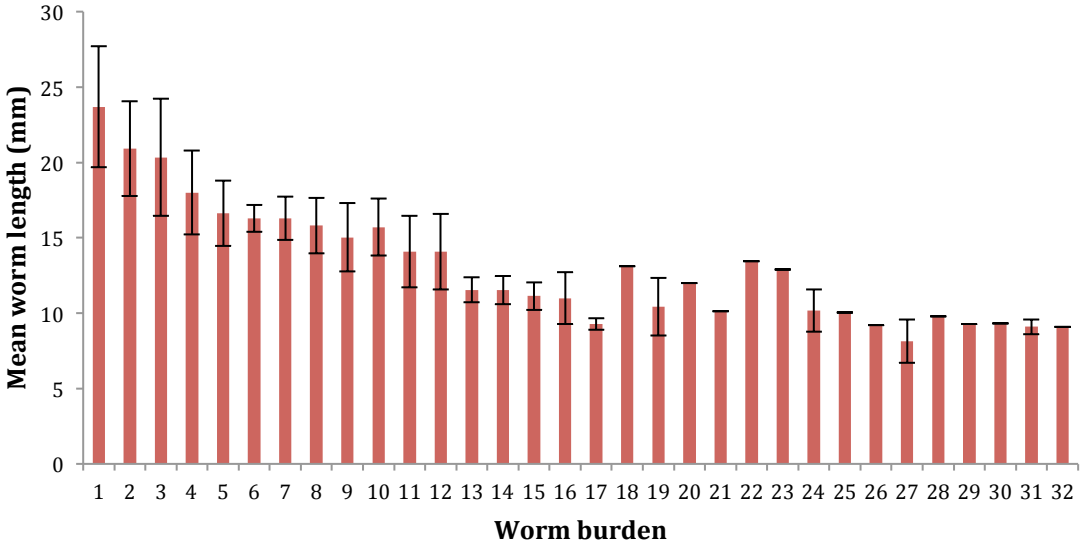

Figure 12 Mean worm length (mm) for varying parasite intensities. (*n* = 1307 worms across 129 hosts).

5.3.3 Presence of nodules, mean worm length and number of adult worms

Crows were excluded from this part of analysis so results are not reported. Retrospective analysis of the Guildford and Herrick (1954) data, and trachea condition in the present study revealed that pheasants with hyperplastic tracheal nodules had fewer adult worms present in the trachea (Mean ± SEM = 5.26 ± 1.01 worms per bird) than birds without nodules (Mean ± SEM = 11.12 ± 1.68 worms per bird) (n = 51, t_{46}= 2.97, p = 0.004). Female worms in birds with evidence of previous exposure were also longer (Mean ± SEM = 16.5 mm ± 1.51) when compared with worms in birds that had no evidence of previous exposure (Mean ± SEM = 13.01 mm ± 0.53) (n = 51, $t_{21.3}$= -2.10, p = 0.04) (Fig.13).

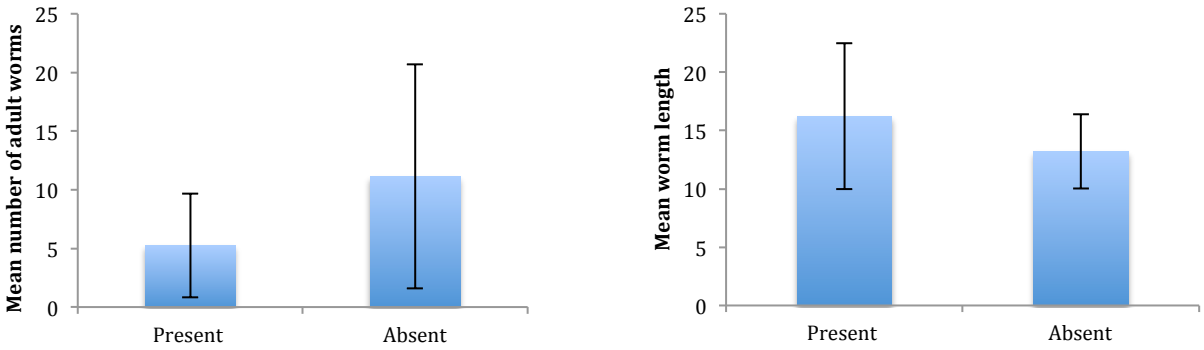

Figure 13 Relationship between the presence of nodules and mean worm burden and mean worm length in Pheasants.

5.3.4 Mean worm length and mean worm burden between species

The mean number of adult worms per trachea differed significantly between species, with crows having a mean worm burden of 11.17 (± SEM = 0.10) and pheasants having an average of 7.72 (± SEM = 1.39) worms per trachea ($t^{72.14}$ = 2.02, p = 0.04). Similarly, mean worm length differed significantly between species, with pheasants having a mean worm length of 17.97 mm (± SEM = 0.85) and crows having a mean worm length of 15.55 mm (± SEM = 0.55) ($t^{66.58}$ = 2.34, p = 0.02).

5.4 Discussion

Density-dependent reductions in worm size and fecundity have been reported in a large number of studies (Michael & Dunby, 1989; Stear *et al.,* 1997; Stear & Bishop, 1999; Tompkins & Hudson, 1999; Walker *et al.,* 2009), and density-dependent reductions in worm length, but not necessarily fecundity, are reported in the vast majority of nematode species studied (Mossinger & Wenk, 1986; Szalai & Dick, 1989; Sinniah & Subramaniam, 1991; Skorping *et al.*, 1991; Marcogliese, 1997; Dezfuli *et al.,* 2002; Irvine *et al*. 2001; Richards & Lewis, 2001). A vast majority of studies concerning density dependence have been laboratory-based experimental infections, which do not accurately represent conditions facing free-living wild animal populations in terms of parasite load and encounter rates. The present study provides reliable information concerning apparent density-dependent regulation of fecundity in both an intensively-managed pheasant population, and a free-living wild population of corvids. Although the fact that immune status is responsible for regulating the establishment of *S. trachea* in ring-necked pheasants is not novel, this is first mention of both parasite and host-mediated factors regulating *S. trachea* populations in any bird species.

In agreement with previous studies (Olivier, 1944; Guildford and Herrick, 1954), immune function appears to be predominantly responsible for the establishment of *S. trachea* within the ring-necked pheasant, however other factors such as exposure rates and larval viability were not taken into account in this study. This is demonstrated by a reduction in parasite abundance in birds that had evidence of previous exposure. There was however, a greater number of adult worms in crows, which suggests that worm establishment is not constrained by size or length of the trachea, and therefore overall host size, however is perhaps a function of host immunity**.** Indeed, Olivier (1944), found that *S. trachea* establishment was dose dependent. He found that the number of worms establishing was inversely proportional

to the size of the infective dose, and attributed this to the strength of the immune response (Olivier, 1944). This result is in stark contrast to the findings of Michael and Dunby (1989), who found that *Trichuris muris* establishment in the murine host is believed to be regulated by density-dependent infraspecific competition, owing to the finite space in the caecum. It is unlikely however, that *S. trachea* establishment is regulated in a similar manner as more worms have been found in crows with a shorter trachea**.** This apparent immune-mediated inhibition on worm establishment has also been identified for *S. trachea* in chickens, with a lower mean worm burden generally identified in older, previously exposed chickens (Crawford, 1940). If establishment were merely a result of parasite-mediated competition, worm establishment, and therefore burden, would be similar in both immunological naïve and previously exposed birds (Luong *et al.,* 2011).

The fact that crows had significantly higher worm burdens when compared with pheasants is interesting. Pheasants are known to develop moderate immunity to *S. trachea* (Olivier, 1944)*,* however, no such work has been conducted in wild crow populations. Being a known reservoir for *S. trachea,* it may be that crows have a higher parasite threshold for the stimulation of an immune response or they do not develop significant immunity to subsequent infections. Indeed, pheasants appear to be more susceptible to infection early on in the rearing process, whereas *S. trachea* adults have been recovered from crows of varying ages (Personal unpublished data). Further work is however, required in order to determine whether wild crows develop any immunity to *S. trachea*.

Although density-dependent reduction of worm fecundity was present in both species, the fact that the effect of crowding on mean worm length was more profound within the crow population is interesting. Mean worm burden explained 82% of the variation in mean worm length in crows, compared with 64% in pheasants, with crows having a tendency for a greater mean worm burden when compared with pheasants. Even so, the fact that worms tended to be shorter in crows, in response to higher mean worm burdens, suggests that these effects are indeed density-dependent. The negative association between worm length and worm burden was present in both species, and appears to be a result of parasite-mediated competition, for either space or resources. Indeed, these effects were even observed in pheasants with no history of previous exposure. Similarly, as there was a vast number of birds of different ages, it is unlikely that age-dependent acquired-immunity was responsible for the manifestation of density dependence within these birds, as the effects were identified in juveniles, as well as adult birds, with little to no acquired immunity. Conversely, Paterson and Viney (2002), observed the absence of density-dependent

mechanisms at regulating survivability and fecundity of *Strongyloides ratti* infra-populations in immuno-compromised hosts. These mechanisms were however, present in later primary infections, suggesting that host mediated heterogeneities in immuno-competence are regulating population dynamics before intraspecific competition for space and nutrients ever occurs in experimentally infected rats (Paterson & Viney, 2002). Alternatively, worm length has been shown to be related to levels of local parasite-specific immunoglobulin A (IgA) (Stear *et al.,* 1997). These responses are however, often absent in immunologically-naïve animals, and only generally manifest in animals that have been previously exposed (Craig *et al.,* 2014) so it is unlikely to be occurring within these study populations.

The parasite threshold for the manifestation of density-dependence within this study was low compared with other studies. For instance, the threshold for density-dependent reductions in fecundity in the caecal nematode, *Heterakis gallinarum,* in pheasants is 96 worms (Tomkins & Hudson, 1999). Similarly, this threshold for *Tricostrongylus colubriformis* in sheep is around 3000 worms per host (Dobson *et al.,* 1990). It is generally believed that density-dependent effects are of greater importance for parasites that are large compared with their host (Poulin & Morand, 2000). Indeed, *S. trachea* adults can grow up to ~33 mm in length in an 80-100mm long trachea (Crow). In comparison, mean worm length of *H. gallinarum* adults in the caeca of pheasants is around 9.64 mm (±0.11) (Tompkins & Hudson, 1999), in caeca ranging from 240.11 for male and 213.84 for female pheasants respectively. Similarly, *Pterygodermatites peromysci,* a nematode parasite of mice, is regulated by tight density-dependent restrictions on the number and length of adult worms in the small intestine (Luong *et al.,* 2011). Similarly to *S. trachea, P. peromysci* can grow up to 33 mm in a 250 mm mouse intestine (Luong *et al.,* 2011).

The findings of the present study are in agreement with previous work that pheasants do indeed develop immunity to *S. trachea* (Olivier, 1944; Guildford & Herrick, 1954). However, nematode length and fecundity appear to be a function of parasite density, and therefore parasite-mediated competition and not host-mediated heterogeneities in immunocompetence.

Chapter 6. Body condition is negatively associated with infection with *Syngamus trachea* in the Ring-necked Pheasant (*Phasianus colchicus).* [4]

6.1 Introduction

Parasites are well known to play an important role in regulating host population dynamics (Tompkins & Begon, 1999; Granoth-Wilding *et al.,* 2005; Irvine *et al.,* 2006; Dunn *et al.,* 2012; Watson, 2013), although there has been some debate as to the relative importance of predators compared with parasites (Moss & Watson, 2001; Irvine, 2006). Recent research however, has demonstrated that parasites are as, if not more, important than predators in regulating host populations (Watson, 2013). Parasites can have direct impacts on host populations through increases in morbidity and mortality, and they can also indirectly affect host populations through reductions in fecundity (Hudson, 1986; Dunn *et al.,* 2012; Granoth-Wilding *et al.,* 2015). Despite this, very little published research exists on the effects of parasite species on condition and host population dynamics in the ring-necked pheasant. One of the few studies by Draycott *et al.* (2002) assessed the effects of *Syngamus trachea* and *Heterakis gallinarum* on pheasant populations and concluded that infection with these species did not negatively affect host body condition. One major issue with this study is that body condition was assessed in April, whereas the infectious stages of *S. trachea,* and therefore infection pressure do not reach their peak until around June/July (Gethings *et al.,* 2015a). A similar issue was observed by Irvine *et al.* (2006). Previous studies failed to find any effect of gastrointestinal nematodes on host condition in Reindeer populations in the High Arctic, however Irvine *et al.* (2006), through the use of delayed-release anthelmintic boluses, demonstrated reductions in host fitness in winter. Reindeer populations were sampled previously when populations were more accessible, which highlights the importance of timing research protocols to account for seasonal dynamics in the transmission of parasites, and therefore any parasite-mediated effects.

