

AN INTEGRATIVE APPROACH TO TESTING DENSITY EFFECTS IN A MIGRATORY  
SONGBIRD, THE PROTHONOTARY WARBLER (*PROTONOTARIA CITREA*)

BY

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DISSERTATION

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## ABSTRACT

Animal populations often fluctuate around local carrying capacity through density-dependent processes such as competition, predation, and disease. Once subject to debate, ecologists now recognize density dependence as a conceptual cornerstone of population biology. Yet despite >50 years of research, key questions persist about the nature and generality of density effects for many animal populations. For my dissertation, I took an integrative approach to test for potential effects and mechanisms of density on the reproduction, behavior, and physiology of a population of cavity-nesting songbirds, the Prothonotary Warbler (*Protonotaria citrea*), in southern Illinois, USA. From 2008-2011, I experimentally manipulated the availability of nest boxes by establishing two low-density and two high-density subplots on each of two 40 ha sites. Previous work in this system has shown that high reproductive success leads to increased site and territory fidelity so I eliminated nest predation and removed the eggs of a common brood parasite, the Brown-headed Cowbird (*Molothrus ater*), to improve warbler nesting success and increase their densities. The resultant range of densities (1-27 neighbors/pair) were at or beyond that seen under natural conditions in this system.

Each year, I estimated warbler reproductive output, nestling provisioning rates, and nestling body condition prior to fledging to test for effects of density on the annual fecundity of warblers. My results suggested that warbler annual fecundity was unaffected by local conspecific densities. Warbler parents in high-density neighborhoods provisioned their young at similar rates and raised a similar number of offspring with similar body condition prior to fledging compared to warblers in lower-density areas.

During 2010 when neighbor densities were at their greatest observed levels, I selected focal males for behavioral observations and I estimated their song, food search movement (i.e.,

hops, walks, and flights), and prey attack rates in relation to density. Male behaviors varied somewhat in relation to neighbor density, particularly during specific stages. Males with more neighbors sang at a greater rate during the preincubation and incubation stages but this trend disappeared by the nestling stage as males reduced their song rates overall, presumably because they were busy providing parental care to their young. Males also increased their food search movement rates during the nestling stage when they had more neighbors but there was no concomitant increase in prey attack rates. Thus, crowded males appeared to be working harder to find food compared to males in lower density neighborhoods.

Finally, I also collected blood samples from focal pairs each year to test for density effects on baseline plasma corticosterone (CORT, the primary glucocorticoid stress hormone in birds), white blood cell (WBC) counts, and WBC ratios. Male and female warblers had similar baseline CORT concentrations, WBC ratios, and WBC counts regardless of the number of neighbors surrounding them. Further, baseline CORT concentrations were weakly correlated with the indices of immune function that I estimated.

Collectively, my results suggest that increased conspecific density did not lead to increased competition for food, reduced reproductive success, or altered stress physiology in this population of Prothonotary Warblers. The bottomland hardwood forests and swamps of southern Illinois represent a very productive habitat type and the interference mechanism that appears to regulate songbirds in many other systems (i.e., density-dependent competition leading to food limitation) does not appear to be important for warblers in this system. Future research should incorporate a larger spatial scale to determine if a site-dependent mechanism of density dependence is operating on this population. Evaluating juvenile recruitment and adult survival in relation to conspecific density would also be helpful. Finally, integrative work is needed to better

understand how stress physiology and immunosuppression may mediate density effects in systems where density-dependent regulation has previously been documented.

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# CHAPTER 1

## GENERAL INTRODUCTION

The regulation of populations around local carrying capacity via density-dependent processes is a well-recognized cornerstone of population biology. Yet even with a significant body of theoretical and empirical work on the subject (e.g. Lack 1954, Sinclair 1989, Turchin 1990, Turchin 1999, Krebs 2002), key questions persist about the nature and generality of density effects for some taxa. For example, even with a well-studied group such as birds, clear evidence for density dependence remains elusive (e.g., Brawn 1987, Torok and Toth 1988, Dhondt et al. 1992). Further, although most studies of density effects have focused on understanding just one density-dependent mechanism (e.g., competition, predation, disease; but see Rodenhouse et al. 2003), multiple mechanisms likely interact to regulate populations (Krebs 2002).

Intensified competition has often been invoked as the main process through which density dependence occurs (reviewed in Hixon et al. 2002). In birds, density-dependent resource competition (e.g., for food, space) leading to variation in fecundity has been particularly well-studied (Lack 1954, Newton 1998). Increased competition with increased conspecific density may reduce overall reproductive output through effects on clutch size, hatching success, or fledging success (e.g., Kluijver 1951, Perrins 1965, Alatalo and Lundberg 1984, Both 1998a, Sillett et al. 2004). Competition for food may also manifest in more subtle ways such as reduced nestling food provisioning rates (Sillett et al. 2004) or body condition prior to fledging (Alatalo and Lundberg 1984, Torok and Toth 1988, Both 1998b) which can lead to density-dependent fledgling survival rates (Perrins 1965, Tinbergen and Boerlijst 1990, Both et al. 1999) and hence recruitment rates.

Less is known about the behavioral responses that can occur when territorial animals experience crowding (but see Sillett et al. 2004, Dobbs et al. 2007). In particular, male attentiveness may have a large impact on overall reproductive output for species in which males are the territorial sex and provide a portion of the parental care. If increased conspecific density negatively influences a male's ability to provision his mate or offspring either directly through food competition or indirectly through mate or territory defense, then this may translate to reduced overall fecundity (i.e., competitive neglect). Alternatively, a male may adjust his behavior accordingly to cope with the increased intensity of competition.

Further, physiological mechanisms of density dependence were proposed >50 years ago (Christian 1950, Christian 1963), but only relatively recently have advances in the field of ecophysiology allowed field biologists to incorporate the physiological responses of animals to density (e.g., Sapolsky 1992, Boonstra et al. 1998, Belden et al. 2007, Leary et al. 2008). In particular, understanding the stress response of vertebrates to their environment has become increasingly important in explaining patterns of behavior and fitness not previously recognized (Romero 2002, McEwen and Wingfield 2003, Korte et al. 2005). Vertebrates secrete glucocorticoids (GCs) via the hypothalamic-pituitary-adrenal (HPA) axis when faced with a stressful event (e.g., fighting, predator attack, short-term food shortage; Sapolsky et al. 2000, Bortolotti et al. 2008). This stress response can be adaptive over the short-term, but chronically stressed vertebrates with continually high concentrations of circulating GCs may show impaired function (e.g., adverse behavioral responses, reduced reproductive output, immunosuppression; Boonstra et al. 1998, Sapolsky et al. 2000, Martin et al. 2005, Bortolotti et al. 2008). Chronic stress and immunosuppression can increase in response to increased competition (Vleck et al. 2000, Ruiz et al. 2002). Under a physiological mechanism of density dependence, increased

intensity of competition or other stressors with density should lead to chronic stress and reduced reproductive output; however, life-history theory predicts a trade-off between reproduction and self-maintenance (e.g., immunocompetence) may occur. Indeed, such a trade-off may help explain the lack of negative density effects on correlates of reproductive success observed in empirical studies. Only a handful of studies to date have investigated a physiological mechanism of density dependence in the field (e.g., mammals: Sapolsky 1992, Boonstra et al. 1998; amphibians: Belden et al. 2007, Leary et al. 2008; hatchery fish: Barton and Iwama 1991, Montero et al. 1999), but to my knowledge none have focused on wild birds.

Wild birds are particularly useful for individual- and population-level studies because of the ease with which they can be captured, individually marked, and observed. Many birds have discrete reproductive seasons in individual nests, making it easy to estimate individual reproductive rates (Newton 1998). For these reasons, they have often been textbook examples for density dependence in vertebrates, and long-term avian studies have provided significant foundation and precedent for questions and assumptions about population regulation (Lack 1954, Newton 1998).

Despite this utility for study, surprisingly few avian studies have used an experimental approach to test ideas about density dependence (but see Tompa 1967, Alatalo and Lundberg 1984, Brawn 1987, Both 1998, Sillett et al. 2004). Even the classic studies by Kluijver and Lack were conducted without experimental control (Kluijver 1951, Lack 1966). Moreover, studies that have experimentally manipulated density are often limited in interpretation due to confounding factors such as habitat (e.g., Torok and Toth 1988) or year effects (e.g., Alatalo and Lundberg 1984).

Cavity-nesting songbirds are particularly ideal for experimentation because their densities can be more readily manipulated through the use of nest boxes (e.g., Kluijver 1951, Lack 1954, Alatalo and Lundberg 1984, Torok and Toth 1988). The Prothonotary Warbler (*Protonotaria citrea*) is a migratory songbird that winters in the neotropics and breeds in bottomland hardwood and swamp forests of the eastern and central U.S. Although this species is a secondary-cavity nester, breeding adults readily accept nest boxes (Fleming and Petit 1986). These warblers are territorial, socially monogamous and exhibit high site and territory fidelity between breeding seasons, particularly following years of high reproductive success (Hoover 2003a). Previous research has provided significant foundation for understanding Prothonotary Warbler behavior, reproduction, and survival throughout its range (e.g., Petit et al. 1990, Petit and Petit 1996, Hoover 2003a, Hoover 2006, Hoover and Reetz 2006, Schelsky 2010).

I designed an experiment to test potential effects and mechanisms of density dependence on the behavior, reproduction, stress physiology, and immune function of Prothonotary Warblers while controlling for many of the confounding factors that have limited the interpretation of previous experimental studies of avian density dependence. Additionally, I conducted my work in the bottomland hardwood and swamp forests of southern Illinois where a high diversity of flora and fauna is found (Kellison and Young 1997, Kozlowski 2002). This system is one of the most productive habitat types in North America, supporting a diverse suite of breeding neotropical migratory birds (Wakeley and Roberts 1996, Sallabanks et al. 2000) and contrasts with existing studies on songbird density effects conducted in relatively homogeneous habitats (e.g., pine, oak, or beech/birch hardwoods; Kluijver 1951, Lack 1954, Torok and Toth 1988, Sillett et al. 2004). This contrast in habitat type allowed me to test the robustness of the

prevailing theory that conspecific competition over food resources is the main factor regulating songbird populations.

By experimentally controlling nest box density, parasitism by a common brood parasite [Brown-headed Cowbird (*Molothrus ater*)], and predation by nest predators, I manipulated the local densities of warblers through time. Local conspecific densities initially ranged from 2-10 neighbors/breeding pair in 2008 but the manipulation more than doubled that range (1-27 neighbors/pair) by 2010 and 2011. The experimental densities created often exceeded those found naturally in this population (Hoover 2009). If density effects via a food competition mechanism help regulate warbler reproductive output through behavioral or physiological mechanisms, I should have been able to detect these effects with my experiment.

In Chapter 2, “Local neighbor density does not influence reproductive output in a cavity-nesting songbird, the Prothonotary Warbler,” I test the effects of density on correlates of warbler reproductive output. I hypothesized that increased warbler densities would lead to reduced total reproductive output through some combination of effects on hatching success, fledging success, and attempts at second broods. I also estimated nestling provisioning rates and nestling body condition prior to fledging to detect more subtle effects of food limitation that may not be apparent when looking at overall fledgling production.

In Chapter 3, “Behavioral responses of breeding male Prothonotary Warblers (*Protonotaria citrea*) to increased conspecific density,” I present the results from focal behavioral observations in which I estimated the song, foraging movement, and prey attack rates of male warblers. I predicted that male song rates would increase with neighbor density but that these rates would vary according to stages of the nest cycle due to conflicting demands on the male’s time (e.g., territory establishment and mate attraction/retention early in the nesting cycle;

parental care responsibilities during the nestling stage). Further, I assumed that competition for food would increase with conspecific density through a combination of direct and indirect effects. Based on that assumption, I predicted that males would increase their rate of foraging movements (i.e., hops, walks, flights) but decrease their rates of prey attacks (gleans, probes, and sallies) with increasing densities. Again, I also hypothesized that these rates would vary according to nest stage due to the peak demand for food at the nestling stage.

I discuss the effects of density on the physiology of warblers in Chapter 4, “Baseline corticosterone and indices of immune function do not vary with conspecific density in a migratory songbird.” I hypothesized that increased conspecific densities would lead to chronically stressed birds as indicated by elevated baseline plasma corticosterone (CORT, the primary GC in birds) and heterophil/lymphocyte (H/L) ratios (as estimated from blood smears). I used the H/L ratio as an additional indicator of chronic stress as birds show increased numbers of heterophils and decreased numbers of lymphocytes in response to prolonged stressors such as social stress and reduced food intake (Gross and Siegel 1983, Vleck et al. 2000, Ruiz et al. 2002). Because I found little evidence for density effects on fecundity in Chapter 2, I predicted that I would instead see a difference in indices of immune function across the range of neighbor densities that I observed. I used white blood cell (WBC) counts as an index of immune function. These counts offer an easy and affordable way to monitor one of many indices of immune function, but they can be difficult to interpret (Sheldon and Verhulst 1996, Norris and Evans 2000, Davis et al. 2008). For example, an increased count could indicate an individual who is immunocompetent or it could indicate an individual who is not immunocompetent and is fighting a current infection (Sheldon and Verhulst 1996). Thus, I did not predict the direction of the relationship between density and WBC counts. I simply sought to describe differences, if any, in

WBC counts among warblers at different neighbor densities. My study represents one of the first to test a physiological mechanism of density dependence on free-living birds.

In Chapter 5, “General Conclusions,” I briefly summarize the results from each chapter and discuss them as a whole. I also attempt to integrate the theoretical aspects of my research with applied management by briefly discussing how my results fit into bottomland hardwood and swamp forest restoration efforts in southern Illinois.

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## CHAPTER 2

### LOCAL NEIGHBOR DENSITY DOES NOT INFLUENCE REPRODUCTIVE OUTPUT IN A CAVITY-NESTING SONGBIRD, THE PROTHONOTARY WARBLER

#### ABSTRACT

Although ecologists recognize density dependence as a conceptual cornerstone of population biology, uncertainties about the generality of density effects remain. Intraspecific competition for resources due to crowding is considered to be a major density-dependent factor, but the way in which competition leads to density effects can vary greatly depending on mobility, territoriality, and behavioral decision-making. Even within a well-studied taxon such as territorial songbirds, evidence for density-dependent processes remains equivocal. Cavity-nesting birds are ideal subjects for experimental tests of density effects because their numbers can be readily manipulated with nest boxes. During 2008-2011, I experimentally tested the effects of density on measures of productivity in the Prothonotary Warbler (*Protonotaria citrea*). I increased local conspecific densities more than two-fold during my experiment ( $\bar{x} = 6.6 \pm 0.24$  neighbors/breeding pair in 2008;  $\bar{x} = 12.6 \pm 0.64$  neighbors/breeding pair in 2011), resulting in a maximum range of densities of 1 to 27 neighbors/breeding pair. Each year, I monitored breeding pairs and recorded their reproductive output, rates of nestling provisioning, and nestling body condition prior to fledging. I found no strong effect of conspecific density on correlates of reproductive success or total fledgling production. Further, nestling provisioning rates and nestling body condition were unaffected by conspecific density. Food availability as mediated by intraspecific competition during breeding does not appear to be a mechanism of density dependence in this population. Future work should attempt to integrate data on a larger spatial scale to better understand the dynamics of mobile species in open populations.

## INTRODUCTION

Ecologists have long noted that many natural animal populations tend to fluctuate around a long-term average abundance (Lack 1954, Nicholson 1954). This stability is thought to be regulated through density-dependent factors such as competition, predation, and disease. Once subject to debate, ecologists now recognize density dependence as a conceptual cornerstone of population biology (Murdoch 1994). Key questions persist, however, about the nature and generality of density effects. For example, even within a well-studied group such as songbirds, clear evidence for density dependence remains elusive (e.g., Brawn 1987, Both 1998a, Torok and Toth 1988, Dhondt et al. 1992, Sillett et al. 2004). Although understanding the regulatory mechanisms controlling some populations has proven difficult, it is considered to be one of the most important tasks still facing ecologists (May 1999). Indeed, modern conservation issues (e.g., habitat fragmentation, climate change) make it a priority to understand the processes influencing the dynamics of populations.

Competition for resources is a major way in which density effects may be exerted on a population but broad generalizations about the outcome of density-dependent competition are difficult because populations are rarely isolated (Hixon et al. 2002) and the life-history traits of a species and current ecological conditions (e.g., food supply or predator population) often interact (May 1999, Carrete et al. 2008). Further, the ability to move to new habitats or use behavior to mediate density effects also helps determine the strength of competition that individuals experience within a population. For example, sessile species such as non-clonal plants cannot escape their neighbors and are subject to interference competition that can reduce their fitness (e.g., asymmetric competition between neighboring adults and seedlings for limiting nutrients) (Crawley and Ross 1990). Conversely, even species with relatively limited dispersal abilities can

select new habitats based in part on resource competition with conspecifics (e.g., rotifers, Kuefler et al. 2012; unicellular algae, Moses et al. 2013) to help maximize their fitness.

Intraspecific competition leading to variability in reproductive output of individuals is a concept that was proposed long ago (Lack 1954); however, disagreement on the mechanism responsible for this relationship remains. Two main hypotheses have been proposed to help explain the density-fecundity pattern. The individual adjustment hypothesis (IAH; also known as the interference competition hypothesis) emphasizes the importance of direct and indirect competitive interactions among individuals in a population and proposes that the intensity of competition increases with crowding (Rodenhouse et al. 2003, Both 1998a, Kruger et al 2012). The IAH is directly applicable to sessile organisms due to their inability to select a different habitat when conditions are too crowded but evidence for IAH also comes from highly vagile species such as territorial songbirds (Both 1998a, Sillett et al. 2004). The second hypothesis, termed the Habitat Heterogeneity Hypothesis (HHH) (Both 1998a, Kruger et al. 2012) implies a spatial context to variability in fecundity and is based on the ideal-despotic and ideal-preemptive models developed for territorial animals by Fretwell and Lucas (1970). Under HHH, the best quality territories are occupied first. As density increases, a greater proportion of lower-quality territories are occupied, leading to reduced fecundity for those poor-quality territory holders without any impacts on the higher-quality territory holders; however, the overall population response shows a negative relationship between density and fecundity similar to IAH. Rodenhouse et al. (1997) outlined a site-dependent regulation hypothesis similar to HHH but further specified that site-dependent regulation is not necessarily exclusive of IAH and may indeed complement or encompass a local crowding mechanism.

