QUANTIFIYING SEASONAL VARIATION IN PHYSIOLOGICAL CONDITION OF ADULT FRANKLIN'S GULL (*LUECOPHAEUS PIPIXCAN*) DURING NESTING

A Thesis
Submitted to The Graduate Faculty
of the
North Dakota State University
of Agriculture and Applied Science

By

Shawn Edward Weissenfluh

In Partial Fulfillment of the Requirements
for the Degree of
MASTER OF SCIENCE

Major Department:
Biological Sciences

November 2011

Fargo, North Dakota

North Dakota State University
Graduate School

Title

Quantifying seasonal variation in physiological condition of adult Franklin's gull (Luecophaeus pipixcan) during nesting

By

Shawn Edward Weissenfluh

The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

SUPERVISORY COMMITTEE:

Chair

Approved by Department Chair:

21 November 2011
Date

Signature

ABSTRACT

Weissenfluh, Shawn Edward, M. S., Department of Biological Sciences, College of Science and Mathematics, North Dakota State University, November 2011. Quantifying Seasonal Variation in Physiological Condition of Adult Franklin's Gull (*Luecophaeus pipixcan*) During Nesting. Major Professor: Dr. Mark E. Clark.

Understanding seasonal variation in adult physiological condition is important for developing hypotheses on how nest initiation, adult condition, chick development and recruitment are related in Franklin's gull and other migratory species of the northern plains. The purpose of this study was to profile physiological condition during the breeding season in nesting Franklin's gull (*Luecophaeus pipixcan*) adults.

Physiological condition was quantified in nesting adults through four metrics: body measurements recorded from live-trapped birds, the corticosterone stress response measured from blood samples collected serially from live-trapped birds, and two measures of immune function (antimicrobial capacity of plasma from blood samples and heterophil/lymphocyte ratios based on blood smears, both taken from live-trapped birds).

Physiological condition declined across the breeding season, as shown by a decline in body condition, stress tolerance and immune performance. Specifically, residual body mass decreased and exposure of the sternum keel increased with the progression of the breeding season. Additionally, birds nesting later in the season showed greater maximum corticosterone concentrations in the stress profile along with lower antimicrobial capacity.

These results suggest two hypotheses: 1) that timing of nesting has a significant impact on the physiological condition of Franklin's gull and 2) that birds in poorer condition initiate breeding later in the season. Seasonal variation in condition

may be related to time constraints observed in temperate latitudes and whether these birds are capital (i.e., acquiring resources outside the breeding area) or income (i.e., acquiring resources locally) breeders. Thus, determining physiological condition during the breeding season is an important step in elucidating how nest initiation, adult condition, chick development and recruitment are related in Franklin's gull.

ACKNOWLEDGEMENTS

I thank my major advisor, Dr. Mark Clark, for his assistance and guidance regarding my research. I also thank the members of my committee, Dr. Wendy Reed, Dr. Mark Sheridan, and Dr. Larry Reynolds, for their comments, suggestions, and advice.

I thank Peter Martin, Nathan Cross and Jacob Froehlich for their fieldwork assistance. I thank Dr. Penelope Gibbs, Heather Vinson, Jacob Froehlich, Michelle Gastecki, Nick Galt, Peter Martin, Nathan Cross, Shanessa Ellis, Andrew Nyhus, Molly Slaby, Michele Heilveil and Petar Mijkovich for their laboratory assistance. I thank Suzy Schmoll, Phyllis Murray and Rita Slator as well as the rest of the department's staff for providing the administrative support necessary to successfully complete this study. I thank the staff at J. Clark Salyer National Wildlife Refuge for providing housing. I thank the North Dakota Game and Fish Department for funding this study.

On a personal level, I thank my parents, Steven and Diana Weissenfluh, and the rest of my family and friends for all of their support and encouragement throughout my study.

TABLE OF CONTENTS

LIST OF FIGURES

INTRODUCTION

Intercontinental migratory birds experience significant time constraints on nesting, rearing and fledging their young and preparing for the long migration back to their wintering grounds. Because of this, arrival time at the breeding grounds can constrain the time available to rear chicks to fledging. Furthermore, factors such as environmental cues that affect arrival time may have significant implications for recruitment. Many environmental cues also vary seasonally and are known to alter the physiology of breeding adults. For example, in the northern hemisphere, photoperiod changes such that day-length increases as the Julian day approaches the summer solstice. Photoperiod (i.e., increasing day length) initiates an array of physiological changes in birds including gonad recrudescence, feather molt, and fat deposition (Wingfield 2005; Wikelski et al. 2008). Along with photoperiod, temperature can directly affect physiology and the timing of breeding. Trumpeter finches (*Bucanetes githagineus*) have been shown to delay egg-laying dates up to 40 days due to lower temperatures (Barrientos et al. 2007).

Adult physiological condition affects both current and future reproductive success. Body condition of female great tits (*Parus major*) is positively correlated with reproductive output (Norte et al. 2010). However, studies have also shown that physiological condition is positively correlated to nutritional reserves critical for adult survival (Brown 1996) and therefore future offspring production (Nager et al. 2001). It is hypothesized that changes in adult physiological condition across the breeding season are a manifestation of trade-offs between investments to offspring

versus self-maintenance predicated on nest initiation date, because offspring survival to recruitment generally declines across the nesting season (Drent 2006).

The physiological condition of nesting adults can be quantified through a number of measures, including body measurements recorded from live-trapped birds. Body mass often fluctuates within the breeding season. Female kittiwakes (*Rissa tridactyla*) exhibit an increase in body mass during incubation and a decrease in body mass during the chick-rearing period (Moe et al. 2002). Similarly, female arctic skuas (*Stercorarius parasiticus*) retain body fat reserves during incubation but shed those reserves, which reduces wing loading and improves flight efficiency after nesting (Phillips and Furness 1997). The ratio between body mass and tarsus or wing length can be an indicator of whole body condition, fat storage or lean mass (Gosler et al. 1998), and is typically used to evaluate nestling condition (Richner et al. 1993). Keel depth, a measure of the protrusion of the keeled sternum above breast muscle tissue, is another measure of whole-organism condition but more specific to flight proficiency that is also known to vary in migratory birds during breeding (Hatch et al. 2010).

The corticosterone stress response in birds provides another measure of physiological condition. Perceived threats or short-term stressors illicit an increase in the production (and circulating levels) of corticosterone that precipitates the vertebrate fight/flight response. As such, capture and handling of free-living birds can induce a rapid elevation of plasma corticosterone concentrations above the background baseline concentration. Because repeated exposure to stress may alter the corticosterone response (Cyr and Romero 2006), integrating the corticosterone

concentration versus time curve has been used to quantify stress-induced changes to physiological condition (Breuner and Hahn 2003). Indeed, the magnitude of the corticosterone response was negatively correlated to parental effort in mourning doves (*Zenaida macroura*) (Miller et al. 2009).