Reproductive success of released ring-necked pheasants is generally poor compared with their 'wild' counterparts (Leif, 1994), but it is currently unclear as to why this is the case (Leif, 1994; Draycott *et al.,* 2000; Millan *et al.,* 2002; Draycott *et al.,* 2006; Villanua *et al.,* 2006). A number of factors such as increased parasitic worm infections and reduced food availability/quality are believed to be major components governing life-history traits in game birds (Hudson *et al.,* 1992b). *Syngamus trachea,* for example, is a parasitic-

[4] Published in *Veterinary Parasitology,* 228, (2016), 1-5.

tracheal nematode that commonly affects managed pheasant and poultry populations. Morbidity rates are generally very high, particularly when birds are managed under high stocking densities and when proper sanitary measures are not incorporated into management programs (Gethings *et al.,* 2015a,b). There is marked pathology associated with syngamosis (Fernando *et al.,* 1971; Nevarez *et al.,* 2002; Atkinson *et al.,* 2008) and mortality rates of affected birds can be as high as 80 % (Wojcik *et al.,* 1999). Experimental infections with *S. trachea* have demonstrated negative associations between larval challenge and weight gain and condition. Hwang (1964) experimentally infected turkey poults with varying numbers of *S. trachea*-infected earthworms to observe their effect on weight gain and packed cell volume (PCV). Significant differences were identified in weight gain between birds infected with an average of 0.2 worms (n = 10) compared with an average of 55 worms (n = 55), with the heavily infected group gaining an average of 51g compared with 1482g for the lightly infected group (Hwang, 1964). This significant effect on weight gain and condition could potentially influence fecundity directly through parasite-mediated competition for resources or indirectly if the host invests more resources into mounting an immune response (Delahay *et al.,* 1995; Shutler *et al.,* 2012). Indeed, Draycott *et al.* (2006) demonstrated increased breeding success in pheasants treated with Flubendazole, which suggests a possible relationship between fecundity and parasitic infection in the ring-necked pheasant.

Similarly, other studies have demonstrated significant negative parasite-mediated effects on host fitness. Many of these associations are however, correlational, and it is often difficult to disentangle cause and effect in the parasite-host relationship. Tompkins *et al.* (2000), demonstrated a negative relationship between the caecal nematode *H. gallinarum* and body condition in partridges. More relevant perhaps, is the negative association identified between *H. gallinarum* and body mass, breast muscle mass and cloacal fat in ring-necked pheasants (Sage *et al* 2002).

The aim of the present study was to evaluate what effect, if any, significant infections with the nematode, *Syngamus trachea* have on pheasant body condition under natural circumstances.

6.2. Materials and method

6.2.1 Study sites

Two pheasant estates were selected in the South West of England due to regular problems with clinical syngamosis, as reported by managers. Site 1 was located approximately at grid reference ST 97502 39837 and consisted of seven release pens. Site 2 was situated approximately at grid reference SU 17769 30326 and similarly consisted of seven release pens. Both sites release ~15,000 birds annually, undertake thorough predator control measures and provide supplementary grain via feed hoppers. Anthelmintic treatment (Flubendazole – at manufacturer's dosage recommendations) ceased after birds were released in June 2015. Sites were matched in order to ensure that any effects on body condition would be parasite-mediated and not a result of intra/inter specific competition for food resources or other environmental factors.

6.2.2 Carcass recovery

One hundred and eighty adult pheasants were recovered following release from June 2015 through April of 2016. Birds were recovered by professional game managers, either as part of crop-protection programs or were shot during the shooting season. Pheasants were either shot whilst flying, or occasionally found dead upon the estate (n = 4). Carcasses that had been scavenged were not included in the analysis and recovered birds were examined for non-parasite related disease that could influence the results. Carcasses were processed immediately upon recovery and assessed for the presence of *S. trachea, Ascaridia galli* and *H. gallinarum* by dissection of the trachea, gastrointestinal tract and abdominal cavity and caeca respectively. Other nematode species were recorded but were not differentiated by species, as they were too few in number. In two instances, severely emaciated pheasants with bulbous, fluid-filled intestines consistent with clinical hexamitiasis and confirmed by the presence of motile protozoa on wet slide preparation were recovered. These birds were excluded from the analyses as they were found to be free of nematode infection, but are mentioned in the discussion.

6.2.3 Worm recovery and body condition assessment

Adult pheasants were weighed to the nearest 0.1g using a digital weighing scales and tarsal length was measured using a digital calliper with accuracy to 0.01 mm. A body condition index was then obtained by dividing body mass by tarsal length (Yom-Tov, 2001), which controlled for body size.

6.2.4 Statistical analysis

To determine whether the data were aggregated, adult worm counts were compared with an estimated Poisson distribution ($\mu = \sigma^2$) with n−1 d.f. using the chi.sq test function in R available in the MASS package. Data were then compared with both an estimated Poisson and a Negative Binomial distribution using the fitdistr function (fitdistrplus package) and goodness of fit was assessed using Maximum Likelihood Estimation using AIC as a determinant. As the number of factors in each model were equal, the model with the lowest AIC score was considered a better fit. All data were analysed using *R* for Macintosh. The effect of parasite burden on host body condition was assessed using ordinary least-squares regression using log(*n+1)* transformed parasite count data. Though the inclusion of the four dead-found birds could be a potential confounder, there was no change in model accuracy when they were excluded. Non-constant error variance was assessed using the Breusch-Pagan test and 'ratio' data were transformed to the appropriate power transformation (*y*^0.15). The transformed data were then assessed for non-constant error variance, which confirmed that the power transformation was successful (x^2 = 0.54, *df* = 1, *p* = 0.51). Differences in parasite burden between sexes (including zero counts) were assessed using Welch's *t-test* for unequal samples.

6.3. Results

6.3.1 Prevalence of S. trachea – Pheasants

Parasite count data were significantly different from the estimated Poisson distribution (x^2 = 2175, d.f = 153, *p* = <0.001) and comparison of models demonstrated the data were aggregated, and consistent with the negative binomial distribution (x^2 = 4.87, d.f = 3, *p* = 0.18). The overall prevalence of *S. trachea* within this study population was 33%, with 32% of males (*n* = 148, *n* = 48 infected) and 38% of females (*n* = 32, *n* = 12 infected) being infected with at least 1 pair of worms. Males had a mean (± SEM) worm burden of 3.01 ± 0.54, and females had a mean (± SEM) worm burden of 4.78 ± 1.68, however no significant differences were found between sexes in mean worm burden ($t^{35.78}$ = -1.26, *p* = 0.21).

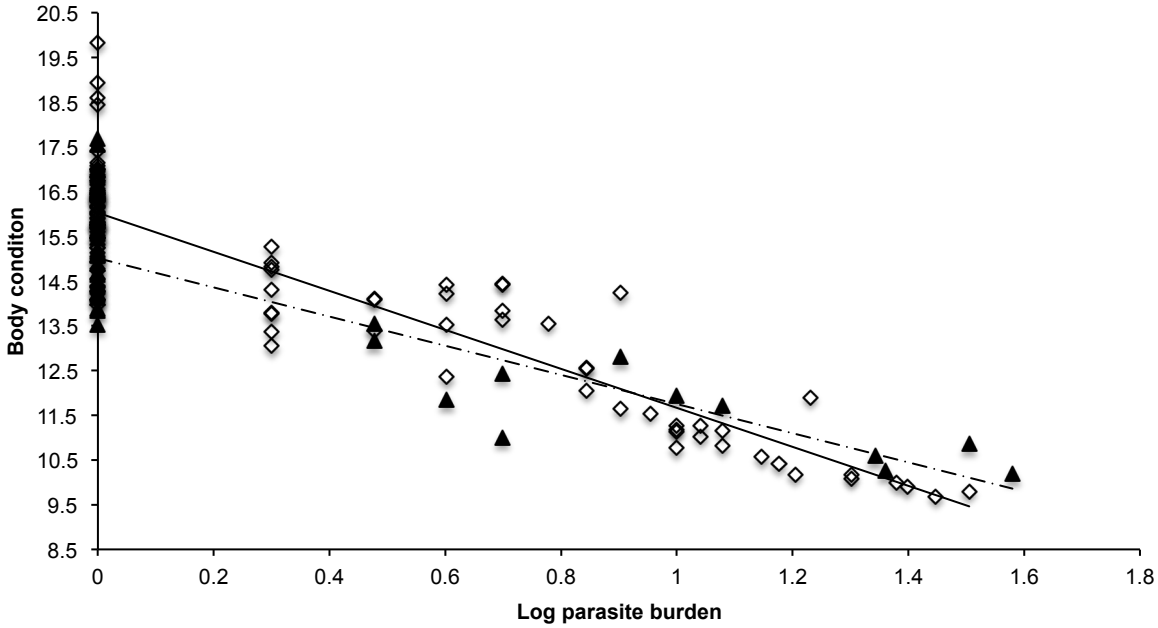

Figure 14 Regression of log parasite burden on body condition for individual sexes. Dashed line, = Females ▲ . Solid line, = Males ◇ .

6.3.2 Effect of S. trachea on body condition

Worm burden and the associated effects on pheasant body condition are presented in tables 5 and 6. The regression of log parasite burden on body condition revealed a significant inverse relationship between increasing worm burden and body condition in pheasants ($F^{3,176}$ = 216.2, R^2 = 0.78, P = < 0.001). Similarly, the effect of 'sex' was significant (coef = 1.1002, t = 2.84, p = 0.005) with regression slopes of b = -4.18 (95% CI = -4.034) and b = -3.23 (95% CI = -2.67) for males and females respectively, with slopes being statistically different from each other (t^{176} = 2.38, p = 0.01). Comparison of regression coefficients for minimum worm burden requirements for identifiable reductions in body condition revealed 1 and 3 pairs were required for males (coef = -1.73, t = -4.69, p = <0.001) and females (coef = -3.24, t =-2.80, p = 0.04) respectively. Stepwise deletion of the lowest parasite burdens suggested a 'flattening off' in body condition reduction above 11 worms per bird.

Table 5 Regression coefficients for the effect of worm burden on body condition in male pheasants. Percentage change is the difference in body condition with zero worms as a comparison. (0 '*' 0.001 '**' 0.01 '*' 0.05).**

Pairs of worms	Difference (-)	Percentage change	*t* value	*P* value
0	15.96		148.592	***
1	-1.74	-10.89	-4.649	***
2	-1.32	-8.24	-2.402	*
3	-2.33	-14.61	-4.259	***
4	-1.86	-11.64	-3.775	***
5	-2.42	-15.18	-2.244	*
6	-3.58	-22.40	-5.681	***
7	-3.03	-18.96	-3.944	***
8	-2.96	-18.57	-3.865	***
9	-4.88	-30.55	-8.899	***
10	-4.82	-30.17	-6.278	***
11	-4.97	-31.15	-6.481	***
13	-5.39	-33.75	-4.989	***
14	-5.54	-34.73	-5.135	***
15	-5.80	-36.33	-5.371	***
16	-4.07	-25.47	-3.766	***
19	-5.84	-36.57	-7.608	***
13	-5.98	-37.46	-5.538	***
24	-6.06	-37.96	-5.613	***
27	-6.28	-39.34	-5.817	***
31	-6.17	-38.67	-5.717	***

6.4. Discussion

Pheasants infected with *Syngamus trachea* demonstrated significantly reduced body condition when compared with uninfected birds, and a negative association was identified between the number of *S. trachea* pairs per bird and body condition. This is the first study to observe such profound reductions in host body condition in pheasants naturally infected with *S. trachea.* Although Hwang *et al.* (1964) found that infection with *S. trachea* negatively affected weight gain in turkey poults, this was a result of an experimental infection, which does not accurately represent conditions faced by wild birds in terms of encounter rates and parasite load. Similarly, the provision of feed *ad libitum* in an experimental setting could increase host fitness and enable the production of a stronger immune response compared with birds under natural conditions.