Intensified competition for food in particular is thought to be a major factor leading to density-dependent fecundity in territorial songbirds (Newton 1998) and can occur in two ways. First, food resources may be reduced directly through increased consumption of the food supply with increased numbers of conspecifics. Alternatively, individuals may spend more time in agonistic interactions when crowded and have less time to spend on foraging, indirectly reducing the amount of food available to them. Both direct and indirect decreases in food availability can negatively influence reproductive output through some combination of effects on clutch size, hatching success, and fledging success (e.g., Kluijver 1951, Perrins 1965, Lack 1966, Alatalo and Lundberg 1984, Both 1998a). Intensified competition may not be apparent early in the nesting cycle but instead manifest during the peak of breeding season food demands (i.e., the nestling stage), and nestling provisioning rates (Silleet et al. 2004) and nestling body condition (e.g., body mass, tarsus length; see Alatalo and Lundberg 1984, Torok and Toth 1988, Both 1998b) prior to fledging can be used to help elucidate food competition that occurs during this time. Young that leave the nest in worse condition (e.g., body mass, body mass corrected for body size) often have reduced survival rates compared to those that fledge in better condition (Perrins 1965, Tinbergen and Boerlijst 1990, Both et al. 1999). Thus, nestling provisioning rates and body condition measurements may identify possible density effects on fledgling survival rates and hence recruitment rates that other correlates of reproductive output may not. Finally, decreased food availability under crowded conditions can also influence female condition and a decision to double-brood (i.e., initiate a second nest after successfully fledging a first nest) (Kluijver 1951, Dhondt 1977, Both 1998b, Silleet et al. 2004). Experiments have shown that providing supplemental food can increase the likelihood that a female will double-brood (Arcese

and Smith 1988, Nagy and Holmes 2005), further strengthening the case that food availability during the breeding season can limit annual fecundity.

Difficulty in gathering evidence for density effects in studies of wild bird populations may stem in part from confounding factors that are unaccounted for in observational studies. For example, a population may be regulated in a density-dependent way but this may interact with density-independent factors (e.g., weather; Higgins et al. 1997, Turchin 1999) that vary across habitats or breeding seasons. Experimental approaches offer the ability to explicitly test for potential effects and mechanisms of density dependence (Krebs 2002) while also controlling for confounding factors. Experimental field studies focused on density dependence have increased in recent years (see reviews by Harrison and Cappuccino 1995, Hixon and Webster 2002; see also Both 1998b, Sillett et al. 2004), but expanding the breadth and variety of studies is still needed to determine predictive relationships (or lack thereof) between populations and ecological mechanisms across a diverse suite of species (Hixon et al. 2002, Krebs 2002).

Over four breeding seasons, I adjusted nest box availability to experimentally manipulate Prothonotary Warbler (*Protonotaria citrea*) densities and quantify potential effects and mechanisms of density dependence. Here I present results on the effects of increased local conspecific density on warbler reproductive output. I assumed that food competition would become important when nest predation was removed and that warbler reproductive output would be negatively affected by increased competition with greater local densities. In particular, I predicted that total fledgling production by females would be lower with increased conspecific density through a combination of density effects on correlates of reproductive success (reduced hatching and/or fledging success, reduced nestling provisioning rates, reduced nestling body condition prior to fledging, fewer double-brooding attempts by females).

## **METHODS**

### **Study area and focal species**

I conducted my research within the Cache River Watershed (CRW) in southern Illinois, USA from 2008-2011. The CRW drains >1900 km<sup>2</sup> of land into the Ohio and Mississippi Rivers at the southern tip of Illinois (Demissie et al. 2008) and contains a diverse suite of habitats within an agricultural matrix. Wet forest habitats, including bald cypress (*Taxodium distichum*) and water tupelo (*Nyssa aquatica*) swamps, occupy ~9% of the landcover (Mankowski 1997).

The Prothonotary Warbler is a neotropical migrant that breeds in the eastern and central U.S. (Petit 1999). The species is a cavity-nesting habitat specialist that associates closely with water in bottomland hardwood and swamp forests (Petit and Petit 1996). Prothonotary Warblers are territorial, socially monogamous, readily accept nest boxes (Fleming and Petit 1986), and exhibit high site fidelity between breeding seasons (Hoover 2003b), especially following years of reproductive success.

### **Weather data**

Abiotic factors such as extremes in temperature or precipitation can cause excessive drying or flooding of habitats, and bottomland forests are especially impacted by such weather events. I obtained total precipitation amounts (in) and average minimum, maximum, and average daily temperatures (°F) for each month of the breeding season (April – July) during 2008-2011 from the National Oceanic and Atmospheric Administration (NOAA) website (<http://cdo.ncdc.noaa.gov/qclcd/QCLCD>). I used data from the Carbondale, Illinois weather station as it represented the station closest to my field sites. I also retrieved long-term average monthly precipitation and temperature data from NOAA's 1981-2010 Climate Normals database (<http://www.ncdc.noaa.gov/land-based-station-data/climate-normals/1981-2010-normals-data>).

## Density manipulation

In March 2008, I established a grid-system of 170 nest boxes on each of two 40 ha study sites in the CRW. At each site, I randomly established two 10 ha low-density and two 10 ha high-density subplots to control for any habitat differences (e.g., wetter or drier areas) within sites. Low-density subplots had boxes spaced 80-100 m apart; high-density subplots had boxes spaced 35-50 m apart. During 2008-2011, I inspected and monitored the status of each box weekly. I wanted to control for the effects of brood parasitism by Brown-headed Cowbirds (*Molothrus ater*) and nest predation so that I could increase warbler densities and focus on a potential food competition mechanism of density dependence. I removed cowbird eggs during early incubation to eliminate confounding effects that occur when adult warblers raise cowbird young (Hoover 2003a; Hoover and Reetz 2006). Because previous work has shown that raccoons (*Procyon lotor*) are the major nest predator in this system (Hoover 2006), I placed all active boxes onto greased poles to prevent raccoons from accessing boxes. By removing the effects of cowbird parasitism and nest predation, I was able to increase the nesting success of the warblers, in turn increasing their site and territory fidelity between years (Hoover 2003a). High site fidelity of returning birds combined with settlement of new birds on each site led to increasing warbler densities each successive year (see results below).

I captured all warblers using nest boxes at each site and banded each bird with a unique combination of a numbered aluminum leg band (U.S. Geological Survey) and colored plastic leg bands. I captured females during incubation by placing a small plastic bag over the opening of nest boxes. I captured males by placing a male decoy warbler paired with a playback of a Prothonotary Warbler song in front of a mist-net within each male's territory. During capture, I measured body mass, wing chord length, and tarsus length of each bird and determined the age

of each individual [second-year (SY; i.e., 1 y old and entering its first breeding season) vs. after second-year (ASY; i.e.,  $\geq 2$  y old; see Kowalski 1986, Pyle et. al 1987).

### **Local neighbor densities**

I georeferenced the location of every nest box using a Trimble GPS unit (Trimble Navigation Ltd., Sunnyvale, California) and transferred the coordinates into ArcGIS (ESRI, Redlands, California) to quantify the distances between nest boxes. Previous work in the CRW has shown that warbler behavior is most influenced by interactions with neighbors within a 200-m radius of their own nest box [e.g., mating decisions (approximately 75% of extra-pair young are sired by males who hold territories within 200 m of a cuckolded male's nest box), breeding dispersal decisions; Schelsky 2010]. Therefore, I calculated neighbor density as the number of warbler pairs within a 200-m radius of each active nest box.

### **Reproductive output**

From late April through the beginning of August each year, I checked nest boxes every 5-7 d to determine when new nests were initiated. I monitored active nests in boxes every 3-7 d as needed. I also monitored natural cavity nests when they were found, but this was a rare occurrence (i.e., only 2 warbler pairs used natural cavities on the plots across the 4 y of the study). I recorded host clutch size and cowbird egg status during each nest check. I also noted whether adults were present at the box or in the area. The frequency of my box- and nest-monitoring efforts allowed me to precisely determine nest initiation and hatch dates, and I always checked nests within 2 d of hatching. When nestlings were present, I noted their physical appearance to age them (Podlesak and Blem 2002). On day 6, 7, or 8 post-hatching, I measured each nestling's tarsus (mm) and body mass (to the nearest 0.01 g) and banded it with a numbered

aluminum leg band (U.S. Geological Survey). I monitored breeding pairs after nestlings fledged to determine whether pairs made additional nesting attempts.

### **Nestling provisioning**

I used video cameras to conduct 1-h nestling provisioning observations on day 6, 7, or 8 post-hatching. All observations were completed between 0700-1100 h and prior to any other human activity at the nest box on that day. Cameras were placed >20 m away from an active box and hidden behind a tree to minimize any potential disturbance to the adult warblers as they flew to/from the nest. I checked nests immediately after videotaping to count the number of nestlings. I later transcribed videos, and the first and last 10 min of video were censored to eliminate any potential bias resulting from human disturbances when entering and leaving the territory. I recorded the number of feeding trips made per hour by each parent, and summed these values to obtain the overall provisioning rate per nestling (i.e., total number of trips/hour/nestling by both adults). I noted the size and type of prey items being delivered to nestlings whenever possible.

### **Statistical analyses**

I used generalized linear mixed models (GLMM) in Proc GLIMMIX using SAS 9.1 (SAS Institute 2003) to model each reproductive output variable (clutch size, hatching success, fledging success, likelihood of second brood attempts) in relation to neighbor density, year, ordinal date, and the neighbor density x year and neighbor density x ordinal date interactions. Ordinal date was defined as the date of nest initiation for each individual nest. I included the interaction terms to explore the potential for density effects to be conditional on annual or seasonal fluctuations in environmental conditions (e.g., weather; Alatalo and Lundberg 1984, Torok and Toth 1988). Hatching success was defined as the number of eggs that hatched divided by the number of eggs that were laid. Fledging success was defined as the number of nestlings

that fledged divided by the number of eggs that hatched. I included cowbird parasitism status as a fixed effect for the clutch size and hatching success analyses because I knew that receiving cowbird eggs could affect these metrics even if cowbird eggs were removed in early incubation (Hoover 2003a). I specified subplot nested within site as a random effect and female identity as a repeated measure for each of these analyses except the fledging success analysis. I used female identity as the repeated measure because high site fidelity led to repeated observations of the same females in successive years. For the fledging success analysis, I specified breeding pair identity as the repeated measure to incorporate the influence of male and female parental care efforts as a whole on nestling growth and development. I specified a Poisson distribution for the clutch size model and a binomial distribution for the hatching success, fledging success, and likelihood of second brood attempts models.

I used mixed models (Proc MIXED; SAS Institute 2003) to model total annual fledgling production in relation to neighbor density, year, ordinal date, and the neighbor density x year and neighbor density x ordinal date interactions. I calculated total annual fledgling production by summing the number of young fledged per female across all nesting attempts within each year. For this analysis, I used the date of initiation of a female's first nesting attempt as the ordinal date. I nested subplot within site as a random effect and used female identity as the repeated measure. Approximately 23% (n=72) of first nest attempts were made by females who were first detected on site during the latter half of the breeding season (on/after 10 June). I had no knowledge of whether these females had nested elsewhere before arriving on site and exploratory analyses showed that double-brooding had a significant effect on the number of young produced but the likelihood of initiating a second nest attempt dropped sharply after the beginning of June. Therefore, I censured these late-arriving females from my analysis.

I also used Proc MIXED to model nestling provisioning rates in relation to neighbor density, year, ordinal date, and the neighbor density x year and neighbor density x ordinal date interactions. I nested subplot within site as a random effect. I did not specify a repeated measure because I repeated recordings on only 9.1% (n=10) of videotaped pairs across the four years of my study. This approach did not qualitatively change my results compared to a repeated-measures model.

I considered density effects on nestling body condition by first calculating the residuals of nestling mass (g) on nestling tarsus (mm) to correct for body size (Adams and Frederick 2009). I then used Proc MIXED to model the residuals in relation to neighbor density, year, ordinal date, and the neighbor density x year and neighbor density x ordinal date interactions. I also included the number of host nestlings as a fixed effect in this model as brood size can influence nestling growth (Podlesak and Blem 2002). I used subplot nested within site as a random effect, and specified breeding pair identity as the repeated measure to incorporate male and female parental care efforts as a whole on nestling body condition.

I applied a Bonferroni correction to control for the familywise error rate when a significant effect was detected for multiple comparisons. I report untransformed means and standard errors in the text and all figures.

## **RESULTS**

### **Weather data**

Total monthly precipitation and average daily minimum, maximum, and average temperatures are provided in Table 2.1. The most notable departures from average include a hotter and drier-than-normal breeding season in 2010 and a wetter-than-normal start to the breeding season in 2011.

## **Density manipulation**

In 2008, conspecific densities ranged from 2-10 neighbors/warbler pair and averaged  $6.6 \pm 0.24$  neighbors/pair. By 2010, local densities increased to  $14.9 \pm 0.51$  neighbors/pair (range: 1-27 neighbors). In 2011, warbler densities ranged from 2-23 neighbors with an average of  $12.6 \pm 0.64$  neighbors/pair. In general, densities built up more in the high-density subplots over time because high annual survival and nest box fidelity by male warblers meant that fewer unoccupied boxes were available in the low-density subplots each successive year.

## **Reproductive output**

As predicted, clutch size did not vary significantly in relation to neighbor density, year, ordinal date, neighbor density  $\times$  year, or neighbor density  $\times$  ordinal date (Table 2.2; Fig. 2.1). Mean clutch size was 4.2 eggs (SE = 0.03; range 2-5) across all years and nest attempts. A density  $\times$  year interaction had a weak effect on hatching success (Table 2.2; Fig. 2.2), but this pattern was contrary to my prediction (i.e., hatching success would decrease with increasing densities) and was driven by differences between 2010 and the first two years of the study. Specifically, hatching success was reduced when birds had fewer neighbors in 2008 and 2009 compared to 2010, but these differences disappeared when local densities were  $\geq 14$  neighbors. Hatching success was significantly affected by Brown-headed cowbird parasitism status (Table 2.2). Cowbirds parasitized 38.3% of all nests overall, and nests that received a cowbird egg had reduced hatching success ( $\bar{x} = 81.4\%$ , SE = 0.02) compared to non-parasitized nests ( $\bar{x} = 92.0\%$ , SE = 0.01). Fledging success was not significantly influenced by neighbor density (Table 2.2; Fig. 2.3), year, ordinal date, neighbor density  $\times$  year, or neighbor density  $\times$  ordinal date (Table 2.2). On average, warbler pairs successfully fledged 86.7% (SE = 0.02) of their young.

Female attempts at second broods were unaffected by neighbor density (Table 2.2; Fig. 2.4). The likelihood of attempting a second brood was significantly affected by ordinal date (Table 2.2), and decreased as the breeding season progressed. Females had an 18% likelihood of attempting a second brood at the beginning of June but this likelihood dropped sharply by 10 June (3%) and starting a second brood attempt became highly unlikely (<1%) at the beginning of July. Second-brood attempts did not differ among the four years of the study.

Total annual fledgling production showed little variation among the range of neighbor densities observed (Table 2.2; Fig. 2.5) and females produced an average of 4.5 fledglings (SE = 0.66) across all neighbor densities. Ordinal date had a significant effect on total annual fledgling production (Table 2.2) even after censoring data for late-arriving females. Females that initiated nesting at the start of the breeding season produced more young each year ( $6.6 \pm 0.72$  fledglings) compared to females that began nesting during the middle of the nesting season ( $4.0 \pm 0.39$  fledglings). The total number of fledglings produced did not vary among years. Neither density x year nor the density x ordinal date interactions were important in the model.

### **Nestling provisioning**

Nestling provisioning rates did not differ across the range of neighbor densities observed (Table 2.3; Fig. 2.6). Pairs averaged 2.8 feeding visits/hour/nestling (SE = 0.11). Provisioning rates were marginally different among years (Table 2.3). Pairs made fewer provisioning trips in 2011 ( $\bar{x} = 2.3$ , SE = 0.31) compared to the previous three years of the study ( $\bar{x} = 2.8$ ; SE = 0.19).

Also contrary to my predictions, nestling body condition prior to fledging did not decline significantly with increasing neighbor densities (Table 2.3, Fig. 2.7). The number of nestmates did affect nestling body condition (Table 2.3). Nestlings with more nestmates had significantly reduced body condition compared to those with fewer nestmates. Nestlings raised with 1

nestmate had the best overall body condition scores prior to fledging ( $\bar{x} = 0.51$ ,  $SE = 0.26$ ) whereas nestlings raised with 3 nestmates had the poorest overall body condition ( $\bar{x} = -0.27$ ,  $SE = 0.08$ ). Ordinal date had a slight negative effect on nestling body condition (Table 2.3) with nestling condition decreasing as the breeding season progressed. Nestling body condition did not vary significantly among years (Table 2.3).

## **DISCUSSION**

Despite more than doubling the range of neighbor densities on the experimental plots over the course of my field study (i.e., 2-10 neighbors/breeding pair initially, increased to 1-27 neighbors/breeding pair), I found little evidence for density effects on Prothonotary Warbler reproductive output. Warbler pairs with more neighbors were able to provision their young at the same rate and produce similar numbers of fledglings in similar body condition as their less-crowded conspecifics. Overall, my results do not support either the IAH or the HHH which both predict an overall negative relationship between density and fecundity.

