# Blood parasite infection linked to condition of spring-migrating Lesser Scaup (*Aythya affinis*)

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Blood parasite infection linked to condition of spring-migrating Lesser Scaup

(Aythya affinis)

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Blood parasite infection linked to condition of spring-migrating Lesser Scaup (*Aythya affinis*)

Abstract:

Numerous organisms exhibit carry-over effects, in which previous environmental conditions impact current performance. For example, reproductive output for many migratory birds can be impacted by events during the preceding migration. Lesser Scaup (*Aythya affinis* (Eyton, 1838)—hereafter scaup) declined dramatically during 1970s–2000s, and there is evidence linking reduced reproductive output to reduced body condition during spring migration. In addition to food availability and quality, haemosporidian parasites (*Plasmodium* spp., *Haemoproteus* spp.) may be associated with condition of spring-migrating birds. We examined whether haemosporidian parasite infection status was linked to measures of size (mass, wing, tarsus and keel length) and condition (body fat, size-corrected mass, wing-load) in female spring-migrating scaup. Infection prevalence varied by year (21.7% in 2014; 47.1% in 2015), and percent body fat was negatively associated with the probability of infection. Body fat levels declined from 2014 to 2015, but at a similar rate for infected and uninfected birds. This pattern suggests that the increased prevalence in 2015 may have been related to the greater proportion of poor-condition birds being more susceptible to infection or recrudescence of latent infections. In light of forecasted range shifts/expansions of avian malaria vectors, the impact of haemosporidian parasites on migratory waterfowl condition warrants further investigation.

Keywords: avian malaria, *Aythya affinis*, blood parasite, body fat, Lesser Scaup, migration, spring condition hypothesis, waterfowl
Introduction:

An organism’s performance at any given time is influenced by current environmental conditions, as well as previous environmental conditions that resulted in persisting changes to the organism’s somatic or epigenetic state. These “carry-over effects” can occur across life-history or developmental stages, and physiological states, and are widespread among wild organisms (O’Connor et al. 2014; Sedinger and Alisauskas 2014). For migratory species, conditions or experiences during migration can have persistent effects, especially when the energetically demanding breeding period follows migration. Waterfowl in particular face myriad challenges during migration, including elevated energetic demands, predation, disturbance from hunting and other sources, inclement weather, and finding suitable stopover sites in regions with high wetland loss rates (Klaassen et al. 2012). Body condition during spring migration can have important fitness ramifications for birds, especially for species that depend heavily upon nutrient reserves for egg production, such as ducks and geese (Ryder 1970; Afton and Ankney 1991; Alisauskas and Ankney 1992; Anteau and Afton 2004). Many species of waterfowl acquire some or most of the resources they will use during the breeding periods during winter and spring migration and may be particularly sensitive to carry-over effects (Heitmeyer and Fredrickson 1981; Anteau and Afton 2009; Sedinger and Alisauskas 2014). Waterfowl arriving on the breeding grounds in poor condition may be unable to lay as many eggs (Anteau and Afton 2004) or may have delayed nesting dates (Afton and Anderson 2001) with resulting negative effects on reproductive output (e.g. Rohwer 1992). There is some indication that species-level declines in North American waterfowl are linked to decreased body condition during spring migration, with a subsequent reduction in reproductive output (Austin et al. 2000; Anteau and Afton 2004; 2006; 2009).
The breeding population of Lesser Scaup (*Aythya affinis* (Eyton, 1838); hereafter scaup) has undergone a dramatic reduction over the past 50 years, from an estimated size of ~8 million in 1972, to a low of 3.2 million in 2006. Work by Anteau and Afton (2004; 2009) revealed that female scaup body condition, particularly lipid reserves, had declined in the upper Midwest from the early 1980’s to the early 2000’s. Based on prior work demonstrating that lipid reserves are an important nutrient limiting scaup reproductive output (Afton and Ankney 1991; Esler et al. 2001), the decline in foraging habitat quality likely resulted in reduced reproductive output (Anteau and Afton 2004; 2009; 2011). A few non-mutually exclusive hypotheses have been proposed to explain the decline in condition and reproductive output of scaup over time, including reduced quantity and quality of spring stopover sites (Anteau and Afton 2004), increases in contaminants (Custer et al. 2003; Anteau et al. 2007; Weegman and Weegman 2007), and elevated helminth burdens (England et al. 2017). However, no single factor has been identified as the definitive cause of the population decline (DeVink et al. 2008).