Table 6 Regression coefficients for the effect of worm burden on body condition in female pheasants. Percentage change is the difference in body condition with zero worms as a comparison. (0 '*' 0.001 '**' 0.01 '*' 0.05)**

Pairs of worms	Difference (-)	Percentage change	*t* value	*P* value
	15.0895			
2	-1.7407	-11.53	-2.077	.
3	-3.2432	-21.49	-2.801	*
4	-3.3739	-22.35	-4.025	***
7	-2.2857	-15.14	-1.974	*
9	-3.1462	-20.85	-2.717	*
11	-3.3748	-22.36	-2.914	**
21	-4.511	-29.89	-3.895	***
22	-4.8288	-32	-4.17	***
31	-4.239	-28.09	-3.66	**
37	-4.9051	-32.50	-4.236	***

The presence of parasites within these study populations, and the apparent parasite-mediated effects on body condition in post-release adult pheasants could have far-reaching ecological consequences (Delahay *et al.,* 1995). It is well documented that reared pheasants have reduced survival and reproductive success compared with their wild counterparts (Leif, 1994; Draycott *et al.,* 2000; Millan *et al.,* 2002; Draycott *et al.,* 2006; Villanua *et al.,* 2006), and the high occurrence of *S. trachea* on pheasant estates could be a significant limiting factor on populations; especially considering that even relatively low numbers of adult worms (well below those at which clinical effects would be observed) are sufficient to produce statistically-significant reductions in host condition. Infection with *S. trachea* could affect host populations directly; though parasite or host-mediated reductions in fecundity via responses to developing and established worms; direct competition for resources; or via (hypothetically) disruption of yolk proteins synthesised in the liver. Similarly, indirect effects such as increased predation rates in infected birds could significantly affect population structure and size (Hudson *et al.,* 1992a). Though the overall prevalence of disease was low, it may still be exerting a negative pressure on population density if infected hosts have reduced life expectancy compared with uninfected hosts (Anderson, 1995). Indeed, *S. trachea* has a high mortality rate among juvenile, immunologically-naïve birds (Wojcik *et al.,* 1999) and an increase in parasite-induced mortality in juvenile birds could stabilise the parasite-host interaction by a net loss of parasites from the system (Anderson & May, 1978). Similarly, as pheasants are extremely susceptible to infection with *S. trachea*, the small proportion of the population susceptible to infection could ensure the persistence of the disease through constant reseeding of infectious stages (Anderson, 1995).

It has been suggested that in order to initiate egg laying, birds must reach a body condition threshold, and that individual host body condition necessarily delays or advances threshold attainment (Drent & Daan, 1980). For example, parasites that undergo hepato-pulmonary migration and/or cause anaemia via exsanguination compete with the host for protein during the time when energy input is concentrated on egg production (Allander & Bennett, 1995). Proteins required for yolk production are synthesised within the liver, and the migration of *S. trachea* larvae across the liver parenchyma could impair the production of these proteins (Allander & Bennett, 1995) thus potentially affecting the onset of laying. Indeed, Jones and Ward (1976) demonstrated that reduced yolk proteins delayed the onset of breeding in Red-Billed Quaeleas. Female pheasants generally lay their eggs between April and June, incidentally when *S. trachea* larval availability is increasing (Gethings *et al.,* 2015a). The development and migration of *S. trachea* across the liver parenchyma could disrupt the formation of vital proteins responsible for chick development directly, or indirectly through competition for host-resources during a period when hen body condition is already reduced (Breitenbach & Mayer, 1959). Indeed, a number of empirical studies have demonstrated increased survival and reproductive success of birds treated with anthelmintics compared with control birds (Hudson, 1986; Draycott *et al.,* 2006). Woodburn *et al.* (2002) demonstrated that birds dosed with anthelmintics reared twice as many chicks as un-dosed controls. It is unknown however whether the anthelmintic had a direct effect on breeding success by reducing parasite challenge, or because the treatment was associated with greater bird survival due to reduced predation (Hudson, *et al.,* 1992a; Millan, *et al.,* 2002; Woodburn *et al.,* 2002). Similarly It has been shown in red grouse populations, that the number of eggs laid is directly related to host body condition and energy intake in the preceding weeks (Delahay *et al.,* 1995). Delahay *et al.* (1995) showed that infection with *Trichostrongylus tenuis* reduced host body condition and could explain poor breeding performance of wild birds. Furthermore, Newborn and Foster (2002) demonstrated that birds with access to grit medicated with Fenbendazole had lower *T. tenuis* burdens and higher body condition scores than control birds. Interestingly, birds from the treated plots had significantly higher breeding success and reared twice as many chicks as birds from control plots. Chick survival was also significantly greater in treated plots compared with control (Newborn & Foster, 2002). This, in conjunction with the findings of the present study appear to suggest that parasite infection does indeed have some measurable effect on host populations, whether that be through parasite-mediated competition, reductions in host fecundity mediated by effects on body condition, or other factors is currently unclear. Although currently only speculative, the tentative link between *S. trachea* infection and pheasant fecundity warrants further consideration. Indeed, Holand *et al.* (2015), found that house sparrows (*Passer domesticus)* infected with *S. trachea* demonstrated reduced

reproductive success compared with uninfected controls. They found a reduction in the proportion of eggs within a nest to hatch as faecal egg counts of mothers increased. Similarly, juvenile females with high faecal egg counts demonstrated significantly reduced lifetime reproductive success compared with uninfected birds.

Visually, infected birds were emaciated with reduced breast muscle mass and prominent keel bones, however no quantitative measurements were taken. Although it is not overly surprising, given the highly pathogenic nature of this parasite, that reductions in host body condition were observed, it is surprising that just one pair of worms was associated with an 11% reduction in body condition compared with uninfected birds. Similarly, these effects were observed in immunologically naïve birds and birds with evidence of previous exposure. The threshold for detectable reductions in host body condition in the present study was particularly low, which could implicate sub-clinical infections as a causal factor of the poor breeding status of released pheasants.

Although there was no detectable difference in mean worm burden between males and females, there was a significant difference in the magnitude of the effect of increasing worm burden. Females, in contrast to males, appear to be able to withstand relatively low worm burdens not suffer any negative effect on body condition below three pairs of worms per host. In contrast, males were often found with single pairs of worms (whereas single infections were not identified in females in this study) and that level of infection already began to affect body condition. The differences between sexes could be explained by differences in resource allocation, and it has been demonstrated that immunocompetence is often sacrificed in favour of the expression of sexual ornaments, particularly in males (Hamilton & Zuk, 1982; Verhulst *et al.,* 1999). Whether females are able to successfully mount an immune response in the presence of one or two pairs of worms requires further investigation. The apparent “flattening off” of parasite-mediated reductions in body condition above eleven worms per bird can perhaps be attributed to the density-dependent reductions in worm length observed at higher densities (Gethings *et al.,* 2016). Density-dependent reductions in worm length peaked at eleven worms per bird before flattening off. This appears to provide evidence of a reduced *per capita* effect above the density threshold.

The findings presented here are in stark contrast to the results of Draycott *et al* (2002), who found that *S. trachea*, along with *H. gallinarum* and *Capillaria* spp. had no real

observable effect on pheasant body condition. This can, however, be explained by the fact that body condition in the Draycott *et al* (2002) paper was assessed in spring, whereas *S. trachea* larval availability, and therefore clinical cases of syngamosis, generally do not reach their peak until June/July (Gethings *et al.,* 2015a). All birds used in the present study were recovered between March and October, when stress levels are likely to be elevated due to release (Villanua *et al.,* 2006), which also coincides with peak larval availability (Gethings *et al.,* 2015a). These conditions are typical of the vast majority of pheasant estates, so results presented here are likely to be comparable with and representative of other intensively reared pheasant populations. Although other parasite species were quantified, there were no similar reductions in pheasant body condition: with the exception of *Hexamitia* spp., *Heterakis gallinarum,* along with a few cestoda, were the only other parasite species recovered from these pheasant populations; however, no effect was observed between their densities and body condition, even when total worm burden included *S. trachea.*

Although it is intuitively likely that the reduction in body condition was a result of significant *S. trachea* infections, it is difficult to disentangle cause and effect. The problem with cross-sectional studies is that it is difficult to establish whether these negative effects were a result of *S. trachea* infection as opposed to other forms of competition, or, whether birds acquired these parasites because they had reduced condition (Irvine, 2006) and less ability to mount an effective immune response. The abundance of supplementary feed, predator control and reduced stocking densities suggests however, that parasites may have been the underlying cause of the observed reductions in body condition. Birds are known to lose a considerable amount of body condition during egg laying and incubation (Breitenbach & Mayer, 1959), however, the reductions in body condition in infected birds were still apparent when compared with uninfected birds, which would likely be facing similar environmental stressors.

6.5. Conclusion

The findings presented here appear to suggest a difference in the magnitude of the effect of worm burden on adult pheasant condition, with females able to withstand higher worm burdens before suffering any negative effects. Similarly, the results of the present study, in conjunction with the findings of Sage *et al.* (2002) demonstrate significant parasite-mediated effects on pheasant condition in birds following release and could be the cause for poor breeding success. However, it should be noted that these birds were examined

out of the breeding season, and subsequent stress and alternative resource allocation during the breeding season could reduce this threshold in female pheasants.

Chapter 7. General discussion

The aims of the present study were 1) to determine the factors influencing disease dynamics of clinical syngamosis; 2) to determine the extent of environmental contamination with the infectious stages of the tracheal parasite, *Syngamus trachea* on pheasant estates; 3) to evaluate, what effect, if any, infection with *S. trachea* is having on pheasant populations, both pre and post release.

Previous researchers have established the importance of temperature in the development and hatching of *S. trachea* eggs (Wehr, 1937; Barus, 1965), however these experiments were conducted in the laboratory, which makes it difficult to draw inferences about disease dynamics in the field. Barus, (1966b), found that the incidence of disease generally reached a peak around July and August, and attributed the increased incidence following rainfall on the appearance of paratenic hosts. However, results from the first year study demonstrated that temperature and humidity were associated with increased movement and abundance of hatched larvae on herbage, which in turn, facilitated transmission and influenced clinical cases of syngamosis in pheasants. Infection status, i.e. positive faecal egg counts, was associated with larval abundance, demonstrating that that these infections were a result of birds consuming hatched larvae and not a paratenic host. The relationship between larval migration and rainfall has been demonstrated for other nematode species (Khadijah *et al.,* 2013a,b), however this was the first mention of such effects within *S. trachea* populations.

Given that *S. trachea* has relatively predictable development behaviour within a set temperature range, it may be possible to predict larval emergence by using simple differential equations. This predictive ability could facilitate the development of forecasting tools to advise gamekeepers on periods of high and low risk in order to develop more targeted approaches to disease management. This could enable gamekeepers to reduce their reliance on anthelmintics, increasing both the profitability and sustainability of pheasant rearing. Though the temperature requirements for the development of L3 have been established (Wehr, Barus), both researchers noted that the eggs hatched 'spontaneously', which is unlikely to be the case. Indeed Otrlepp, (1923), found that eggs did not hatch below 20 °C. Similarly, *Haemonchus contortus*, a *Strongylid* nematode of sheep only hatches between 9 and 36 °C, with hatching times reduced at increasing temperatures (Crofton, 1965). Knowing that the minimum temperature for *S. trachea* larval hatch is 20 °C, it would be fairly simple to determine this hatching threshold by incubating

embryonated eggs at various temperatures and quantifying the proportion of eggs hatching. These data could then be used to develop predictive models to quantify disease risk at a given temperature range, which gamekeepers could use to inform their decision making regarding treatment or management options.

Several studies suggest that population size and stocking densities are major components in contributing to observed disease patterns, and there is a direct relationship between host abundance and the health status of a population (Kellogg & Prestwood, 1968; Permin *et al.,* 1998; Kjaer, 2004; Gortazar *et al.*, 2006; Heckendorn *et al.,* 2009; Sherwin *et al.,* 2013). For infectious diseases that rely on ingestion of larval stages, the increased density and aggregation of a population will facilitate disease transmission by allowing a greater contact time between a host and parasite. The two biggest factors in determining the variation in the numbers of eggs per pen in this system were the age of a pen, i.e. how long it had been in constant use, and, average pen stocking density. Birds that were housed in pens with fewer birds per acre had lower faecal egg counts compared with birds managed at higher densities. Similarly, the continued use of a release pen over time, in some cases for more than 40 years, facilitates the build-up of the infectious stages in the environment (Goldova *et al.,* 2002). This finding is in stark contrast to previous understanding that paratenic hosts predominantly facilitate the between-year transmission, and that larvae do not survive for long in soil (Barus, 1966c). The continued use of release pens has implications for successive generations of birds by not allowing enough time for the natural mortality of eggs and larvae through desiccation. Pasture rotation is a common practice in sheep farming, and allows for the natural mortality of infectious stages between years in order to provide a 'fresh' pasture for the next cycle (Abbott *et al.,* 2012). Anecdotal evidence suggests that this practice could be effective in reducing parasite burden in pheasants (Simon *et al.,* 2011; *Pers. Comms* – VLA Game Fair), however it may not be viable for small estates due to time and cost implications of moving release pens. Similarly, the validity of this practice requires further testing in regards to pheasant rearing as it is generally not known how long eggs and larvae remain viable in the environment. Indeed, results from the first year indicate that the extent of environmental contamination was directly proportional to the number of years a pen had been in constant use (Gethings *et al.,* 2015a). This suggests that eggs and larvae are able to remain viable for at least a year, as previous rearing cycles directly influenced subsequent disease occurrence. A potential alternative would be to reduce stocking density, either by reducing the number of birds per release pen, or, by increasing the overall size of the pen (Kjaer, 2004). These findings are in direct comparison with Barus

(1966a,b), who determined that eggs could not remain viable for long and that earthworms were the primary source of between-year infection pressure.