Competition for resources, especially food, is thought to be an important mechanism through which density-dependent reproduction can occur in territorial songbirds (Lack 1954, Lack 1966, Newton 1998) and evidence for the negative effects of density on reproduction in songbirds has been previously documented (e.g., Kluijver 1951, Dhondt et al. 1992, Arcese and Smith 1988, Sillett et al. 2004, McKellar et al. 2014). Reduced fecundity due to density-dependent intraspecific food competition has also been shown for a diverse array of taxa [e.g., grasshoppers (Class Orthoptera), Joern and Klucas 1993, Liu et al. 2007; arctic ground squirrels, Karels and Boonstra 2000]. However, the importance of density-dependent food availability for other territorial animals is still under debate (e.g., microtine rodents; see Batzli 1996 and references therein). Further, results of studies have not been consistent even within taxa. For

example, some authors have failed to document density effects on reproduction in insects (e.g., tropical butterflies; Bauerfeind and Fischer 2005) while others have concluded that factors other than food are affected by density and more important in regulating populations (e.g., parasitoids on cyclic forest Lepidoptera; Berryman 1996). My results further highlight that it is difficult to make broad generalizations regarding the effects of density on reproduction even within a specific taxon.

I assessed nestling body condition and food provisioning rates in an attempt to detect sublethal effects of density-dependent food availability. Several songbird studies have documented reduced nestling mass or nestling tarsus measurements with increased density (Alatalo and Lundberg 1984, Both 1998b, Sillett et al. 2004; but see Arcese and Smith 1988, Torok and Toth 1998) but I am aware of only one previous experimental field study that has documented provisioning rates in relation to density. Sillett et al (2004) found that Black-throated Blue Warbler adults in reduced neighbor density treatments tended to provision their nestlings at a greater rate and fledged heavier young than adults in control treatments during one year of their study. Similar to many breeding songbird species, Prothonotary Warblers rely heavily on Lepidopteran larvae and spiders (Class Arachnida) to feed their nestlings (N. Davros, unpublished data). Prothonotary Warblers, however, are also able to take advantage of other prey items such as dragonflies (Order Odonata; N. Davros, unpublished data) and mayflies (Order Ephemeroptera; Petit et al. 1990) that emerge from the water at different times during the season. These additional food resources may not be readily available to songbird species that do not nest over water. The availability of additional protein-rich food sources may explain why Prothonotary Warblers were able to provision their young and fledge them at similar weights

regardless of conspecific density compared to these other studies where food became limiting during peak demands within the breeding season.

Density-dependent effects are most likely to be observed when biotic and abiotic factors interact (Higgins et al. 1997, Newton 1998, Turchin 1999, Smallegange et al. 2011). Evidence for an interaction between density-dependent and density-independent factors (e.g., weather, climatic variation) has been well documented in ungulates (e.g., Portier et al. 1998, Coulson et al. 2000, Bardsen and Tveraa 2012) and has been implicated in several songbird studies (e.g., Alatalo and Lundberg 1984, Torok and Toth 1988, Sillett et al. 2004). Indeed, for birds that rely on insects as a primary food source during the breeding season, extremes in temperatures (specifically, prolonged heat or cold) and precipitation (drought, flooding) can interfere with the life-cycle development of the insects they feed heavily upon (Newton 1998). The resulting reduction or mismatch in the timing of food availability in poor weather years may drive any interaction between negative density effects on reproduction and environmental conditions. My study occurred across four years, the last two of which had the highest warbler densities but experienced environmental conditions (e.g., air temperature, precipitation) that departed from normal. Southern Illinois experienced an unusually hot, dry summer in 2010 which led to each of my sites drying up almost completely by early July. The drought was followed by historic flooding in spring 2011 at the start of the breeding season. Prothonotary Warblers showed altered behaviors during May 2011 (e.g., many males drastically reduced their singing activity for several days; N. Davros, personal observation) and females delayed nest initiation by an average of 8 days (N. Davros, unpublished data). If density effects are most pronounced when conditions are challenging, I expected to detect such effects during these latter two years when warbler

densities were at their greatest levels during seemingly adverse abiotic conditions. Density effects were not, however, more pronounced during 2010 and 2011.

The local neighbor densities I obtained in my experiment were at the extreme end of the density ranges observed under natural conditions. Increased densities of Prothonotary Warblers in response to hydrologic restoration with gully plugs were previously documented within this system (pre-treatment swamps without plugs:  $1.1 \pm 1.10$  warblers/ha; post-treatment swamps with plugs:  $2.1 \pm 1.15$  warblers/ha; Hoover 2009). Using the same definition of density as the Hoover (2009) study, the densities achieved in my study were 1.3 warblers/ha and 2.1 warblers/ha across the entire plot for each of my two study sites, respectively; however, the densities were twice as high in localized areas within each of my sites. Thus, even at the extreme neighbor densities achieved with my experiment, food did not appear to limit the fecundity of Prothonotary Warblers in this bottomland hardwood forest system compared with songbird studies conducted in other habitat types (Kluyver 1951, Lack 1954, Lack 1966, Torok and Toth 1988, Sillett et al. 2004). Notwithstanding, I acknowledge that territoriality may mediate potential density effects (Brown 1969, Fretwell and Lucas 1970). I did not estimate territory size in my study; however, variation in male territory size with density has previously been experimentally documented for Prothonotary Warblers (Petit and Petit 1996) as well as other songbirds (e.g., Both and Visser 2000, Sillett et al. 2004 and references therein). As a cavity nester, Prothonotary Warblers may be limited by the availability of nest cavities under natural conditions. If warbler males did contract their territories once competition for nest sites was eliminated on my study plots, it was not to the extent that allowed food resources to become limiting for reproduction. Indeed, the bottomland hardwood forests and swamps of North America support a high diversity of flora and fauna (Kellison and Young 1997, Kozlowski

2002), including breeding neotropical migratory birds (Wakeley and Roberts 1996, Sallabanks et al. 2000). Competition for food may not be an important density-dependent factor in this diverse ecosystem and other factors such as nest predation may ultimately limit the fecundity of Prothonotary Warblers in southern Illinois. In particular, raccoons are the primary nest predator of Prothonotary Warblers in southern Illinois under natural conditions and their nest predation rates are dependent on abiotic factors, specifically standing water depth (Hoover 2006).

All habitats are heterogeneous and vary in quality across space. One advantage of experimental field studies is that they offer control over potential confounding variables compared to descriptive studies. My nest box addition experiment controlled for the potential confounds of habitat and territory quality by randomly assigning the location of low- and high-density subplots within each site. My study occurred across just two study plots, however, and although I had experimental control within my sites, I did not have control across all sites within the CRW. This is an important distinction to make given that Prothonotary Warblers are highly mobile and able to prospect and select breeding habitat across the entire CRW. Density-dependent habitat selection to maximize fecundity has been documented for a diverse suite of animal groups, including gerbils (Rosenzweig and Abramsky 1985), birds (Farrell et al. 2012, Nicolaus et al. 2012), rotifers (Kuefler et al 2012), and algae (Moses et al. 2013). Indeed, the site-dependent mechanism of population regulation outlined by Rodenhouse et al. (1997) proposes that site-dependent regulation operates over a broad spatial scale for species that are highly mobile. Although my results do not show support for an IAH (interference) mechanism within my sites, such regulation may nonetheless be occurring on a vital rate that I did not measure (see Nevoux et al. 2010). Alternatively, a site-dependent mechanism of regulation (e.g., site preemption, density-dependent habitat selection) may preclude the crowded population sizes

at which IAH mechanisms become evident (Rodenhouse et al. 1997). Therefore, studies at the metapopulation level that incorporate a greater degree of spatial heterogeneity may be more useful if both high- and low-density populations occur at the same time with varying degrees of density-dependent habitat selection and reproduction (Morris 1996, Ray and Hastings 1996).

Despite the history of debate surrounding the concept of density dependence, most ecologists today would not question “if” but “how” density-dependent processes work to regulate populations (Turchin 1999). Further, most ecologists would also agree that multiple mechanisms likely interact to regulate populations (Krebs 2002, Rodenhouse et al. 2003), and there may not be one clear answer to understanding how density influences the behavior and reproduction of different species or sub-populations. My future work will include an analysis of adult survival to determine if survival is influenced by local crowding. An evaluation of recruitment in relation to density would also be useful; however, my dataset of re-sighted juveniles is limited despite short-distance natal dispersal of warblers in this system (McKim-Louder et al. 2013).

Additionally, future studies should continue to manipulate populations experimentally across space and time to test for multiple potential density-dependent mechanisms and to account for abiotic factors that may be important in explaining the dynamics of open populations. Integration of long-term monitoring efforts are also needed as longitudinal data can help elucidate delayed density dependence and carry-over effects important in population dynamics (Beckerman et al. 2002, Ratikainen et al. 2008). Ultimately, the habitat issues that we face today (e.g., fragmentation, restoration, climate change) make it a priority to understand the basic processes underlying the dynamics of animal populations if we are to manage these populations effectively.

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## TABLES AND FIGURES

Table 2.1. Total monthly precipitation (in) and average daily minimum, maximum, and average temperature (°F) for the city of Carbondale in southern Illinois during the Prothonotary Warbler (*Protonotaria citrea*) breeding season during 2008-2011. Thirty-year climatological averages (1981-2010) are also shown.

	Total				Minimum				Maximum				Average Daily			
	Precipitation				Temperature				Temperature				Temperature			
	April	May	June	July	April	May	June	July	April	May	June	July	April	May	June	July
2008	na <sup>a</sup>	6.25	1.21	7.95	na	52.8	66.1	66.9	na	73.9	87.1	88.2	na	63.4	76.6	77.6
2009	7.79	3.57	2.71	6.23	45.9	56.3	67.0	65.8	66.9	76.6	87.5	84.1	56.4	66.5	77.3	75.0
2010	3.87	3.79	3.37	1.50	50.1	59.1	70.0	71.1	74.1	78.5	89.9	91.5	62.1	68.8	80.0	81.3
2011	13.51	6.99	0.00	4.03	49.2	55.8	64.5	72.2	70.9	74.9	86.5	93.2	60.1	65.4	75.5	82.7
30-year average	4.14	5.16	3.99	3.59	45.1	55.6	64.0	67.8	67.5	76.8	85.5	88.5	56.3	66.2	74.7	78.2

<sup>a</sup>Data from the Carbondale, Illinois weather station were not available for April 2008.

Table 2.2. Results of generalized linear mixed models comparing correlates of Prothonotary Warbler (*Protonotaria citrea*) annual reproductive output to conspecific neighbor density, year, ordinal date, density x year, and density x ordinal date.

	Clutch Size (n = 377)		Hatching Success (n = 371)		Fledging Success (n = 365)		Attempts at 2nd Brood (n = 286)		Annual Fledgling Production (n = 207)	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Neighbor density	0.04	0.84	0.12	0.73	1.97	0.16	0.48	0.49	0.11	0.75
Year	0.01	0.99	3.66	<b>0.014</b>	0.41	0.74	1.02	0.39	0.70	0.56
Ordinal date	0.89	0.35	0.04	0.84	2.59	0.11	11.92	<b>0.001</b>	6.08	<b>0.02</b>
Density x year	0.01	0.99	2.24	<b>0.09</b>	0.66	0.58	0.18	0.91	0.30	0.83
Density x ordinal date	0.08	0.77	0.08	0.78	1.97	0.16	0.54	0.46	0.11	0.75
Cowbird parasitism <sup>a</sup>	0.11	0.74	18.73	<b>&lt;0.0001</b>	-	-	-	-	-	-

<sup>a</sup>Cowbird parasitism status was included as a fixed effect for clutch size and hatching success models only.

Table 2.3. Results of generalized linear mixed models comparing Prothonotary Warbler (*Protonotaria citrea*) nestling provisioning rates (total feeding visits/hour/nestling) and nestling body condition in relation to conspecific neighbor density, year, ordinal date, density x year, density x ordinal date, and the number of nestmates.

	Nestling Provisioning Rates (n = 120)		Nestling Body Condition (n = 339)	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Neighbor density	0.18	0.67	0.54	0.46
Year	2.56	<b>0.06</b>	1.98	0.13
Ordinal date	1.28	0.26	2.77	<b>0.10</b>
Density x year	2.07	0.11	1.30	0.28
Density x ordinal date	0.18	0.67	0.21	0.64
Number of nestmates <sup>a</sup>	-	-	14.18	<b>&lt;0.001</b>

<sup>a</sup>Number of nestmates was included as a fixed effect for the nestling body condition model only.

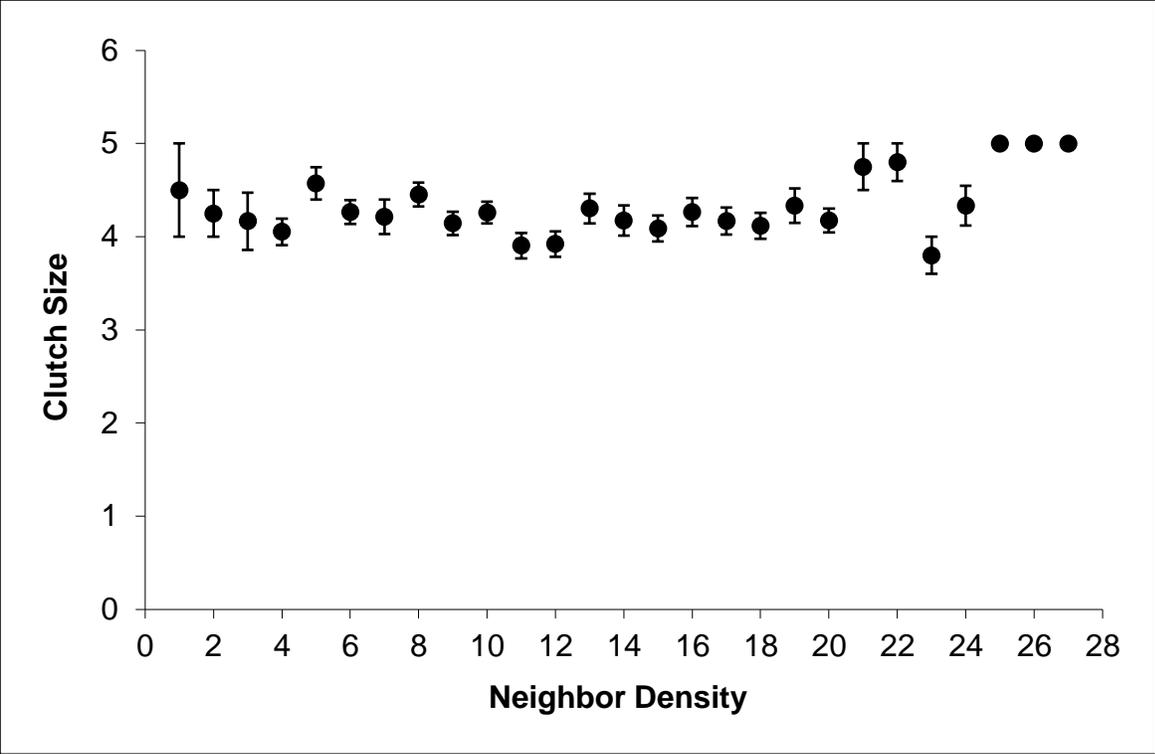


Figure 2.1. Clutch size of Prothonotary Warblers in southern Illinois in relation to conspecific neighbor density during 2008-2011.

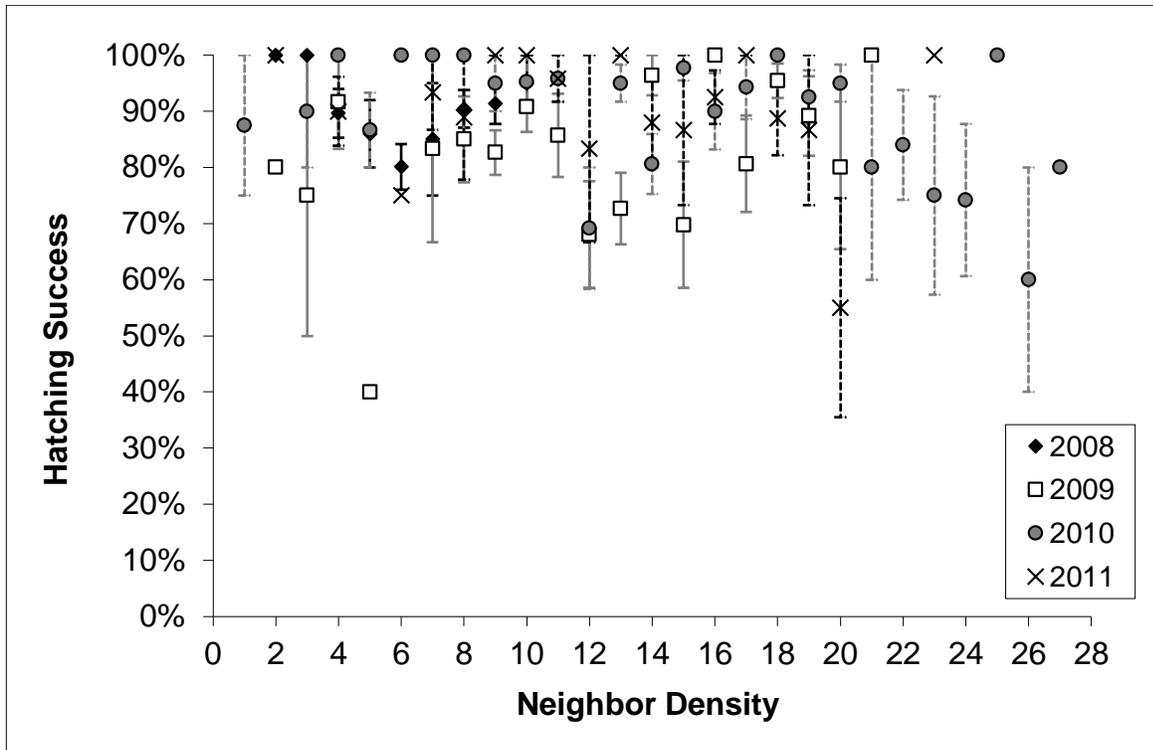


Figure 2.2. Hatching success of Prothonotary Warblers in southern Illinois in relation to conspecific neighbor density and year. Hatching success was defined as the number of eggs hatched divided by the number of eggs laid in each nest. Nests that were parasitized by Brown-headed Cowbirds are included in the analysis.