Physiological condition can also be assessed through measures of immune function. In free-living birds, two particular measurements have been used to characterize the status of the immune system: antimicrobial capacity of plasma (Millet et al. 2007) and heterophil/lymphocyte ratio (Gross and Siegel 1983; Vleck et al. 2000). Bactericidal capacity of plasma assesses multiple components of constitutive immunity in vertebrates, and has been correlated with handling stress in birds (Millet et al. 2007), but not used to assess immunocompetence of nesting adults. In tropical birds, one hour of acute stress has been shown to reduce bacteria killing ability by up to 40% (Matson et al. 2006). In chronically-stressed individuals, the heterophil/lymphocyte ratio increases. Greater investment in offspring compromised investments in immune constituents of adult great tits (Ots and Hõrak 1996). Moreover, Dubiec et al. (2005) have shown that early-breeding female great tits have greater blood counts of total leukocytes, lymphocytes and heterophils than birds starting their clutches late in the season.

Evidence of effects on timing of nesting has been observed in Franklin's gull (*Leucophaeus pipixcan*), an intercontinental migrant that nests in the northern Great Plains. Berg (2009) found that Franklin's gull chicks hatched early in the season were structurally larger than chicks hatched later in the season. Furthermore, preliminary data on Franklin's gull eggs artificially incubated under alternative photoperiods

indicated that both photoperiod and maternal effects (i.e., maternal environment and phenotype) associated with timing of nesting affect embryonic development of chicks (Clark and Reed, *unpublished data*). It is not known if adult condition varies within the nesting season in Franklin's gull. Changes in adult physiology could be responsible for the seasonal maternal effects observed in Franklin's gull chick development. Thus, understanding seasonal variation in adult condition will provide critical information for developing hypotheses on how nest initiation, adult condition, chick development and recruitment are related in Franklin's gull. We hypothesize that the timing of nesting is critical for Franklin's gull and that early-nesting birds are in better physiological condition then late-nesting individuals.

METHODS

Bird Collection Site

I monitored adult Franklin's gulls during nesting from early May through late June at Rush Lake Waterfowl Production Area in north-central North Dakota. In late April I observed numerous adult Franklin's gulls near the Rush Lake Waterfowl Production Area, and began searching areas within the Rush Lake marsh each week to locate nests for monitoring. When a new nest was found, I marked the location with a handheld GPS, placed a small (approximately 3 cm diameter) float with a unique nest identification code near the nest, recorded the number of eggs present, marked the eggs on their blunt end with a permanent marker and revisited nests on subsequent days to determine the final clutch size and the onset of continuous incubation by the adults. Newly initiated nests (i.e., those for which the female was still in the process of laying eggs) were determined by the presence of a single egg in the nest or confirmation of laying by the appearance of an additional egg within 48 hours. For nests found with one egg present, I used the flotation method to determine whether the egg was recently laid. Eggs that did not float in the water were considered recently laid (Nol and Blokpoel 1983; Ackerman and Eagles-Smith 2010). Nests determined to be within the first days of continuous incubation (or not yet undergoing continuous incubation) were then targeted for subsequent adult capture of the adults within the first week of incubation to measure physiological condition.

Bird Collection

I captured and quantified physiological condition of incubating Franklin's gull adults that initiated nests early, middle and late in the nesting period in 2010. All

gulls were trapped within the first week of initiating continuous incubation. Adults were captured using nest traps (Burger 1971) placed on targeted nests. Larger traps were sometimes used to acclimate a nesting bird, by allowing them to fly in and out of the trap without disturbance. Smaller traps, which prevented birds from flying out, were then set to capture the nesting bird. Traps were set daily and monitored for captures at 30-45 minute intervals. Once an adult was captured, I approached the nest rapidly and recorded the time of startle based on the bird's behavior (i.e., attempting to fly out of the nest trap). The startle response generally started as soon as a bird made visual contact with us as I approached the nest. Upon capture, I collected a small blood sample (approximately 600 µl) from the brachial vein within approximately three minutes of the initial startle response, then collected subsequent blood samples (approximately 300 µl each) at 20 minutes and 30 minutes after the initial startle response, which is the protocol for obtaining samples necessary to profile a stress response via plasma corticosterone (Wingfield et al. 1982). Following collection, blood samples were temporarily placed in a cooler of ice for transport to the laboratory. In the interval between collection of the first and second blood samples, I measured body mass using a spring scale (± 5.0 grams), tarsus length, culmen length, nares length, breast width and keeled sternum depth using digital calipers (± 0.1 mm), and wing chord length using a wing rule (± 0.5 mm). During blood sample collection, we also made a blood smear for counting heterophil and lymphocyte numbers, prepared by spreading a drop of blood across a microscope slide producing a single layer of cells and also set aside a small drop of blood in

Queen's lysis buffer (Seutin et al. 1991) to preserve DNA for sexing (Franklin's gulls are not sexually dimorphic in size or plumage; Burger and Gochfeld 1994).

Plasma Sample Preparation

Blood samples were taken to the laboratory for processing within approximately four hours of collection. I centrifuged blood samples at 3000 rpm for 10 minutes to separate red blood cells from plasma, and plasma samples were separated into approximately 100 µl aliquots and stored at -20 C until further analysis (corticosterone radioimmunoassay or bactericidal assay).

Evaluation of Body Condition

Body condition was computed from several metrics of skeletal size in relation to body mass. As a simple metric of body condition, I computed the residual of body mass from an orthogonal regression of body mass and tarsus length (Schulte-Hostedde et al. 2005). Keel depth was also used to quantify condition (Schmidt-Wellenburg et al. 2008). In addition, I computed the total wingspan from twice wing chord length added to breast width to estimate wing loading (the ratio between wingspan and body mass) (Mueller et al. 2002). Residuals of body mass regressions are expected to be positively correlated to body condition (Green 2001; Schulte-Hostedde et al. 2005), keel depth is expected to be positively correlated to body condition and flight muscle performance (Schmidt-Wellenburg et al. 2008), while wing loading values are expected to be negatively correlated to flight capability (Gosler et al. 1998; Hatch et al. 2010).

Radioimmunoassay

I measured plasma corticosterone concentrations using radioimmunoassay to

quantify stress response in nesting adults. I used 10 µl of plasma for the corticosterone radioimmunoassay following the protocol described in Wingfield and Farner (1975). Briefly, a small amount of ^{3}H corticosterone (~2000 cpm) was added to each plasma sample to estimate extraction efficiency. We extracted steroids from the plasma by adding 5 ml of distilled dichloromethane and dried the supernatant at 40 C under a stream of dried nitrogen gas. Dried extracts were resuspended in PBSg buffer and refrigerated overnight at 4 C. I split these samples into duplicate vials for the radioimmunoassay and estimated corticosterone levels based on competitive binding between known amounts of labeled corticosterone and unknown amounts of corticosterone in samples on a corticosterone specific antibody (cross-reactivity binding affinity for corticosterone antibody ab7798: 11-dehydrocorticosterone 0.67%, deoxycorticosterone 1.5%, 18-OH-DOC <0.01%, cortisone <0.01%, cortisol <0.01% and aldosterone 0.2%, as reported by the manufacturer). Assay values were corrected for plasma volume and individual recoveries after extraction (recoveries after extraction, 78-90%). Intra-assay coefficient of variation was 28.9%; inter-assay coefficient of variation was 18.7%. The corticosterone stress response is expected to be positively correlated with short-term stress exposure (Cyr and Romero 2006).

