

# Seasonal and Age-Related Changes in Blood Parasite Prevalence in Dark-Eyed Juncos (*Junco hyemalis*, Aves, Passeriformes)

PIERRE DEVICHE,<sup>1\*</sup> ELLIS C. GREINER,<sup>2</sup> AND XAVIER MANTECA<sup>3</sup>

<sup>1</sup>Department of Biology, Arizona State University, Tempe, Arizona, 85287-1501

<sup>2</sup>Department of Pathobiology, University of Florida, Gainesville, Florida, 32611-0880

<sup>3</sup>School of Veterinary Science, Universitat Autònoma de Barcelona, Barcelona, Spain

**ABSTRACT** We determined seasonal changes in blood parasite infections in a free-living population of Dark-eyed Juncos (*Junco hyemalis*) breeding in interior Alaska (65 °N; 148 °W). The common parasites found in blood smears were *Leucocytozoon fringillinarum* (56%), *Trypanosoma avium* (33%), and *Haemoproteus fringillae* (9%). In males, parasite prevalences were relatively high at arrival on breeding grounds and increased during the breeding season. Intensity of infection with *Leucocytozoon* also increased between spring and summer, and then decreased at the time of migration (September). This decrease did not occur in adult females. Elevated prevalences during the breeding season probably reflected the addition of new cases via vector activity to positive status resulting from spring relapse. We observed neither an association between parasite species nor a consistent relationship between parasite intensity and body condition. To further study relationships between reproductive system activity and parasite infections, we compared prevalences in adult males that were undergoing their first cycle of gonadal development and regression (males in their second calendar year, or SY) with those of older males (males in their third or more calendar year, i.e., after-second-year males or ASY). Circulating testosterone concentrations declined in both groups between arrival on breeding grounds (end of April–early May) and the end of the reproductive period (July), and they were higher in May in ASY than in SY males. At the peak of the breeding season (June), ASY males also had a higher parasite prevalence than SY males. This difference may have resulted from immunosuppressive effects of gonadal hormones and/or from behavioral differences between SY and ASY males such that older males were exposed to more insect vectors than younger males. *J. Exp. Zool.* 289:456–466, 2001. © 2001 Wiley-Liss, Inc.

Interest in the ecological and evolutionary implications of parasite infections has grown rapidly in recent years, largely as a result of Hamilton and Zuk's ('82) hypothesis that parasites play a role in sexual selection. This role currently remains contentious (Clayton et al., '92) because results that are both consistent (Møller, '90; Zuk et al., '90; Clayton, '91) and inconsistent (Hausfater et al., '90; Weatherhead, '90; Weatherhead et al., '91) with Hamilton and Zuk's ('82) hypothesis have been obtained. Studies on this subject are complicated by the fact that parasite infections can vary as a function of numerous factors including age, sex, densities of vectors, long-term habitat changes and other environmental variables, as well as breeding effort (see below), that potentially interact with the host's susceptibility to infections (Bennett et al., '82; Norris et al., '94; Weatherhead

and Bennett, '91, '92; Seutin, '94; Merilä et al., '95; Wiehn and Korpimäki, '98).

One underlying assumption of Hamilton and Zuk's hypothesis is that parasites exert deleterious effects on their hosts. Indeed, the traditional view that parasites are relatively benign (Cox, '89) has been repeatedly challenged (Toft and Karter, '90), and parasites are now regarded as having potentially negative effects on the survival and fitness of both mammalian (Toft and Aeschlimann, '91) and avian (Atkinson and van Riper, '91; Raidal and Jaensch, '00) hosts. For example, chronic infections may cause nutritional stress and mortal-

\*Correspondence to: Pierre Deviche, Department of Biology, Arizona State University, PO Box 871501, Tempe, AZ 85287-1501.  
E-mail: deviche@asu.edu

Received 16 December 1999; Accepted 6 November 2000

ity by decreasing foraging ability (Jenkins et al., '63). Parasites can also increase susceptibility to predation (Vaughn and Coble, '75) and high parasite intensity is in some cases associated with reduced expression of sexually selected traits (Thompson et al., '97; but see Seutin, '94). Overall, however, the impact of parasites on their hosts and the physiological mechanism underlying this impact remain poorly known (Toft, '91).

Experimental evidence has been obtained supporting the hypothesis of a trade-off between reproductive effort and the efficiency of the immune responses that control parasite infection. Specifically, these responses are apparently compromised and parasite infections increase during the reproductive period, when breeding adults devote considerable time and energy to activities such as food provisioning to the young and nest and territory defense, or when breeding effort is experimentally increased (Ots and Hõrak, '96; Norris et al., '94; Weatherhead and Bennett, '91, '92; Rintimäki et al., '99). The idea of a trade-off between breeding effort and efficiency of immune responses is consistent with results showing that mounting these responses can be metabolically costly (Demas et al., '97). Reproductive (gonadal) hormones may play an important role in the modulation of immune response that is associated with the reproductive period. In some studies these hormones apparently depressed the immune system activity (Hillgarth and Wingfield, '97; Mooradian et al., '87; Folstad and Karter, '92; Casto et al., '99; but see Braude et al., '99 for an alternative hypothesis), and in most middle and high latitude bird species gonadal hormone secretion is elevated during the breeding season (Deviche et al., '00; Morton et al., '90; Wingfield and Farner, '78a,b).

To investigate the relationships between reproductive system activity and parasite infections, we studied haematozoan parasites in male and female Dark-eyed Juncos (*Junco hyemalis*, hereafter juncos) belonging to a population breeding in interior Alaska. Juncos from this population return to their breeding areas at the end of April-early May. Males arrive, on average, earlier than females, and most breeding activities (pair formation, courtship, nest construction, egg laying, incubation, and raising of young) take place between mid-May and early July. At this time the reproductive system rapidly involutes as birds become photorefractory (Deviche, '95). Prebasic molt takes place between early-mid-July and the end of August-early September, while birds are still on their breeding grounds. Most adults migrate during September.

We investigated whether prevalence and intensity of parasite infection change through the breeding season and we determined the distribution of parasites in the host population. As in another species (White-crowned Sparrow, *Zonotrichia leucophrys oriantha*: Morton et al., '90), adult male juncos differ with respect to their reproductive system development based on their age (Deviche et al., '00). Second-year males (SY: males sampled during their second calendar year and entering their first breeding season; see Pyle, '97 for terminology) have lower plasma testosterone (T) concentrations at the beginning of the breeding season and smaller testes and cloacal protuberances (CP, an androgen-dependent secondary sexual characteristic: Deviche, '92; Schwabl and Farner, '89) than older (ASY: males sampled during their third or more calendar year, i.e., after-second-year) males. Based on the results described above and on evidence that gonadal hormones can exert immunosuppressive effects, we hypothesized that parasite prevalence would be higher in both sexes during than before or after the breeding season and higher in ASY than SY males.