It was evident from the literature review that there was a large emphasis on paratenic hosts in the transmission dynamics of *S. trachea*, and this reasoning may have influenced gamekeeper's perceptions of disease dynamics. Previous experimental design inadvertently overestimated the importance of earthworms in the maintenance and transmission of syngamiasis by using Petri dishes or discrete plots of land in order to infect earthworms. This would not only increase the likelihood of an earthworm ingesting a *S. trachea* egg, but would also concentrate the number of eggs in such a way that is atypical of infections under natural conditions. Indeed, Barus (1965) only found 6.3% of earthworms to be infected when obtained from a large pheasant pen, and then only 1-12 larvae were found per worm. Campbell (1935) obtained a large number of earthworms from a release pen that had seen significant losses from syngamosis in the previous years. As the onset of clinical symptoms generally coincides with the commencement of adult worm egg production, it can be assumed that viable eggs would have been shed onto the ground by infected birds. Out of eight pheasant chicks fed eleven earthworms each, only two birds were found to be harbouring 1 pair of worms upon *post-mortem* examination (Campbell, 1935). This was then repeated with forty pheasant chicks being fed seven earthworms each, however only two birds were found to harbour one and six pairs of worms (Campbell, 1935). Although it is evident that the earthworm can act as a source of transmission to immunologically naïve pheasants, the disproportionally high numbers of worms that are required to result in clinical disease demonstrates that it is a poor method of transmission over direct ingestion of infectious eggs and larvae from the ground. Indeed, the data collected from the second year study indicated that the contamination of soil with eggs and larvae is the most viable and important source of infection to pen-reared birds. The high concentration of the infectious stages around points of non-random congregation, i.e. feeders and drinkers, are a major source of infection to immunologically naïve birds. However, as egg and larval survival is highly climate dependent, paratenic hosts *may* play a great role in disease transmission when climatic conditions are less favourable for larval survival. Similarly, though researchers have successfully transmitted *S. trachea* from a number of wild bird species to domestic flocks, success was always greater when this was facilitated via an earthworm. Paratenic hosts, may, therefore be important for facilitiating inter-specific transmission from wild birds to managed populations, however once infections are established within a population, it appears that direct contact with eggs and larvae is the primary method of intra-specific transmission.

From speaking with gamekeepers, it is apparent that many leave their feeders in the same location between years, often for ease of access and little attention is paid to managing the environmental worm burden, beyond the frequent use of anthelmintics (*Pers comms* The Game Fair, 2015). This environmental contamination not only poses a threat to the sustainability of pheasant rearing, but as many avian parasites show little host specificity, the potential disease transfer between penned and wild populations warrants serious consideration. Analysis between the distance from the feeder and the abundance of eggs and larvae demonstrated that there was an average reduction of between 73 and 93% within the first two metres. The frequent movement of feeders could, theoretically, reduce the incidence and severity of disease, however this requires further research in order to draw any definitive conclusions. Similarly, if feeders are moved too rapidly, this could potentially lead to a greater level of environmental contamination by spreading eggs and larvae more evenly in space and time. Feeders that limit the amount of faecal-contamination of grain could be a more suitable option where the movement of feeders is not feasible. Spring hoppers, that tend to spill grain out on to the floor, only facilitate the transmission of disease by increasing the contact time between the host and the infectious stages.

Birds are generally treated within the release pens, and as anthelmintic treatment is usually suppressive in nature, due to the high levels of environmental contamination (Gethings *et al.,* 2015a,b), birds are rapidly re-infected. It has been demonstrated that released pheasants generally disperse <1km from release sites (Leif, 1994), with birds often returning to nest and roost in the release pens over night. As anthelmintic supplementation must cease as soon as birds are released, the continued use of heavily infected pens will result in the rapid reinfection of pheasants, facilitating the dissemination of infectious stages. This lends itself to the possible transmission of drug-resistant generalist-parasites between species, enabling the dissemination of resistant genes across species home ranges whilst reducing survival and productivity of released pheasant populations. Indeed, pheasants infected with *S. trachea* demonstrated significantly reduced body condition compared with uninfected controls and the extent of this reduction generally increased as worm burden increased. Although there was no difference in the prevalence or mean worm burden between sexes, interestingly, there were differences in the magnitude of the effect of increasing worm burden. Females appear to be able to withstand low worm burdens, with birds not demonstrating any reductions below three pairs of worms. In contrast, males demonstrated significant reductions when infected with just one pair of worms. A sex difference in the prevalence of *S. trachea* has been noted previously in partridges, with Whitlock (1937) and Clapham

(1939) finding a higher proportion of females infected among confined populations. Whitlock, (1937) attributed this difference to the strain of egg-laying, which may have reduced immunocompetence. One reason for the differences observed between this study and previous studies could be differences in sexual behaviour. Pheasants will form harems, with one male to several females that he then protects from rival males. In contrast, partridges are monogamous and it could be that resource allocation for sexual ornamentation in male pheasants is favoured over immunocompetence (Hamilton & Zuk, 1982).

Regardless of the cause of these differences, the effect of *S. trachea* on pheasant body condition could have wide ranging implications for population stability and could be the reason for the poor breeding success and survival of released pheasants.

Chapter 8. Conclusions

From the review of past literature, and results presented herein, it is clear that parasites are a significant limiting factor for pheasant survival and productivity, and current management techniques could be improved to ensure survival post-release. It is clear that *Syngamus trachea* is an important parasite within pheasant populations and its effects on production and population dynamics can be pervasive.

The continued use of discrete releasing areas is maintaining and even exacerbating the levels of disease in pheasant populations, particularly when feeders remain in place between years. The number of years a pen had been in constant use was one of the biggest factors explaining the variation in egg abundance between pens. This high level of concentration of infectious material within pens, particularly around feed hoppers appears to be the primary factor driving intra-specific disease transmission within pheasant pens.

The identification of the importance of feed hoppers, pen age and stocking density can potentially be translated into practical, low cost solutions for disease management by interrupting the parasite's lifecycle. The prophylactic use of anthelmintics on the study sites appears to be effectively controlling disease incidence and severity for the time being. However, the more or less permanent provision of anthelmintic-treated grain in lieu of adequate environmental sanitation/removal of faeces or feeders that limit the amount of faecal–grain contamination could have serious implications for disease management in the coming years. As only one class of anthelmintics, the benzimadazoles, are licensed for use in game birds, there is a real potential for the development of drug resistance.

Syngamus trachea was previously thought to be relatively benign, however it was demonstrated that even sub-clinical infections were sufficient to reduce body condition in managed pheasants. It is clear that *S. trachea* is pervasive among managed pheasant populations, and the implications for survival and reproduction are likely to be significant in the absence of anthelmintic treatment. The identification of spatial *foci* upon estates provides a simple, low-cost solution to managing environmental contamination that should be incorporated into gamekeeper's disease management plans.

Reference list

Abbott, K.A., Taylor, M., Stubbings, L.A. (2012) *Sustainable worm control strategies for sheep.* Available: http://www.scops.org.uk/alert_pdfs/SCOPSTechnicalmanual4thEditionJune20121307201217552 1.pdf.

Aebischer, N.J. (2003) The national game bag census: recent trends and the effect of foot and mouth disease. The Game Conservancy Trust Review of 2002 34, 61–65

Aebischer, N. J., Davey, P. D., & Kingdon, N. G. (2011) National gamebag census: mammal trends to 2009. *Game & Wildlife Conservation Trust, Fordingbridge*.

Allander, K., & Bennett, G. F. (1995) Retardation of breeding onset in Great Tits (*Parus major*) by blood parasites. *Functional Ecology*. 9(4), 677-682.

Anderson, R. M. (1995) Evolutionary pressures in the spread and persistence of infectious agents in vertebrate populations. *Parasitology*. 111, S15-S31.

Anderson, R. M., & May, R. M. (1978) Regulation and stability of host-parasite population interactions: I. Regulatory processes. *Journal of Animal Ecology*. 47(1), 219-247.

Andreopoulou, M., Tsiouris, V., Georgopoulou, I., & Papadopoulos, E. (2011) Case of *syngamosis* in partridges of a backyard farm. *Journal of the Hellenic Veterinary Medical Society.* 62, 327-333.

Atkinson, C.T., Thomas, N.J., & Hunter, B. 2008. *Parasitic Diseases of Wild Birds.* Wiley-Blackwell: Iowa, USA.

Bandelj, P., Blagus, R., Trilar, T., Vengust, M., & Rataj, A. V. (2015) Influence of phylogeny, migration and type of diet on the presence of intestinal parasites in the faeces of European passerine birds (Passeriformes). *Wildlife Biology*, *21*(4), 227-233.

Barbour, A.D., & Pugliese, A. (2000) On the variance-to-mean ratio in models of parasite distributions. *Advances in Applied Probability.* 32, 701-719.

Barus, V. (1966a). The effect of temperature and air humidity on the development and the resistance of eggs of the nematode *Syngamus trachea* (Montagu, 1811). *Helminthologia.* 7, 103-106.

Barus, V. (1966b). Seasonal dynamics of the invasion extensity of the nematode *Syngamus trachea* (Montagu, 1811) in breeding turkeys (*Melagris gallopavo f. domestica*). *Helminthologia.* 132(7), 29-32.

Barus, V. (1966c). The longevity of the parasitic stages and the dynamics of eggs production of the nematode *Syngamus trachea* (Montagu, 1811) in chicken and turkeys. *Folia Parasitologica*, *13*(3), 274-277.

Benskin, C. M., Wilson, K., Jones, K., & Hartley, I. R. (2009) Bacterial pathogens in wild birds: a review of the frequency and effects of infection. *Biological Reviews*, *84*(3), 349-373.

Bignal, E., Bignal, S., & Still, E. (1987) Gapeworm infection in Choughs. *Ringing & Migration*. 8(1), 56-58,

Bird, A. F., & Bird, J. (2012). *The structure of nematodes*. Academic Press.

Borovkov, K., Day, R., & Rice, T. (2013) High host density favors greater virulence: a model of parasite-host dynamics based on multi-type branching processes. *Journal of Mathematical Biology.* 66, 1123-1153.

Breitenbach, R. P., & Meyer, R. K. (1959) Effect of incubation and brooding on fat, visceral weights and body weight of the hen pheasant (*Phasianus colchicus*). *Poultry Science*. 38, 1014-1026.

Brockhurst, M.A., Buckling, A., & Rainey, P.B. (2006) Spatial heterogeneity and the stability of host-parasite coexistence. *Journal of Evolutionary Biology.* 19, 374-379.

Brunner, J.L., & Ostfeld, R.S. (2008) Multiple causes of variable tick burdens on small-mammal hosts. *Ecology*. 89, 2259–2272.

Burn, P.R. (1980) Density dependent regulation of a fish trematode population. *J. Parasitology.* 66, 173–174.

Callinan, A.P.L., & Westcott, J.M. (1986) Vertical distribution of trichostrongylid larvae on herbage and in soil. *International Journal for Parasitology.* 16, 241-244.

Campbell, J. W. (1935) The gapeworm (*Syngamus*) in wild birds. *The Journal of Animal Ecology*, 4(2), 208-215.

Chastel, O., Weimerskirch, H., & Jouventin, P. (1995) Body condition and seabird reproductive performance: A study of three Petrel species. *Ecology*. 76, 2240–2246.

Churcher, T.S., Ferguson, N.M., & Basanez, M-G. (2005) Density dependence and overdispersion in the transmission of helminth parasites. *Parasitology.* 131, 121-132.

Clapham, P.A. (1935) On nodules occasioned by gapeworm in pheasants. *Journal of Helminthology.* **13**, 9-12.

Clapham, P. A. (1934). Experimental studies on the transmission of gapeworm (Syngamus trachea) by earthworms. *Proceedings of the Royal Society of London. Series B*. *115*(791), 18-29.

Clapham, P. A. (1938) Are there host strains within the species of *Syngamus trachea*?. *Journal of Helminthology*, *16*(1), 49-52.

Clapham, P. A. (1939a) On the larval migration of *Syngamus trachea* and its causal relationship to pneumonia in young birds. *Journal of Helminthology*, *17*(3), 159-162.

Clapham, P. A. (1939b) On a sex difference in the infection rate of birds with Syngamus trachea. *Journal of Helminthology*, *17*(4), 192-194.

Clapham, P. A. (1940a) On wild birds as transmitters of helminth parasites to domestic stock. *Journal of Helminthology*, *18*(1), 39-44.

Clapham, P. A. (1940b) On the helminths of corvid birds in the British Isles. *Journal of Helminthology*, *18*(2-3), 89-94.

Coles, G.C., Bauer, C., Borgsteede, F.H.M., Geerts, S., Klei, T.R., Taylor, M.A., & Waller, P.J. (1992) World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) methods for the detection of anthelmintic resistance in nematodes of veterinary importance. *Veterinary Parasitology* 44, 35- 44.

