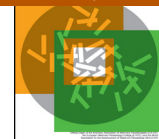




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## Veterinary Parasitology

journal homepage: [www.elsevier.com/locate/vetpar](http://www.elsevier.com/locate/vetpar)Spatio-temporal factors influencing the occurrence of *Syngamus trachea* within release pens in the South West of EnglandO.J. Gethings<sup>a,\*</sup>, R.B. Sage<sup>b</sup>, S.R. Leather<sup>a</sup><sup>a</sup> Department of Crop and Environment Sciences, Harper Adams University, Edgmond, Newport TF10 8NB, UK<sup>b</sup> Game & Wildlife Conservation Trust, Burgate Manor, Fordingbridge SP6 1EF, UK

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## ABSTRACT

*Syngamus trachea* is a pathogenic tracheal nematode that causes syngamiasis in wild and game birds, especially when birds are managed at high densities. Despite its pathogenic nature, very little is known about its epidemiology and relationship with ambient temperature and humidity. The spatial and temporal modelling of disease was undertaken on two pheasant estates within the South West of England from April 2014 to August 2014. Significant differences between the mean numbers of eggs per gram of soil were identified between pens at both site 1 and site 2 but did not differ significantly between sites. Egg abundance was significantly associated with soil moisture content, with greater egg survival between years in pens with higher average volumetric soil moisture content. Previous years stocking density and pen age were also associated with greater egg survival between years with more eggs being recovered in pens with greater stocking densities, and pens that had been sited longer. The greatest model to explain the variation in the numbers of eggs per gram of soil per pen was a combination of soil moisture content, stocking density and pen age.

Larval recovery differed significantly between sites. Larval abundance was significantly and positively associated with temperature and relative humidity at site 1. Similarly, temperature and humidity were also positively and significantly associated with larval abundance at site 2. Rainfall did not influence larval recovery at either site 1 or site 2. The model with the greatest ability to explain larval abundance at both sites, was a combination of temperature, humidity and rainfall. Infection status (positive faecal egg counts) was significantly and positively associated with larval abundance at both sites, but rainfall was only positively associated at site 1. Temperature and humidity were positively associated with infection status at site 2, but not at site 1. The present study highlights the influence of climatic variables on both egg survival and larval abundance, and could therefore be used to develop more targeted treatment strategies around periods of higher disease risk. The frequent use of release pens is a clear factor in the epidemiology of syngamiasis, and it is recommended that pens be rested and/or rotated in order to reduce infection pressure in subsequent flocks.

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## 1. Introduction

An understanding of the factors influencing infectious disease dynamics is fundamental to the control and

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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 and 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 and 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<sup>TM</sup>, 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<sup>TM</sup>) 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/km<sup>2</sup> (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 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 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 paratenic 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 and van Dijk, 2012), with temperature, humidity and rainfall being the most significant factors (Pullan et al., 2012; Dybing et al., 2013; Morgan and 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 and Herrick, 1952; Khadijah et al., 2013a, 2013b). As *S. trachea* larvae are extremely susceptible to desiccation (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 and 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., 2000) 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.

## 2. Materials and methods

### 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 67340 48539 and consisted of seven release pens. Site 1 undertook Corvid control via the use of Larsen. Site 2 was situated approximately at grid reference – SU 17769 30326, 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<sup>TM</sup>) in the feed as a prophylactic. 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.

## 2.2. Sample collection

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

### 2.2.1. 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 cm × 20 cm surface area was marked out and excavated to a depth of 1.5 cm totalling 600 cm<sup>3</sup> before collecting in a sealable plastic bag. 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 h.