Another important factor that could influence scaup condition during spring migration and the subsequent breeding period is infection with haemosporidian blood parasites (*Haemoproteus* spp. and *Plasmodium* spp.). Haemosporidian parasites are ubiquitous among most wild bird populations (Valkiunas 2005), but their effects on hosts can vary widely depending on the specific host-parasite pairing as well as the sex and condition of the host (Knowles et al. 2009; Isaksson et al. 2013; Ellis et al. 2014; Calero-Riestra and Garcia 2016). There is evidence that waterfowl infected with hematozoan parasites (haemosporidians and leukocytozoans) are smaller and have reduced body condition (Fleskes et al. 2017), but the direction of cause and effect is unclear (Flint and Franson 2009; Fleskes et al. 2017). The association between blood parasite infection and body condition in waterfowl reflects one of two
non-mutually exclusive scenarios: 1) birds in poor condition are more susceptible to infection or recrudescence of a latent infection (e.g. Valkiunas 2005) due to reduced immunocompetence (sensu Flint and Franson 2009), or 2) infection with the parasites leads to reduced condition as a result of elevated energetic requirements involved in fighting (or tolerating) the infection (sensu Latorre-Margalef et al. 2009). Under both scenarios, infection status can provide information about the condition of the bird that may be important for its fitness prospects.

We examined aspects of size and condition (morphometrics, percent body fat, wing-loading, mass) and haemosporidian infection in spring-migrating female scaup at sites in Illinois and Wisconsin to help determine if infection could be an important driver of female condition, and subsequently important for reproductive output. Based on prior work demonstrating negative relationships between blood parasites and morphometric measures in wintering ducks (Fleskes et al. 2017), we predicted that birds infected with haemosporidian parasites would be smaller and in poorer body condition. To date, little work has been conducted on blood parasites of spring-migrating waterfowl and none has been done on scaup (c.f. Reeves et al. 2015; Meixell et al. 2016). Data linking parasite infection status and indices of condition may provide important insight into the factors that are negatively impacting scaup populations.

Methods:

We collected female scaup \((n=130)\) at 24 traditionally important stopover sites along the Illinois and upper Mississippi rivers, USA, from 10 February – 20 April 2014 and 11 March – 12 April 2015 (Fig. 1). We selected collection sites using historical data, ebird reports, available aerial survey data, and consultation with natural resource managers throughout the Midwest. Collection
sites were grouped into four distinct regions based on habitat type, physiography, and latitude (Fig. 1). Apparently healthy scaup that were observed (i.e., via spotting scope or binoculars) foraging were experimentally collected using a shotgun and sneak boat, without the aid of decoys or bait (Callicutt et al. 2011). Blood was collected immediately after collection by cardiac puncture, placed into a 1.5 ml microcentrifuge tube treated with EDTA and centrifuged for 10 minutes (1500 rpm). The plasma was removed by pipetting, and the red blood cells were frozen at approximately −20 °C for subsequent screening of haemosporidian parasites. We measured keel, tarsus and wing length (length of the closed wing chord to nearest mm), and post-eviscerated body mass (using a Pesola scale ±20g) to control for differences due to recent foraging bouts. After removal of the gastrointestinal tract, bill, feet, and feathers, each carcass was milled, flash frozen, and homogenized in an industrial blender. Following Novakofski et al. (1989), a 10g subsample of the homogenized, powdered carcass was oven dried at 110°C for 24-hr to obtain percent moisture. Body fat levels (measured as % lipids) were determined by extracting lipids from the oven dried sample using an azeotropic mixture of chloroform:methanol, drying the sample, and weighing again. We divided post-eviscerated mass by wing length² to estimate the energetic requirements of flight (i.e., wing-loading). Additionally, we divided the post-eviscerated mass by tarsus length² to create an index of body condition (Labocha and Hayes 2012). Age (i.e., second year [SY] or after second year [ASY]) was determined based on iris color (Trauger 1974) and wing characteristics (Carney 1992).