Bactericidal Assay

Immune system strength was quantified using a bactericidal assay. Antimicrobial activity was measured following the protocol described by Millet et al. (2007). Briefly, I streaked out a positive (A1) and negative (V1) control on trypic soy agar (TSA) and incubated overnight at 37 C. After incubation, I inoculated 3.0 ml of TSB with a single colony from each of the controls and incubated overnight at 37 C

while shaking at 200 rpm. Following overnight incubation, 10 µl of each of the controls were used to inoculate 3.0 ml of TSB, which were then incubated at 37 C while shaking at 200 rpm for 45 minutes. Next, two 100 µl aliquots of each sample were centrifuged at 8000 rpm at 4 C for five minutes. I then decanted the supernatant and resuspended each sample with 100 µl of PBS, vortexed each sample, then repeated centrifugation. The samples were again decanted and resuspended with 60 µl of PBS and 20 µl of plasma sample. After vortexing, 5 µl of each sample was removed and placed into a separate vial containing 45 µl of PBS. Serial dilutions (1:10) were then performed for each sample and diluted to 10^{-7} and plated on TSB in 3 x 10 µl aliquots for each dilution. This step occurred at 0, two, and four hours during incubation. After each sample was plated, the plates were incubated at 37 C overnight. After incubation, I counted and recorded the number of colonies and assigned the sample categorically as sensitive (a decrease in bacteria over time) or resistant (an increase in bacteria over time). Antimicrobial capacity is expected to be positively correlated with chronically stressed individuals (Millet et al. 2007).

Heterophil:Lymphocyte Counts

I further quantified immune system suppression using heterophil/lymphocyte counts. I counted heterophil and lymphocyte cells from prepared blood smears as described in Vleck et al. (2000). Blood smears were fixed and stained with a Harleco Hemacolor staining kit, and then magnified 100x (oil immersion) on a compound microscope, and heterophils and lymphocytes were manually counted. A total of 100 cells (heterophils and lymphocytes combined) were counted to provide the ratio

between cell types. The ratio of heterophils:lymphocytes is expected to be positively correlated with long-term stress exposure (Gross and Siegel 1983).

Statistical Analysis

I used general linear models to provide a seasonal physiological profile of Franklin's gull nesting adults. Specifically, I modeled the measures of condition (i.e., residual body mass, keel depth and wing loading), as linear function of trap date (expressed as Julian day). I verified that successive plasma corticosterone concentrations for an individual increased and used both the maximum concentration as well as the difference between the maximum and baseline concentration to quantify the stress response, which I then modeled as a linear function of trap date. For analysis of immune system components, I used a principal components analysis (PCA) of the A1 bacteria colony count, V1 bacteria colony count and heterophil/lymphocyte ratio to reduce dimensionality and modeled the first and second principal components as linear functions of trap date. All statistical analyses were conducted using JMP statistical software (JMP 2002).

RESULTS

I captured 61 adults from 61 nests determined to be in the first week of continuous incubation from 14 May 2010 to 16 June 2010 at the Rush Lake Waterfowl Production Area in North Dakota. Of the 61 adults captured, I was able to obtain body mass measurements from 54 individuals, tarsus length measurements from 55 individuals, keel depth measurements from 49 individuals and wing loading from 49 individuals. I was able to obtain at least one plasma sample from all 61 captured adults, but were only able to determine plasma corticosterone concentrations for profiling the individual corticosterone stress response (i.e., concentrations from plasma samples collected at less than three, 20 and 30 minutes from startle response) from 55 of the individuals. I recovered plasma and cultured the A1 and V1 bacteria assays from 56 individuals and obtained blood smears for determining heterophil/lymphocyte ratio for 54 individuals.

Residual body mass, wing loading (the ratio of body mass to wingspan), body mass and breast width are negatively related to trap date whereas keel depth is positively related to trap date. Adult body mass and right tarsus length are positively correlated (Pearson's $r = 0.45$, $p = 0.001$, $n = 49$), and we determined residual body mass from an orthogonal regression fit to the observations (Figure 1). Residual body mass decreases with trap date ($F_{1,47} = 18.43$, $p < 0.001$, $r^2 = 0.28$; Figure 2). Keel (i.e., the exposure of the keel bone above the pectoral muscle) increases with trap date ($F_{1,47} = 21.01$, $p < 0.001$, $r^2 = 0.31$; Figure 3). Estimated wing loading (i.e., body mass divided by wingspan) declines with trap date ($F_{1,47} = 12.47$, $p < 0.001$, $r^2 = 0.21$), because body mass and breast width decrease with trap date

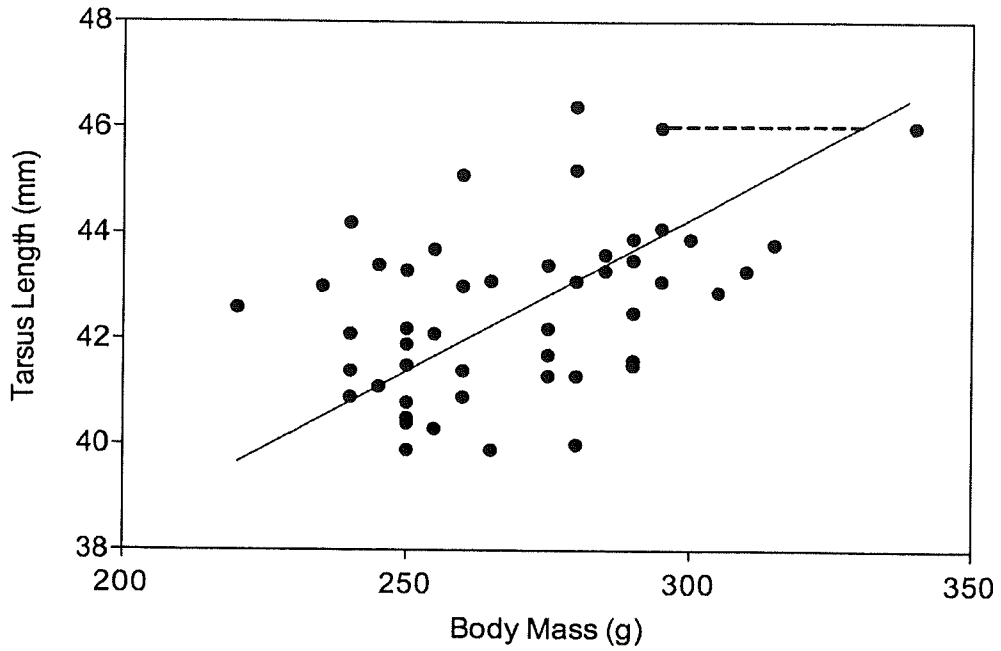

Figure 1. Positive correlation between tarsus length and body mass. The solid line represents an orthogonal regression fit to the data ($r^2 = 0.45$). Residual body mass is indicated by the dashed line for one observation.