### 2.2.2. 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 and Herrick \(1952\)](#). Samples were collected into 30 g containers to ensure uniformity. After removing large rocks and debris, the 30 g sample of soil was mixed with 100 ml of water and 1 drop of anionic surfactant (Fairy Liquid™) in order to ensure egg and soil separation. The sample was thoroughly mixed and left to stand for 24 h in order to ensure maximum egg recovery. The sample was then passed through sieves of decreasing apertures (500, 250, 125 and 40 µm) to filter out large soil particles and retain eggs. The eggs retained on the 40 µm sieve were recovered using a fine spray of water and transferred to a 500 ml-measuring beaker. The egg-water mixture was then transferred to a 100 ml-measuring cylinder in order to facilitate sedimentation. The sample was left to settle for 2 h before removing the excess water with a 100 ml syringe until a 15 ml water–soil mixture remained. This was then transferred to a 15 ml conical centrifuge tube before being centrifuged at 1500 rpm (554rcf (G)) for 3 min (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 15 ml volume. The sample was then spun again at 1500 rpm for 3 min to allow eggs and larvae to float to the top of the test tube. A 1 ml aliquot was then transferred to a Nematode Counting Slide (Chalex Corp.) before examination under a microscope at 100× magnification. The number of eggs/ml was then multiplied by 30(g) to give an estimation of the total number of eggs in the original sample.

### 2.2.3. 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.

### 2.2.4. 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<sup>2</sup>.

### 2.2.5. 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 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 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. \(1992\)](#).

### 2.2.6. 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 three to four herbage plucks were collected every 2 feet. Black plastic bags were filled with enough grass to total 1 kg 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 h to ensure optimum larval separation. After 2 h, larger plant material was removed and the remaining liquid was poured through sieves of decreasing apertures (250, 125 and 40 µm). The larvae-containing residue on the 40 µm sieve was washed and collected into a 15 ml conical test tube and shaken for 10 s. After several seconds, a 1 ml 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.

### 2.2.7. 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

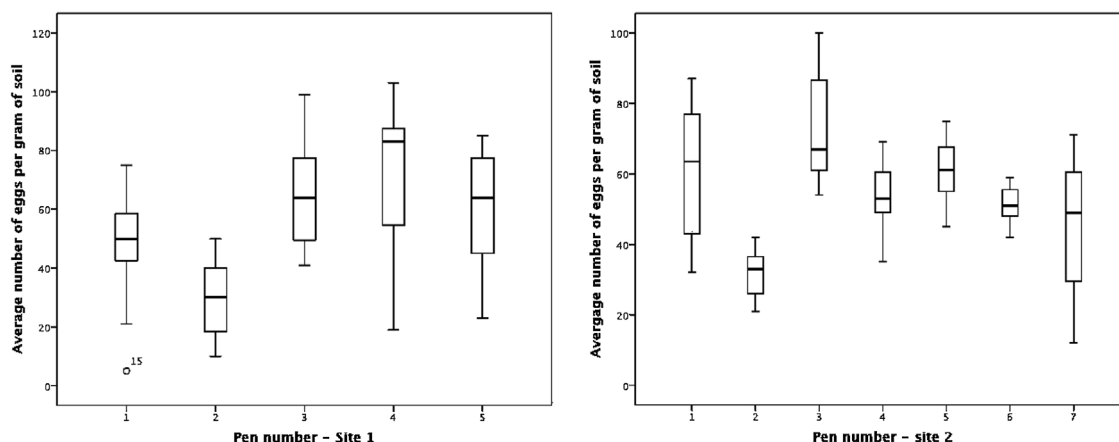


Fig. 1. Average number of eggs per gram of soil within release pens between sites.

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.

### 2.2.8. Climatological factors

Air and soil temperature were recorded at both sites using Tinytag™ Plus 2 (TGP-4020) Data Loggers measuring at 15-min intervals throughout the duration of the study. Soil temperature was measured at a depth of 2-in. using a Tinytag™ (PB-5002-1M5) Thermistor Probe. Relative humidity was also measured at 15-min 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.

### 2.3. Statistical analysis

All data were analysed using SPSS (20) for Macintosh. Data were tested for normality using the standard One-Sample Kolomogrov–Smirnov 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 (Fig. 1).