Cooper, C. L., & Crites, J. L. (1974. Helminth parasites of the robin from South Bass Island, Ohio. *Journal of wildlife diseases*. *10*(4), 397-398.

Cornell, S.J., Isham, V.S., & Grenfel, B.T. (2004) Stochastic and spatial dynamics of nematode parasites in farmed ruminants. *Proceedings of the Royal Society; B.* 271, 1243-1250.

Cote, I. M., & Sutherland, W. J. (1997). The effectiveness of removing predators to protect bird populations. *Conservation Biology*, *11*(2), 395-405.

Craig, N.M., Smith, D.W., Pate, J.A., Morrison, I.W., & Knight, P.A. (2014) Local cytokine transcription in naïve and previously infected sheep and lambs following challenge with *Teladorsagia circumcincta. BMC Veterinary Research.* 10**.** doi:10.1186/1746-6148-10-87.

Crawford, M. (1940) Infection of adult fowls with *Syngamus trachealis. Indian Journal of Veterinary Science and Animal Husbandry.* 10**,** 293-294.

Crofton, H. D. (1965) Ecology and biological plasticity of sheep nematodes. I. The effect of temperature on the hatching of eggs of some nematode parasites of sheep. *Cornell Veterinarian*, *55*(2), 242-250.

Crofton, H.D. (1971) A quantitative approach to parasitism. *Parasitology.* 62, 179-193.

Davies, N. B., & Lundberg, A. (1985) The influence of food on time budgets and timing of breeding of the Dunnock Prunella modularis. *Ibis*, *127*(1), 100-110.

Delahay, R. J., Speakman, J. R., & Moss, R. (1995) The energetic consequences of parasitism: effects of a developing infection of *Trichostrongylus tenuis* (Nematoda) on red grouse (*Lagopus lagopus scoticus*) energy balance, body weight and condition. *Parasitology*. 110, 473-482.

Devada, K., & Sathianesan, V. (1988) Pathogenesis and clinical signs of *Syngamus trachea* infection in chicken. *Kerala Journal of Veterinary Science.* 19, 66-70.

Devada K., % Sathianesan V., 1989: Comparative anthelmintic efficacy of mebendazole thiabendazole and albendazole against syngamus trachea infection in chicken. Kerala Journal of Veterinary Science 20(1): 59-64

De Wit, J. J. (1995) Mortality of rheas caused by a *Syngamus trachea* infection. *Veterinary Quarterly*, *17*(1), 39-40.

Dezfuli, B.S., Volponi, S., Beltrami, I., & Poulin, R. (2002) Intra- and interspecific density-dependent effects on growth in helminth parasites of the cormorant, *Phalacrocorax carbo sinensis. Parasitology.* 124, 537-544.

Dobson, A. P., Waller, P. J. & Donald, A. D. (1990) Population dynamics of *Trichostrongylus colubriformis* in sheep: the effect of infection rate on the establishment of infective larvae and parasite fecundity. *International Journal for Parasitology.* 20, 347–352.

Dolton, C.S. & Brooke, M.de L. (1999) Changes in the biomass of birds breeding in Great Britain, 1968–88. *Bird Study*. 46, 274-278.

Donald, A.D. (1968) Population studies on the infective stage of some nematode parasites of sheep. *Parasitology.* 58, 951-960.

Dowell, S. D. (1990) Differential behaviour and survival of hand-reared and wild grey partridge in the United Kingdom. In *Perdix V: Gray Partrige and Ringnecked Pheasant Workshop (eds KE Church, RE Warner & SJ Brady)* (pp. 230-241).

Draycott, R. A., Parish, D. M., Woodburn, M. I., & Carroll, J. P. (2002) Spring body condition of hen pheasants *Phasianus colchicus* in Great Britain. *Wildlife Biology*. 8, 261-266.

Draycott, R.A.H., Parish, D.M.B., Woodburn, M.I.A., & Carroll, J.P. (2000) Spring survey of the parasite *Heterakis gallinarum* in wild-living pheasants in Britain. *Veterinary Record.* 147, 245-246.

Draycott, R.A.H. & Sage, R.B. (2005) Parasite control and breeding success in pheasants (*Phasianus colchicus*). In: Pohlmeyer, K. (ed.) Extended Abstracts of the XXVIIth Congress of the International Union of Game Biologists, Hannover 2005: 76-77. DSV-Verlag, Hamburg

Draycott, R.A.H., Woodburn, M.I.A., Ling, D.E., & Sage, R.B. (2006) The effect of an indirect anthelmintic treatment on parasites and breeding success of free-living pheasants (*Phasianus colchicus). Journal of Helminthology*. 80, 409-415.

Drent, R. H., & Daan, S. (1980) The Prudent Parent: Energetic Adjustments in Avian Breeding 1). *Ardea*. 68, 225-252.

Ducheyne, E., Charlier, J., Vercruysse, J., Rinaldi, L., Biggeri, A., Demeler, J., & Kaba, J. (2015) Modelling the spatial distribution of Fasciola hepatica in dairy cattle in Europe. *Geospatial health*. *9*(2), 261-270.

Dunn A.M., Torchin M.E., Hatcher M.J., Kotanen P.M., Blumenthal D.M., Byers J.E., Coon C.A.C., Frankel V.M., Holt R.D., Hufbauer R.A., Kanarek A.R., Schierenbeck

K.A., Wolfe L.M., & Perkins S.E. (2012) Indirect effects of parasites in invasions. *Functional Ecology*. 26, 1262–1274.

Dwight, D., & Bowman, M.S. (2013) *Georgis' Parasitology for Veterinarians.* Tenth Edition. Saunders, Elsevier: Philadelphia, USA.

Dybing, N.A., Fleming, P.A., & Adams, P.J., (2013) Environmental conditions predict helminth prevalence in red foxes in Western Australia. *International Journal for Parasitology; Parasites and Wildlife.* 2, 165-172.

Egbetade, A. O., Olatundun, O. A., & Gadsby, E. L. (2014) Fatal *Syngamus trachea* Infection in a Barn Owl (*Tyto alba*) in Cross River State, Nigeria. *Nigerian Veterinary Journal*, *35*(3), 1015-1017.

Elston, D.A., Moss, R., Boulinier, T., Arrowsmith, C., & Lambin, X. (2001) Analysis of aggregation, a worked example: numbers of ticks on red grouse chicks. *Parasitology.* 122, 563-569.

Elton, C., & Buckland, F. (1928) The gape-worm (*Syngamus trachea* Montagu) in Rooks (*Corvus frugilegus L.). Parasitology*, *20*(04), 448-450.

Enigk, K., & Dey-Hazra, A. (1970) Zur Wirtsspezifität von *Syngamus trachea* (Strongyloidea, Nematoda). *Zoonoses and Public Health*, *17*(8), 840-847.

Fernando, M.A., Stockdale, P.H.G., & Remmler, O. (1971) The route of migration, development and pathogenesis of *Syngamus trachea* (Montagu, 1811), Chapin 1925, in pheasants. *The Journal of Parasitology.* **57**, 107-116.

Foreyt, W.J. (2001) *Veterinary Parasitology: Reference Manual* Fifth Edition. Blackwell: Iowa, USA.

Formenti, N., Vigano, R., Rotelli, L., Ferrari, M., Cerutti, M.C., & Lanfranchi, P. (2013) Effect of suboptimal environment and host age on helminth community of black grouse (*Tetrao tetrix*). *European Journal of Wildlife Research.* 59, 351-358.

Gethings, O. J., Sage, R. B., & Leather, S. R. (2015a) Spatio-temporal factors influencing the occurrence of *Syngamus trachea* within release pens in the South West of England. *Veterinary Parasitology*. 207, 64-71.

Gethings, O. J., Sage, R. B., & Leather, S. R. (2015b). Spatial distribution of infectious stages of the nematode *Syngamus trachea* within pheasant (*Phasianus colchicus*) release pens on estates in the South West of England: Potential density dependence?. *Veterinary parasitology*, *212*(3), 267-274.

Gethings, O. J., Rose, H., Mitchell, S., Van Dijk, J., & Morgan, E. R. (2015c) Asynchrony in host and parasite phenology may decrease disease risk in livestock under climate warming: *Nematodirus battus* in lambs as a case study. *Parasitology*, *142*(10), 1306-1317.

Gethings, O. J., Sage, R. B., & Leather, S. R. (2016) Density-dependent regulation of fecundity in *Syngamus trachea* infrapopulations in semi-naturally occurring ring-necked pheasants (*Phasianus colchicus*) and wild Carrion Crows (*Corvus corone*). *Parasitology*. 143, 716-722.

Goble, F.C. & Kutz, H.L. (1945) Notes on the Gapeworms (Nematoda: Syngamidae) of galliform and passerifrom birds in New York State. *The Journal of Parasitology.* **31,** 394-400.

Goldova, M., Letkova, V., Curlik, J., Misko, J., & Soroka, J. (2002) The ecological aspects of endoparasitoses of game birds in cage-breeding systems. *Folia veterinaria*. 46, 34-35.

Goldova, M., Palus, V., Letkova, V., Kocisova, A., Curlik, J., & Mojzisova. (2006) Parasitoses in pheasants (*Phasianus colchicus*) in confined systems. *Veterinarski Arhiv.* 76, 83-89.

Gortázar C, Acevedo P, Ruiz-Fons F., & Vicente J. (2006) Disease risks and overabundance of game species. *European Journal of Wildlife Research.* 52, 81-87.

Granoth-Wilding, H.M.V., Burthe, S.J., Lewis, S., Herborn, K.A., Takahashi, E.A., Daunt, F., & Cunningham, E.J.A. (2005) Indirect effects of parasitism: costs of infection to other individuals can be greater than direct costs borne by the host. *Proceedings of the Royal Society; B.* 282. http://dx.doi.org/10.1098/rspb.2015.0602.

Griffith, R., Yaeger, M., Hostetter, S., Tell, L. A., Wetzlich, S., Vickroy, T., & Dzikamunhenga, R. (2013) Safety of fenbendazole in Chinese ring-necked pheasants (Phasianus colchicus). *Avian diseases*, *58*(1), 8-15.

Guildford, H.G., & Herrick, C.A., (1952) Seasonal fluctuations in the numbers of coccidia oocysts and parasite eggs in the soil of pheasant shelter pens. *Transactions of the Wisconsin Academy of Sciences, Arts, and Letters.* 41, 153-262.

Guilford, H. G. & Herrick, C. A. (1954) The effect of gapeworm disease in pheasants. *Transactions of the Wisconsin Academy of Sciences Arts and Letters*. 43 25-50

Hamilton, W.D. & Zuk, M. (1982) Heritable true fitness and bright birds: A role for parasites? *Science*. 218, 384-387.

Harris, J. E., & Crofton, H. D. (1957). Structure and function in the nematodes: internal pressure and cuticular structure in Ascaris. *Journal of Experimental Biology*, *34*(1), 116-130.

Heckendorn, F., Haring, D.A., Amsler, Z., & Maurer, V. (2009) Do stocking rate and a simple run management practice influence the infection of laying hens with gastronintestinal helminths? *Veterinary Parasitology.* 159, 60-68.

Hillgarth, N. & Wingfield, J.C. (1997) Parasite-mediated sexual selection: endocrine aspects. pp. 78–104 in Clayton, D.H. & Moore, J. (Eds) Host parasite evolution – general principles and avian models. Oxford, Oxford University Press.

Hofle, U., Gortazar, C., Ortiz, J.A., Knispel, B., & Kaleta, E.F. (2004) Outbreak of trichonmoniasis in a woodpigeon (*Columba palumbus*) wintering roost. *European Journal of Wildlife Research.* 50, 73-77.

Holand, H., Jensen, H., Tufto, J., Pärn, H., Sæther, B. E., & Ringsby, T. H. (2015) Endoparasite infection has both short-and long-term negative effects on reproductive success of female house sparrows, as revealed by faecal parasitic egg counts. *PloS*. 10, e0125773.

Holand, H., Jensen, H., Tufto, J., Saether, B.E., & Ringsby, T.H. (2013) Temporal and spatial variation in prevalence of the parasite *Syngamus trachea* in a metapopulation of House Sparrows (*Passer domesticus*). *Parasitology.* 140, 1275-1286.

Holmes, J.C. & Bethel, W.M. (1972) Modification of intermediate host behaviour by parasites. Behavioural Aspects of Parasite Transmission (eds E.U. Canning & C.A. Wright), pp. 123-149. Academic Press, London.

Hudson, P.J. (1986) The effect of a parasitic nematode on the breeding production of red grouse. *Journal of Animal Ecology*. 55, 85-92.

Hudson, P. J., Dobson, A. P., & Newborn, D. (1992a) Do parasites make prey vulnerable to predation? Red grouse and parasites. *Journal of animal ecology*. 681-692.