_Haemosporidian parasite assessment:_

Approximately 15µl of the thawed blood was added to a 500µl lysis buffer (Longmire 1997). We then added 5µl of 20mg/ml proteinase K solution (MidSci cat# IB05406) to each sample, and
incubated them overnight at 60°C overnight in a hot water bath. We extracted genomic DNA from the samples using ammonium acetate-isopropanol (Svensson and Ricklefs 2009). For details on PCR technique, see Fecchio et al. (2013). Briefly, we screened the samples for the presence of avian haemosporidian blood parasites of the genera *Plasmodium* and *Haemoproteus* (order Haemosporida) using a PCR protocol designed to amplify a 154bp fragment of rRNA-coding parasite mitochondrial DNA (Fallon et al. 2003). Samples were run out on a 1.0% agarose gel stained with GelGreen® (Biotium, Fremont, CA, USA), and imaged under UV light to obtain infection status.

**Statistical Analyses:**

To examine the associations between each measure of body condition (as the dependent variable) and parasite status (as the focal independent variable), we ran individual general linear models (GLMs) that included ordinal date, year, age, and region as fixed effects (Table S1). We performed simple model reductions in which non-focal fixed effects that were significant (\(p \leq 0.05\)) were retained in the final model along with parasite status (Table 1). For models in which region was retained, we compared values among the four regions using Tukey’s post-hoc pairwise tests for multiple comparisons. We assessed levels of covariance between parasite status and the other fixed effects using Pearson’s chi-squared tests and nominal logistic regressions. Year significantly covaried with parasite status (see below), so we performed secondary analyses within year for dependent variables in which parasite status was significant in order to assess if parasite status could be confounded with some aspect of year. We confirmed normality of residuals and homogeneity of variance per model assumptions and designated \(\alpha = 0.05\). All analyses were conducted in SAS 9.4 and JMP 10.0 (Cary, NC).
Results:

We collected 60 scaup in 2014 (9 SY, 51 ASY) and 70 scaup in 2015 (12 SY, 58 ASY). Due to logistical constraints, we were unable to assess percent body fat for the 11 birds collected from one region (SI-Fig. 1), thus sample size for percent fat is 119, and for all others is 130 (Table 1). The proportion of scaup classified as SY did not differ between years (15% in 2014, and 17.1% in 2015: Pearson’s test: $\chi^2=0.11, p=0.74$). Haemosporidian parasite prevalence averaged 35.4% across years, but was greater in 2015 (47.1%) than in 2014 (21.7%) (Pearson’s test: $\chi^2=9.17, p<0.01$). Infection status did not differ by age (35.8% for ASY, 33.3% for SY: Pearson’s test: $\chi^2=0.05, p=0.83$), region (Pearson’s test: $\chi^2=1.59, p=0.66$), or ordinal date (Nominal logistic regression: $\chi^2=0.01, p=0.92$).

Body fat levels were associated with both parasite infection status and ordinal date (Table 1), in which infected individuals had lower fat levels (Fig. 2), and birds collected earlier had greater fat levels (Fig. 3). When the data were divided by year, fat levels did not significantly differ by parasite status in either year (2014: $t$-test: $t=0.66, p=0.51$; 2015: $t$-test: $t=1.40, p=0.17$), although the trend was in the same direction both years (Fig. 2). There was also a trend for fat levels to decline between years (15.8% in 2014, and 13.6% in 2015), and this decline was similar for both infected and uninfected birds (Infected: 2.1% decline; Uninfected: 1.6% decline) (Fig. 2). Mass did not vary by parasite status (Table 1), but there were significant associations between mass and ordinal date, age, and region (Table 1), in which birds collected earlier in the season were heavier (Fig. 3), and ASY birds were heavier than SY birds (SY=521.5g, ASY=549.0g). There was no difference in ordinal date for the age classes ($t$-test: $t=0.23, p=0.82$), indicating temporal variation in mass was not a result of more SY birds being collected later in the season.
Post-hoc analyses of mass by region indicated there were no significant differences in mass between collection regions ($p>0.2$ for all comparisons). Wing length was positively associated with parasite status (Table 1), and this pattern appears to be driven by scaup collected in 2015, in which infected birds had significantly longer wings than uninfected birds ($t$-test: $t=2.51$, $p=0.01$; Fig. 4), whereas there was no difference in 2014 ($t$-test: $t=0.37$, $p=0.72$; Fig. 4). Tarsus length differed by year (Table 1), in that birds collected in 2015 had longer tarsi (39.77 mm in 2014, and 41.26 mm in 2015), and keel length was inversely associated with ordinal date (Table 1). Wingload was associated with parasite status (Table 1), in which infected birds were lighter per unit wing. This pattern was the same in both years, although not significant in either year (2014: $t$-test: $t=1.73$, $p=0.09$; 2015: $t$-test: $t=1.66$, $p=0.10$). The final body condition model indicated there were no associations between any of the fixed effects and body condition (Table 1).