## MATERIALS AND METHODS

### *Subjects and sample collection*

We caught 255 adult juncos (males [M]:  $n = 211$ ; females [F]:  $n = 44$ ) from a local population around Fairbanks, Alaska, USA ( $65^{\circ}\text{N}$ ;  $148^{\circ}\text{W}$ ) using either Japanese mist nets or seed-baited Potter traps in 1997 ( $n = 209$ ) and 1998 ( $n = 46$ ). Birds were caught between the end of April, when they return from migration, and September, when they migrate from their breeding grounds. They were divided into five groups based on overall breeding stage of the sampled population: April to 5 May: arrival on breeding grounds and establishment of breeding territories (only males were captured,  $n = 49$ ); 5–31 May: beginning of the breeding season, when pairs are formed and nests built, and females lay eggs (only males were also captured during this phase;  $n = 51$ ); June: middle of the breeding season, when females incubate and young hatch (M:  $n = 56$ ; F:  $n = 11$ ); July: end of the breeding period, when young fledge and prebasic molt starts (M:  $n = 36$ ; F:  $n = 19$ ); September, fall migration (M:  $n = 19$ ; F:  $n = 14$ ). Hatching-year, fully grown juveniles were caught 3–9 August 1997 (M:  $n = 3$ ; F:  $n = 1$ ; unknown sex:  $n = 21$ ) and during fall migration (12–16 September 1997; M:  $n = 31$ ). Since blood parasites may show a diurnal periodicity (Gore et al., '82), birds were caught at approximately the same time

of day (09:00 to 13:00). Within minutes of capture, a blood sample (approximately 200  $\mu$ l) was collected from a brachial vein into heparinized microhematocrit tubes. Approximately 5  $\mu$ l of blood was used to prepare a blood smear on a microscope slide according to Harrison and Harrison ('86) and Bennett ('70). The remaining blood (adult males caught in summer 1997 only) was centrifuged (10 min at 3000 rpm in a refrigerated centrifuge) within hours, and the supernatant plasma was harvested using a Hamilton glass syringe and stored at  $-20^{\circ}\text{C}$ . Slides were individually labeled, air-dried, fixed for 5–10 min in 100% methanol, stained using the Giemsa procedure (Bennett, '70), cleared with xylene, and cover-slipped using Cytoseal 60. Representative slides were deposited in the U.S. National Parasite Collection, Beltsville, Maryland 20705 (accession no. 088310.00, 088311.00, 088317.01, and 088317.02). Birds were weighed to the nearest 0.1 g using a spring balance, adult male CP widths were measured to the nearest 0.1 mm with calipers, and time of capture to the nearest 10 min was noted. When possible, males caught between April and July were classified as SY or ASY based on plumage characteristics (Pyle, '97). In the fall, SY and ASY males have the same plumage and the two age classes could not be differentiated. Most adult females could not be aged.

#### *Smear examination*

Smears were examined at low (250 $\times$ ; 5 min) and high (400 $\times$ ; 10 min) magnification and birds were classified as negative or positive for various parasite types (see below). Parasite species were identified by examination of smears at 1000 $\times$  magnification under oil immersion, using previously published terminology (Peirce, '81; Bennett and Peirce, '88; Burrey-Caines and Bennett, '92; Bennett et al., '94). Prevalence of infection was defined as the percentage of infected individuals in a sample. To assess reliability of parasite detection between observers, 25 smears collected from adult males caught in June and July 1997 were analyzed independently by two of the authors. There was no difference between the two observers in the detection either of all parasites or of any parasite type ( $\chi^2$  or Fisher exact probability tests:  $P$ 's all  $> 0.15$ ). Intensity of infection with *Leucocytozoon* was defined as the number of these parasites per 100 visual fields at 400 $\times$  magnification; at least 200 different visual fields were counted in each slide. Thickness of blood smears on slides is often un-

even and sampled fields were, therefore, selected in a line extending from one end of the smear to the other (Weatherhead and Bennett, '91). We determined the prevalence, but not the intensity of *Trypanosoma* infection because the number of these parasites per slide was generally very low and counting these hematozoa on blood smears may not reliably estimate their density, as they tend to remain in the bone marrow (Levine, '85). *Haemoproteus* was detected in a relatively small proportion of the samples (see Results) and prevalence, but not intensity of infection with this parasite was, therefore, also measured.

#### *Plasma testosterone concentrations*

Total plasma T concentrations were measured using a commercial solid-phase radioimmunoassay system (Diagnostic Products Co., Los Angeles, CA) in samples collected from 98 adult males caught in 1997. Briefly, plasma (25  $\mu$ l) was added to T antibody-precoated plastic tubes.  $^{125}\text{I}$ -labelled T solution (1 ml; approximately 44,000 dpm) was added to all the tubes that were then incubated in a water bath at  $37^{\circ}\text{C}$  for three hours. The content of the tubes was decanted and the antibody-bound radioactivity was measured in a  $\gamma$  counter. Plasma T concentrations were calculated by reference to a standard curve generated by incubating samples containing known T concentrations (0.1 to 16 ng/ml) in the same conditions as the junco plasma samples. The antibody used in the assay has low ( $<5\%$ ) cross-reactivity with corticosterone, estradiol, progesterone, and  $5\alpha$ -dihydrotestosterone. A junco plasma dilution curve was parallel to the standard curve. T was undetectable in samples obtained from captive males that were either castrated or chronically exposed to artificially short photoperiod, as well as from most field-sampled adult breeding females, but levels of the steroid were elevated in males that were artificially photostimulated or received subcutaneous T-filled Silastic capsules (Gulledge and Deviche, '97; Deviche and Gulledge, '00; personal observations). Thus, the assay specifically detects T. All samples were assayed in duplicate and in a single series.

#### *Statistical analyses*

$\chi^2$  tests for independent samples and Fisher exact probability tests were used to determine differences in prevalence between age and sex groups and between months, as well as to study associations between parasite types. To analyze differences in *Leucocytozoon* infection intensity across

seasons within one sex, groups were compared using one-way analysis of variance (ANOVA) on ranks followed with multiple comparison tests (Dunn's method). Sex- or (in adult males) age-related differences in infection intensity across seasons were analyzed using two-way ANOVAs on ranked data followed with Student-Newman-Keuls multiple comparison tests. Two-way ANOVAs were used also to analyze age- and season-related variations in plasma T concentrations, body mass (BM), and CP widths. Data were presented as percentages of birds infected (prevalence), medians  $\pm$   $\frac{1}{2}$  interquartile interval (intensity of *Leucocytozoon* infection, CP), and means  $\pm$  standard deviation (BM, plasma T concentrations). Statistical significance level was in all cases set at  $\alpha = 0.05$ .

## RESULTS

### *Parasite identification*

The following parasite species were observed: *Trypanosoma avium*, *Leucocytozoon fringillinarum*, and *Haemoproteus fringillae*. Microfilariae were not identified any further.