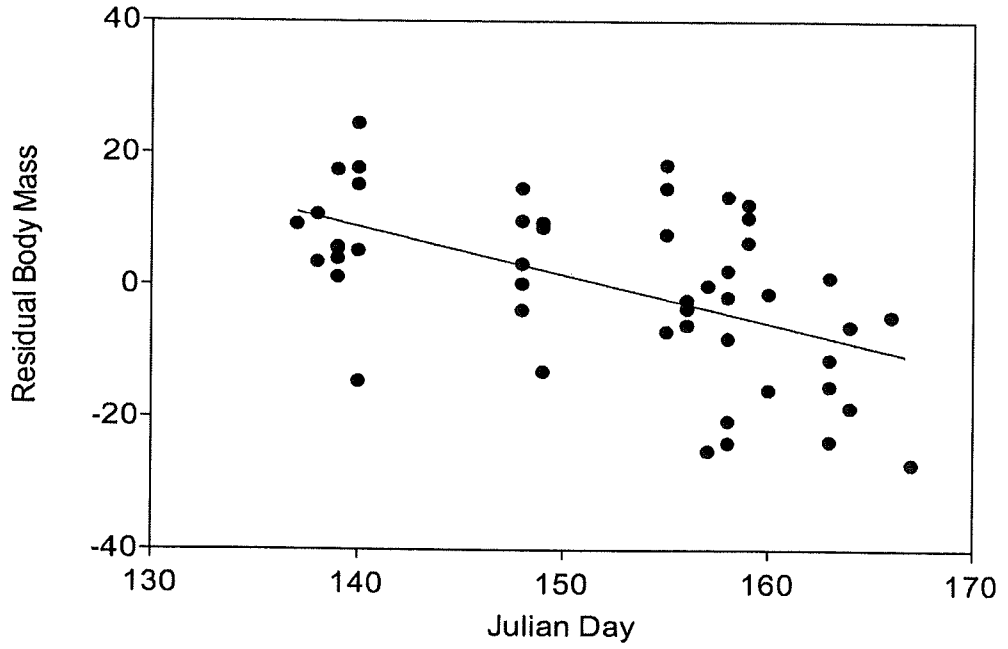

Figure 2. Residual body mass (from the orthogonal regression of body mass and tarsus length) is negatively related to trap date. Filled circles indicate observed data and the solid line represents the general linear regression fit to the data ($r^2 = 0.28$).

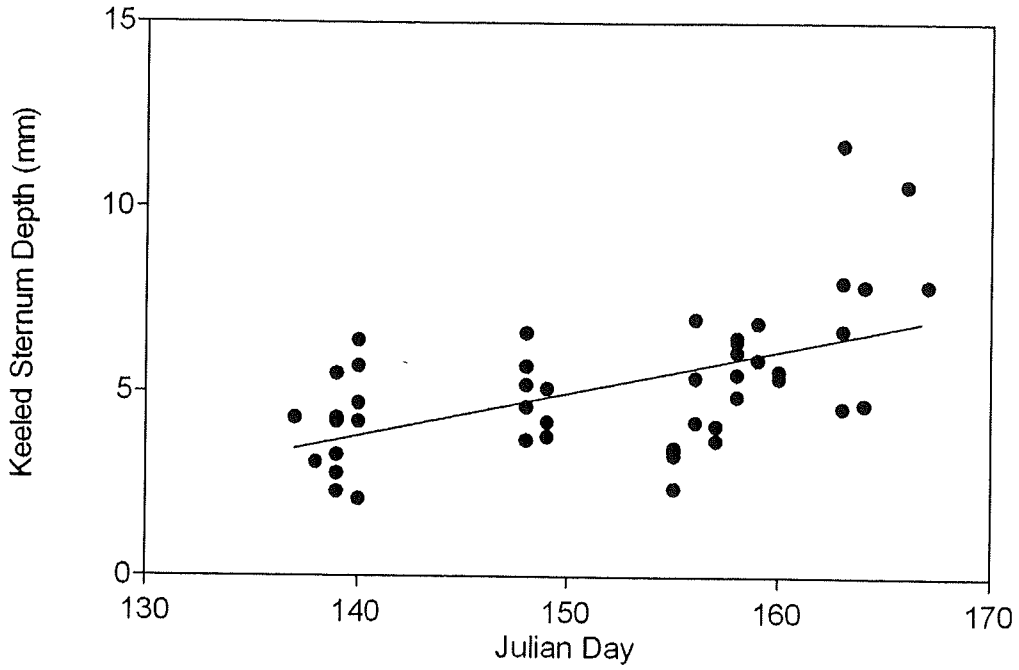

Figure 3. Keeled sternum depth (i.e., protrusion of the keel bone above the pectoral muscle) is positively related to trap date. Filled circles indicate observed values and the solid line represents the general linear regression fit to the data ($r^2 = 0.31$).

(mass: $F_{1,52} = 9.05$, $p = 0.004$, $r^2 = 0.15$; breast width: $F_{1,53} = 4.60$, $p = 0.04$, $r^2 = 0.08$).

Serially-collected plasma samples exhibited corticosterone concentrations that increased from baseline concentrations to higher asymptotic concentrations collected 20 min or more after startle. Maximum corticosterone concentrations occurred in either the second or third serial plasma sample in 54 of the 55 individuals for which three serial plasma samples were available. Moreover, concentrations from the first serial sample (mean and standard error of 7.44 ± 0.95) were lower than concentrations from the second serial sample (19.65 ± 0.95; $t = 12.86$, $p < 0.001$, $n = 54$), whereas concentrations from the second serial sample are lower than concentrations from the third serial sample (21.97 ± 0.95; $t = 2.20$, $p = 0.032$, $n = 54$) but did not differ by as much (Figure 4). Later nesting adults show a seasonal

increase in the stress response. The rise in corticosterone concentration during the stress response, as measured by the difference in the maximum concentration observed in an individual and the baseline concentration (i.e., the concentration associated with the first serial sample) increased with trap date ($F_{1,53} = 10.41$, $p = 0.002$, $r^2 = 0.16$, Figure 5). Maximum corticosterone concentrations also increase with trap date ($F_{1,53} = 9.46$, $p = 0.003$, $r^2 = 0.15$), as did the concentrations from the second ($F_{1,53} = 14.92$, $p < 0.001$, $r^2 = 0.22$) and third ($F_{1,53} = 3.97$, $p = 0.051$, $r^2 = 0.07$) serial samples. However, baseline corticosterone concentrations did not differ across the breeding season ($F_{1,53} = 0.13$, $p = 0.720$, $r^2 = 0.002$).