Discussion:

Lesser Scaup have experienced a dramatic population decline during the past half century, but the specific mechanisms leading to this reduction are not well understood (Austin et al. 2000; 2006; Anteau et al. 2014). We explored infection rates of scaup with haemosporidian blood parasites compared to body condition during the important period of spring migration. We found evidence that parasite infection status was linked to multiple size and condition-related traits in spring-migrating scaup. Thus infection with haemosporidian blood parasites could be associated with reduced reproductive output through the mechanism of reduced body condition consistent with the spring condition hypothesis. Whether infection status alone or in conjunction with changes in body condition due to other factors is more important is not clear, but they likely act in an additive fashion to negatively impact reproductive output (England et al. 2018).
Previous work found that body fat levels were the most important nutrient determining scaup egg output (Afton and Ankney 1991; Esler et al. 2001). We determined that birds infected with haemosporidian blood parasites had reduced body fat levels compared to uninfected birds. This association between infection status and body fat levels indicates that there could be a link between haemosporidian infection and egg production. However, we were unable to determine whether infection resulted in reduced body fat content or if birds with reduced body fat were more likely to be infected, but we have circumstantial evidence to support the latter. For example, fat levels were lower in 2015 (13.6% body fat) than in 2014 (15.8% body fat), concomitant with an increase in haemosporidian prevalence from 2014 to 2015 (21.7% to 47.1%, respectively). If infection status was driving variation in fat levels, we would expect to see comparable fat levels in uninfected birds in 2014 and 2015, but fat levels were similarly reduced in infected and uninfected birds in 2015 compared to 2014 (Fig. 2). This suggests that the population-level reduction in body fat from 2014 to 2015 was indicative of birds being in poorer condition in 2015, and thus more susceptible to becoming infected or to experiencing recrudescence of latent infections (sensu Flint and Franson 2009). Vernal recrudescence is a widespread phenomenon in temperate species, in which dormant infections re-emerge in the spring during periods of elevated energetic expenditure and reduced immune function (Valkunias 2005).

If birds in poor body condition are more likely to become infected with haemosporidian parasites or experience recrudescence, this could exacerbate the impacts of their reduced body condition. Recent work indicates that haemosporidian parasites can have deleterious effects on wild birds (e.g. Knowles et al. 2010; Lachish et al. 2011), and that even in cases where effects may not be obvious, infection may lead to cryptic decreases in fitness via elevated rates of
telomere shortening and potentially reduced longevity (Asghar et al. 2015). Such parasite-related fitness effects would be in addition to the negative effects of low body fat composition and could result in scaup experiencing even greater reductions in reproductive output, although prior work on reproductive output and lipid reserves in scaup did not account for infection status (Afton and Ankney 1991; Anteau and Afton 2004). More controlled or experimental studies would be needed to firmly establish a cause and effect relationship.

In addition to body fat levels, haemosporidian infection was associated with wing length, although in the opposite direction to what we predicted. Our prediction was based on prior work in waterfowl documenting negative associations between wing length and blood parasite infection status (Fleskes et al. 2017). There is evidence that larger individuals can be infected at higher rates by vectored parasites (Port et al. 1980; Logan et al. 2010) due to their larger surface area and potentially increased heat and CO$_2$ signatures (sensu Fernandez-Grandon et al. 2015). We do not believe that to be the case here because none of the more direct measures of body size (mass, tarsus or keel length) were associated with infection status. Another possibility is that wing length is linked to age and exposure risk. SY birds have shorter wings than ASY birds in many species (e.g. Alatalo et al. 1984), and younger birds can exhibit lower parasite prevalence as a result of less exposure time to parasites and vectors (e.g. Deviche et al. 2001). However, we found no age-related differences in either wing length or parasite infection status. Individuals with longer wings may exhibit different migration patterns, and could be longer-distance migrants (e.g. Bowlin and Wikelski 2008; Flockhart et al. 2017). If so, these birds may have higher energetic demands, which could lead to reduced immune function, and elevated risk of being infected or experiencing recrudescence of the malaria parasites (Owen and Moore 2006; 2008). We also found that infected birds had reduced wing-loads, but this was not surprising
given the positive association between infection status and wing length and the negative association between infection and body fat.