Hudson, P.J., Newborn, D. & Dobson, A.P. (1992b) Regulation and stability of a free-living host-parasite system − *Trichostrongylus tenuis* in red grouse. I. Monitoring and parasite reduction experiments. *Journal of Animal Ecology*. 61, 477–486.

Hunter, G. C. & Quenouille, M. H. (1952) A Statistical examination of the worm egg count sampling technique for sheep. *Journal of Helminthology.* 26, 157-70.

Hutchings, M.R., Gordon, I.J., Kyriazakis, I., % Jackson, F. (2001) Sheep avoidance of faeces-contaminated patches leads to a trade-off between intake rate of forage and parasitism in subsequent foraging decisions. *Animal Behaviour.* 62, 955-964.

Hwang, J. C. (1964) Hemogram of turkey poults experimentally infected with *Syngamus trachea*. *Avian Disease*. 8, 380-390.

Irvine, R. J. (2006) Parasites and the dynamics of wild mammal populations. *Animal Science*. 82, 775-781. doi:10.1017/ ASC2006106

Irvine, R. J., Corbishley, H., Pilkington, J. G., & Albon, S. D. (2006) Low-level parasitic worm burdens may reduce body condition in free-ranging red deer (*Cervus elaphus*). *Parasitology*. 133, 465-475.

Irvine, R. J., Stien, A., dallas, J. F., Halvorsen, O., Langvatn, R. & Albon, S. D. (2001) Contrasting regulation of fecundity in two abomasal nematodes of Svalbard reindeer (*Rangifer tarandus platyrhynchus*). *Parasitology.* 122, 673–681.

Ives, A. R. (2015) For testing the significance of regression coefficients, go ahead and log-transform count data. *Methods in Ecology and Evolution.* 6**,** 828-835. doi: 10.1111/2041-210X.12386

Johnson, P.T.J., & Hoverman, J.T. (2014) Heterogeneous hosts: how variation in host size, behaviour and immunity affects parasite aggregation. *Journal of Animal Ecology.* 83, 1103-1112.

Jones, P. J., & Ward, P. (1976) The level of reserve protein as the proximate factor controlling the timing of breeding and clutch-size in the red-billed Quelea (*Quelea quelea*). *Ibis.* 118, 547-574.

Kaplan, R. M. (2004) Drug resistance in nematodes of veterinary importance: a status report. *Trends in parasitology*, *20*(10), 477-481.

Kahn, C. M., Line, S., & Aiello, S. E. (2005) The Merck Veterinary Manual. Merck & Co. *Inc., Whitehouse Station, NJ*.

Kellogg, F. E., & Prestwood, A. K. (1968) Gastrointestinal helminths from wild and pen-raised bobwhites. *The Journal of Wildlife Management*. 32(3), 468-475.

Khadijah, S., Kahn, L.P., Walken-Brown, S.W., Bailey, J.N., & Bowers, S.F. (2013a). Effect of stimulated rainfall timing on faecal moisture and development of *Haemonchus contortus* and *Trichostrongylus colubriformis* eggs to infective larvae. *Veterinary Parasitology.* 192, 199-210

Khadijah, S., Kahn, L.P., Walken-Brown, S.W., Bailey, J.N., Bowers, & S.F. (2013b). Soil moisture influences the development of *Haemonchus contortus* and *Trichostrongylus colubriformis* to third stage larvae. *Veterinary Parasitology.* 196, 161-171.

Kirsch, R. (1984). Treatment of nematodiasis in poultry and game birds with fenbendazole. *Avian diseases*, 311-318.

Kjaer, J. B. (2004) Effects of stocking density and group size on the condition of the skin and feathers of pheasant chicks. *The Veterinary Record*. *154*(18), 556-558.

Kocan, R.M. (1969) Various grains and liquid as potential vehicles of transmission for *Trichomonas gallinae. Journal of Wildlife Diseases.* 5, 148-149.

Kotrlá, B., Černý, V., Kotrlý, A., Minář, J., Ryšavý, B., & Šebek, Z. (1984) Wild animals parasitoses. *Academia, Praha, Czech Republic*.

Krone, O., Friedrich, D., & Honisch, M. (2007) Specific status and pathogenicity of syngamid nematodes in bird species (Ciconiformes, Falconiformes, Gruiformes) from Germany. *Journal of Helminthology.* 81, 67-73.

Lees, A. C., Newton, I., & Balmford, A. (2013) Pheasants, buzzards, and trophic cascades. *Conservation Letters*, 6: 141–144. doi: 10.1111/j.1755-263X.2012.00301.x

Leif, A.P. (1994) Survival and reproduction of wild and pen-reared ring-necked pheasant hens. *Journal of Wildlife Management*. 58, 501-506.

Lewis, A. (1928) Observations on the morphology of *syngamus trachea* from some wild and domestic birds. *Journal of Helminthology.* 6**,** 99-112.

Linden, M., Gustafsson, L., & Part, T. (1992) Selection on fledging mass in the collared flycatcher and the great tit. *Ecology*, *73*(1), 336-343.

Loman, J. (1980) Reproduction in a population of the hooded crow *Corvus cornix. Ecography.* 3, 26-35.

Luong, L.T., Vigliotti, B.A., & Hudson, P.J. (2011) Strong density-dependent competition and acquired immunity constrain parasite establishment: Implications for parasite aggregation. *International Journal for Parasitology.* **41,** 505-511.

McGregor, J. K., Kingscote, A. A., & Remmler, F. W. (1961) Field trials in the control of gapeworm infections in pheasants. *Avian Diseases*, *5*(1), 11-18.

Magwisha, H.B., Kassuku, A.A., Kyvsgaard, N.C., & Permin, A. (2002) A comparison of the prevalence and burdens of helminth infections in growers and adult free-range chickens. *Tropical Animal Health and Production*. 34, 205-214.

Marcogliese, D. J. (1997) Fecundity of sealworm (*Pseudoterranova decipiens*) infecting grey seals (*Halichoerus grypus*) in the Gulf of St. Lawrence, Canada: lack of density-dependent effects. *International Journal for Parasitology.* 27, 1401–1409.

McCraw, B. M., & Lautenslager, J. P. (1971) Pneumonia in calves associated with migrating Ascaris suum larvae. *The Canadian Veterinary Journal*, *12*(4), 87-90.

McCann, C. M., Baylis, M., & Williams, D. J. (2010) The development of linear regression models using environmental variables to explain the spatial distribution of Fasciola hepatica infection in dairy herds in England and Wales. *International Journal for Parasitology*, *40*(9), 1021-1028.

McGregor, J. K. (1963). The efficacy of thiabendazole in the control of gapeworm infection in pheasants. *The Canadian Veterinary Journal*, *4*(8), 206.

MEA, EMEA/MRL/267/97-FINAL: The European Agency for the Evaluation of Medicinal Products. Flubendazol, Summary Report 2; Veterinary Medicines Evaluation Unit. London, UK: Committee for Veterinary Medicinal Products; 1997.

Michael, E. & Dunby, D.A.P. (1989) Density dependence in establishment, growth and worm fecundity in intestinal helminthiasis: the population biology of *Trichuris muris* (Nematoda) infection in CBA/Ca mice. *Parasitology.* 98, 451-458.

Millán, J., C. Gortazar, P. Tizzani, & F. J. Buenestado. (2002) Do helminths increase the vulnerability of released pheasants to fox predation? *Journal of Helminthology*. 76, 225–229.

Molina, X., Casanova, J. C., & Feliu, C. (1999) Influence of host weight, sex and reproductive status on helminth parasites of the wild rabbit, *Oryctolagus cuniculus*, in Navarra, Spain. *Journal of helminthology*, *73*(03), 221-225.

Morgan, D.O. & Clapham, P.A. (1934) Some observations on gape-worm in poultry and game birds. *Journal of Helminthology.* **12,** 63-70.

Morgan, E.R., & Wall, R. (2009) Climate change and parasitic disease: farmer mitigation? *Trends in Parasitology*. 25, 308-313.

Morgan, E.R., & van Dijk, J. (2012) Climate and the epidemiology of gastrointestinal nematode infections of sheep in Europe. *Veterinary Parasitology.* 189, 8-14.

Morley, F. H. W., & Donald, A. D. (1980) Farm management and systems of helminth control. *Veterinary Parasitology*. *6*(1-3), 105-134.

Moss, R. *&* Watson, A. (2001) Population cycles in birds of the grouse family (*Tetraonidae*)*. Advances in Ecological Research.* 32*,* 53–111.

Moss, R., Watson, A., Trenholm, I.B. & Parr, R. (1993) Caecal threadworms *Trichostrongylus tenuis* in red grouse *Lagopus lagopus scoticus*: effects of weather and host density upon estimated worm burdens. *Parasitology*. 107, 199–209.

Mossinger, J & Wenk, P. (1986) Fecundity of *Litomosoides carinii* (Nematoda, Filarioidea) *in vivo* and *in vitro*. *Parasitology Research.* 72, 121–131.

Moynihan, I. W., & Musfeldt, I. W. (1950) Gapeworm infestation of pheasants. *Canadian Journal of Comparative Medicine and Veterinary Science*, *14*(9), 308-310.

NADIS. (2014) *NADIS - National Animal Disease Information Service*. [online] Available at: http://www.nadis.org.uk/bulletins/diseases-of-game-birds/part-4-diseases-from-10-days-to-7-weeks-of-age.aspx [Accessed 4 Nov. 2014].

Nevarez, J.G., Gamble, K.C., & Tully, T.N. (2002) *Syngamus trachea* infection in two red and yellow barbets *Trachyphonus erythocephalus*. *Journal of Avian Medicine and Surgery.* 16, 31–33.

Newborn, D. & Foster, R. (2002) Control of parasite burdens in wild red grouse *lagopus lagopus scoticus* through the indirect application of anthelmintics . *Journal of Animal Ecology*. 39, 909-914.

Nielsen, M.K., Vidyashankar, A.N., Anderson, U.V., DeLisi, K., Pilegaard, K., & Kaplan, R.M. (2010) Effects of fecal collection and storage factors on strongylid egg counts in horses. *Veterinay Parasitology.* 167, 55-61.

Nikolaou, S., & Gasser, R. B. (2006) Prospects for exploring molecular developmental processes in *Haemonchus contortus*. *International Journal for Parasitology*, *36*(8), 859-868.

NOAH. (2014) *NOAH Compendium of Animal Medicines: Overview - Introduction*. [online] Available at: http://www.noahcompendium.co.uk/Compendium/Overview/-21790.html [Accessed 4 Nov. 2014].

O' Connor, L.J., Walken-Brown, S.W., & Kahn, L.P. (2006) Ecology of the free-living stages of major trichostrongylid parasites of sheep. *Veterinary Parasitology.* 142, 1-15.

Olivier, L. (1944) Acquired resistance in chickens, turkeys, and ring-necked pheasants to the gapeworm, *Syngamus trachea*. *Journal of Parasitology.* 30, 64-76.

Onbaşilar, E. E., Poyraz, Ő., Erdem, E., & Őztűrk, H. (2008) Influence of lighting periods and stocking densities on performance, carcass characteristics and some stress parameters in broilers. *Archiv Fur. Geflűgelk*, *72*, 193-200.

Ortlepp, R. J. (1923) The life-history of *Syngamus trachealis* (montagu) v. siebold, the gape-worm of chickens. *Journal of Helminthology*. *1*(03), 119-140.

Public and Corporate Economic Consultants (PACEC). (2014) *The Value of Shooting Report*. [ONLINE] Available at: https://basc.org.uk/the-value-of-shooting/. [Accessed 26 April 2017].

Paterson, S. & Viney, M.E. (2002) Host immune responses are necessary for density dependence in nematode infections. *Parasitology.* 125, 283-292.

Pavlović, I., Đorđević, M., & Kulišić, Z. (2012) Endoparasites of farm-reared pheasants (Phasianus colchicus L.) in Serbia. In *Modern aspects of sustainable management of game population. International symposium on hunting, Zemun-Belgrade, Serbia* (pp. 125-129).

Pennycott, T.W. (2000) Causes of mortality and culling in adult pheasants. *Veterinary Record.* 146, 272-278.

Perrins, C. M. (1970) The timing of birds 'breeding seasons. *Ibis*, *112*(2), 242-255.

Permin, A., Nansen, P., Bisgaard, M., Frandsen, F., & Pearman, M. (1998) Studies on *Ascaridia galli* in chickens kept at different stocking rates. *Avian Pathology.* 27, 382-389.

Persson, I., & Göransson, G. (1999) Nest attendance during egg laying in pheasants. *Animal Behaviour*. *58*(1), 159-164.

Poulin, R. & Morand, S. (2000) Parasite body size and interspecific variation in levels of aggregation among nematodes. *The Journal of Parasitology.* 86**,** 642-647.

Poulin, R. (2013) Explaining variability in parasite aggregation levels among host samples. *Parasitology.* 140, 541-546.

Price, T., Kirkpatrick, M., & Arnold, S. J. (1988) Directional selection and the evolution of breeding date in birds. *Science*. *240*(4853), 798-799.