### *Total and specific prevalence in adults*

Blood parasites were found in 67% of the smears prepared from adults. The most prevalent species was *L. fringillinarum* (56%) followed by *T. avium* (33%), *H. fringillae* (9%), and microfilariae (3%). Due to their low prevalence, microfilariae will not be discussed further.

Intensity of *L. fringillinarum* infection did not follow a normal distribution (Kolmogorov-Smirnov one-sample test:  $P < 0.001$ ). This parasite was detected in 49% of the individuals. Among the parasitized individuals, approximately 70% had one or two parasites/100 visual fields and only 5% of the birds had more than 10 parasites/100 visual fields (Fig. 1). The percentages of birds that were infected either with *L. fringillinarum* plus *T. avium*, with *L. fringillinarum* plus *H. fringillae*, with *T. avium* plus *H. fringillae*, or with the three parasite species were 23.1%, 5.5%, 3.5%, and 3.1%, respectively. These percentages did not differ statistically from those calculated assuming no association between parasite species (18.4%, 4.3%, 2.7%, and 1.6%, respectively;  $\chi^2$  tests,  $P$ 's  $> 0.2$ ).

### *Annual variation in parasite prevalence*

To determine whether parasite prevalence varied across years, we compared prevalence in adult birds sampled during two periods in 1997 and 1998: April to 10 May (1997, males only:  $n = 47$ ;

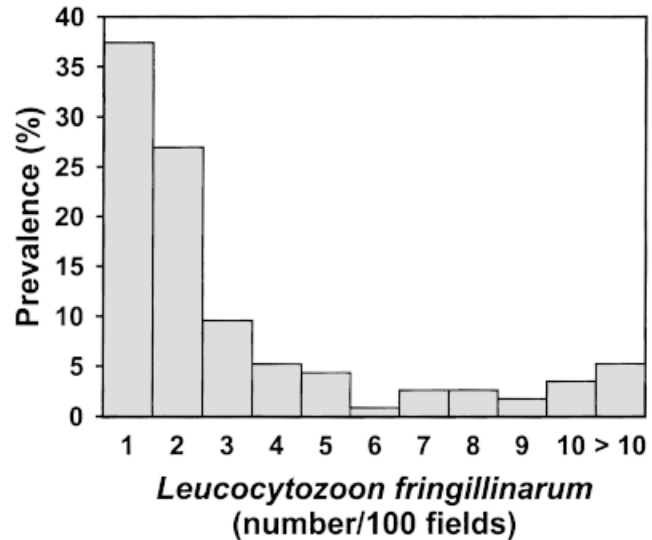


Fig. 1. Prevalence of infection (percentage of parasitized individuals) by *Leucocytozoon fringillinarum* (number of infected cells per 100 microscope individuals at 400 $\times$  magnification) in adult male and female Dark-eyed Juncos (*Junco hyemalis*;  $n = 255$ ) sampled in interior Alaska between April and September.

1998:  $n = 26$ ) and 20–30 June (1997:  $n = 21$ ; 1998:  $n = 20$ ). During the April-May period, birds had a higher overall parasite prevalence in 1998 (69.2%) than in 1997 (34.0%;  $\chi^2 = 6.98$ , d.f. = 1,  $P = 0.008$ ). This difference resulted from a higher *T. avium* prevalence in 1998 (30.8%) than in 1997 (4.3%; Fisher exact probability test:  $P = 0.003$ ). We observed no other difference between years for data collected April-10 May and parasite prevalence was similar in 10–20 June 1997 and 1998. Because year-to-year differences were limited to one sampling period and one parasite (*T. avium*), data obtained for the two years were combined in all subsequent analyses.

### *Seasonal changes in parasite prevalence and intensity in adults*

In adult males, overall parasite prevalence changed seasonally ( $\chi^2 = 12.64$ , d.f. = 3,  $P = 0.013$ ; Fig. 2). Pairwise comparisons between data obtained for consecutive sampling periods revealed one nearly significant difference (July  $>$  September: Fisher exact probability test:  $P = 0.054$ ). However, males had a higher parasite prevalence in July than April-early May ( $\chi^2 = 7.17$ , d.f. = 1,  $P = 0.007$ ) or mid-May (id.:  $\chi^2 = 6.45$ , d.f. = 1,  $P = 0.011$ ). Analysis of results obtained for each parasite indicated that *Leucocytozoon fringillinarum* and *Trypanosoma avium* prevalence varied sea-

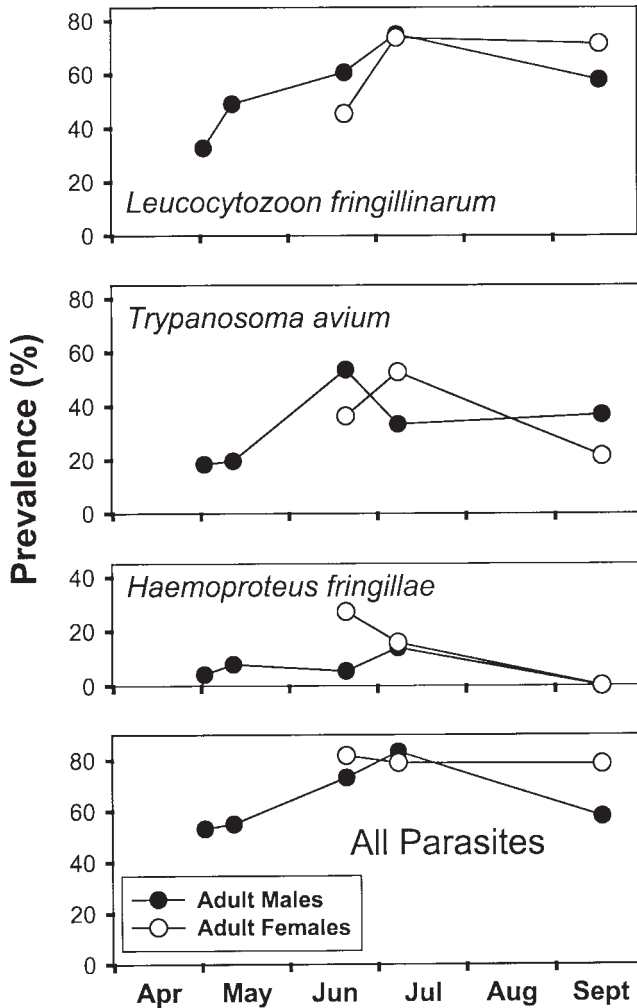


Fig. 2. Seasonal changes in prevalence of *Leucocytozoon fringillinarum*, *Trypanosoma avium*, *Haemoproteus fringillae*, and total blood parasites in adult male ( $n = 19$  to 56 individuals/point) and female ( $n = 11$  to 19/point) Dark-eyed Juncos caught in interior Alaska between April and September. For each sampling period (April-5 May, 6-31 May, June, July, and September) data are grouped as a function of the corresponding median capture date.

sonally ( $P$ 's  $\leq 0.002$ ). *Leucocytozoon fringillinarum* prevalence increased between April-early May and June ( $\chi^2 = 7.16$ , d.f. = 1,  $P = 0.007$ ), whereas *T. avium* prevalence increased between mid-May and June ( $\chi^2 = 11.74$ , d.f. = 1,  $P < 0.001$ ). Prevalence of neither parasite changed between June and July or between July and September.