Scaup collected earlier in migration were larger (based on keel length), and weighed more than those sampled later. This pattern is consistent with work done in many species in which earlier-migrating individuals are in better condition that later-arriving ones (Marra et al. 1998; Kokko 1999). Timing of migration has also been linked to timing of nest initiation and reproductive success (Lozano et al. 1996; Kokko 1999; Gienapp and Bregnballe 2012), and work done with scaup and other waterfowl suggests that late-arriving, and late-breeding birds produce fewer offspring than earlier-arriving birds (Afton and Anderson 2001; Rohwer 1992). We did not find a direct relationship between infection status and collection date, but the strong links between infection status and both body fat and wing-loading indicate there could be an indirect relationship between infection status and timing of migration.

There was a significant difference in tarsus length between years, in which tarsi were longer in 2015. This was the only size-related trait in which we found a significant difference between years, although there was a trend for increased wing length as well (Table S1). The lack of a concurrent increase in mass from 2014 to 2015 is likely the result of overall lower fat levels in 2015. Indeed, there were trends for both body fat and body condition to decrease in 2015 (Table S1). It is possible that the overall decline in body condition resulted in size-specific effects in which smaller birds were less able to cope with poor food resources and did not undertake migration, or experienced higher mortality rates between years. Increasing the sample sizes and tracking morphological and condition parameters during winter, spring and summer would be needed to gain better insight into these patterns.
Here we have demonstrated that female scaup infected with haemosporidian parasites were in poorer condition than those that were not infected, and this pattern is likely driven by changes in susceptibility or recrudescence as a result of condition-related reduced immune function. If birds in poor condition are more likely to become infected or exhibit recrudescence (or both), and scaup are in poorer condition than they were historically, haemosporidian parasite infections could impact scaup populations in multiple ways. In addition to impacting the breeding success of birds already infected, an increase in the proportion of parasite-positive birds can lead to increased rates of transmission for the population and community. Moreover, many climate-change predictions indicate that avian blood parasite vectors will experience range shifts and expansions, and that many birds may face new and/or greater pressure from vectored pathogens (Garamszegi 2011; Perez-Rodriquez et al. 2014). The interplay between food resources, condition and parasite status may play an important role in regulating scaup population dynamics and should be the focus of future research (sensu England et al. 2017).

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Table 1. Results from general linear models examining associations between measures of size and body condition, and haemosporidian parasite (Plasmodium spp. and Haemoproteus spp.) infection status in female spring-migrating Lesser Scaup (Aythya affinis) collected throughout the upper Midwest USA during 2014 and 2015. All initial models also included ordinal date, year, age (second year or after second year), and capture region (see Fig. 1) as fixed effects, and those that were significant ($p \leq 0.05$) were retained in the final model. Effects that are significant in the reduced models are bolded.

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Figure captions:

Figure 1. Map depicting the four regions where female Lesser Scaup (*Aythya affinis*) were collected during spring migration in 2014 and 2015 across the upper Midwest (A), and detailed maps showing collection locations in each region (B), including Southern Illinois (SI), Illinois River Valley (IRV), Central Mississippi River Valley (CMRV), and the Upper Mississippi River Valley (UMRV).

Figure 2. Mean (± SE) percent body fat by year and haemosporidian infection status in spring-migrating female Lesser Scaup (*Aythya affinis*) collected throughout the upper Midwest USA during 2014 and 2015.

Figure 3. Percent body fat by ordinal date and haemosporidian parasite infection status (dashed line reflects the slope for uninfected birds, and the solid line reflects the slope for infected birds) in spring-migrating female Lesser Scaup (*Aythya affinis*) collected throughout the upper Midwest USA during 2014 and 2015.

Figure 4. Mean (± SE) wing length by haemosporidian infection status in spring-migrating female Lesser Scaup (*Aythya affinis*) collected throughout the upper Midwest USA during 2014 and 2015.
% Body Fat

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<td>n=33</td>
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</tbody>
</table>

https://mc06.manuscriptcentral.com/cjz-pubs
Wing Length (mm)

Uninfected
Infected

2014
n=47
n=13

2015
n=37
n=33

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