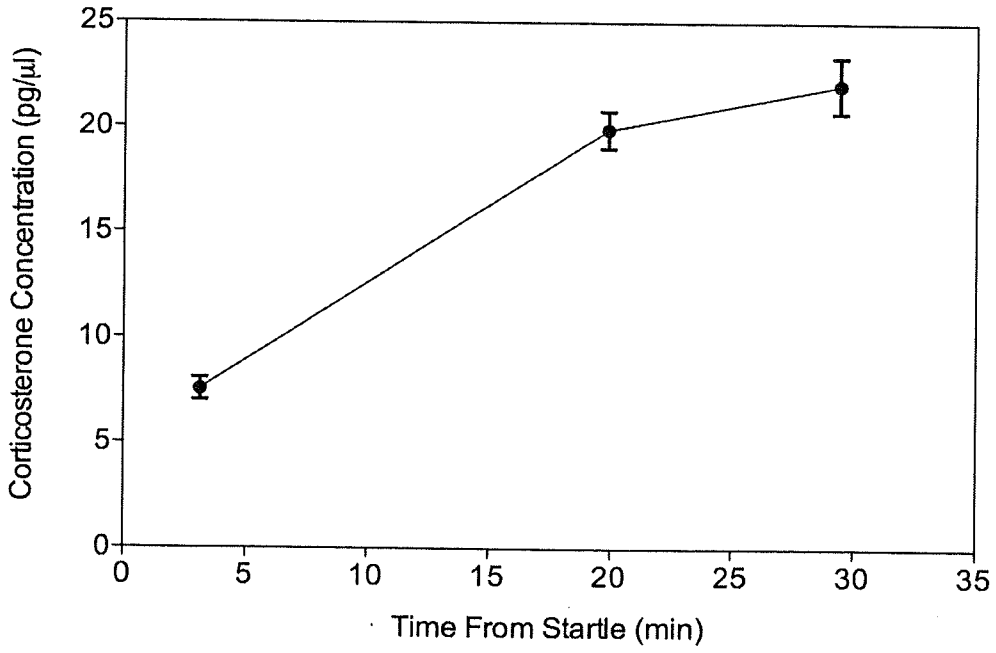

Figure 4. Plasma corticosterone concentrations determined from serial plasma samples. Time from startle indicates the time elapsed from startle to the time when the plasma was collected, and each point represents the mean corticosterone concentrations for which three plasma samples were collected ± SE (n = 54).

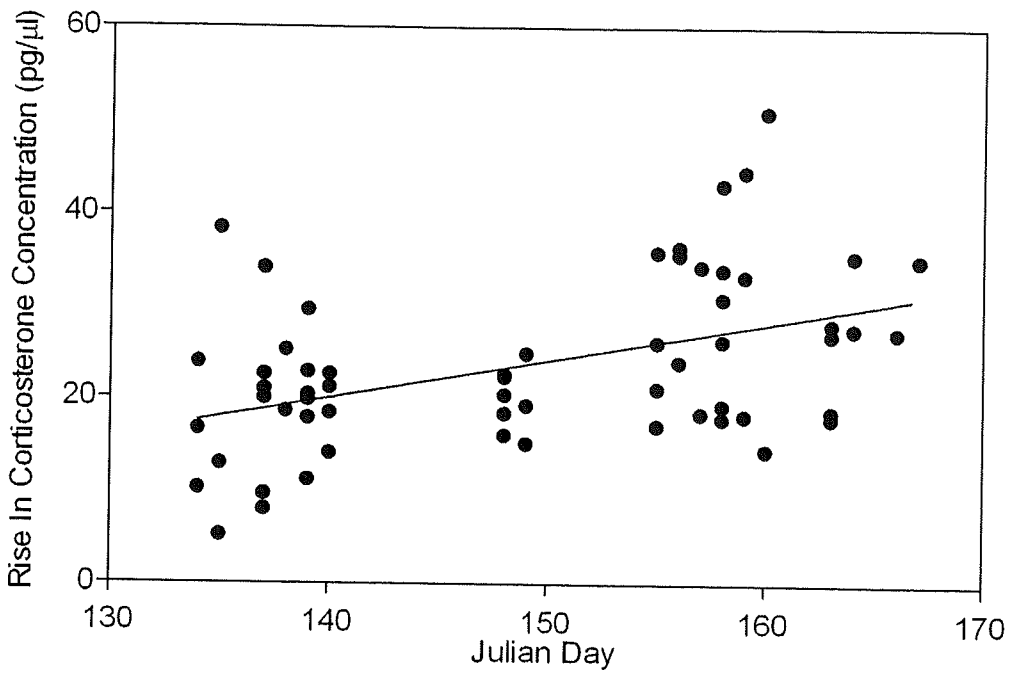

Figure 5. Rise in corticosterone concentration (i.e., maximum concentration – baseline concentration) is positively related to trap date. Filled circles indicate observed values and the solid line represents the linear regression fit to the data (r^2 = 0.21).

The first two principal components for the A1 bacteria colony count, V1 bacteria colony count and heterophil/lymphocyte ratio are linearly related to trap date. A1 bacteria colony count, V1 colony count and heterophil/lymphocyte ratio were all log-transformed to achieve normality. The eigenvector for the first principal component weights the log-transformed A1 colony count highest (coefficient of 0.933), followed by log-transformed heterophil/lymphocyte ratio (coefficient of 0.329) and log-transformed V1 colony count (coefficient of -0.139), accounts for 51.71% of the variance and the components are positively related to trap date ($F_{1,49}$ = 6.93, p = 0.011, r^2 = 0.123; Figure 6). The eigenvector for the second principal component weights the log-transformed heterophil/lymphocyte ratio highest

(coefficient of 0.895), followed by log-transformed A1 colony count (coefficient of -0.356) and log-transformed V1 colony count (coefficient of -0.268), accounts for 36.19% of the variance and the components are negatively related to trap date ($F_{1,49}$ = 10.63, p = 0.002, r^2 = 0.178; Figure 6).