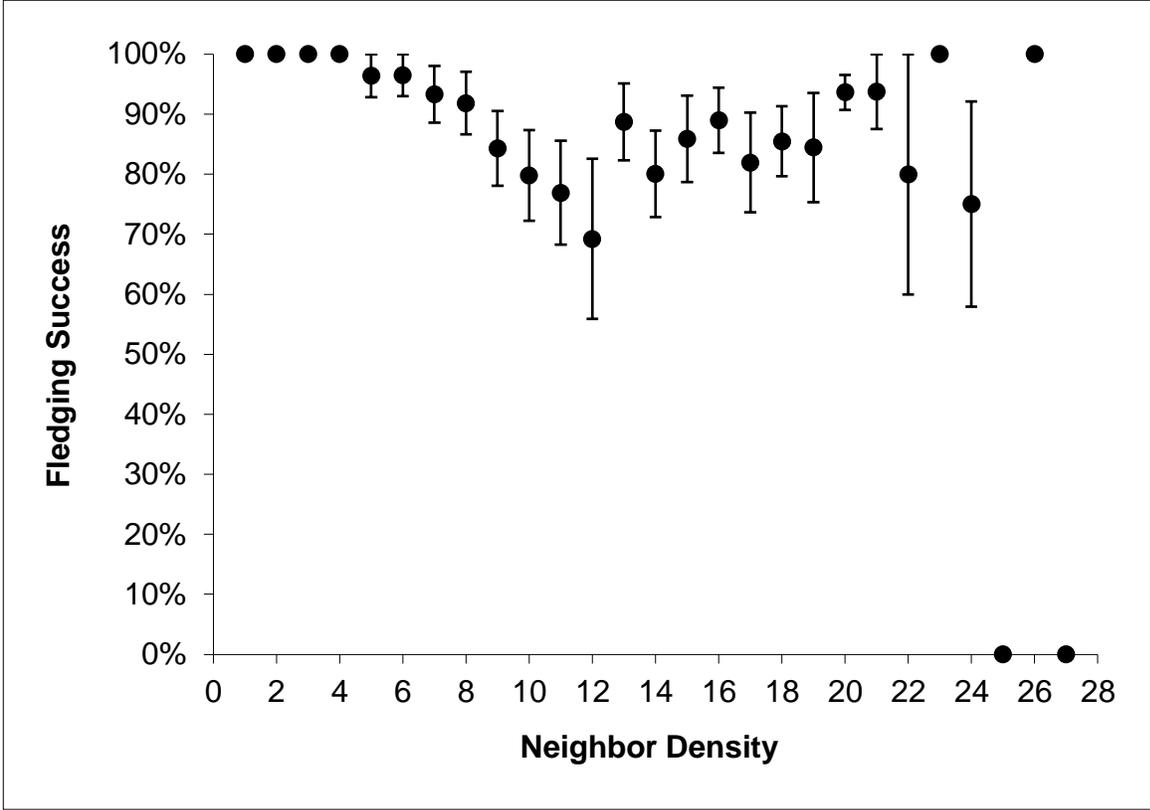


Figure 2.3. Fledging success of Prothonotary Warblers in southern Illinois in relation to conspecific neighbor density during 2008-2011. Fledging success was defined as the number of nestlings fledged divided by the number of eggs that hatched in each nest.

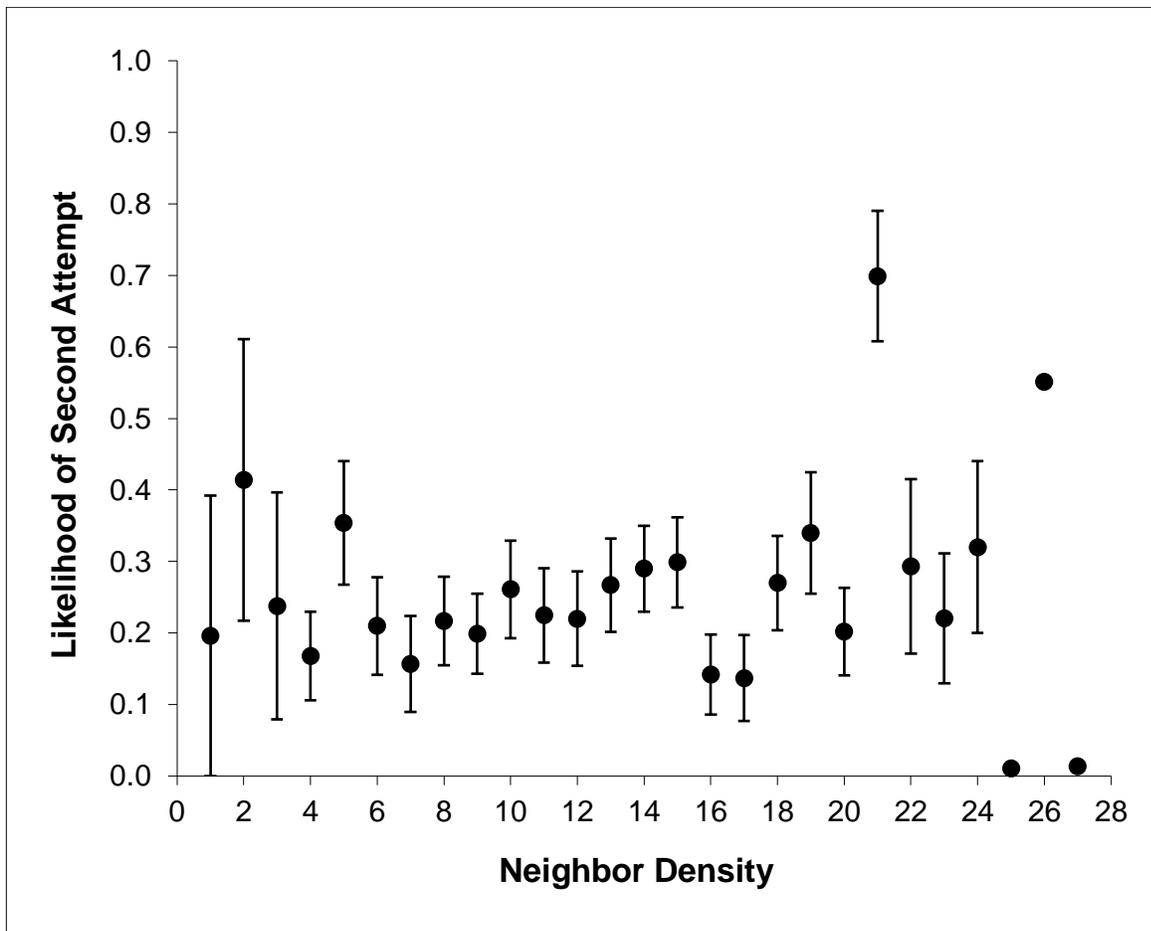


Figure 2.4. The likelihood of a female Prothonotary Warbler attempting a second brood in relation to conspecific neighbor density during 2008-2011 in southern Illinois.

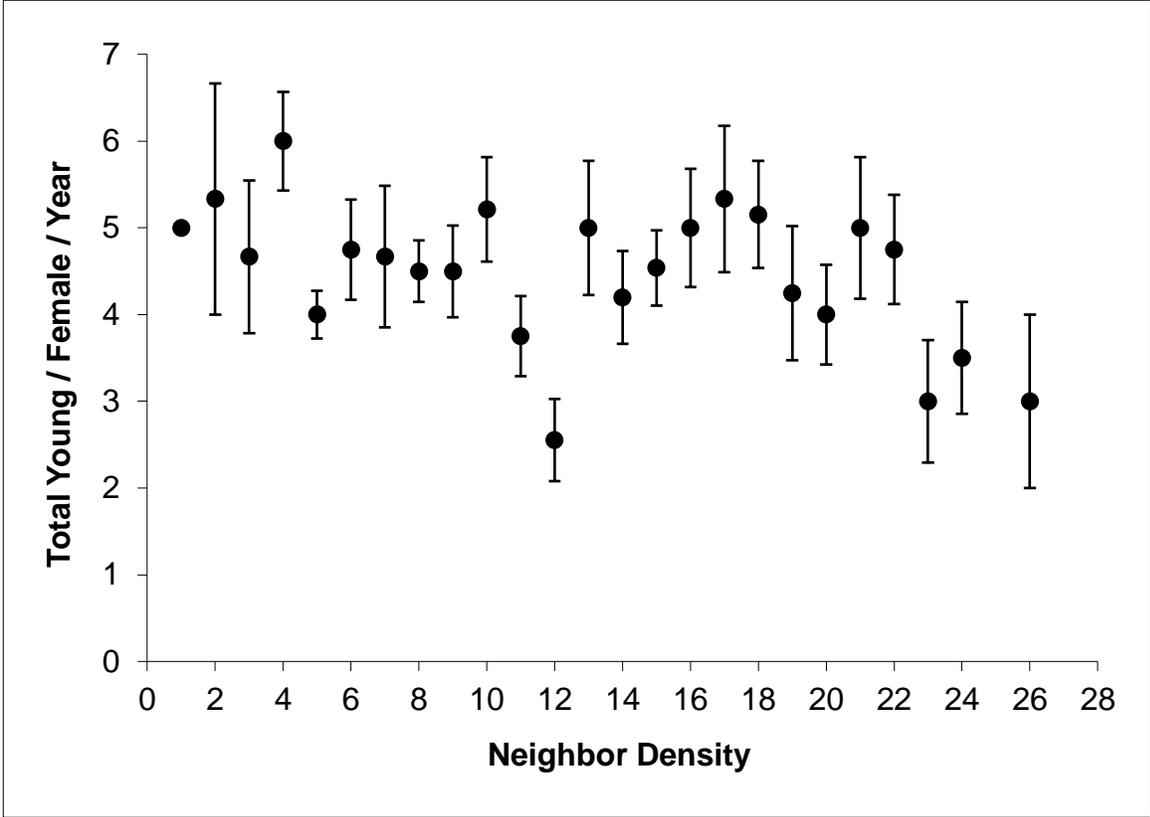


Figure 2.5. Total number of nestlings produced per Prothonotary Warbler female per year in relation to conspecific neighbor density during 2008-2011.

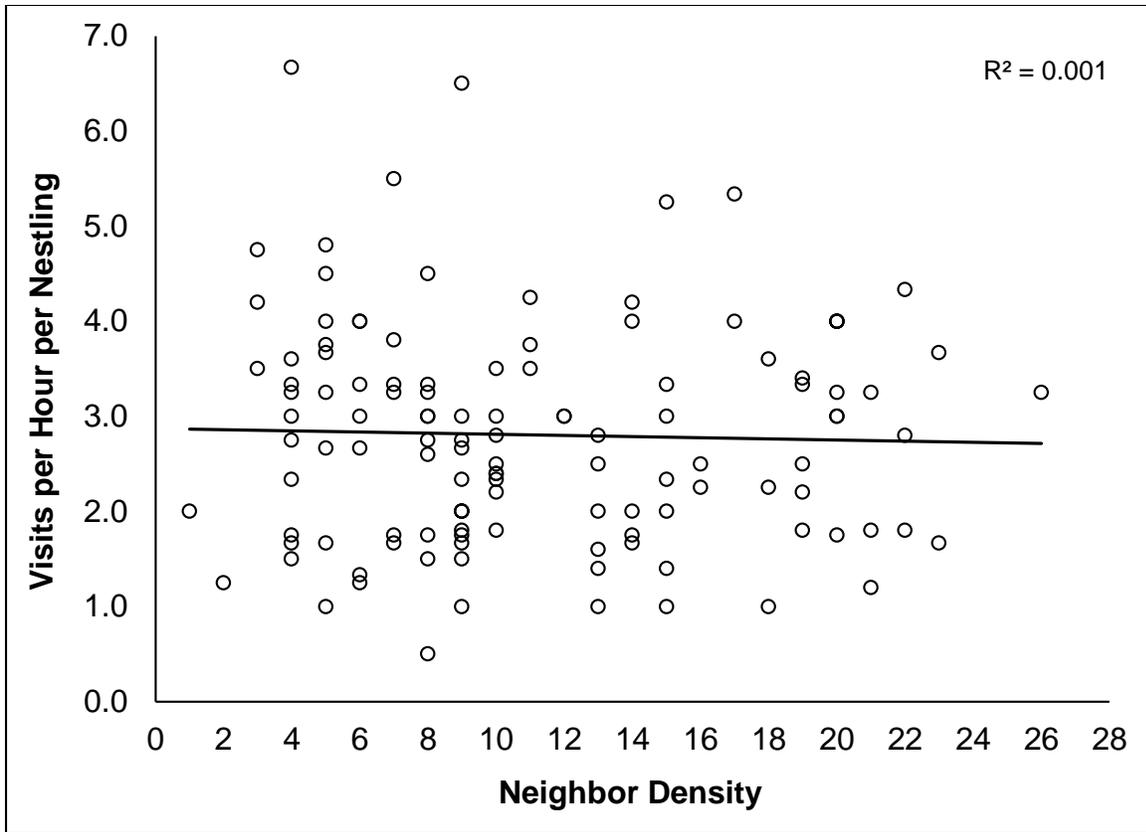


Figure 2.6. Nestling provisioning rates (total feeding visits/hour/nestling) by Prothonotary Warbler adults in relation to conspecific neighbor density during 2008-2011 in southern Illinois.

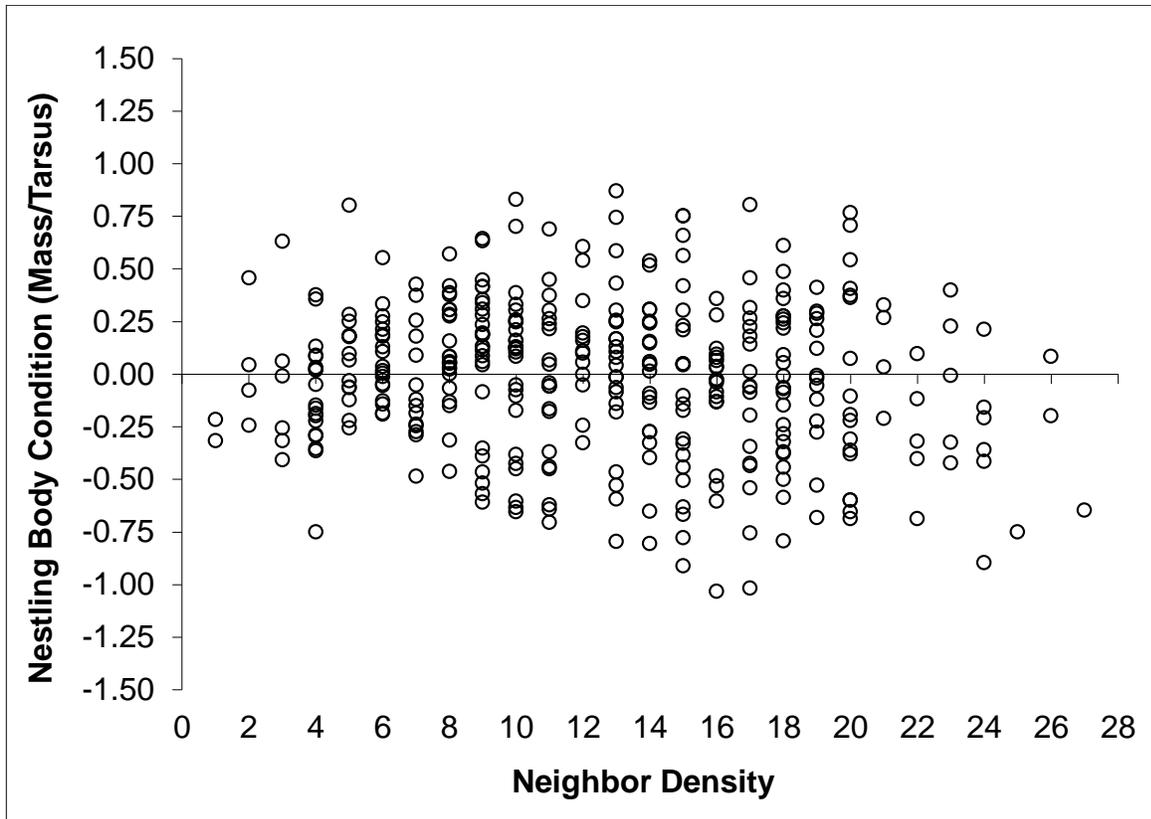


Figure 2.7. Body condition [residuals of mass (g) on tarsus (mm)] of Prothonotary Warbler nestlings in relation to conspecific neighbor density during the 2008-2011 breeding seasons in southern Illinois.

## CHAPTER 3

### BEHAVIORAL RESPONSES OF BREEDING MALE PROTHONOTARY WARBLERS (*PROTONOTARIA CITREA*) TO INCREASED CONSPECIFIC DENSITY

#### ABSTRACT

Conspecific density and resource competition can affect a suite of behavioral traits in territorial animals. I experimentally increased local densities of a migratory songbird, the Prothonotary Warbler (*Protonotaria citrea*), from  $6.6 \pm 0.24$  neighbors/breeding pair in 2008 to  $14.9 \pm 0.51$  neighbors/breeding pair in 2010 and then observed male behaviors during the 2010 breeding season when densities were at their peak. I estimated song, foraging search, flight, and prey attack rates to assess how males responded to increased densities and competitive load (e.g., for space, mates, food). Male song rates generally increased with density but this response was not significant and varied across stages of the nesting cycle. Flight rates of males did not vary significantly across the range of local densities that I observed. Rates of movements associated with searching for food increased more than 5-fold during the nestling stage with increasing densities, but there was no concomitant increase in prey attack rates. Therefore, crowded males appeared to devote more effort to finding similar amounts of food during the nestling stage compared to less-crowded males. Overall, I found little evidence that song and foraging behaviors of males were affected by increased local conspecific densities. Although this population of Prothonotary Warblers appears to be able to cope with conspecific crowding, a critical examination of other potential mechanisms of density dependence is still needed to better understand how this population is regulated.