Neither overall parasite prevalence nor prevalence of any parasite in adult females varied significantly between June and September. During this period, males and females did not differ except that more females than males tended to be infected with *H. fringillae* in June (27.4 vs. 5.4%; Fisher exact test:  $P = 0.051$ ).

In males, *Leucocytozoon fringillinarum* infection intensity changed seasonally (one way ANOVA on ranks:  $P = 0.001$ ; Fig. 3), being higher in June and July (but not September) than in April-early May, but similar in mid-May and September. No difference was detected between June (and July) and May, possibly due to the fact that there was a large individual variation in intensity during this month (see Fig. 3). Intensity of infection in females did not vary significantly between June and September. Between June and September there was no overall sexual difference in infection intensity (two-way ANOVA on ranked data:  $P > 0.25$ ), but there was a Time  $\times$  Sex interaction ( $F_{2,154} = 3.25$ ,  $P = 0.041$ ). As shown by multiple pair-wise comparisons, females had higher infection intensity than males in September, but not earlier.

#### Age-related differences in reproductive function and parasite prevalence in adult males

Males sampled shortly after arrival (end of April and May) had elevated circulating T levels (average approx. 6 ng/ml; Fig. 4) that gradually decreased during the reproductive season ( $F_{3,133} = 32.79$ ,  $P < 0.001$ ). By July circulating T had become undetectable in all but one of 36 sampled males. Plasma T levels were on average higher in ASY than in SY males ( $F_{1,133} = 4.02$ ,  $P = 0.047$ ).

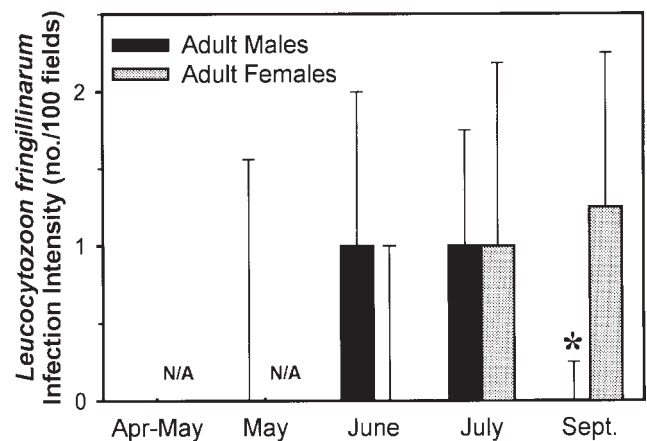


Fig. 3. Seasonal changes in intensity of blood cell infection by *Leucocytozoon fringillinarum* (number of infected cells per 100 microscope visual fields at 400 $\times$  magnification; medians + 1/2; interquartile intervals) in adult male ( $n = 19$  to 56/point) and female ( $n = 11$  to 19/point) Dark-eyed Juncos sampled on their interior Alaska breeding grounds between April and September. \* Indicates a significant difference between sexes (Student-Newman-Keuls test:  $P < 0.05$ ). N/A: data not available.

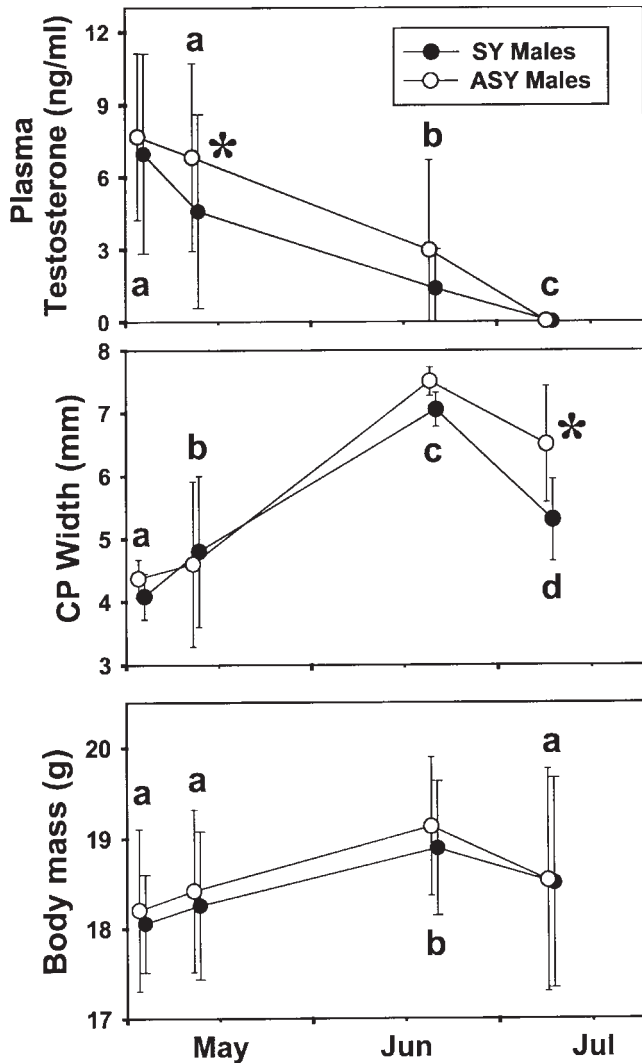


Fig. 4. Seasonal changes in plasma testosterone concentrations (mean  $\pm$  s.d.;  $n = 7$  to  $30$ /point), cloacal protuberance widths (CP; medians  $\pm$   $\frac{1}{2}$  interquartile intervals;  $n = 12$  to  $34$ /point), and body masses (means  $\pm$  s.d.;  $n = 12$  to  $34$ /point) in adult Second-Year (SY) and After-Second-Year (ASY) male Dark-eyed Juncos between April and July. \* Indicates a significant difference between ages, and for a given parameter, data (ages combined) with a different letter differ significantly (Student-Newman-Keuls test:  $P < 0.05$ ).

This difference was statistically significant in May, but not at other times.