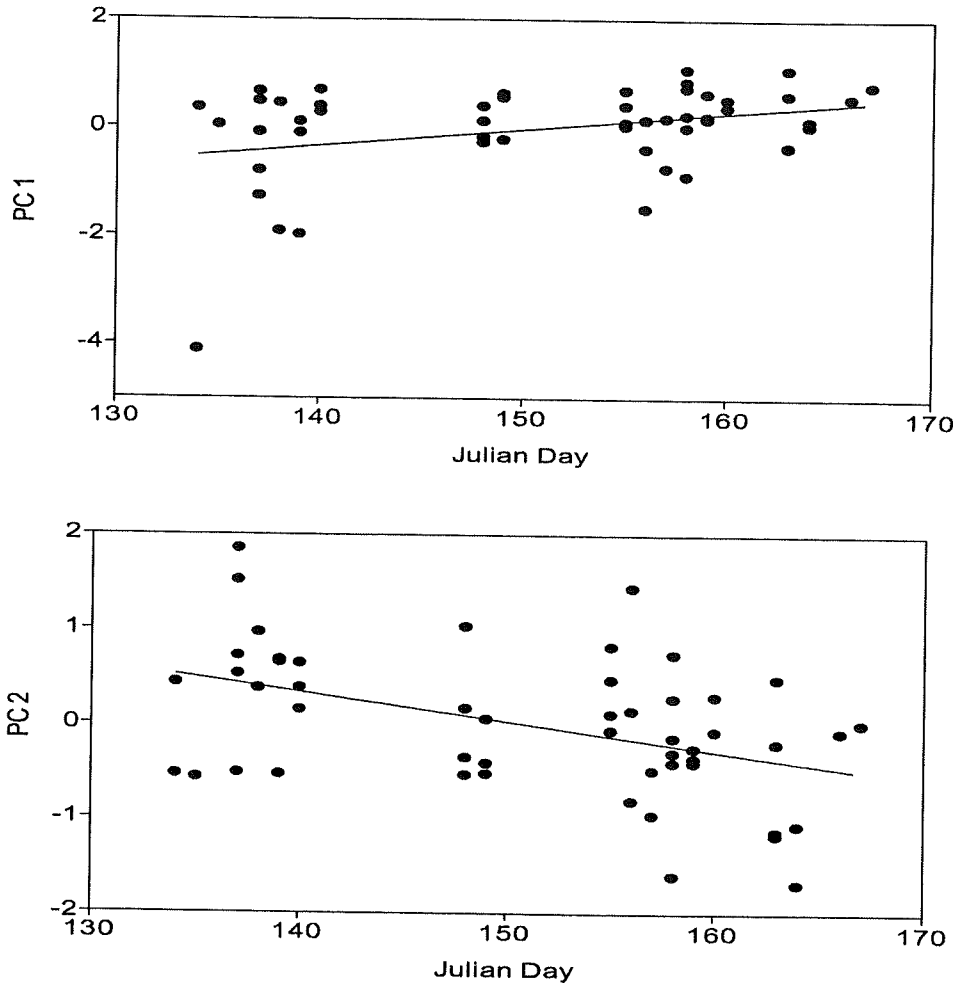

Figure 6. (PC1) The first principal component for log-transformed A1 colony count, V1 colony count and heterophil/lymphocyte ratio (filled cirlces) is positively related to trap date. Large values for the first principal component are associated with large A1 colony counts, large heterophil/lymphocyte ratios and low V1 colony counts. (PC2) The second principal component for log-transformed A1 colony count, V1 colony count and heterophil/lymphocyte ratio (filled circles) is negatively related to trap date. Large values for the second principal component are associated with large heterophil/lymphocyte ratios, large A1 colony counts and low V1 colony counts. The solid line in each plot represents the linear regression fit to the principal component values.

DISCUSSION

Understanding seasonal variation in body condition of breeding adults is critical to understanding how factors affecting the timing of nesting in migratory birds impact reproductive success. Structural size of Franklin's gull chicks varies seasonally (Berg 2009), due to both photoperiod (an environmental signal) effects and maternal effects (Clark and Reed, *unpublished data*). Environmental cues vary seasonally and can produce changes in adult physiology; however, no information is available on how adult physiology changes during the breeding season in Franklin's gull, and many other migratory species.

In this study, I found that residual body mass (i.e., body mass corrected for skeletal size) of nesting adult Franklin's gull decreased as the breeding season progressed (Figure 2). Other studies have reported similar trends in body mass or body condition. For example, Golet and Irons (1999) found that body mass of adult kittiwakes (*Rissa tridactyla*) decreases during the chick-rearing period, and hypothesized that this occurs as a trade off between chick survival and adult survival. In other words, Golet and Irons suggest that breeding adult kittiwakes compromise their body condition because they are investing resources in chicks. I captured nesting adult Franklin's gulls in the first half of incubation prior to the onset of food provisioning of offspring, yet still observed a decline in body condition (i.e., residual body mass). Similarly, Kitaysky et al. (1999) found that body condition decreased in late-nesting kittiwakes, and hypothesized that local ecological factors (e.g., declining food availability) were the proximate mechanisms responsible for the pattern. Information on the temporal dynamics of macro-invertebrates associated with the

northern plains is unavailable but would, however, help provide correlative information in support or against a local resource limiting condition.

Keel depth, another measure of body condition, also varied seasonally. Adult birds exhibited an increase in keel bone exposure with the progression of the breeding season (Figure 3). Keel depth is a measure of pectoral muscle (the principle flight muscle) size (Bolton et al. 1991), indicating that Franklin's gull adults that initiate nesting later in the season have reduced flight muscle. Similarly, Neto and Gosler (2010) found that breast muscle size and protein reserves decreased across the breeding season in Savi's warblers (*Locustella luscinioides*), which has been hypothesized to influence future survival. Again, this could be due to breeding adults compromising their own body condition to offset diminished resources for chicks associated with late-season nests. However, several alternative hypotheses could also explain the pattern. For instance, if later nesting birds arrive at breeding sites later because they are in poorer condition and took longer to complete migration, a similar pattern in body condition and timing of nesting might be observed. Both et al. (2005) showed that both arrival and breeding dates in pied flycatchers (*Ficedula hypoleuca*) were dependent on temperatures on their main staging grounds. Inclement weather may delay migration and could result in birds arriving in poorer condition.

In addition to seasonal declines in overall body condition, I also found evidence of seasonal variation in physiological sensitivity to stress in Franklin's gull. Birds that initiate nesting later in the season show higher maximum plasma corticosterone concentrations in the stress profile compared to birds that initiate nesting earlier in the season (Figure 5). The vertebrate stress response is

characterized by an increase in concentrations of plasma corticosterone following a stress, and the peak in plasma corticosterone concentration is considered a measure of sensitivity to an induced stress (Beuving and Vonder 1978). Hence, later nesting Franklin's gull adults are more sensitive to a perceived threat (i.e., stress) than are earlier nesting adults. A recent study investigating stress response changes during the breeding season found an increase in response in birds during late incubation (Adams et al. 2005), which is similar to findings that variation in corticosterone levels during the breeding season are related to reproductive stage of the adult (Pereyra and Wingfield 2003). My observations indicate there is seasonal variation in stress sensitivity (measured via rise in corticosterone concentration) not related to differences in the reproductive cycle of adults in Franklin's gull.