## INTRODUCTION

Studies of density dependence in songbirds have focused on associations between density and reproductive success under the assumption that competition is likely the most important mechanism regulating territorial songbird populations (e.g., Alatalo and Lundberg 1984, Torok and Toth 1988, Both 1998). Less attention has been paid to more subtle and sex-specific behavioral responses that can occur when territorial birds experience increased conspecific densities (but see Sillett et al. 2004, Dobbs et al. 2007). For species in which males are the territorial sex and provide a portion of the parental care, male attentiveness may play a large role in determining overall reproductive output. In particular, conspecific density may influence a male's ability to provision his mate or nestlings either directly through food competition, or indirectly through mate or territory defense. This may translate to reduced overall reproductive output for birds that experience increased competition due to crowding (i.e., competitive neglect). Alternatively, male behavior might change in response to density in a way that allows him to cope with the increased intensity of competition without having any adverse effects (e.g., reproduction, survival). Thus, better understanding subtle behavioral responses to density can provide additional insight into the mechanisms of competition often assumed to be important for density dependence in songbirds.

The rate at which a male sings may also reflect the intensity of sexual competition that is often a function of conspecific density. Bird song functions in territorial defense against intruding males as well as mate attraction and retention (Catchpole 1982, Catchpole 1987). Increased densities can lead to increased agonistic interactions over territorial space and mates, and song is often used to settle disputes before they escalate to fighting (Smith 1991). Regardless of the purpose of the song (i.e., territory defense or mate attraction), males are expected to

increase their song activity in response to increased conspecific density (Penteriani 2003, Goretkaia 2004, Sexton et al. 2007, Hamao et al. 2011).

Although increased conspecific densities can reduce food consumption indirectly through time spent on other activities (e.g., agonistic interactions), increased densities may also directly reduce food availability for breeding birds. Because precise measures of the amount of prey available to birds are difficult and time-consuming to quantify in natural settings, using bird foraging behavior as a proxy can be useful when direct information cannot be obtained (Hutto 1990). Food availability can be reflected in the foraging behavior of birds through search rates (e.g., hops and walks within a patch being searched), flight distances and rates, and prey attack rates in relation to prey density and diversity (Hutto 1990, Martin and Karr 1990, Dobbs et al. 2007). Birds may also begin to include more energetically-expensive prey attack maneuvers (e.g., more aerial attacks from birds that traditionally glean) in response to reduced food availability or increased food demands (e.g., feeding nestlings; Martin and Karr 1990, Dobbs et al. 2007). Thus, if competition for food intensifies with increased conspecific densities, the foraging behavior of breeding males may reflect this change.

I assessed the effects of local crowding on the behavior of breeding male Prothonotary Warblers (*Protonotaria citrea*). I sequentially increased warbler densities over three consecutive breeding seasons and observed male behaviors in the third season when densities were at their peak. Assuming that conspecific density influences competition for resources among territorial males, I predicted that males would sing at faster rates with increased conspecific density but that these rates would decline overall as the nesting stage progressed (i.e., as social mates became less receptive and male parental care responsibilities increased). I also assumed that increased conspecific density would reduce food availability through a combination of direct and indirect

effects and therefore: 1) increase food search and flight rates of foraging males, and 2) reduce prey attack rates of males, especially during the nestling stage when demands to acquire food were at their peak. Alternatively, I predicted that males might switch to atypical foraging maneuvers (i.e., from glean and probes to sally strikes and sally hovers; see Table 1 for definitions) in response to increased conspecific density in order to keep up with nestling food demands.

## **METHODS**

### **Study area and focal species**

I conducted my research in the Cache River Watershed (CRW) in southern Illinois, USA from 2008-2010. The CRW drains 1909 km<sup>2</sup> of land into the Ohio and Mississippi Rivers (Demissie et al. 2008) at the southern tip of Illinois. The area contains a unique diversity of habitat types, including bottomland hardwood forests, wetlands, and bald cypress (*Taxodium distichum*) and water tupelo (*Nyssa aquatica*) swamps, all within an agricultural matrix.

The Prothonotary Warbler is a neotropical migratory songbird that breeds in the central and eastern U.S. (Petit 1999). These warblers are habitat specialists that often nest over water (Petit and Petit 1996) in bottomland and swamp forests. They are territorial, socially monogamous, and nest in secondary and natural tree cavities but readily use nest boxes (Fleming and Petit 1986). In southern Illinois, this species also exhibits increased site and territory fidelity between breeding seasons, especially following years of reproductive success (Hoover 2003a).

### **Density manipulation**

In 2008, I established a grid-system of 170 nest boxes on each of two 40 ha study sites. I established two 10 ha low-density and two 10 ha high-density subplots on each site to control for potential habitat differences within each site (e.g., drier vs. wetter locations). Nest boxes in low-

density subplots were spaced 80-100 m apart; boxes in high-density subplots were spaced 35-50 m apart. During the 2008-2010 breeding seasons, I monitored all nest boxes weekly and recorded the contents of each box; active boxes were monitored more frequently as needed. I also searched for and monitored nests in natural cavities, but only 2 warbler pairs used natural cavities on the plots during my study. Raccoons (*Procyon lotor*) are the major nest predator in this system (Hoover 2006) so I placed active boxes onto greased poles to minimize nest predation. Brown-headed Cowbirds (*Molothrus ater*) introduce confounding effects on warbler reproductive success (Hoover 2003b, Hoover and Reetz 2006). Therefore, I removed cowbird eggs during early incubation to further increase warbler reproductive success. With the effects of nest predation and cowbird parasitism effectively removed, I was able to increase warbler nesting success, thereby also increasing their site and territory fidelity between years (Hoover 2003a). High fidelity of returning birds combined with settlement of new birds led to increasing warbler densities each year, particularly on the high density subplots where more unoccupied boxes were available (see below).

### **Capture and marking of birds**

I captured all adult warblers using nest boxes and color-banded each bird with a unique combination of colored plastic leg bands and a numbered aluminum leg band (U.S. Geological Survey) to facilitate field identification of individuals. I captured incubating females by placing a small plastic bag over the opening of nest boxes. Males were captured in mist-nets by placing a male decoy warbler paired with a playback of a Prothonotary Warbler song in front of the net within his territory. I measured body mass and other standard morphometric measurements and determined the age of each bird [second-year (SY; i.e., 1 y old and entering its first breeding season) vs. after second-year (ASY; i.e.,  $\geq 2$  y old)] (Kowalski 1986, Pyle et al. 1987).

### **Estimation of local breeding densities**

I used a global positioning system (GPS) receiver to determine the coordinates of every nest box, and then transferred the coordinates into ArcGIS (ESRI, Redlands, California) to map distances among nest boxes. I defined neighbor density as the number of warbler pairs within a 200-m radius of each active nest box. I felt this was the best measure of local density for a given breeding pair as previous work in this system has shown that warbler behaviors (e.g., breeding dispersal and mating decisions) are heavily influenced by their interactions with neighbors within this distance (Schelsky 2010).

### **Male behavioral observations**

In 2010, I randomly chose banded males from low- and high-density subplots for focal behavioral observations. I observed males during their initial nesting attempt of the breeding season and I conducted observations throughout the nesting cycle (i.e., pre-incubation, laying, and nestling stages) when possible. Due to logistical and time constraints, all males were not observed across all stages. I conducted observations between 0630-0930 h and prior to any other human activity at the male's nest box on that day. During a focal observation, I first located and observed a male for 30 s prior to the start of data collection. This helped prevent biasing data towards conspicuous movements (e.g., flying; see Holmes and Robinson 1988) that first drew my attention. I used binoculars to observe each male, and I continuously dictated all of his behaviors into an mp3 voice recorder. I used a stopwatch to keep time and I threw out any observation bouts lasting <30 s. I followed focal birds until they were lost from sight for more than 30 s, until I became an apparent disturbance, or until I had observed the male for >12 min in a day. I later transcribed my recordings and tallied counts for the following behaviors: song, walk, hop, flight, sally strike, sally hover, glean, or probe. I did not differentiate between songs

used for territorial defense (e.g., countersongs) and songs used for attracting females. I based food search movements and prey attack maneuvers on definitions provided by Holmes and Robinson 1988 (Table 3.1). I lumped flights of all distances into one category. Finally, I calculated rates for each behavior for each male per observation period; rates are expressed as number per minute.

### **Statistical analyses**

Males were consistent in the type of prey attack maneuvers that they used while foraging. I observed 203 attempts at attacking prey; the majority of attempts were made using gleanings (75.4%) and probes (17.2%) (Fig. 3.1). Sally hovers and sally strikes combined (7.4%) were rarely observed. Therefore, I combined all prey attack maneuvers into one “prey attack rate” category for analysis. Hopping and walking rates were highly positively correlated with each other and were combined into a “food search rate” category.

I used generalized linear mixed models (GLMMs) using Proc GLIMMIX in SAS 9.1 (SAS Institute 2003) to model rates of male behaviors in relation to conspecific density. For song rate, food search rate, flight rate, and prey attack rate models, I used neighbor density, nest stage (pre-incubation, incubation, nestling), male age (SY, ASY), and the neighbor density x stage interaction as explanatory variables, and subplot nested within site as a random effect. I specified individual males as a repeated measure. I included nest stage in the models to account for changes in the fertility status of the male’s social mate and demands on his parental care responsibilities through time (Sexton et al. 2007). I included male age to control for any differences in breeding experience between SY and ASY individuals (Forsslund and Part 1995). Each behavioral response count was modeled as a Poisson distribution with the log of observation time specified as an offset variable for the linear predictor. This is appropriate for

rate data when the counts have been obtained in time intervals of varying lengths (GLIMMIX Procedure, SAS Institute 2003). I report untransformed means and standard errors in the text for ease of interpretation.

## **RESULTS**

### **Density manipulation**

In 2008, warbler pairs had  $6.6 \pm 0.24$  neighbors (range = 2-10 neighbors per pair). By 2010, densities increased to  $14.9 \pm 0.51$  neighbors per pair (range = 1-27 neighbors) (Fig. 3.2). Overall, densities built up more in the high-density subplots because more boxes were available compared to the low-density subplots.

### **Male behavioral observations**

I observed 41 focal males during 55 different observation bouts for a total of 3:43:50 h of observation. Twenty-three observations were made during the pre-incubation stage, 15 observations during incubation, and 17 observations during the nestling stage. Observation bouts ranged from 00:30 to 11:53 min; the mean observation time was  $04:09 \pm 0:38$  min.

Males sang  $2.3 \pm 0.19$  songs per minute across all observations. Although I found male song rates were positively correlated to neighbor density, this relationship was generally weak (Table 3.2, Fig. 3.3, Appendix 1). I did find that this pattern reversed during the nestling stage as predicted, but the density  $\times$  stage interaction was not significant.

I found a significant neighbor density  $\times$  stage interaction on food search rates (Table 3.2, Fig. 3.4, Appendix 2). Males with more neighbors searched for food at a greater rate during the nestling stage compared to their less-crowded counterparts. This was opposite the pattern I observed during the incubation stage when crowded males searched at a slower rate compared to less-crowded males. Flight rate, my other movement rate metric, did not vary in relation to

neighbor density or nest stage (Table 3.2, Fig. 3.5, Appendix 3). On average, males made  $6.8 \pm 0.65$  food search movements and  $2.7 \pm 0.23$  flights per minute across all observations.

Males made  $1.1 \pm 0.27$  prey attacks per minute across all observations. Contrary to my predictions, prey attack rates did not vary significantly in relation to neighbor density or stage of the nest cycle (Table 3.2, Fig. 3.6, Appendix 4). Males with more neighbors generally had a greater prey attack rate during the pre-incubation stage, but this trend reversed during the incubation stage and disappeared by the nestling stage.

## **DISCUSSION**

Overall, I found limited evidence that the song and foraging behavior of territorial male Prothonotary Warblers was affected by local conspecific densities. I predicted that male-male competition for territorial space and potential mates would increase with conspecific density. Using song rates as a measure of this competition, I found a positive but non-significant trend for increased song rates with density during the first two stages of the nesting cycle (i.e., pre-incubation and incubation). Song rates declined slightly with neighbor density during the nestling stage, presumably because males became more occupied with providing parental care during this stage. Several observational studies have found increased singing activity (e.g., song rate, length, frequency) with greater densities of conspecifics during the breeding season (Penteriani 2003, Goretskaia 2004, Sexton et al. 2007, Ripmeester et al. 2010, Hamao et al. 2011), but only Sexton et al (2007) differentiated among stages of the nesting cycle. I am aware of only one other songbird study that experimentally tested the effects of neighbor density on song rates. Sillett et al (2004) reduced Black-throated Blue Warbler (*Dendroica caerulescens*) densities to test a competition mechanism of density dependence. They did not find a significant effect of neighbor density on countersinging rates, although reduced-density males tended to sing

less than control males in the pre-incubation stage (i.e., nest building and egg-laying; Sillett et al. 2004).

Prothonotary Warbler males sing relatively simple songs compared to most songbirds (Bryan et al. 1987); therefore, I focused my efforts on quantifying rates rather than other aspects of their songs (e.g., structure). If song rates function primarily to defend resources from other males, then males should sing at a similar rate throughout the nesting cycle to prevent intrusions from rivals (Titus et al. 1997). Alternatively, if song is used primarily to attract a fertile female to pair with, then song rates should be highest during the pre-incubation period (Titus et al. 1997). My finding that males sang at slightly higher rates during the incubation period compared to the pre-incubation stage with increasing density suggests another possible alternative. A male with many neighbors may have increased opportunities for extra-pair matings. By increasing his singing rate once his social mate is sitting on eggs, he may be signaling his quality or that of his territory (Penteriani 2003, Clarkson 2007) to new females arriving on-site. Although I am confident that my experimental design and random selection of focal males adequately controlled for potential differences in territory or male quality that could influence song rates (Petit and Petit 1996, Clarkson 2007), further work on the effect of density on mating strategies and their associated behaviors (e.g., extra-pair paternity, parental care; Kokko and Rankin 2006) is still needed in this system.

I found mixed evidence for my hypothesis that increased density would lead to increased competition for food. Males searched for food by hopping and walking along branches and other substrates at a greater rate during the period of peak demands (i.e., nestling stage) when they had more neighbors, but I did not find an associated increase in flight rates or prey attack rates with increasing density. Taken together, my results suggest that crowded males were working harder

within a patch to find the same amount of food as their less-crowded counterparts (Hutto 1990). Conversely, male Black-throated Blue Warblers in both control and reduced-density treatments increased their food search rates without a concomitant increase in prey attack rates, suggesting that all males regardless of neighbor density were responding to increased time or energy constraints (Dobbs et al. 2007). My results also conflict with those of Lyons (2005) who found that Prothonotary Warblers did not change their foraging speed during the nestling stage; however, Lyons (2005) compared foraging behavior of Prothonotary Warblers in two different habitat types and did not directly measure conspecific density. Although I do not have direct measures of prey sizes and types to go along with male foraging observations, I found that nestling provisioning rates and body condition prior to fledging did not vary with density (see Chapter 2). Thus, even if crowded males were working harder to find similar amounts of food during peak demands, it did not translate into food limitation during the nestling stage.

Interestingly, males did use sally-type maneuvers more during the nestling stage (18%) compared to the other nest stages (pre-incubation = 3.8%, incubation = 2.3%) (Fig. 3.1). Their overall use of sally maneuvers was relatively low compared to gleans and probes, however, and many males were never observed using a sally strike or sally hover. Others have reported similar shifts in foraging maneuvers through time, but without accompanying invertebrate data it is difficult to determine whether the warblers' increased use of sally maneuvers during the nestling stage was a compensatory response to density during a time of high food demand (Martin and Karr 1990, Lyons 2005, Dobbs et al. 2007) or simply a temporal response to the types of prey available (Hutto 1990, Martin and Karr 1990, Petit et al. 1990a). Incorporating methods designed to tease these two responses apart would benefit studies attempting to understand whether intensified food competition is occurring at greater conspecific densities.

The food search and prey attack rates I quantified are below the rates documented in other studies of Prothonotary Warblers (Lyons 2005 and references within) and other Parulids (Lovette and Holmes 1995, Dobbs et al. 2007) whereas the frequencies of foraging maneuvers were comparable to the percentages previously reported for Prothonotary Warblers (Petit et al. 1990a, Lyons 2005). I was interested in quantifying behavioral differences among males with different neighbor densities during a year of peak densities in my field experiment, but summer 2010 was also the hottest and driest year of my study (see Chapter 2) and it is unknown whether this drought affected prey availability and reduced overall foraging rates. It would have been interesting to compare how male foraging rates varied during other years with similar neighbor densities but different environmental conditions (Wiens et al. 1987, Petit et al. 1990b).

Despite more than doubling neighbor density in an experimental fashion, I did not find that conspecific density influenced the male behaviors I quantified. However, positive density dependence may be more important in this system as Prothonotary Warblers can actually gain fitness benefits from using public information they obtain from their neighbors in addition to their own private information to make behavioral decisions (e.g., breeding dispersal, mate choice; Schelsky 2010). Future work should focus on how density might influence the decisions they make (e.g., extra-pair mating, parental care, dispersal) based on neighbor information. Additionally, more studies are needed to better understand how crowding can influence songbird behavior in a way that scales up to the level of density-dependent competition in systems where it has been documented. The bottomland hardwood and swamp forests of southern Illinois provide some of the most productive habitat in North America, supporting a high diversity of flora and fauna (Kellison and Young 1997, Kozlowski 2002), and I believe that food limitation

and density-dependent reproduction is likely a more important factor for songbird populations in other, less diverse and less productive systems.

## **ACKNOWLEDGMENTS**

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## TABLES AND FIGURES

Table 3.1. Classification<sup>a</sup> of foraging movements and prey attack maneuvers made by male Prothonotary Warblers (*Protonotaria citrea*) during focal behavioral observations in 2010.