Cloacal protuberance (CP) widths were small at the time of arrival, rapidly increased until June, and decreased in July, as males became photorefractory. On average, ASY males had larger CP sizes than SY males (two-way ANOVA:  $F_{1,185} = 4.65$ ,  $P = 0.032$ ), but as shown by multiple comparison tests the two age groups differed from each other only in July. Body masses changed seasonally (two-way ANOVA:  $F_{3,185} = 9.03$ ,  $P < 0.001$ ),

but were not age-class dependent ( $P > 0.2$ ). They increased between mid-May and June, but in July decreased and did then not differ from those in April and May (Fig. 4). Thus, age-class differences in CP sizes apparently were specific to the reproductive system rather than due to differences in body size or condition.

We investigated age-related differences in parasite prevalence between April and July by comparing the percentages of infected SY and ASY males caught during each sampling period. The two age classes of males had similar parasite prevalence at the beginning (May) and end (July) of the breeding season (Fig. 5). In June, however, ASY males had a higher overall parasite prevalence than SY males ( $\chi^2 = 4.07$ , d.f. = 1,  $P = 0.044$ ). This difference primarily resulted from more ASY than SY males being infected with *L. fringillinarum* ( $\chi^2 = 5.16$ , d.f. = 1,  $P = 0.023$ ). It was not associated with differences in intensity of infection with this parasite type (two way ANOVA on ranked data: age effect and age  $\times$  time interaction:  $P$ 's  $> 0.20$ ). The two age classes had similar *T. avium* and *H. fringillae* prevalence (maximum 25% in June in ASY males; data not shown) throughout the study period.

#### Parasite prevalence in hatching-year birds

*Leucocytozoon fringillinarum* and *Trypanosoma avium* were found in two out of 25 (8%) HY juncos sampled in August. This prevalence was lower than that of adults caught either in July (81.8%;  $\chi^2 = 35.66$ , d.f. = 1,  $P < 0.001$ ) or September (66.7%;  $\chi^2 = 17.84$ , d.f. = 1,  $P < 0.001$ ). In contrast, *L. fringillinarum*, *T. avium*, and *H. fringillae* were detected in 45.2%, 35.5%, and 3.2%, respectively, of the samples obtained from HY males caught in September. These percentages, as well as total parasite prevalence of these males (77.4%), were similar to those of adult males sampled during the same month (Fig. 6).

## DISCUSSION

The present investigation documented seasonal variations in blood parasite infection in a free-living breeding population of Dark-eyed Juncos. We found that in adult males, prevalence was relatively high when birds arrived on their breeding grounds (April and early May), before emergence of most local insect vectors. Prevalences increased during the breeding period, coincident with the time when vectors also become abundant. Furthermore, the intensity of infection with one parasite

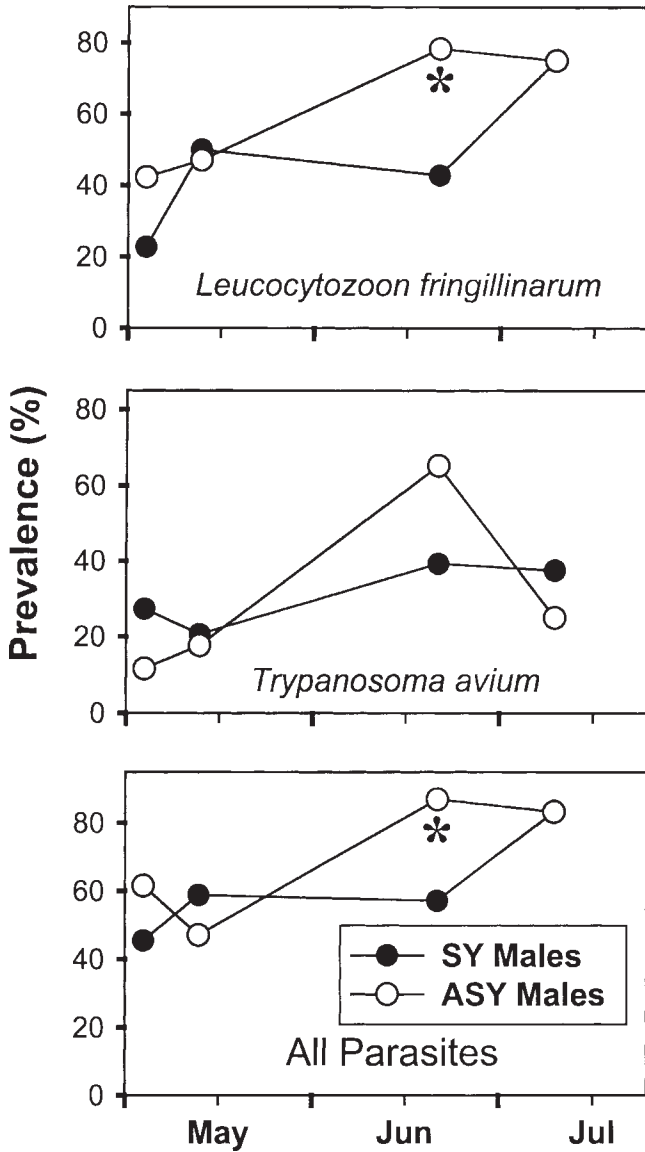


Fig. 5. Seasonal changes in prevalence of *Leucocytozoon fringillinarum*, *Trypanosoma avium*, and all blood parasites in adult Second-Year (SY; n = 24 to 34/point) and After-Second-Year (ASY; n = 12 to 26/point) male Dark-eyed Juncos caught on their interior Alaska breeding grounds between April and July. \* Indicates a significant age difference ( $\chi^2$  test;  $P < 0.05$ ). See legend of Figure 2 for additional comments.

species (*Leucocytozoon fringillinarum*) did not follow a normal distribution. Rather, this parasite was not detected in a large proportion of the sampled birds and relatively few individuals were heavily infected. These findings are in general agreement with other studies on passerine birds including juncos (Bennett and Fallis, '60; Young et al., '93; Weatherhead and Bennett, '91, '92; Kucera, '81).

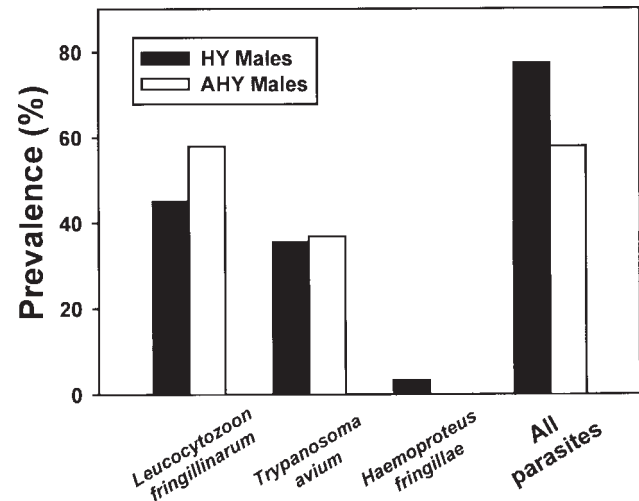


Fig. 6. Prevalence of blood parasites in young (Hatching-Year, HY, n = 31) and adult male (AHY, n = 19) Dark-eyed Juncos caught in interior Alaska in September.