Immune function represents another aspect of the physiological condition of an individual. Constitutive immunity in vertebrates can be assessed by the bacterial capacity of plasma (Millet et al. 2007). Immune function can also be measured through heterophil/lymphocyte ratios (Gross and Siegel 1983), because lymphocytes are a primary component of the acquired immune system (Bone and Moore 2008) and an increase in the number of heterophils relative to lymphocytes can indicate a chronic stress or an increase in vulnerability to infection (Gross and Siegel 1983; Vleck et al. 2000). Using principal component analysis to consider multiple aspects of variation in the immune system components, we found that bacteria-killing capability was lower in late-nesting gulls compared to early nesting gulls (Figure 6). However, variation in heterophil/lymphocyte ratios of nesting Franklin's gull adults is more complex, with some variation indicating a positive relationship with trap date (and

therefore nest initiation) (Figure 6a) but other elements of the variation indicating a negative correlation with trap date (Figure 6b). Moller et al. (2003) found that birds experience seasonal changes in the impact of parasites, resulting in changes in immune function that are related to nest type and location. Other studies have indicated that incubating adults exhibit an increase in heterophil/lymphocyte ratios compared to non-incubating adults during the breeding season (Ots and Horak 1996; Horak et al. 1998; Hanssen et al. 2005). My findings suggest that later nesting Franklin's gull adults exhibit some immune suppression. However variation in immune system performance is not explained by season alone.

Timing of nesting has a significant impact on the physiological condition of migratory birds. Individuals nesting later in the breeding season are known to be in poorer condition than individuals nesting earlier in the breeding season (Perrins 1970; Verhulst et al. 1995), and I found that multiple indices of condition of adult Franklin's gulls are lower in later nesting individuals. Migratory birds nesting in temperate latitudes are under time constraints to find nesting sites, lay eggs, fledge their young and prepare for the migration back to non-breeding areas.

Seasonal variation in condition may be related to these time constraints through resource allocation for self-maintenance and offspring. Many researchers have hypothesized that postmigratory residual body stores are critical for successful breeding (Ryder 1970; Ankney and MacInnes 1978; Drent and Daan 1980; Ebbinge et al. 1982; Davidson and Evans 1988; Sandberg and Moore 1996). Perrins (1970) hypothesized that breeding birds laying eggs later in the season experienced a shortage of food during egg formation, resulting in offspring that are unable to fully

profit from the seasonal peak in food availability seen in most temperate ecosystems. The relative importance of each factor (residual fat stores versus local food resources) has been more broadly expanded to contrast species in which a capital (e.g., fat stores) versus income (e.g., local food resources) breeding strategy is evident in the reproductive life history (Drent and Daan 1980). At present not enough information is available to determine if Franklin's gull adults are capital or income breeders.

I found that body condition, stress tolerance and immune system performance decreased with progression of the breeding season in Franklin's gull, which is consistent with both capital and income breeding strategies. Structural size at hatching and post-hatching survival of Franklin's gull chicks decline as the breeding season progresses (Berg 2009), therefore, the timing of nesting has significant fitness consequences for both parents and offspring in this species. We observed seasonal differences in multiple metrics of condition during the first part of incubation (and therefore before costs of parental care in chick provisioning are incurred), but it is not known if these seasonal differences are present during the latter stages (e.g., the rapid yolk development stage) of egg development. Little is known about physiological condition before and during migration, how food resources vary seasonally, how egg constituents vary seasonally, and how all of these relate to the timing of nesting in Franklin's gull. However, my results indicate that late-nesting adults are in poorer physiological condition compared to early-nesting adults, which likely compounds differences in survival of late-season versus early-season chicks. Thus, determining whether resources acquired outside the breeding area (i.e., capital) or locally (i.e., income) underlie the seasonal patterns in adult condition and chick characteristics is

critical to directing management efforts in Franklin's gull and other long-lived birds nesting in the northern plains.

LITERATURE CITED

Ackerman, J. T. and C. A. Eagles-Smith. 2010. Accuracy of egg flotation throughout incubation to determine embryo age and incubation day in waterbird nests. Condor 112:438-446.

Adams, N. J., J. F. Cockrem, G. A. Taylor, E. J. Candy and J. Bridges. 2005. Corticosterone responses of grey-faced petrels (*Pterodroma macroptera gouldi*) are higher during incubation than during other breeding stages. Physiological and Biochemical Zoology 78:69-77.

Ankney, C. D. and C. D. MacInnes. 1978. Nutrient reserves and reproductive performance of female lesser snow geese. Auk 95:459-471.

Barrientos, R., A. Barbosa, F. Valera and E. Moreno. 2007. Temperature but not rainfall influences timing of breeding in a desert bird, the trumpeter finch (*Bucanetes githagineus*). Journal of Ornithology 148:411-416.

Berg, E. A. 2009. Effects of nest site environment and timing of breeding on reproductive success in Franklin's gulls (*Larus pipixcan*). M. S. Thesis. North Dakota State University, Fargo, ND.

Beuving, G. and G. M. A. Vonder. 1978. Effect of stressing factors on corticosterone levels in the plasma of laying hens. General and Comparative Endocrinology 35:153-159.

Bolton, M., P. Monaghan and D. C. Houston. 1991. An improved technique for estimating pectoral muscle protein condition from body measurements of live gulls. Ibis 133:264-270.

Bone, Q. and R. H. Moore. 2008. Biology of Fishes (3rd Ed.). Taylor & Francis Group, New York.

Both, C., R. G. Bijlsma and M.E. Visser. 2005. Climatic effects on timing of spring migration and breeding in a long-distance migrant, the pied flycatcher (*Ficedula hypoleuca*). Journal of Avian Biology 36:368-373.

Breuner, C. W. and T. P. Hahn. 2003. Integrating stress physiology, environmental change, and behavior in free-living sparrows. Hormones and Behavior 43:115-123.

Brown, M. E. 1996. Assessing body condition in birds. Current Ornithology 13:67-135.

Burger, J. 1971. A method for marsh-trapping breeding Franklin's gulls. Bird Banding 42:123-124.

Burger, J. and M. Gochfeld. 1994. Franklin's gull: *Larus pipixcan*. In A. Poole and F. Gill [eds.], Birds of North America. American Ornithologists' Union & Academy of Natural Sciences, Washington, D.C. & Philadelphia, PA.

Cyr, N. and L. M. Romero. 2006. Chronic stress in free-living European starlings reduces corticosterone concentrations and reproductive success. Journal of General and Comparative Endocrinology 151:82-89.

Davidson, N. C. and P. R. Evans. 1988. Prebreeding accumulation of fat and muscle protein by arctic-breeding shorebirds. In: Ouellet H (ed) Acta XIX Congr Int Ornithol, University of Ottawa Press, Ottawa, pp 342-352.

Drent, R. H. 2006. The timing of birds' breeding seasons: the Perrins hypothesis revisited especially for migrants. Ardea 94:305-322.

Drent, R. H. and S. Daan. 1980. The prudent parent: energetic adjustments in avian breeding. Ardea 68:225-252.