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Foraging Movements	
Walking	Used at least one leg at a time for support
Hopping	Both feet off the ground but wings were not used; includes jumps
Flight	Used their wings and took flight; includes flights of all distances
Prey Attack Maneuvers	
Sally strike	Caught prey in flight and consumed it while continuing to fly
Sally hover	Caught prey during a hover-like flight
Glean	Removed prey item from surface of a plant, fallen log, or the ground
Probe	Inserted bill into a tree cavity or the ground

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<sup>a</sup>Based on definitions provided by Holmes and Robinson 1988.

Table 3.2. Results of generalized linear mixed models (GLMM) comparing behavioral responses (song, foraging search, flight, and prey attack rates) of male Prothonotary Warblers (*Protonotaria citrea*) in relation to neighbor density, nest stage, male age and the density x stage interaction during first broods in 2010 in southern Illinois. Results in bold are significant at the  $P < 0.10$  level or less.

	Song		Foraging		Flight		Prey	
	Rate		Search Rate		Rate		Attack	
	(n = 55)		(n = 55)		(n = 55)		(n = 55)	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Neighbor density	1.25	0.29	2.87	0.12	0.75	0.41	0.23	0.64
Nest stage	1.27	0.33	11.14	<b>0.003</b>	1.47	0.28	2.30	0.15
Male age	3.58	<b>0.09</b>	1.32	0.28	0.30	0.60	1.81	0.21
Density x stage	2.28	0.15	6.09	<b>0.021</b>	0.77	0.49	2.64	0.12

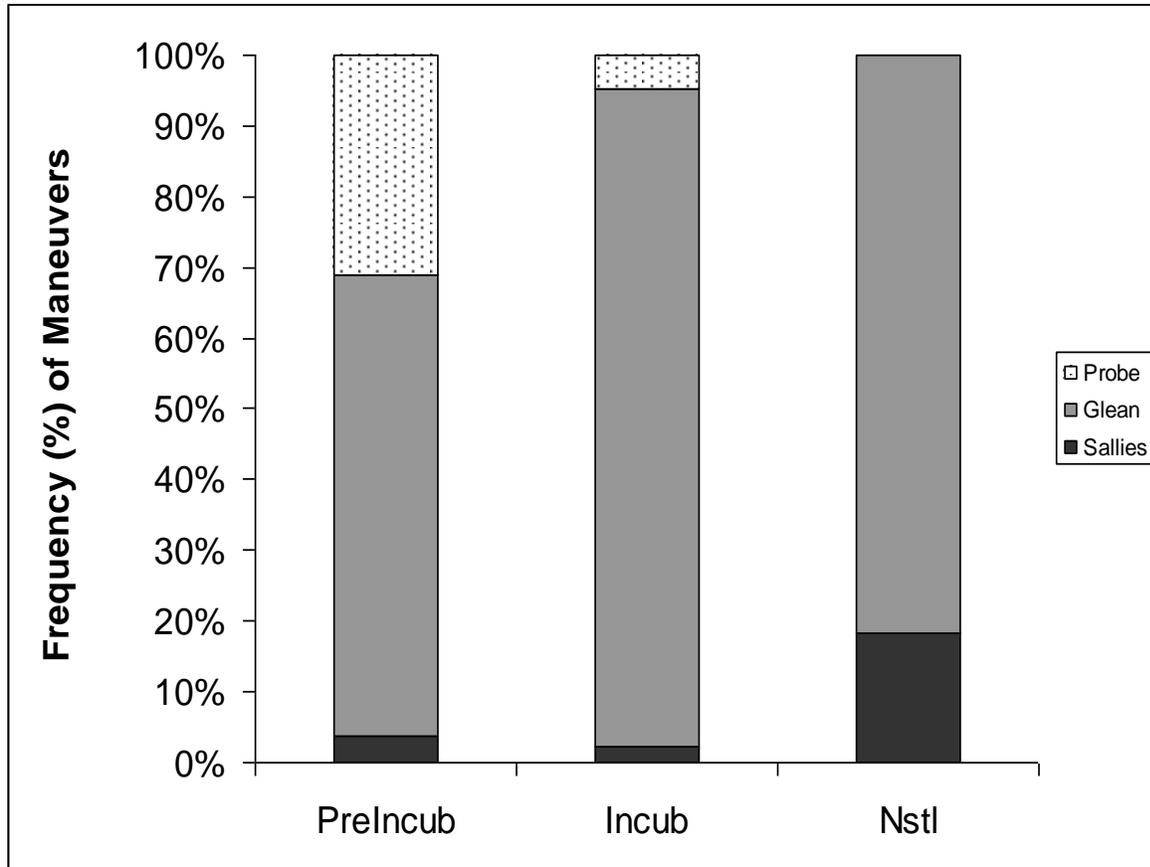


Figure 3.1. Frequency (%) of three prey attack maneuvers used by male Prothonotary Warblers during behavioral observations across all stages (pre-incubation, incubation, and nestling) of the nest cycle in 2010.

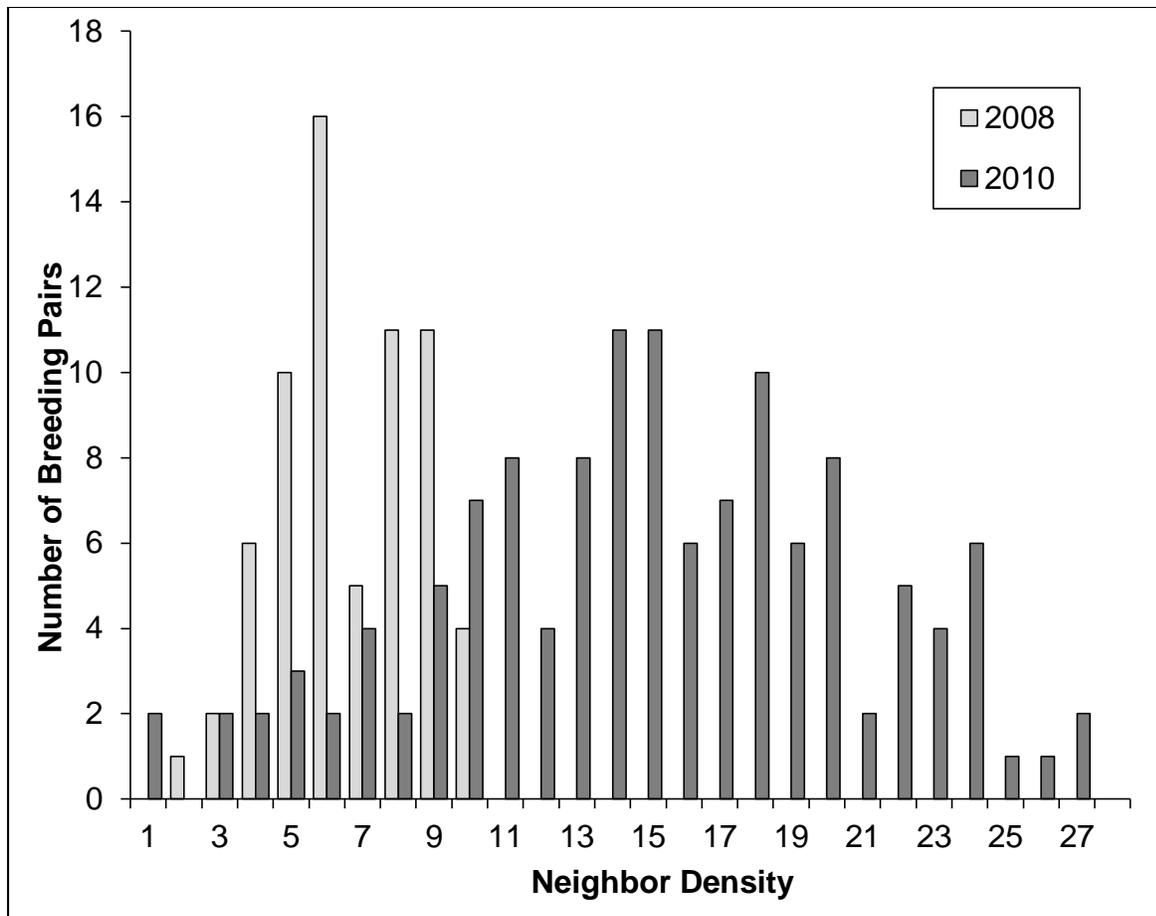


Figure 3.2. The number of breeding Prothonotary Warbler pairs at each conspecific neighbor density in 2008 compared to 2010. Neighbor density was defined as the number of warbler pairs within a 200-m radius of each active nest box.

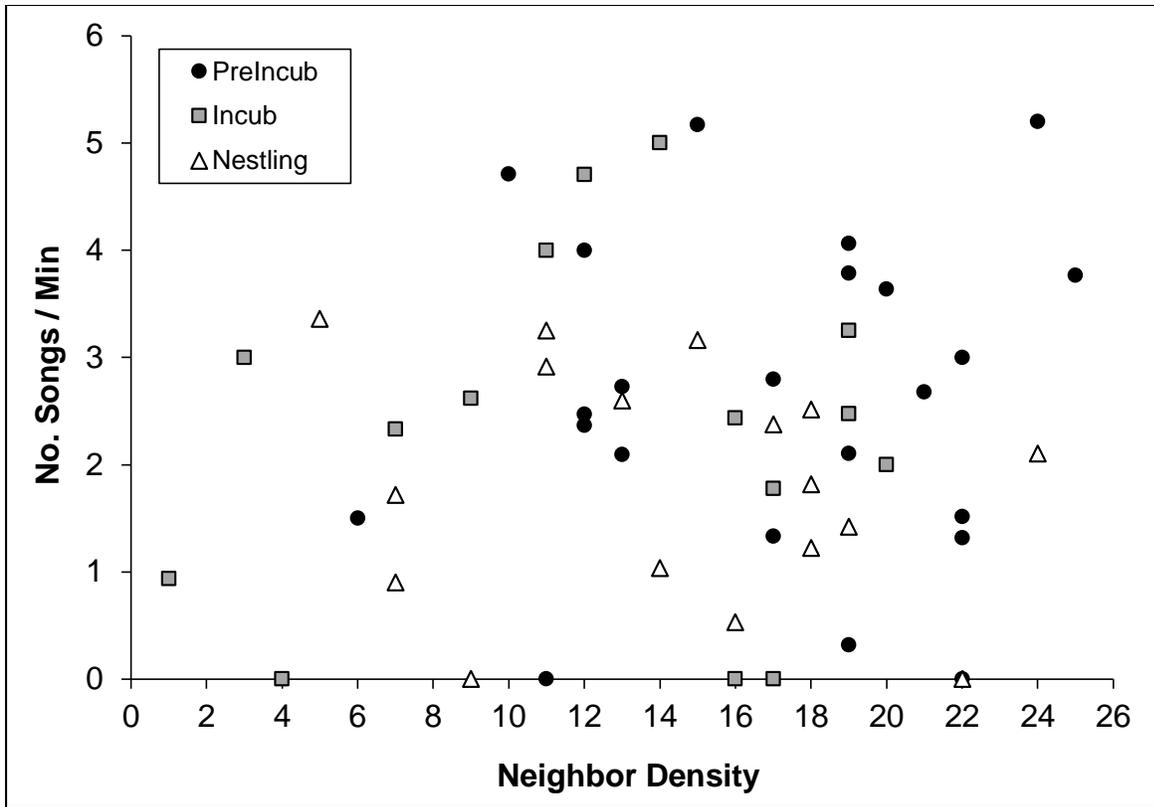


Figure 3.3. Song rates of male Prothonotary Warblers (*Protonotaria citrea*) in relation to neighbor density and nest stage during behavioral observations in 2010.

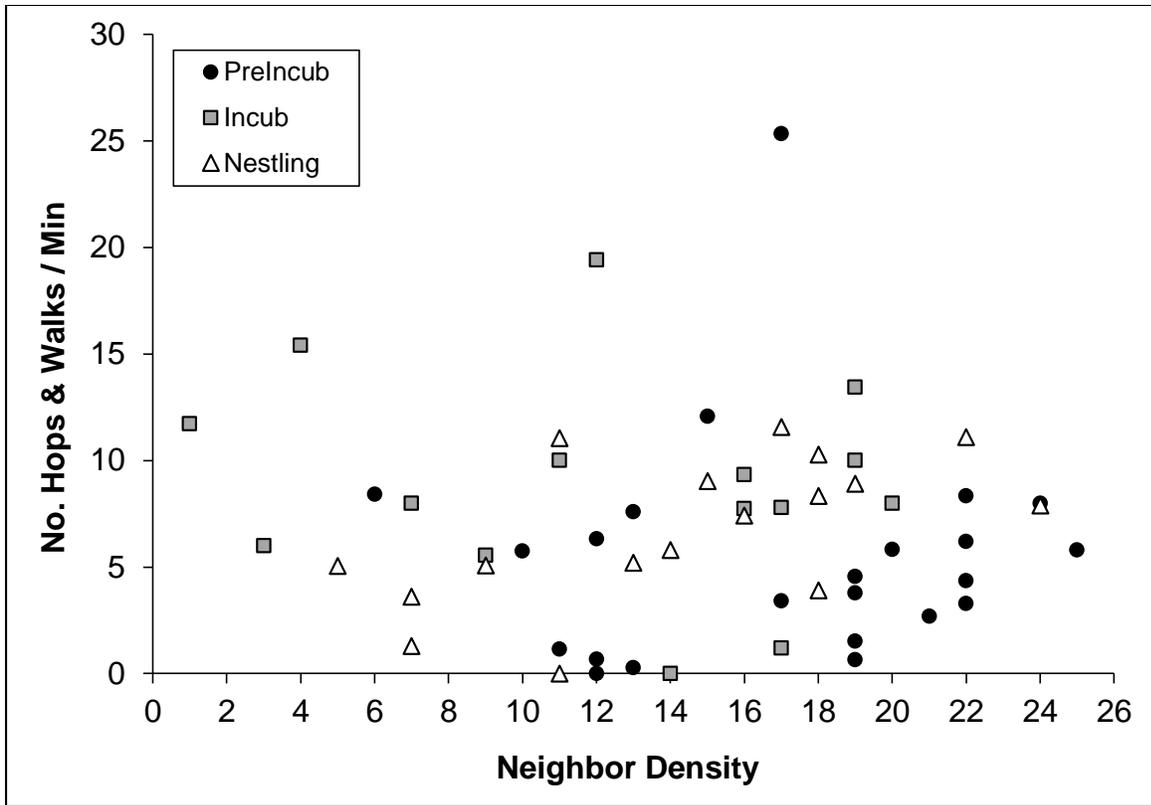


Figure 3.4. Food search rates of male Prothonotary Warblers (*Protonotaria citrea*) in relation to neighbor density and nest stage during behavioral observations in 2010. Hops and walks were combined into one “search rate” category.

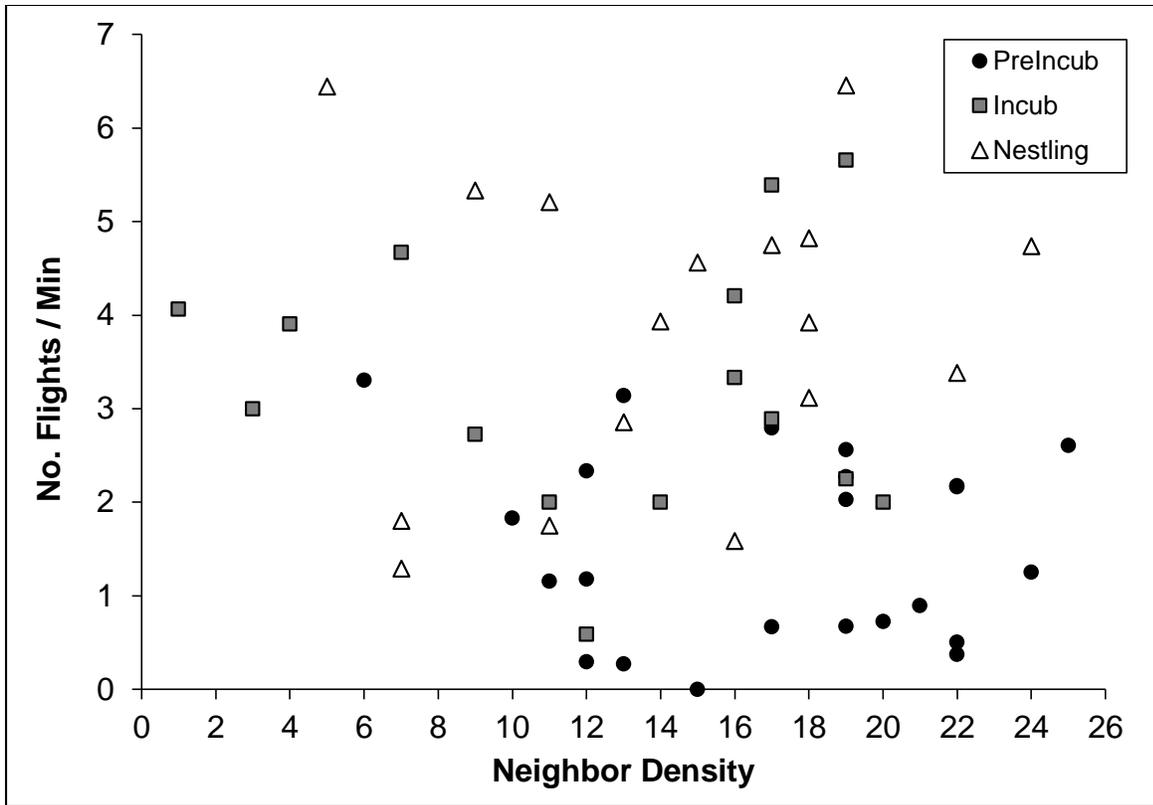


Figure 3.5. Flight rates of male Prothonotary Warblers (*Protonotaria citrea*) in relation to neighbor density and nest stage during behavioral observations in 2010. Flights of all distances were combined together.