Pullan, R.L., Sturrock, H.J.W., Soares magalhaes, R.J., Clements, A.C.A., & Brooker, S.J. (2012) Spatial parasite ecology and epidemiology: a review of methods and applications. *Parasitology.* 139, 1870-1887.

Putaala, A., & Hissa, R. (1995) Effects of hand-rearing on physiology and anatomy in the grey partridge. *Wildlife Biology*. *1*(1), 27-31.

Real, L.A., & Biek, R. (2007) Spatial dynamics and genetics of infectious diseases on hetergenous landscapes. *Journals of the Royal Society Interface.* 4, 935-948.

Reed, T. E., Daunt, F., Hall, M. E., Phillips, R. A., Wanless, S., & Cunningham, E. J. A. (2008) Parasite treatment affects maternal investment in sons. *Science*. *321*(5896), 1681-1682.

Reid, A. J., Blake, D. P., Ansari, H. R., Billington, K., Browne, H. P., Bryant, J., & Malas, T. B. (2014) Genomic analysis of the causative agents of coccidiosis in domestic chickens. *Genome Research*. *24*(10), 1676-1685.

Richards, D. T. & Lewis, j. w. (2001) Fecundity and egg output by *Toxocara canis* in the red fox, *Vulpes vulpes*. *Journal of Helminthology.* 75, 157–164.

Robertson, P. A. (1991) Estimating the nesting success and productivity of British pheasants *Phasianus colchicus* from nest-record schemes. *Bird Study*. *38*(2), 73-79.

Rossanigo, C.E., & Gruner, L. (1994) Relative effect of temperature and moisture on the development of strongyle eggs to infective larvae in bovine pats in Argentina. *Veterinary Parasitology.* 55, 317-325.

Ruff, M.D. (1999) Important parasites in poultry production. *Veterinary Parasitology.* 84, 337-347.

RVC/FAO Guide to Veterinary Diagnostic Parasitology. Available at http://www.rvc.ac.uk/Review/Parasitology/poultrEggs/syngamus.htm.

Sage, R. B., Putaala, A., & Woodburn, M. I. (2002) Comparing growth and condition in post release juvenile common pheasants on different diets. *Poultry Science*. 81, 1199-1202.

Sage, R.B., Ludolf, I.C., & Robertson, P.A. (2005) The ground flora of ancient semi-natural woodlands in pheasant release pens in England. *Biological Conservation*. 122, 243-252.

Sage, R.B., Putaala, A., Pradell-Ruiz, V., Greenall, T.L., Woodburn, M.I.A., & Draycott, R.A.H. (2003) Incubation success of released hand-reared pheasants *Phasianus colchicus* compared with wild ones. *Wildlife Biology.* 9, 179-184.

Santilli, F., & Bagliacca, M. (2012) Occurrence of eggs and oocysts of intestinal parasites of pheasant (*Phasianus colchicus*) in droppings collected in differently managed protected areas of Tuscany (Italy). *European Journal of Wildlife Research.* 58, 369-372.

Saunders, L.M., Tompkins, D.M., & Hudson, P.J. (2000a) Role of oxygen availability in the embryonation of *Heterakis gallinarum* eggs. *International Journal of Parasitology.* 30, 1481-1485.

Saunders, L.M., Tompkins, D.M., & Hudson, P.J. (2000b) Spatial aggregation and temporal migration of free-living stages of the parasitic nematode *Trichostrongylus tenuis. Functional Ecology*. 14, 468-473.

Sharpe, G.I. (1964). Effects of Thiabendazole on Syngamus trachea in pheasants. Nature, 201: 315-316.

Shaw, J. L., & Moss, R. (1990). Effects of the caecal nematode Trichostrongylus tenuis on egg-laying by captive red grouse. *Research in veterinary science*, *48*(1), 59-63.

Shaw, D.J., & Dobson, A.P. (1995) Patterns of macroparasite abundance and aggregation in wildlife populations: a quantitative review. *Parasitology.* 111, 111-133.

Sherrard-Smith, E., Perkins, S.E., Chadwick, E.A., & Cable, J. (2015) Spatial and seasonal factors are key determinants in the aggregation of helminthes in their definitive hosts: *Pseudamphistomum truncatum* in otters (*Lutra lutra*). *International Journal for Parasitology.* 45, 75-83.

Sherwin, C.M., Nasr, M.A.F., Gale, E., Petek, M., Stafford, K., Turp, M., & Coles, G.C., (2013) Prevalence of nematode infection and faecal egg counts in free-range laying hens: relations to housing and husbandry. *British Poultry Science.* 54, 12-23.

Shutler, D., Alisauskas, T. & McLaughlin, J.D. (2012) Associations between body composition and helminths of lesser snow geese during winter and spring migration. *International Journal for Parasitology*. 42, 755-760.

Silangwa, S.M., & Todd, A.C. (1964) Vertical migration of trichostrongylid larvae on grasses. *The Journal for Parasitology.* 50, 278-285.

Simon, F., Germain, K., Bain, O., & Cabaret, J. (2011) Organic broilers on new runs: dynamics of helminth parasites. *Journées de la Recherche Avicole, 9*. Presented at 9.

Journées de la Recherche Avicole, Tours, FRA (2011-03-29 - 2011-03-30). Paris, FRA : ITAVI - Institut Technique de l'Aviculture.

Sinniah, B. & Subramaniam, K. (1991) Factors influencing the egg production of *Ascaris lumbricoides*: relationship to weight, length and diameter of worms. *Journal of Helminthology.* 65, 141–147.

Skorping, A., Read, A. F. & Keymer, A. E. (1991) Life history covariation in intestinal nematodes of mammals. *Oikos.* **60**, 365–372.

Stear, M.J. & Bishop, S.C. (1999) The curvilinear relationship between worm length and fecundity of *Teladorsagia circumcincta. International Journal for Parasitology.* **29,** 777-780.

Stear, M.J., Bairden, K., Duncan, J.L., Holmes, P.H., McKellar, Q.A., Park, M., Strain, S., Murray, M., Bishop, S.C.,& Gettinby, G. (1997) How hosts control worms. *Nature.* 389*,* 27.

Stromberg, B.E**.** (1997) Environmental factors influencing transmission. *Veterinary Parasitology.* 72, 247-264.

Sutherland, I., & Scott, I. (2009). *Gastrointestinal nematodes of sheep and cattle: biology and control*. John Wiley & Sons.

Szalai, A. J. & Dick, T. A. (1989) Differences in numbers and inequalities in mass and fecundity during the egg- producing period for *Raphidascaris acus* (Nematoda: Anisakidae). *Parasitology.* **98,** 489–495.

Tapper, S.C. (1999) A question of balance: game animals and their role in the British countryside. 288 pp. The Game Conservancy Trust, Hampshire UK

Taylor, E. L. (1928) *Syngamus trachea* from the starling transferred to the chicken and some physiological variation observed. *Annals of Tropical Medicine & Parasitology*, *22*(3), 307-318.

Taylor, E.L. (1935) *Syngamus trachea*. The longevity of the infective larvae in the earthworm, slugs and snails as intermediate hosts. *The Journal Of Comparative Pathology And Therapeutics.* 48, 149-156).

Taylor, E.L. (1939) Technique for the estimation of pasture infestation by strongyloid larvae. *Parasitology.* 31, 473-478.

Taylor, L.R., Woiwod, I.P., Perry, J.N. (1978) The density-dependence of spatial behavior and the rarity of randomness. *Journal of Animal Ecology.* 47, 383-406.

Temple, S.A. (1987) Do predators always capture substandard individuals disproportionately from prey populations? *Ecology.* 68, 669–674.

Thienpont, D. C., Vanparus, O. F. J., & Hermans, L. C. (1973) Mebendazole, a new potent drug against *Syngamus trachea* in turkeys. *Poultry science*, *52*(5), 1712-1714.

Tompkins, D.M. & Begon. M. (1999) Parasites can regulate wildlife populations. *Parasitology Today*. 15, 311-316.

Tompkins, D.M. & Hudson, P.J. (1999) Regulation of nematode fecundity in the ring-necked pheasant (*Phasianus colchicus*): not just density dependence. *Parasitology.* 118, 417-423.

Tompkins, D.M., Greenman, J.V., & Hudson, P.J. (2001) Differential impact of a shared nematode parasite on two gamebird hosts: implications for apparent competition. *Parasitology*. 122, 187-193.

Tompkins, D. M., Parish, D. M., & Hudson, P. J. (2002). Parasite-mediated competition among red-legged partridges and other lowland gamebirds. *The Journal of wildlife management*, 445-450.

Uriate, J. & Gruner, L. (1994) Development and distribution of trichostronglye infective larvae on a pasture irrigated by flooding or by spraying. *Parasitology Research.* 80, 657-663.

Vanparijs, O. (1984) Anthelmintic activity of flubendazole in naturally infected geese and the economic importance of deworming. *Avian Diseases*, 526-531.

Van Dobben, W.H. (1952) The food of the cormorants in the Netherlands. *Ardea* 40, 1-63.

Van Dijk, J., & Morgan, E. R. (2010) Variation in the hatching behaviour of *Nematodirus battus*: Polymorphic bet hedging?. *International Journal for Parasitology*, *40*(6), 675-681.

Van Dijk, J., & Morgan, E. R. (2012) The influence of water and humidity on the hatching of *Nematodirus battus* eggs. *Journal of Helminthology*, *86*(03), 287-292.

Varga, I. (1971) The pathophysiological effect of *Syngamus trachea* infection in turkeys. *Journal of comparative pathology*, *81*(1), 63-69.

Varga, I., Banbidi, G., Szell, Z., & Balint, C. (1998) Efficacy of albendazole against Syngamus trachea in experimentally infected turkeys. *Magyar Allatorvosok Lapja*, *120*(6), 336-338.

Vaughan, G.E. & Coble, P.W. (1975) Sublethal effects of three ectoparasites on fish. *Journal of Fisheries Biology*. 7, 283-294.

Verhulst, S., & Tinbergen, J. M. (1991) Experimental evidence for a causal relationship between timing and success of reproduction in the great tit *Parus m. major*. *The Journal of Animal Ecology*, 60(1) 269-282.

Verhulst, S. Dieleman, S.J. & Parmentier, H.K. (1999) A tradeoff between immunocompetence and sexual ornamentation in domestic fowl. *Proceedings of the National Academy of Science*. 96, 4478-4481.

Villanúa, D., Acevedo, P., Toledo, R., Höfle, U., Rodríguez, O., & Gortázar, C. (2006) Changes in parasite transmission stage excretion after pheasant release. *Journal of helminthology.* 80, 313-318.

Villanúa, D., Pérez-Rodríguez, L., Rodríguez, O., Viñuela, J., & Gortazar, C. (2007). How effective is pre-release nematode control in farm-reared red-legged partridges Alectoris rufa?. *Journal of helminthology*, *81*(1), 101-103.

Villanua, D., Perez-Rodriguez., Casa, F., Alzaga, V., Acevedo, P., Vinuela, J., & Gortazar, C. (2008) Sanitary risks of red-legged partridge releases: Introduction of parasites. *European Journal for Wildlife Research.* 54, 199-204.

Villanúa, D., Pérez-Rodríguez, L., Rodríguez, O., Viñuela, J., & Gortazar, C. (2007). How effective is pre-release nematode control in farm-reared red-legged partridges Alectoris rufa?. *Journal of helminthology*, *81*(1), 101-103.

Vincente, J., Hofle, U., Garrido, J.M., Fernandez-De-Mera, I.G., Acevedo, P., Juste, R., Barral, M., & Gortazar, C. (2007) Risk factors associated with the prevalence of tuberculosis-like lesions in fenced wild boar and red deer in South Central Spain. *Veterinary Research.* 38, 451-464.

Walker, M., Hall, A., Anderson, R. M., & Basáñez, M. G. (2009). Density-dependent effects on the weight of female Ascaris lumbricoides infections of humans and its impact on patterns of egg production. *Parasites & vectors*, *2*(1), 11. https://doi.org/10.1186/1756-3305-2-11

Watson, A., Moss, R., Parr, R., Trenholm, I. & Robertson, A. (1988) Preventing a population decline of red grouse *(Lagopus lagopus scoticus)* by manipulating density. *Experientia.* 44, 274-5.

Watson, M.J. (2013) What drives population-level effects of parasites? Meta-analysis meets life-history. *International Journal for Parasitology; Parasites and Wildlife.* 2, 190-196.

Wehr, E.E. (1937) Observations on the development of poultry gapeworm Syngamus trachea. *Transactions of the American Microscopical Society*. 56,72–78.

Wehr, E. E., & Hwang, J. C. (1967) Anthelmintic activity of thiabendazole against the gapeworm (*Syngamus trachea*) in turkeys. *Avian Diseases*, *11*(1), 44-48.