Two mutually nonexclusive factors may account for the increase in parasite intensity observed during the reproductive season. First, this increase may have resulted from breeding taking place when insect vectors are most abundant and the probability of acquiring new infections is highest. This possibility is supported by the fact that in natural populations of Greenfinches (*Carduelis chloris*) sampled in geographically separated areas, prevalence of blood parasites was generally correlated with local vector abundance (Merilä et al., '95). Second, and as discussed in more detail below, breeding effort may induce a relapse of chronic infection and/or increase susceptibility to new parasite infections, irrespective of changes in vector abundance or pathogenicity. It should be noted that a relatively high (over 50%) proportion of male juncos returning to their breeding grounds at the end of April and early May had parasitemias. Further, hematozoa were found in 77% hatching-year males sampled during fall migration, indicating that many males are infected within a few months of hatching. Thus, a relapse of chronic infections acquired during previous year and/or vernal migration may have contributed to the increased parasite prevalence associated with reproduction.

In June, ASY males had an overall parasite prevalence 52% higher than SY males. This difference resulted primarily from older males having a higher (82%) *L. fringillinarum* prevalence than younger males. Similarly, blood parasite infections increase with age in adult male Great Tits, *Parus major* (Norris et al., '94), Red-winged

Blackbirds, *Agelaius phoeniceus* (Weatherhead and Bennett '91), and redpolls, *Carduelis flammea* (Seutin '94). As was the case for *L. fringillinarum* in juncos, overall parasite infection *intensity* did not increase with age in adult Red-winged Blackbirds (Weatherhead and Bennett, '92). It should be pointed out that SY and ASY male juncos had similar parasite prevalence when arriving on breeding grounds (April and early May) as well as at the end of the reproductive period (July). This observation indicates that the age-class difference in prevalences was specific to the breeding season and did not result from a pre-existing difference between SY and ASY males.

Plasma T concentrations in males were high shortly after arrival on breeding grounds and they gradually declined to basal levels at the end of the reproductive season (July), when birds initiated prebasic (postnuptial) molt. This seasonal pattern resembles that described in other seasonal species (White-crowned Sparrow: Wingfield and Farner ['78a,b]; Song Sparrow, *Melospiza melodia*: Wingfield ['84]). In addition to changing seasonally, plasma T concentrations were age-class dependent as ASY males had higher T levels than SY males in May. At the end of the breeding season (July), ASY males also had larger CP sizes than SY males and in another study, we found ASY males to have larger testes than SY males during the summer (Deviche et al., '00). These results provide evidence for age-class differences in the seasonal activity of the reproductive system. Similar observations were made in White-crowned Sparrows (Morton et al., '90). We hypothesize that an age-class difference in reproductive system activity may have been directly or indirectly (see below) responsible for the fact that ASY males suffered a transiently higher prevalence than SY males. Sex hormones may in some conditions exert immunosuppressive effects (Folstad and Karter, '92; Mooradian et al., '87; Hillgarth and Wingfield, '97). Higher plasma T levels in ASY than in SY males in May were, therefore, possibly associated with age-specific differences in immune responses such that SY males were either physiologically more susceptible to a relapse of existing hematozoa infection or suffered a higher rate of new infections than older males. This hypothesis is consistent with the fact that age-class differences in plasma T levels (May) *preceded* differences in parasite infection rates (June). In addition, T administration to male juncos (Casto et al., '99) and Superb Fairy-wrens, *Malurus cyaneus* (Peters, '00) exerted immunosuppressive effects.

Alternately, T may have influenced parasite prevalences indirectly, i.e., through changes in behavior. Chandler et al. ('94) administered T to male Dark-eyed Juncos during the incubation and nestling stages of the nesting cycle and found that hormone-treated birds occupied larger home ranges and made more long-distance movements than controls. T treatment to juncos also stimulates singing behavior and enhances the frequency of courtship displays (Enstrom et al., '97). Higher circulating T concentrations in ASY than in SY juncos may have been associated with behavioral differences between the two age groups such that older males were exposed to insect vectors and infected at a higher rate than younger males.

Finally, differences in parasite prevalence between SY and ASY male juncos may have resulted from a difference in breeding effort between the two age classes. SY male White-crowned Sparrows are less successful in obtaining mates than older males, although when paired, males of the two age classes raise equal numbers of young (Morton et al., '90). In Great Tits, clutch size and, thus, presumably breeding effort, increase with age (Norris et al., '94). We found that SY male juncos initiate pre-basic molt earlier than ASY males (Deviche et al., '00). In most passerine species, the onset of this molt signals the end of the reproductive period. With results obtained in other species, we suggest that the cumulative influence of several factors (less success in finding a mate, smaller clutch size, earlier termination of breeding activities) resulted in SY male juncos having a lesser breeding effort than ASY males. Several recent studies have identified a positive relationship between this effort and hematozoan infections (Richner et al., '95; Oppliger et al., '96; Norris et al., '94; Nordling et al., '98; Gustafsson et al., '94). This may explain why parasite prevalence was higher in ASY than in SY male juncos when reproductive effort was maximal (June, when many young have hatched and are fed by parents.) Studies investigating the relationship between breeding effort and plasma T levels are warranted to test this hypothesis. Indeed, T treatment to male juncos increased the home range of these males and stimulated their singing and courtship displays (Chandler et al., '94; Enstrom et al., '97), but also decreased the rate of food provisioning to the young and the effectiveness of nest defense (Ketterson and Nolan, '92; Ketterson et al., '96).

The potentially detrimental effects of avian hematozoa infections on the fitness and health of their hosts have been the object of considerable

debate (for review, see Møller, '90). Most avian studies have found no association between body mass or condition and parasite infections (passerines: Bennett et al., '88, Weatherhead, '90, Weatherhead and Bennett, '92; Mallard, *Anas platyrhynchos*: Shutler et al., '99). The present study, albeit not designed to directly test relationships between these variables, does not provide evidence for effects of parasite infections on body mass. Indeed, SY and ASY males differed with respect to their *L. fringillinarum* (and overall parasite) prevalence in June, but the two age groups had similar body masses during this month as well as earlier and later in the breeding season. Moreover, seasonal changes in parasite prevalence (and in *L. fringillinarum* infection intensity) and in body mass in males followed independent rather than opposite time courses. Both increased between May and June, but the decrease in body mass that took place in July did not concur with a drop in parasitaemia. A recent investigation on free-living Collared Flycatchers (*Ficedula albicollis*) revealed a negative correlation between *Haemoproteus* infection intensity on the breeding grounds and subsequent overwinter survival (Nordling et al., '98, but see Weatherhead, '90, and Weatherhead and Bennett, '92, for data on Red-winged Blackbirds and Brown-headed Cowbirds, *Molothrus ater*). This correlation suggests that hematozoan infections exert pathogenic effects resulting in increased mortality. Additional research is warranted to elucidate whether this increase results from parasite-induced negative effects on body condition as compared to detrimental influences, e.g., on foraging ability or susceptibility to predation.