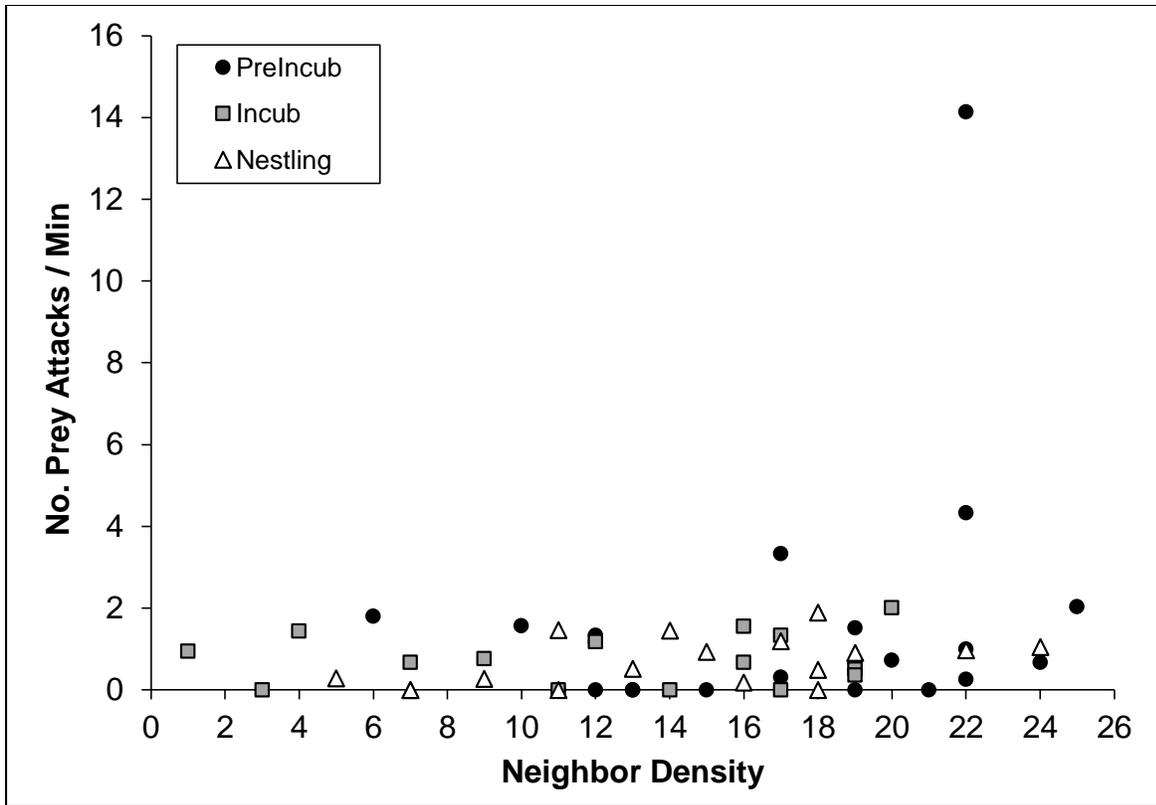


Figure 3.6. Prey attack rates of male Prothonotary Warblers (*Protonotaria citrea*) in relation to neighbor density and nest stage during behavioral observations in 2010. A prey attack consisted of any glean, probe, sally strike, or sally hover movement.

## CHAPTER 4

### BASELINE CORTICOSTERONE AND INDICES OF IMMUNE FUNCTION DO NOT VARY WITH CONSPECIFIC DENSITY IN A MIGRATORY SONGBIRD

#### ABSTRACT

The vertebrate stress response can be stimulated by increased conspecific competition, and chronic elevation of glucocorticoid stress hormones (GCs) can lead to stress-induced reproductive or immunological suppression. Therefore, physiological and immunological responses may underlie density-dependent regulation in some animal populations. I used an experimental approach to test the effects of conspecific density on baseline plasma corticosterone (CORT; the primary GC in birds) concentrations and indices of immunological function in the Prothonotary Warbler (*Protonotaria citrea*), a Neotropical migratory songbird. From 2008-2011, I used nest boxes to manipulate warbler densities during the breeding season. I collected blood samples from adult warblers to determine variation in baseline plasma CORT concentrations, heterophil/lymphocyte (H/L) ratios, white blood cell (WBC) differentials, and total WBC counts in relation to local conspecific density. I did not observe a strong effect of density on baseline CORT concentrations, H/L ratios, or any indices of immune function. Further, CORT concentrations were weakly correlated with H/L ratios and total WBC counts. Baseline plasma CORT concentrations did vary among years. In addition, first-year breeders had greater plasma CORT concentrations and increased proportions of basophils compared to older birds. Whereas I found no associations between local conspecific densities and stress responses or immune function, prior breeding experience did influence stress physiology and indices of immune function in adult warblers.

## INTRODUCTION

Density dependence is widely recognized as an important aspect of population ecology, but a clear understanding of the mechanisms and environmental conditions under which it operates is still lacking for many populations (Turchin 1999, Hixon et al. 2002, Krebs 2002, May 1999).

Integrating physiological and immunological responses of animals into studies of density dependence may help us advance our understanding of population regulation as the vertebrate stress response can be stimulated by increased social competition (see Summers et al. 2005 for review). Commonly called the “fight or flight” response, the vertebrate stress response initiates the secretion of glucocorticoids (GCs) through the hypothalamus-pituitary-adrenal (HPA-) axis which elicits subsequent physiological, hormonal, and/or behavioral changes for coping with stressful, short-term events (e.g., predator attack, severe storm; Sapolsky et al. 2000).

Continually high levels of circulating GCs, however, can lead to chronic stress and impaired function, including adverse behavioral responses, reduced survival or reproductive output, or suppression of immune activity (Boonstra et al. 1998, Sapolsky et al. 2000, Martin et al. 2005, Bortolotti et al. 2008).

Although the concept of a physiological mechanism of density dependence was proposed some time ago (Christian 1950, Christian 1963), ecologists have only recently begun to explicitly test the relationship between density and levels of GCs in field studies (e.g., mammals: Sapolsky 1992, Boonstra et al. 1998; amphibians: Belden et al. 2007, Leary et al. 2008; hatchery fish: Barton and Iwama 1991, Montero et al. 1999). Several studies using either direct or correlative measures of conspecific density have found that chronic stress or immunosuppression can indeed increase in response to increased densities and resource competition (Silverin 1998, Montero et al. 1999, Vleck et al. 2000, Ruiz et al. 2002, Rogovin et al. 2003), whereas other studies have

found decreasing GC levels with increasing group size or social contact (Stoinski et al. 2002, Machatschke et al. 2004, Mommer and Bell 2013).

In a study of Prothonotary Warblers (*Protonotaria citrea*), I found little evidence of density effects on reproductive output (see Chapter 2). Notwithstanding, associations between density and physiological stress responses may still be occurring. Life-history theory regarding allocation of resources predicts a trade-off between current reproduction, self-maintenance, and survival, and stress-induced immunosuppression may mediate these trade-offs (Sheldon and Verhulst 1996). Immunosuppression may benefit animals that have limited time and/or opportunities to breed, allowing them to concentrate resources towards current reproduction rather than self-maintenance, but it could also further compound any increased risk of exposure to parasites or disease that may occur in high-density populations (Newton 1998). If greater conspecific densities lead to chronically stressed individuals, then resource allocation towards self-maintenance (i.e., immune function) may take priority at the expense of reproduction. Alternatively, the stress response may be modulated to avoid negative reproductive effects with increased density at the expense of immune function. Indeed, such a trade-off could explain the lack of negative density effects on reproduction that I observed in my warbler population.

Evidence for modulation of the stress response during the breeding season to allow for successful reproduction is well documented in birds (Wingfield et al. 1992, Wingfield et al. 1995, Wingfield and Sapolsky 2003), but studies that incorporate physiological and immunological responses to conspecific density during the breeding season are still lacking. Although studies have described the relationships between CORT and immunocompetence (e.g., Ilmonen et al. 2003, Martin et al. 2005, Martin 2009) and between density and immunocompetence (e.g., Moller et al. 2006, Calsbeek et al. 2008), very few studies have

collectively studied all three variables (e.g., Hawley et al. 2006) to examine the potential trade-offs among these variables.

I conducted a field experiment to explore how conspecific density affects baseline GC concentrations [i.e., corticosterone (hereafter, CORT), the primary GC in birds] and indices of immunological function in a migratory songbird. I used baseline CORT concentrations as an indicator of chronic stress. I used nest boxes to manipulate Prothonotary Warbler densities over four breeding seasons and collected blood samples from adults using these boxes to determine baseline plasma CORT concentrations, heterophil/lymphocyte (H/L) ratios, white blood cell (WBC) differentials, and total WBC counts. I derived the H/L ratio as an additional index of chronic stress as birds show increased numbers of heterophils and decreased numbers of lymphocytes in response to prolonged stressors including reduced food intake, injuries, social stress, and increased parental effort (Gross and Siegel 1983, Vleck et al. 2000, Ilmonen et al. 2003). One particular advantage of using the H/L ratio in addition to plasma CORT concentrations is that, unlike plasma CORT, H/L ratios do not change rapidly in response to events just prior to capture (e.g., a predator attack) or handling stress (Wingfield et al. 1982, Davis et al. 2008). I predicted that warblers would show increased baseline CORT concentrations and H/L ratios with increased local densities. I also predicted that total WBC counts would vary significantly in relation to density. I did not have *a priori* predictions about the direction of the relationships between WBC counts and warbler densities due to the difficulty in interpreting this monitoring technique (i.e., an increased count could indicate an individual who is immunocompetent or an individual who is not immunocompetent and fighting an infection; Sheldon and Verhulst 1996, Norris and Evans 2000, Davis et al. 2008).

## **METHODS**

### **Study area & focal species**

I conducted my research during the 2008-2011 breeding seasons within the Cache River Watershed (CRW) in southern Illinois, USA. The CRW drains nearly 2000 km<sup>2</sup> of land into the Cache, Ohio, and Mississippi Rivers at the southern tip of Illinois (Demissie et al. 2008). It contains a unique diversity of habitat types within an agricultural matrix, with wet forest habitats (including bald cypress [*Taxodium distichum*] and water tupelo [*Nyssa aquatic*] swamps) occupying ~9% of the landcover (Mankowski 1997).

The Prothonotary Warbler is a neotropical migrant that breeds in the central and eastern U.S. (Petit 1999) and nests in tree cavities in bottomland hardwood and swamp forests (Petit and Petit 1996). Prothonotary Warblers are territorial, socially monogamous, and readily use nest boxes (Fleming and Petit 1986). They also exhibit high site and territory fidelity between breeding seasons (Hoover 2003a), particularly following years of reproductive success.

### **Density manipulation**

In 2008, I established a grid-system of 170 nest boxes (see Fleming and Petit 1986 for box design) on each of two 40 ha study sites in the CRW. At each site, I randomly established two 10 ha low-density and two 10 ha high-density subplots to control for any habitat differences within each site (e.g., drier vs. wetter locations). Boxes on low-density subplots were spaced 80-100 m apart; boxes on high-density subplots were spaced 35-50 m apart. From late April through early August 2008-2011, I monitored the contents of each box every 3-7 days. Previous work in this system has shown that nest predation by raccoons (*Procyon lotor*) is the main cause of nesting failure (Hoover 2006). Therefore, I placed all active boxes onto greased poles to effectively eliminate nearly all nest predation. Additionally, Brown-headed Cowbirds (*Molothrus ater*) are a

brood parasite that commonly parasitizes the nests of Prothonotary Warblers. I removed cowbird eggs during early incubation to eliminate the confounding effects that occur when adult warblers raise cowbird young (Hoover 2003b, Hoover and Reetz 2006). By effectively removing the effects of nest predation and brood parasitism, I was able to increase the nesting success of the warblers, thereby increasing their site and territory fidelity between years. High fidelity of returning birds combined with settlement of new birds into the sites led to increasing warbler densities each year, particularly on the high-density subplots where more boxes were available (see results below).

### **Local neighbor densities**

I captured all warblers using nest boxes and color-banded each with a unique combination of colored plastic leg bands and a numbered aluminum leg band (U.S. Geological Survey). During initial handling, I also measured body mass, wing chord length, and tarsus length of each bird and determined the age of each individual (Kowalski 1986, Pyle et al. 1987). Each new adult was classified as 1-year old (i.e., entering its first breeding season) or  $\geq 2$ -years old.

I georeferenced the location of each nest box and transferred the coordinates into ArcGIS (ESRI, Redlands, California) to calculate the distances among nest boxes. I then estimated local neighbor density as the number of warbler pairs within a 200-m radius of each active nest box within each year as previous work has determined that Prothonotary Warbler breeding behavior is most influenced by interactions with neighbors within this distance (e.g., approximately 75% of extra-pair young are sired by males within this distance; Schelsky 2010).

### **Baseline corticosterone and immunological measures**

Each year, I randomly selected focal breeding pairs across a range of neighbor densities (1-26 neighbors) for blood sampling. I bled all pairs between 10:30-16:30 h during the late nestling

stage in an attempt to minimize other factors that can affect baseline CORT levels. I bled adults on nestling day 7 (fledging = day 10) whenever possible, but some adults were bled on day 6 or 8 due to logistical and time constraints. I always bled both adults from a focal pair on the same day. I placed mist-nets in front of nest boxes to capture adults as they arrived to feed nestlings and took small blood samples ( $\leq 25 \mu\text{l}$ ) from the brachial vein via heparinized microcapillary tubes within 3 min of capture (Wingfield et al. 1982, Angelier et al. 2010). I also remeasured and reweighed a subset of these adults. During 2010-2011 only, I smeared a drop of blood on a microscope slide using the standard two-slide wedge procedure and air-dried the slide. The remaining blood was contained in the microcapillary tube by a clay plug on one end, stored on ice for  $\leq 8$  h, and then centrifuged for 11 min. I estimated packed cell volume (PCV) using a hematocrit reader card before extracting the plasma from each sample. Plasma samples were stored individually in a freezer until they could be later analyzed for CORT concentration levels.

I fixed and stained blood smears using the Protocol Hema 3 (Fisher Diagnostics) staining system. I had assistants view stained slides under a microscope using an x100 oil immersion lens to estimate total WBC counts, WBC differentials, and H/L ratios. I obtained total WBC counts by counting the number of leukocytes in each of five monolayer fields, then using the equation provided by Campbell and Ellis (2007; pg. 26) to obtain an estimated total WBC count:

$$\text{Estimated Total WBC Count/mm}^3 = \text{Ave \# WBC per 5 fields/1000} \times 3,500,000$$

I did not apply a correction to the estimates because all PCV values were within the normal range (35-55%) found in birds (Campbell and Ellis 2007). For WBC differentials, I counted 100 leukocytes per slide and classified each cell as a lymphocyte, monocyte, heterophil, eosinophil,

or basophil to determine their proportions. Next, I obtained heterophil and lymphocyte counts by multiplying their proportion with total WBC counts. I then used these values to get the H/L ratio for each individual (Ots et al. 1998, Ilmonen et al. 2003). The assistants examining blood smears were unaware of data associated with each bird except for band numbers, color combinations, and sample collection dates.

I estimated plasma CORT levels using Enzyme Immunoassay (EIA) kits (Cat No. 901-097, Assay Designs). For the EIAs, I modified the optimization protocol outlined by Wada et al (2007) to determine the plasma dilution and amount of steroid displacement buffer (SDB) that worked best for Prothonotary Warbler plasma CORT. My first modification involved sacrificing plasma samples from 16 birds for which I had too little plasma to run them independently and instead pooled them into 4 samples (4 birds per pooled sample) for the optimization plate. I did this to avoid losing additional samples from individual birds for which I had very small volumes. Second, I did not strip and spike the plasma as suggested by Wada et al (2007). I did, however, run the pooled samples against a standard curve at plasma dilutions of 1:40, 1:50, and 1:60, each with 0, 1, and 2% steroid displacement buffer (SDB). My results indicated that a plasma dilution of 1:50 with 1% SDB (per raw plasma volume) worked best for the warbler plasma CORT samples. To determine CORT concentrations in individual warbler samples, I added 4  $\mu$ l 1:100 dilution of SDB buffer to 4  $\mu$ l raw plasma. After 5 min, 192  $\mu$ l assay buffer were added to each sample, then samples were vortexed and added to individual wells in the assay plate (100  $\mu$ l/well) with each sample run in duplicate. I measured the standard curve in duplicate, with eight standards ranging from 40,000 to 6.4 pg/ml (100  $\mu$ l/well). I also ran a separate, external standard of 200 pg/ml on every plate. I then followed the assay procedure as outlined by the kit's directions. After adding stop solution, absorbance was read at 405 nm on a Molecular Devices

FilterMax F3 microplate reader (Molecular Devices, Sunnyvale, CA). I optimized the microplate reader prior to reading each plate. Finally, I followed the protocol of Mommer and Bell (2013) to average absorbance data for duplicate well samples and to convert blank-corrected, total-activity-corrected optical densities into final plasma CORT concentrations (ng/ml) for each individual warbler sample. I completely randomized samples within and across four plates, with a roughly equal representation of males and females across sites and years on each plate. Intra- and inter-plate coefficients of variation were 2.76% and 7.86%, respectively.

### **Statistical analyses**

I used mixed models (Proc MIXED; SAS Institute 2003) to model baseline plasma CORT concentrations, H/L ratios, estimated total WBC counts, and proportion of each WBC type in relation to neighbor density, year, sex, adult age, and the neighbor density x year interaction. Each bird was deemed an individual sampling unit and I did not specify a repeated measure because 95% and 100% of the birds retained in my CORT and WBC analyses, respectively, were bled only once across all years of the study. I nested subplot within site as a random effect in each model to account for any variation in habitat conditions (e.g., wetter vs. drier locations, more vs. less herbaceous ground cover) that may have existed across the subplots and sites. Year was included as a fixed effect to account for variation in weather patterns in southern Illinois during my study (e.g., drought in 2010, flooding in 2011) that may have influenced breeding conditions (e.g., food availability) and individual CORT responses to these conditions (e.g., Ouyang et al. 2011). I included sex as a fixed effect because males and females can have differing physiological responses during the breeding season (Ots et al. 1998, Scheuerlein et al. 2001). I included adult age (1-year old vs.  $\geq 2$ -years old) as a fixed effect to account for potential differences in breeding performance and response to stress among breeders of different ages

(Heidinger et al. 2006). Finally, I recognized that body condition can sometimes have an effect on baseline CORT levels (e.g., Angelier et al. 2007*b*, Cornelius et al. 2010) and other hematological measures; however, I did not have tarsus or mass measurements for every adult and therefore could not estimate body condition for each individual. To avoid loss of data, I ran preliminary analyses on body condition and plasma CORT. I found little effect of body condition on baseline CORT values ( $F_{1,8} = 0.13$ ;  $P = 0.73$ ); thus, I ran my full datasets without incorporating adult body condition into any of the models.

