Body condition is negatively associated with infection with Syngamus trachea in the ring-necked pheasant (phasianus colchicus)


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

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Summary

The role that parasites play in regulating animal populations is debated, however recent research hints at their pervasiveness among free-living animal populations. Parasites exert both direct and indirect effects on host populations, and can act to regulate populations. The Ring-necked pheasant is an important game-bird species in the UK, and large numbers of birds are released annually. The impact of the ubiquitous tracheal nematode, *Syngamus trachea* on pheasant populations through effects on host condition was assessed on two pheasant estates in the south west of England. Pheasants infected with *S. trachea* demonstrated a significant reduction in host condition compared with uninfected controls, with as few as one pair of worms per bird. Although there was no difference in worm burden between sexes, analysis of regression slopes revealed there was a significant difference between sexes in the magnitude of the effect of increasing worm burden on host condition, with detectable effects observed in hosts with one and three pairs of worms for males and females respectively. The observed reductions in host condition in birds with even sub-clinical infections could be the cause of poor reproductive success and survival of pheasants post-release.

Keywords

*Phasianus colchicus; Pheasant; Body condition; Gapeworm; Syngamus trachea; Reproduction*
**Introduction**

Parasites are well known to play an important role in regulating host population dynamics (Tompkins & Begon, 1999; Irvine *et al.*, 2006; Dunn *et al.*, 2012; Watson, 2013; Granoth-Wilding *et al.*, 2015), although there has been some debate as to the relative importance of predators compared with parasites (Irvine, 2000; Moss & Watson, 2001). 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.*, 2015). 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., 1992). *Syngamus trachea*, for example, is a parasitic-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., 2015). 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.

### 2. Materials and method

#### 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 manufacturers 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.

#### 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.

2.3 Worm recovery and body condition assessment

Adult pheasants were weighed to the nearest 0.1 g 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.

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.
The transformed data were then assessed for non-constant error variance, which confirmed that the power transformation was successful ($\chi^2 = 0.54$, $df = 1$, $p = 0.51$). Differences in parasite burden between sexes (including zero counts) were assessed using Welch’s $t$-test for unequal samples.

3. Results

3.1 Prevalence of S. trachea – Pheasants

Parasite count data were significantly different from the estimated Poisson distribution ($\chi^2 = 2175$, d.f = 153, $p = <0.001$) and comparison of models demonstrated the data were aggregated, and consistent with the negative binomial distribution ($\chi^2 = 4.87$, 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 ($t_{35.78} = -1.26$, $p = 0.21$).

3.2 Effect of S. trachea on body condition

Worm burden and the associated effects on pheasant body condition are presented in tables 1 and 2. The regression of log parasite burden on body condition revealed a significant inverse relationship between increasing worm burden and body condition in pheasants ($F_{3,176}^{3.176} = 216.2$, $R^2 = 0.78$, $P = <0.001$). 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 ($t_{176}^{176} = 2.38$, $p = 0.01$).
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.

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 poult, 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.

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.*, 1992). 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.*, 2015). 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., 1992; 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 (Getings et al., 2015). 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 (Getings et al., 2015). 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 *Hexamitisa* 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, 200) 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.

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.

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