Studies investigating sexual differences in blood parasite intensity have provided variable results. In Great Tits, parasite prevalence was higher in females than males and increased with clutch size in males, but not in females (Norris et al., '94). In Brown-headed Cowbirds, parasite prevalence increased during the summer in females and in SY males, but not in ASY males (Weatherhead and Bennett, '92). In contrast, adult male Red-winged Blackbirds were more heavily parasitized with leucocytozooids than conspecific adult females (Weatherhead and Bennett, '91). The present investigation did not reveal sexual differences in parasite prevalence. However, intensity of infection by *L. fringillinarum* declined between summer and fall in males, but not in females. As a result, males and females had a similar intensity of infection during the summer, but females had

higher intensities than males in September. Males and females may differ with respect to aspects of their behavior that modify exposure to insect vectors, resistance to new infections, and/or the ability to fight and eliminate acquired infections, perhaps as a result of sex-specific interactions with environmental conditions (Wiehn and Korpimäki, '98). The few data that are currently available do not permit drawing general conclusions regarding sex differences in parasite prevalence in adult passerines. Elucidating the bases of these differences will require investigations examining the relationships between the ecology of parasite vectors and sex-specific aspects of the behavior and physiology of their hosts.

#### ACKNOWLEDGMENTS

The authors thank Dr. John Blake for comments on an early version of the manuscript. Thanks are due to Tanya Carlin and Cynthia Restrepo for assistance with sample collection. Xavier Manteca was supported by a research grant from the Universitat Autònoma de Barcelona, Spain.

#### LITERATURE CITED

- Atkinson CT, van Riper C. 1991. Pathogenicity and epizootiology of avian hematozoa: *Plasmodium*, *Leucocytozoon* and *Haemoproteus*. In: Loya JE and Zuk M, editors. Bird-parasite interactions. Ecology, evolution and behavior Oxford: Oxford University Press. pp 19–49.
- Bennett GF. 1970. Simple technique for making avian blood smears. *Can J Zool* 48:585–586.
- Bennett GF, Fallis AM. 1960. Blood parasites of birds in Algonquin Park, Canada, and a discussion of their transmission. *Can J Zool* 38:261–273.
- Bennett GF, Peirce MA. 1988. Morphological forms in the avian Haemoproteidae and an annotated checklist of the genus *Haemoproteus* Kruse, 1890. *J Nat History* 22:1683–1696.
- Bennett GF, Caines JR, Bishop M.A. 1988. Influence of blood parasites on the body mass of passeriform birds. *J Wild Diseases* 24:339–343.
- Bennett GF, Peirce MA, Earle RA. 1994. An annotated checklist of the valid avian species of *Haemoproteus*, *Leucocytozoon* (Apicomplexa: Haemosporina) and *Hepatozoon* (Apicomplexa: Haemogregarinidae). *Systemic Parasitol* 29:61–73.
- Bennett GF, Thommes F, Blancou J, Artois M. 1982. Blood parasites of some birds from the Lorraine region, France. *J Wild Diseases* 18:81–88.
- Braude S, Tang-Martinez Z, Taylor GT. 1999. Stress, testosterone, and the immunoredistribution hypothesis. *Behav Ecol* 10:345–350.
- Burrey-Caines JR, Bennett GF. 1992. The Haemoproteidae (Apicomplexa: Haemosporina) of the avian families Fringillidae and Emberizidae s.l. *Can J Zool* 70:1149–1160.
- Casto JM, Ketterson ED, Nolan V, Jr. 1999. Elevated testosterone suppresses humoral and cell-mediated immunity in male Dark-eye Juncos. Abstract, Third Annual Meeting of the Society for Behavioral Neuroendocrinology, Charlottesville, Virginia.