## 3. Results

### 3.1. 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 Figs. 2 and 3).

#### 3.1.1. Background levels of disease: influence of soil moisture, years in use and stocking density

**3.1.1.1. Soil moisture.** The relationship between soil moisture content and the number of eggs per gram of soil

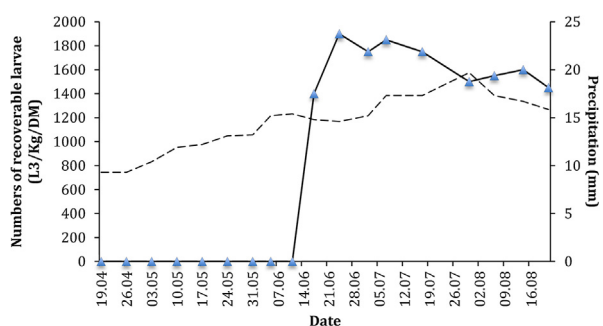
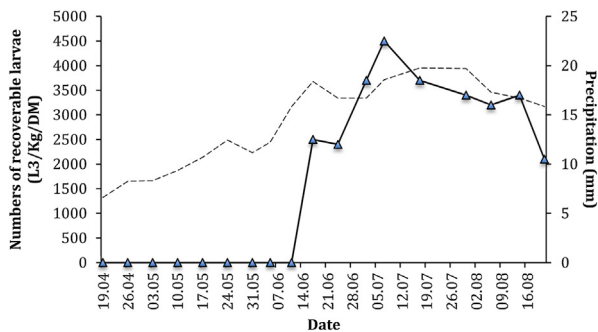


Fig. 2. Numbers of *S. trachea* larvae (solid line) recovered per kg of dry matter (L3/kg/DM) and weekly precipitation (dashed line) in mm at site 1.





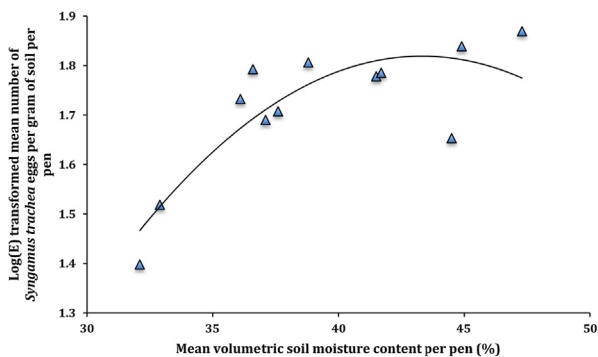
**Fig. 3.** Numbers of *S. trachea* larvae (solid line) recovered per kg of dry matter (L3/kg/DM) and weekly precipitation (dashed line) in mm at site 2.

per pen was not linear, however the fitting of 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 (Fig. 4).

**3.1.1.2. Pen age and stocking density.** Stocking density, expressed as birds/m<sup>2</sup>, 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.

### 3.1.2. 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



**Fig. 4.** Relationship between volumetric soil moisture content and the abundance of Log (Base-E) transformed number of eggs per gram of soil per pen.

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.

### 3.1.3. Numbers of eggs and larvae over time

The numbers of recoverable larvae differed significantly between sites ( $t^{12} = 4.213$ ,  $p = 0.001$ ) with an average of  $1730 \pm 195.57$  L3/kg/DM recovered at site 1, and  $3211 \pm 759.02$  L3/kg/DM at site 2. In general, the numbers of recoverable larvae increased with time at both site 1 ( $R = 0.79$ ,  $p = 0.001$ ) and site 2 ( $R = 0.78$ ,  $p = 0.001$ ), 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).

### 3.1.4. 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 ( $R = 0.636$ ,  $p = 0.017$ ). Similarly, temperature ( $R = 0.751$ ,  $p = 0.001$ ) and humidity ( $R = 0.625$ ,  $p = 0.006$ ) influenced larval development and recovery at site 2. 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.

### 3.1.5. 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 ( $R = 0.642$ ,  $p = 0.013$ ) and site 2 ( $R = 0.951$ ,  $p = 0.001$ ), 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.

### 3.1.6. 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 ( $F_{2,4} = 47.020$ ,  $R = 0.979$ ,  $p = 0.002$ ).

## 4. Discussion

Parasite development, and therefore transmission and infection success is dependant 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 and 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 and Todd, 1964; Rossanigo and Gruner, 1994; Khadijah et al., 2013a, 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 and Todd, 1964; Moss et al., 1993; Saunders et al., 2000; 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 caninum* (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 and +3 °C; however, eggs kept at the lower end of the development threshold did not survive for prolonged periods when exposed to fluctuating temperatures of +12 to +24 °C and humidity ranging from 36% to 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 *Ascaridia 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 the best of 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 and Westcott, 1986; 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 (Uriate and 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 one pair of gapes (Mean  $6.4 \pm 9.6$  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).

## 5. 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.

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