Dubiec, A., M. Witek and M. Cichon. 2005. Seasonal decline in leukocyte concentrations and reproductive output in female great tits (*Parus major*). Auk 122:829-834.

Ebbinge, B. S., A. St. Joseph, P. Prokosch and B. Spaans. 1982. The importance of spring staging areas for Arctic-breeding geese, wintering in western Europe. Aquila 89:249-258.

Golet, G. H. and D. B. Irons. 1999. Raising young reduces body condition and fat stores in black-legged kittiwakes. Oecologia 120:530-538.

Gosler, A. G., J. J. D. Greenwood, J. K. Baker and N. C. Davidson. 1998. The field determination of body size and condition in passerines: a report to the British Ringing Committee. Bird Study 45:92-103.

Green, A. J. 2001. Mass/length residuals: Measures of body condition or generators of spurious results? Ecology 82:1473-1483.

Gross, W. B. and H. S. Siegel. 1983. Evaluation of the heterophil/lymphocyte ratio as a measure of stress in chickens. Avian Diseases 27:972-979.

Hanssen, S. A., D. Hasselquist, I. Folstad and K. E. Erikstad. 2005. Cost of reproduction in a long-lived bird: incubation effort reduces immune function and future reproduction.

Hatch, M. I., R. J. Smith and J. C. Owen. 2010. Arrival timing and hematological parameters in Gray Catbirds (Dumetella carolinensis). Journal of Ornithology 151:545-552.

Horak, P., S. Jenni-Elermann, I. Ots and L. Tegelmann. 1998. Health and reproduction: the sex-specific clinical profile of great tits (*Parus major*) in relation to breeding. Canadian Journal of Zoology 76:2235-2244.

JMP. 2002. JMP, Version 5. SAS Institute, Inc., Cary, North Carolina.

Kitaysky, A. S., J. C. Wingfield and J. F. Piatt. 1999. Dynamics of food availability, body condition and physiological stress response in breeding Black-legged kittiwakes. Functional Ecology 13:577-584.

Matson, K. D., Tieleman, B. I. and Klasing, K. C. 2006. Capture stress and the bactericidal competence of blood and plasma in five species of tropical birds. Physiological and Biochemical Zoology 79:556-564.

Miller, D. A., C. M. Vleck and D. L. Otis. 2009. Individual variation in baseline and stress-induced corticosterone and prolactin levels predicts parental effort by nesting mourning doves. Hormones and Behavior 56:457-464.

Millet, S., J. Bennett, K. A. Lee, M. Hau and K. C. Klasing. 2007. Quantifying and comparing constitutive immunity across avian species. Developmental and Comparative Immunology 31:188-201.

Moe, B., I. Langseth, M. Fyhn, G.W. Gabrielsen and C. Bech. 2002. Changes in body condition in breeding kittiwakes Rissa tridactyla. Journal of Avian Biology 33:225-234.

Moller, A. P., J. Erritzoe and N. Saino. 2003. Seasonal changes in immune and parasite impact on hosts. American Naturalist 161:657-671.

Mueller, H., D. Berger, N. Mueller, W. Robichaud and J. Kaspar. 2002. Age and sex differences in wing loading and other aerodynamic characteristics of merlins. The Wilson Bulletin 114:272-275.

Nager, R. G., P. Monaghan and D. C. Houston. 2001. The cost of egg production: increased egg production reduces future fitness in gulls. Journal of Avian Biology 32:159-166.

Neto, J. M. and A. G. Gosler. 2010. Variation in body condition of Savi's Warblers *Locustella lusciniodies*: the reproductive stress and flight adaptation hypothesis revisited. Journal of Ornithology 151:201-210.

Nol, E. and H. Blokpoel. 1983. Incubation period of Ring-billed gulls and the egg immersion technique. Wilson Bulletin 95:283-286.

Norte, A. C., J. A. Ramos, H. L. Sampaio, J. P. Sousa and B. C. Sheldon. 2010. Physiological condition and breeding performance of the Great Tit. The Condor 112:79-86.

Ots, I. and P. Hõrak. 1996. Great tits Parus major trade health for reproduction. Proceedings of the Royal Society of London Series B-Biological Sciences 263:1443-1447.

Pereyra, M. E. and J. C. Wingfield. 2003. Changes in plasma corticosterone and adrenocortical response to stress during the breeding cycle in high altitude flycatchers. General Comparative Endocrinology 130:222-231.

Perrins, C. M. 1970. The timing of birds' breeding seasons. Ibis 112:242-255.

Phillips, R. and R. Furness. 1997. Sex-specific variation in the loss of mass by breeding arctic skuas. Oikos 28:163-170.

Richner, H., A. Oppliger and P. Christe. 1993. Effect of an ectoparasite on reproduction in great tits. Journal of Animal Ecology 62:703-710.

Ryder, J. P. 1970. A possible factor in the evolution in clutch size in Ross' goose. Wilson Bull 82:5-13.

Sandberg, R. and F. R. Moore. 1996. Fat stores and arrival on the breeding grounds: reproductive consequences for passerine migrants. Oikos 77:577-581.

Schmidt-Wellenburg, C. A., S. Engel and G. H. Visser. 2008. Energy expenditure during flight in relation to body mass: effects of natural increases in mass and artificial load in Rose Coloured Starlings. Journal of Comparative Physiology Biochemical Systemic and Environmental Physiology 178:767-777.

Schulte-Hostedde, A. J., B. Zinner, J. S. Millar and G. J. Hickling. 2005. Restitution of mass-size residuals: Validating body condition indices. Ecology 86:155-163.

Suetin, G., B. N. White and P. T. Boag. 1991. Preservation of avian blood and tissue samples for DNA analysis. Can J Zool 69:82-90.

Verhulst, S., J. H. Van Balen and J. M. Tinbergen. 1995. Seasonal decline in reproductive success of the great tit: variation in time or quality? Ecology 76:2392-2403.

Vleck, C. M., N. Vertalino, D. Vleck and T. L. Bucher. 2000. Stress, corticosterone, and heterophil to lymphocyte ratios in free-living Adelie Penguins. Condor 102:392-400.

Wikelski, M., L. B. Martin, A. Scheuerlein, M. T. Robinsoni, N. D. Robinson, B. Heim, M. Hau and E. Gwinner. 2008. Avian circannual clocks: adaptive significance and possible involvement of energy turnover in their proximate control. Philosophical Transactions of the Royal Society B-Biological Sciences 363:411-423.

Wingfield, J. C. 2005. Flexibility in annual cycles of birds: implications for endocrine control mechanisms. Journal of Ornithology 146:291-304.

Wingfield, J. C., J. P. Smith and D. S. Farner. 1982. Endocrine responses of white-crowned sparrows to environmental stress. Condor 84:399-409.

Wingfield, J. C. and D. S. Farner. 1975. The determination of five steroids in avian plasma by radioimmunoassay and competitive protein binding. Steroids 26:311-327.