- Chandler CR, Ketterson ED, Nolan V, Jr, Ziegenfus C. 1994. Effects of testosterone on spatial activity in free-ranging male Dark-eyed juncos, *Junco hyemalis*. *Anim Behav* 47:1445–1455.
- Clayton DH. 1991. The influence of parasites on host sexual selection. *Parasitol Today* 7:329–334.
- Clayton DH, Pruett-Jones SG, Lande R. 1992. Reappraisal of the interspecific prediction of parasite-mediated sexual selection: opportunity knocks. *J Theor Biol* 57:95–108.
- Cox FEG. 1989. Parasites and sexual selection. *Nature* 341:289.
- Demas GE, Chefer V, Talan MI, Nelson RJ. 1997. Metabolic costs of mounting an antigen-stimulated immune response in adult and aged C57BL/6J mice. *Am J Phys Reg Int Comp Phys* 42:R1631–R1637.
- Deviche P. 1992. Testosterone and opioids interact to regulate feeding in a male migratory songbird. *Horm Behav* 26:394–405.
- Deviche P, Gullledge CC. 2000. Vocal control region sizes of an adult female songbird change seasonally in the absence of detectable circulating testosterone concentrations. *J Neurobiol* 42:202–211.
- Deviche P, Wingfield JC, Sharp PJ. 2000. Year-class differences in the reproductive system, plasma prolactin and corticosterone concentrations, and onset of prebasic molt in male Dark-eyed Juncos (*Junco hyemalis*) during the breeding period. *Gen Comp Endocrinol* 118:425–435.
- Enstrom DA, Ketterson ED, Nolan V, Jr. 1997. Testosterone and mate choice in the dark-eyed junco. *Anim Behav* 54:1135–1146.
- Folstad I, Karter AJ. 1992. Parasites, bright males, and the immunocompetence handicap. *Am Nat* 139:603–622.
- Gore TC, Noblet GP, Noblet R. 1982. Effects of pinealectomy and ocular enucleation on diurnal periodicity of *Leucocytozoon smithi* (Hemosporina) gametocytes in the peripheral blood of domestic turkeys. *J Parasitol* 29:415–420.
- Gullledge CC, Deviche P. Androgen control of vocal control region volumes in a wild migratory songbird (*Junco hyemalis*) is region and possibly age dependent. *J Neurobiol* 32:391–402.
- Gustafsson L, Nordling D, Andersson MS, Sheldon BC, Qvarnström A. 1994. Infectious diseases, reproductive effort and the cost of reproduction in birds. *Phil Trans R Soc London B: Biol Sci* 346:323–331.
- Hamilton WD, Zuk M. 1982. Heritable true fitness and bright birds: a role for parasites? *Science* 218:384–387.
- Harrison GJ, Harrison LR. 1986. *Clinical avian medicine and surgery including aviculture*. Philadelphia: W.B. Saunders Co.
- Hausfater G, Gerhardt HG, Klump GM. 1990. Parasites and mate choice in tree frogs, *Hyla versicolor*. *Amer Zool* 30:299–311.
- Hillgarth N., Wingfield J. 1997. Parasite-mediated sexual selection: endocrine aspects. In: Clayton DH, Moore J, editors. *Host-parasite evolution. General principles and avian models*. Oxford: Oxford University Press. p 78–104.
- Jenkins D, Watson A, Miller GR. 1963. Population studies on Red Grouse, *Lagopus lagopus scoticus* (Lath) in Northeast Scotland. *J Anim Ecol* 32:317–376.
- Ketterson, ED, Nolan V, Jr. 1992. Hormones and Behavior: an integrative approach. *Am Nat* 140:S33–S62.
- Ketterson ED, Nolan V, Jr., Cawthorn JM, Parker PG, Ziegenfus C. 1996. Phenotypic engineering: using hormones to explore the mechanistic and functional bases of phenotypic variation in nature. *Ibis* 138:70–86.
- Kucera J. 1981. Blood parasites of birds in Central Europe. 3. *Plasmodium* and *Haemoproteus*. *Folia Parasitol (Praha)* 28:303–312.
- Levine ND. 1985. *Vertebrate protozoology*. Ames, IA: Iowa State University.
- Merilä J, Bjorkund M, Bennett GF. 1995. Geographic and individual variation in hematozoan infections in the greenfinch, *Carduelis chloris*. *Can J Zool* 73:1798–1804.
- Møller AP. 1990. Parasites and sexual selection: Current status of the Hamilton and Zuk hypothesis. *J Evol Biol* 3:319–328.
- Mooradian AD, Morley JE, Korenman SG. 1987. Biological actions of androgens. *Endocrine Rev* 8:1–28.
- Morton ML, Peterson LE, Burns DM, Allan N. 1990. Seasonal and age-related changes in plasma testosterone levels in mountain White-crowned Sparrows. *The Condor* 92:166–173.
- Nordling D, Andersson M, Zohari S, Gustafsson L. 1988. Reproductive effort reduces specific immune response and parasite resistance. *Proc R Soc London B: Biol Sci* 265:1291–1298.
- Norris K, Anwar M, Read AF. 1994. Reproductive effort influences the prevalence of haematozoan parasites in great tits. *J Anim Ecol* 63:601–610.
- Oppliger A, Christie P, Richner A. 1996. Clutch size and malaria resistance. *Nature* 381:65.
- Ots I, Hörak P. 1996. Great Tits *Parus major* trade health for reproduction. *Proc R Soc London B: Biol Sci* 263:1443–1447.
- Peirce MA. 1981. Distribution and host-parasite checklist of haematozoa of birds in Western Europe. *J Nat History* 15:419–458.
- Peters A. 2000. Testosterone treatment is immunosuppressive in superb fairy-wrens, yet free-living males with high testosterone are more immunocompetent. *Proc R Soc London B: Biol Sci* 267:883–889.
- Pyle P. 1997. *Identification guide to North American birds. Part I. Columbidae to Ploceidae*. Bolinas, CA: Slate Creek Press. 732 p.
- Raidal SR, Jaensch SM. 2000. Central nervous disease and blindness in Nankeen kestrels (*Falco cenchroides*) due to a novel *Leucocytozoon*-like infection. *Avian Pathol* 29:51–56.
- Richner H, Christie P, Oppliger A. 1995. Paternal investment affects prevalence of malaria. *Proc Natl Acad Sci USA* 92:1192–1194.
- Rintimäki PT, Huhta E, Jokimäki J, Squires-Parsons D. 1999. Leucocytozoonosis and trypanosomiasis in redstarts in Finland. *J Wildl Dis* 35:603–607.
- Schwabl H, Farner DS. 1989. Endocrine and environmental control of vernal migration in male White-crowned Sparrows, *Zonotrichia leucophrys gambelii*. *Physiol Zool* 62:1–10.
- Seutin G. 1994. Plumage redness in redpoll finches does not reflect hemoparasitic infection. *Oikos* 70:280–286.
- Shutler D, Ankney CD, Mullie A. 1999. Effects of the blood parasite *Leucocytozoon simondi* on growth rates of anadid ducklings. *Can J Zool* 77:1573–1578.
- Toft CA. 1991. Current theory of host-parasite interactions. In: Loye JE, Zuk M, editors. *Bird-parasites interaction. Ecology, evolution, and behavior*. Oxford: Oxford University Press. pp 3–15.
- Toft CA, Aeschlimann A. 1991. Introduction: coexistence or conflict. In: Toft CA, Aeschlimann A, Bolis L, editors. *Parasite-host interactions: coexistence or conflict?* Oxford: Oxford University Press. pp. 1–12.
- Toft CA, Karter AJ. 1990. Parasite-host evolution. *Tr Ecol Evol* 5:326–329.

- Thompson CW, Hillgarth N, Leu M, McClure HE. 1997. High parasite load in House Finches (*Carpodacus mexicanus*) is correlated with reduced expression of a sexually selected trait. *Am Nat* 149:270–294.
- Vaughn GE, Coble PW. 1975. Sublethal effects of three ectoparasites on fish. *J Fish Biol* 7:283–294.
- Weatherhead PJ. 1990. Secondary sexual traits, parasites, and polygyny in red-winged blackbirds, *Agelaius phoeniceus*. *Behav Ecol* 1:125–130.
- Weatherhead PJ, Bennett GF. 1991. Ecology of Red-winged Blackbird parasitism by haematozoa. *Can J Zool* 69:2352–2359.
- Weatherhead PJ, Bennett GF. 1992. Ecology of parasitism of Brown-headed Cowbirds by haematozoa. *Can J Zool* 70:1–7.
- Wiehn J, Korpimäki E. 1998. Resource levels, reproduction and resistance to haematozoan infections. *Proc R Soc London B: Biol Sci* 265:1197–1201.
- Wingfield J. 1984. Environmental and endocrine control of reproduction in the Song Sparrow, *Melospiza melodia*. I. Temporal organization of the breeding cycle. *Gen Comp Endocr* 56:406–416.
- Wingfield J, Farner DS. 1978a. The annual cycle of plasma irLH and steroid hormones in feral populations of the White-crowned Sparrow, *Zonotrichia leucophrys gambelii*. *Biol Reprod* 19:1046–1056.
- Wingfield J, Farner DS. 1978b. The endocrinology of a natural breeding population of the White-crowned Sparrow (*Zonotrichia leucophrys pugetensis*). *Physiol Zool* 51:188–205.
- Young BE, Garvin MC, McDonald DB. 1993. Blood parasites in birds from Monteverde, Costa Rica. *J Wild Diseases* 29:555–560.
- Zuk M, Thornhill R, Ligon JD, Johnson K. 1990. Parasites and mate choice in Red Jungle Fowl. *Amer Zool* 30:235–244.