Welchman, D. D. B., Ainsworth, H. L., Jensen, T. K., Boye, M., King, S. A., Koylass, M. S., & Dalton, J. R. (2013). Demonstration of Ornithobacterium rhinotracheale in pheasants (Phasianus colchicus) with pneumonia and airsacculitis. *Avian pathology*, *42*(2), 171-178.

Welte, S. C., & Kirkpatrick, C. E. (1986) Syngamiasis in juvenile American robins (*Turdus migratorius*), with a note on the prevalence of other fecal parasites. *Avian Diseases*, 30(4), 736-739.

Whitlock, S. C. (1937). An apparent case of sexual difference in resistance to parasitic infection. *Journal of Parasitology*, *23*(4).

Williams, I.C. & Newton, I. (1969) Intestinal helminths of the bullfinch *Pyrrhula pyrrhula* (L.), in southern England. *Proceedings of the Helminthological Society of Washington.* 36, 76-82.

Williams, A. R., & Palmer, D. G. (2012) Interactions between gastrointestinal nematode parasites and diarrhoea in sheep: Pathogenesis and control. *The Veterinary Journal*, *192*(3), 279-285.

Wissler, K., & Halvorsen, O. (1975) The occurrence of gapeworm (*Syngamus trachea*) in willow grouse. *Journal of wildlife Diseases*, *11*(2), 245-247.

Wójcik, A. R., Wasilewski, I., Grygon-Franckiewicz, B., & Zbikowska, E. (1999) Economic losses in pheasant breeding evoked with endoparasites. *Wiadomosci Parazyltologiczne.* 45, 363 – 368 (In Polish).

Wolstenholme, A. J., Fairweather, I., Prichard, R., von Samson-Himmelstjerna, G., & Sangster, N. C. (2004) Drug resistance in veterinary helminths. *Trends in parasitology*, *20*(10), 469-476.

Woodburn MIA (1995) Do parasites alter pheasant breeding success? *Game Conservancy Annual Review.* 26:96–97

Woodburn, M., Sage, R.B. & Carroll, J.P. (2002) The efficacy of a technique to control parasitic worm burden in pheasants (*Phasianus colchicus*) in the wild. *Zeitschrift Fur Jagdwissenschaft***.** 48, 364–372.

Woog, F., Maierhofer, J., & Haag, H. (2013) Endoparasites in the annual cycle of feral Greylag Geese *Anser anser*. *Wildfowl*. *61*(61), 166-181.

Yazwinski, T.A., Chapman, H.D., Davis, R.B., Letonja, T., Pote, L., Maes, L., Vercruysse, J., & Jacobs, D.E. (2003) World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines for evaluating the effectiveness of anthelmintics in chickens and turkeys. *Veterinary Parastiology.* 116, 159-173.

Yazwinski, T. A., Tucker, C. A., Wray, E., Jones, L., & Clark, F. D. (2013). Observations of benzimidazole efficacies against Ascaridia dissimilis, Ascaridia galli, and Heterakis gallinarum in naturally infected poultry. *Journal of Applied Poultry Research*, *22*(1), 75-79.

Yom-Tov, Y. (2001) Global warming and body mass decline in Israeli passerine birds. *Proceedings of the Royal Society; B*. 268, 947-952.

Appendix A – Release pen data for chapter 3

Site	Age of pen (years)	Average moisture content (%)	Area (acres)	Stocking density (Birds/m^2)	Average number of eggs per gram of soil	Log number of eggs
1	27	37.1	1.97	0.175	49	1.69
1	15	32.1	3.00	0.082	25	1.40
1	25	38.8	3.26	0.189	64	1.81
1	27	41.5	4.07	0.154	60	1.78
1	17	41.7	2.965	0.150	61	1.79
2	35	36.6	2.15	0.184	62	1.79
2	20	32.9	1.062	0.116	33	1.52
2	40	47.3	6.27	0.138	74	1.87
2	25	36.1	2.37	0.209	54	1.73
2	35	44.9	3.71	0.200	69	1.84
2	37	37.6	2.90	0.170	51	1.71
2	20	44.5	4.16	0.125	45	1.65

Appendix B – Climate data and parasite recovery rates for site 1.

Date	Temperature (^{0}C)	Number of larvae per kg	Relative humidity (%)	Precipitation (inches)	Faecal egg counts
19.04	9.3	0	79.79	13	
26.04	9.3	0	81.67	22	
03.05	10.4	0	89.5	12	
10.05	11.9	0	94	15	
17.05	12.2	0	89.7	43	
24.05	13.1	0	87.1	0.5	
31.05	13.2	0	91.2	13	
05.06	15.2	0	81	0	0
11.06	15.4	0	87.5	0	0
17.06	14.8	1400	92.8	5.5	0
24.06	14.6	1900	96.30	19.5	350
02.07	15.2	1750	94.10	16	150
07.07	17.3	1850	90.80	11	150
17.07	17.3	1750	90.10	17.5	450
30.07	19.7	1500	86.30	12	
06.08	17.3	1550	79.50	0	
14.08	16.7	1600	90.50	6	
21.08	15.9	1450	89.50	8	

Appendix C – Climate data and parasite recovery rates for site 2.

Date	Temperature (°C)	Number of larvae per kg	Faecal egg counts	Relative humidity (%)	Precipitation (Inches)
19.04	6.61	0		76.4	11
26.04	8.25	0		79.43	17
03.05	8.32	0		83.43	9
10.05	9.35	0		91	10
17.05	10.7	0		85.56	11
24.05	12.4	0		85.21	12
31.05	11.1	0		87.45	9
05.06	12.2	0		81	19
11.06	15.8	0		87.5	8
17.06	18.4	2500		89.4	4
24.06	16.7	2400		90.20	0
02.07	16.7	3700	100	94.37	13
07.07	18.6	4500	350	96.32	8.5
17.07	19.8	3700	150	90.10	3.5
30.07	19.7	3400	100	92.95	8.5
06.08	17.3	3200	50	89.45	4
14.08	16.7	3400	150	92.76	17
21.08	15.9	2100	350	87.95	12

Appendix D – Faecal egg count data – site 1

Date	Faecal egg counts
24.06	450
	400
	150
	0
	200
	0
	0
	450
	750
	1100
	(350)
2.07	0
	150
	50
	50
	200
	0
	0
	100
	450
	500
	(150)
9.07	0
	200
	0
	0
	0
	0

	0
	50
	600
	650
	(150)
17.06	600
	750
	300
	0
	50
	900
	1100
	0
	300
	500
	(450)

Appendix E – Faecal egg count data - site 2

Date	Faecal egg counts
02-Jul	50
	200
	350
	350
	0
	0
	0
	0
	0
	50
	100
07-Jul	500
	0
	150
	50
	50
	750
	450
	1500
	0
	50
	350
17-Jul	0
	0
	0
	700
	100
	100

	150
	50
	150
	250
	150
30-Jul	50
	0
	450
	100
	100
	150
	50
	0
	0
	100
	100
06-Aug	50
	0
	0
	0
	150
	250
	250
	100
	150
	50
	100
14-Aug	0
	0
	0
	100

	150
	250
	350
	50
	0
	100
	100
21-Aug	0
	50
	1300
	750
	0
	50
	150
	650
	550
	0
	350

Appendix F – Release pen data for chapter 4.

Site	Pen	Age	Moisture	Birds	Density per m	Birds per acre	Size of pen in acres
1	Pen 1	27	37.1	1400	0.18	709.94	1.97
1	Pen 2	15	32.1	1000	0.08	333.23	3.00
1	Pen 3	25	38.8	2500	0.19	765.32	3.27
1	Pen 4	27	41.5	2500	0.15	624.89	4.00
1	Pen 5	17	41.7	1800	0.15	606.98	2.97
2	Pen 1	25	36.1	1300	0.21	548.52	2.37
2	Pen 2	35	36.6	1200	0.18	558.14	2.15
2	Pen 3	20	44.5	2100	0.13	504.81	4.16
2	Pen 4	37	37.6	1200	0.17	413.79	2.90
2	Pen 5	40	47.3	2500	0.14	398.72	6.27

Appendix G – Number of eggs at a given distance from feeder site 1.

Distance from feeder	Pen 1	Pen 2	Pen 3	Pen 4	Pen 5
0	50	45	550	67	87
0	90	72	487	74	51
0	56	39	114	53	55
0	62	68	421	37	21
2	24	12	35	21	19
2	31	14	39	15	14
2	22	11	41	17	11
2	29	19	24	24	5
5	11	3	5	4	0
5	8	5	3	10	0
5	5	4	7	0	0
5	0	2	9	5	4
10	0	0	0	0	0
10	0	0	0	0	0
10	0	0	11	5	0
10	6	7	0	0	0
15	0	0	0	7	6
15	0	0	0	0	0
15	0	0	0	4	0
15	0	5	0	3	8

Appendix H – Number of eggs per gram of soil in quadrants WITHOUT a feeder at site 1.

Sample	Pen 1	Pen 2	Pen 3	Pen 4	Pen 5
1	7	4	11	41	2
2	11	0	2	1	14
3	10	0	0	4	0
4	4	3	4	5	4
5	0	0	5	31	14
6	4	4	6	22	17
7	7	0	4	12	18
8	9	0	2	0	0
9	10	5	10	6	11
10	11	6	6	7	0
11	19	0	8	3	2
12	21	7	9	9	4
13	0	4	5	6	6
14	2	0	7	3	3
15	4	0	8	8	7

Appendix I – Number of eggs at a given distance from feeder site 2.

Distance from feeder	Pen 1	Pen 2	Pen 3	Pen 4	Pen 5
0	1003	112	1500	68	210
0	775	156	995	54	157
0	1450	116	1250	55	114
0	556	97	1867	57	115
2	74	31	25	12	32
2	67	45	32	9	9
2	59	32	19	4	12
2	28	27	25	5	24
5	1	10	3	4	0
5	0	5	6	2	6
5	4	3	9	7	0
5	3	0	0	0	2
10	0	0	0	0	5
10	0	0	0	0	0
10	0	0	0	1	0
10	3	6	8	0	0
15	19	0	0	0	0
15	45	0	0	0	0
15	0	0	0	0	0
15	1	5	0	0	0

Appendix J - Number of eggs per gram of soil in quadrants WITHOUT a feeder at site 2.

Sample	Pen 1	Pen 2	Pen 3	Pen 4	Pen 5
1	4	8	6	14	13
2	2	14	1	15	4
3	2	4	15	3	11
4	9	12	5	0	0
5	4	10	12	12	11
6	7	4	4	4	6
7	5	5	8	6	7
8	3	8	0	8	19
9	5	7	7	22	9
10	9	5	12	15	11
11	10	6	14	12	0
12	0	4	26	5	3
13	0	3	27	5	4
14	0	9	11	11	19
15	0	8	19	8	15

Appendix K – Worm burden and mean worm length for pheasants.

Worm burden	Mean worm length
9	15
28	9
25	9
39	9.09
9	13
20	10.5
8	15
21	12
8	12.5
3	14
4	14
5	15
7	15
1	15.5
11	15.76
6	16
10	16
7	16
2	17.9
10	18.5
5	19.56
1	19.71
3	20.5
5	21.5
2	21.5
2	21.61
2	22
3	22

2	22.67
1	22.97
1	25
3	26
1	27
1	27.31
2	28
4	21.43
8	17.4

Appendix L – Worm burden and mean worm length for crows.

Worm Burden	Mean worm length
28	6
28	8.75
38	8.75
28	8.79
17	9
16	9
20	9
20	9.1
27	9.21
36	9.27
37	9.32
38	9.45
17	9.54
25	9.76
32	9.79
15	9.9
26	10.04
22	10.12
14	10.45
13	10.94
15	10.95
13	11.21
12	11.41
15	11.718
25	11.72
14	11.92
15	11.95
16	11.95

16	12
14	12.2
11	12.41
13	12.5
10	12.518
24	12.9
20	13.08
18	13.12
23	13.45
9	13.94
5	13.95
7	14.21
12	14.39
7	14.49
5	14.95
5	15
5	15.1
6	15.31
10	15.75
10	15.757
10	15.76
8	16
7	16.3
12	16.41
6	16.42
5	16.45
5	16.49
5	16.5
7	16.51
7	16.52
2	16.7

5	17
8	17
8	17
4	17.41
6	17.45
7	17.45
4	17.5
7	17.98
5	18
9	18.2
7	18.5
1	18.71
2	18.76
2	19.54
3	19.54
1	19.7
1	19.7
4	19.7
3	20
2	20.43
1	21
1	21.49
1	22
1	22.17
1	22.45
1	22.73
1	23.95
1	26.54
1	26.7
1	27.24
1	27.31

1	30.09
1	32

Appendix M – *Syngamus trachea* larval stage (3). Sheath is indicated by arrow. Larvae were developed to L3 from eggs obtained by direct dissection of adult female worms.