I used a Pearson correlation analysis to determine the relationship between baseline plasma CORT and H/L ratios and WBC counts. I also ran a simple linear regression using baseline CORT as a main effect to explain total WBC counts.

I arcsine transformed data for WBC proportions prior to analysis because they did not exhibit a normal distribution, but I report untransformed values (means  $\pm$  SEs) for ease of interpretation. All other data were normally distributed.

## **RESULTS**

### **Density manipulation**

Local neighbor densities more than doubled across the four years of my field experiment. In 2008, initial densities at the sites averaged  $6.6 \pm 0.24$  neighbors per breeding pair (range = 2-10). By 2010, local densities had increased to  $14.9 \pm 0.51$  neighbors per pair (range = 1-27). By 2011, densities averaged  $12.6 \pm 0.64$  neighbors per pair (range = 2-23). High nest box fidelity by returning male warblers meant that fewer unoccupied boxes were available in the low-density subplots each year; thus, densities generally built up more in the high-density subplots over time.

### **Baseline corticosterone**

From 2008-2011, I collected 63 samples from 60 individuals in under 3 min ( $\bar{x} = 2:48 \pm 0.02$  min) for baseline plasma CORT analysis. Corticosterone concentrations averaged  $12.37 \pm 0.61$  ng/ml across all samples. Baseline plasma CORT concentrations varied slightly with neighbor density (Table 4.1, Fig. 4.1) and generally increased from  $8.99 \pm 1.02$  ng/ml at the lowest densities to  $12.39 \pm 1.04$  ng/ml at the highest densities. Corticosterone concentrations were different among years (Tables 4.1 & 4.2), but this result was driven by differences between 2010 and 2011 and post-hoc comparisons showed that annual differences were no longer significant once a multiple comparisons test with a Bonferroni correction was applied ( $P \geq 0.21$  for all pairwise comparisons). Corticosterone concentrations were similar between females and males (Tables 4.1 & 4.3), but 1-year old birds had greater baseline plasma CORT concentrations compared to older birds (Tables 4.1 & 4.4). The neighbor density  $\times$  year interaction was unimportant in explaining baseline CORT concentrations.

### **Immunological measures**

During 2010-2011, I collected 64 blood smears from 64 individual birds. Lymphocytes comprised the majority of white blood cells ( $45.0\% \pm 0.5$ ) across all samples, followed by heterophils ( $23.0\% \pm 0.5$ ), eosinophils ( $19.3\% \pm 0.4$ ), basophils ( $10.8\% \pm 0.6$ ), and monocytes ( $1.7\% \pm 0.2$ ). Heterophil/lymphocyte ratios averaged  $0.52 \pm 0.02$  (range = 0.30-0.95) and estimated total WBC counts averaged  $5493.9 \pm 478.7$  (range = 1400.0-24500.0) across all adults.

Warbler H/L ratios increased only slightly with neighbor density (Table 4.1, Fig. 4.2), averaging  $0.42 \pm 0.04$  at the lowest densities to  $0.55 \pm 0.03$  at the highest densities. I found no effects of year, sex, adult age, or the density  $\times$  year interaction on H/L ratios (Tables 4.1-4.4).

The proportions of heterophils and lymphocytes were similar across the range of neighbor densities that I observed (Table 4.1, Figs. 4.4 & 4.5, respectively). Heterophils showed a slight increase with neighbor density ( $\bar{x} = 20.4 \pm 0.01\%$  at lower densities;  $\bar{x} = 24.1 \pm 0.01\%$  at greater densities) whereas lymphocytes decreased slightly with neighbor density ( $\bar{x} = 48.4 \pm 0.03\%$  at lower densities;  $\bar{x} = 43.7 \pm 0.01\%$  at greater densities). Neither year, sex, adult age, nor the density  $\times$  year interaction had an effect on the proportion of heterophils or lymphocytes. Further, the proportion of monocytes, eosinophils, and basophils showed little variation across the range of neighbor densities that I observed (Table 4.1). One-year old birds had a significantly greater proportion of basophils compared to birds  $\geq 2$ -years old (Tables 4.1 & 4.4), but basophils were not significantly affected by year, sex, or the density  $\times$  year interaction (Tables 4.1-4.3). Year, sex, adult age, and the density  $\times$  year interaction did not influence the proportion of monocytes or eosinophils in the warblers (Tables 4.1-4.4).

Neighbor density did not have a strong effect on total WBC counts (Table 4.1, Fig. 4.3). Total WBC counts averaged  $3360.0 \pm 408.2$  in the lowest density neighborhoods and  $5293.8 \pm 736.9$  in the highest density neighborhoods. I found no effects of year, sex, adult age, or the density  $\times$  year interaction on WBC counts (Tables 4.1-4.4).

Although the trends between density and CORT and density and H/L ratios were similar (Figs. 4.1 & 4.2), baseline plasma CORT concentrations were only weakly correlated with H/L ratios ( $|r| = 0.10$ ,  $P = 0.56$ ). Baseline plasma CORT concentrations showed a weak negative correlation with total WBC counts ( $|r| = -0.05$ ;  $F_{1,33} = 0.07$ ,  $P = 0.79$ ; Fig. 4.6).

## **DISCUSSION**

I predicted that increased conspecific densities would lead to chronically stressed birds as measured by baseline plasma CORT concentrations and H/L ratios. Given that I did not find any

adverse effects of density on reproductive output (see Chapter 2), I predicted that warblers would instead show changes in immune function (as estimated by WBC differentials and total WBC counts) with the presumed chronic stress they would experience with increasing neighbor densities. The range of neighbor densities on my sites more than doubled through the course of my experiment [2008: 2-10 neighbors; 2010: 1-27 neighbors (maximum observed range); 2011: 2-23 neighbors], yet I did not find a concomitant increase in baseline CORT concentrations or H/L ratios. I also observed no relationships between density and indices of immune function. My results for the indices of immune function are not altogether surprising considering that I did not find any differences in CORT or H/L ratios across the range of observed neighbor densities. I predicted that immune function would be a resource-based trade-off with CORT suppressing immune function rather than reproduction (Sheldon and Verhulst 1996, Råberg et al. 1998), but my results suggest that increased conspecific densities do not represent a challenge to the stress physiology of Prothonotary Warblers during the nestling stage.

Studies from a variety of taxa have reported positive correlations between population density and CORT concentrations (Jessop et al. 1999, Rogovin et al. 2003, Raouf et al. 2006), no association (Kuznetsov et al. 2004, Belden et al. 2007), or negative correlations (Stoinski et al. 2002, Machatschke et al. 2004, Mommer and Bell 2013). One potential explanation for variability in species' stress responses could be their ability to modulate the stress response (Wingfield and Sapolsky 2003). Modulation of the stress response to allow for successful breeding may be particularly important for species that migrate and/or breed seasonally (Wingfield and Sapolsky 2003). Studies have previously documented modulation of the stress response in migratory songbirds (Wingfield et al. 1992, Wingfield et al. 1995). As a migratory species with a defined breeding season, Prothonotary Warblers may be down-regulating their

stress responses to density to allow for successful reproduction during a period of their life-cycle with increased energetic demands.

Alternatively, increased social contact or population density may increase transmission of information about a particular habitat such as food availability, predation risk, or conspecific reproductive success (e.g., Ryer and Olla 1991, Boulinier and Danchin 1997, Parejo et al. 2006 and references therein, Krama et al. 2012), thereby buffering individuals in groups from potential stressors and reducing their GC levels as a result. Prothonotary Warblers use both public and private information to gain fitness benefits from breeding in a familiar site (Schelsky 2010). My finding that baseline CORT levels were unaffected by density lends support to the idea that the benefits gained from breeding in areas with increased social contact may outweigh any potential disadvantages.

Lack of a chronic stress response in relation to density could also be due to a lack of food competition among warblers in this system. Food competition is often considered to be an important density-dependent factor regulating bird populations and many studies have shown that reduced food availability or foraging success can increase baseline and total CORT response in birds (e.g., Marra and Holberton 1998, Clinchy et al. 2004, Angelier et al. 2007*b*, Schoech et al. 2007, Cornelius et al. 2010). I found little evidence for food limitation in this system as neither nestling provisioning rates nor nestling body condition varied in relation to neighbor density, and overall warbler reproductive output was similar across neighbor densities (see Chapter 2). Although some warblers on the plots experienced dramatically increased neighbor densities and presumably had smaller territories as a result (Petit and Petit 1996), they may not have reduced their territories to the extent that it affected food availability (Brown 1969, Fretwell and Lucas 1970, Petit and Petit 1996) or stress physiology.

Although my primary objective was to focus on density effects on baseline CORT concentrations, I included adult age as a covariate because some studies have found evidence for modulated CORT responses with age (Heidinger et al. 2006, Bókony et al 2009) whereas other studies have not found any pattern (Marra and Holberton 1998, Deviche et al. 2000, Angelier et al. 2007a). I found strong evidence for differences in baseline CORT across the two age classes used in my analysis, with first-year breeders (i.e., 1-year old birds) showing greater CORT levels than older birds. Limited future opportunity to breed has been proposed as one hypothesis to explain why vertebrates might resist stress (Wingfield and Sapolsky 2003). Although apparent survival rates for this warbler population can be high for a migratory bird (i.e., >80% of double-brooded Prothonotary Warblers return to the same sites in subsequent years; Hoover 2003a), migration still poses risks and older warblers may be resisting stress during the breeding season so as not to sacrifice current reproduction (Wingfield and Sapolsky 2003). My sample of older birds included individuals that ranged in age from 3-years old (i.e., entering their second breeding season) up to at least 8-years old, with the majority (72%) of older birds sampled being  $\geq 4$ -years old. The potentially confounding effects of age and experience cannot be determined from my study, however, as none of the 1-year old birds had prior breeding experience on my sites whereas the majority of older birds did have prior experience. Age and prior breeding experience are therefore highly correlated in my analysis. Older birds may simply be better at handling stressors than first-year breeders because they have experience from the previous breeding season(s).

I also found that WBC differentials showed little variation except that 1-year old birds had a significantly greater proportion of basophils than older birds. Basophils function in allergic reactions, acute inflammation, and cutaneous hypersensitivity, and increased numbers have been

observed in birds that are in the initial stage of inflammatory disease or under chronic stress (Maxwell and Robertson 1995). My finding that basophil counts were greater in 1-year old birds compared to older birds concurs with my CORT results. The two results taken together could suggest that younger (or less experienced) birds were chronically stressed. Alternatively, the differences could be due to age-specific maturation or senescence of the immune system (Noreen et al. 2011 and references therein, Palacios et al. 2011).

The fields of eco-physiology and eco-immunology offer exciting opportunities to help field biologists explain patterns of fitness and life-history traits observed in wild animal populations (Sheldon and Verhulst 1996, Romero 2004). In particular, incorporating measures of stress physiology and immune function may help explain the sometimes conflicting evidence for density-dependent reproduction in vertebrate populations. The population of Prothonotary Warblers that I studied inhabit a diverse and productive system compared to systems where evidence for density dependence of songbird populations has been found (e.g., Lack 1954, Alatalo and Lundberg 1984, Both 1998, Sillett et al. 2004), and density effects due to presumably increased competition do not appear to be important in this warbler population. Comparative work in other avian populations is needed, especially in systems where factors known to induce stress responses occur (e.g., decreased food availability, aggression and social dominance, increased vulnerability to predators). Further, the relationship between GC responses and individual behavior can be very complex with the timing, magnitude, and context of physiological responses dependent on both social and environmental conditions (Sapolsky et al. 2000, Summers et al. 2005). Therefore, future studies should also try to incorporate information on individual behavioral responses to conspecific density across multiple stages of the breeding

season to better understand the role of individual behavior in mediating physiological responses to density.

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## TABLES AND FIGURES

Table 4.1. Results of generalized linear mixed models comparing Prothonotary Warbler (*Protonotaria citrea*) baseline corticosterone (CORT) concentrations, heterophil/lymphocyte (H/L) ratios, total white blood cell (WBC) counts, and proportion of WBC types in relation to conspecific neighbor density, year, sex, adult age, and the density x year interaction. Sixty-three samples were collected from 2008-2011 for baseline CORT analysis; 64 samples were collected from 2010-2011 for H/L ratio and WBC determination.

	Neighbor						Adult						Density		
	Density			Year			Sex			Age			x Year		
	F	P	df	F	P	df	F	P	df	F	P	df	F	P	df
Baseline plasma CORT	1.20	0.28	1,50	2.73	<b>0.05</b>	1,50	0.20	0.65	1,50	3.28	<b>0.08</b>	1,50	1.69	0.18	1,50
H/L ratio	0.17	0.68	1,53	0.35	0.56	1,53	1.06	0.31	1,53	0.15	0.70	1,53	0.42	0.52	1,53
Total WBC count	1.28	0.26	1,53	0.49	0.49	1,53	0.50	0.48	1,53	0.19	0.67	1,53	0.01	0.82	1,53
% Heterophils	0.03	0.87	1,53	0.21	0.65	1,53	0.29	0.59	1,53	0.13	0.72	1,53	0.36	0.55	1,53
% Lymphocytes	0.85	0.36	1,53	0.22	0.64	1,53	1.57	0.22	1,53	2.00	0.16	1,53	0.13	0.72	1,53
% Monocytes	0.07	0.79	1,53	0.95	0.33	1,53	0.07	0.79	1,53	0.80	0.37	1,53	0.00	0.97	1,53
% Eosinophils	0.52	0.47	1,53	1.41	0.24	1,53	0.13	0.72	1,53	0.04	0.85	1,53	1.23	0.27	1,53
% Basophils	0.01	0.93	1,53	2.05	0.16	1,53	1.50	0.23	1,53	4.37	<b>0.04</b>	1,53	0.60	0.44	1,53

Table 4.2. Annual mean and standard error (SE) values for baseline plasma corticosterone (CORT), heterophil/lymphocyte (H/L) ratios, estimated total white blood cell (WBC) counts, and proportion of WBC types for Prothonotary Warbler (*Protonotaria citrea*) adults in southern Illinois during 2008-2011.

	2008			2009			2010			2011		
	Mean	SE	<i>n</i>	Mean	SE	<i>n</i>	Mean	SE	<i>n</i>	Mean	SE	<i>n</i>
Baseline plasma CORT	10.07	1.11	17	12.34	1.99	9	12.72	0.88	21	14.36	1.21	16
H/L ratio <sup>a</sup>	-	-	-	-	-	-	0.51	0.02	39	0.53	0.03	25
Total WBC count <sup>a</sup>	-	-	-	-	-	-	4576.9	382.1	39	5656.0	592.5	25
% Heterophils <sup>a</sup>	-	-	-	-	-	-	22.6	0.6	39	23.5	0.8	25
% Lymphocytes <sup>a</sup>	-	-	-	-	-	-	44.8	0.7	39	45.2	1.0	25
% Monocytes <sup>a</sup>	-	-	-	-	-	-	2.3	0.3	39	9.0	0.3	25
% Eosinophils <sup>a</sup>	-	-	-	-	-	-	19.4	0.5	39	19.4	0.8	25
% Basophils <sup>a</sup>	-	-	-	-	-	-	10.8	0.8	39	11.0	1.0	25

<sup>a</sup>Blood smears for determination of WBC counts and proportions were collected in 2010 and 2011 only.

Table 4.3. Mean and standard error (SE) values for baseline plasma corticosterone (CORT), heterophil/lymphocyte (H/L) ratios, estimated total white blood cell (WBC) counts, and proportion of WBC types for female and male Prothonotary Warbler (*Protonotaria citrea*) adults in southern Illinois.

	Females			Males		
	Mean	SE	<i>n</i>	Mean	SE	<i>n</i>
Baseline plasma CORT	12.91	0.84	28	11.94	0.87	35
H/L ratio	0.50	0.02	31	0.53	0.02	33
Total WBC count	4425.8	432.3	31	5536.4	487.8	33
% Heterophils	22.7	0.7	31	23.2	0.7	33
% Lymphocytes	45.6	0.8	31	44.4	0.8	33
% Monocytes	1.8	0.4	31	1.7	0.3	33
% Eosinophils	19.5	0.7	31	19.4	0.6	33
% Basophils	10.4	0.7	31	11.4	1.0	33

Table 4.4. Mean and standard error (SE) values for baseline plasma corticosterone (CORT), heterophil/lymphocyte (H/L) ratios, total white blood cell (WBC) counts, and proportion of WBC types for 1-year old compared to  $\geq 2$ -year old Prothonotary Warbler (*Protonotaria citrea*) adults in southern Illinois.

	1-year old			$\geq 2$ -years old		
	Mean	SE	<i>n</i>	Mean	SE	<i>n</i>
Baseline plasma CORT	13.71	0.98	27	11.36	0.74	36
H/L ratio	0.50	0.03	24	0.53	0.02	40
Total WBC count	4229.2	353.7	24	5460.0	475.7	40
% Heterophils	21.7	1.0	24	23.8	0.5	40
% Lymphocytes	44.4	1.0	24	45.3	0.7	40
% Monocytes	1.3	0.4	24	2.0	0.3	40
% Eosinophils	19.4	1.0	24	19.5	0.4	40
% Basophils	13.2	1.1	24	9.5	0.7	40

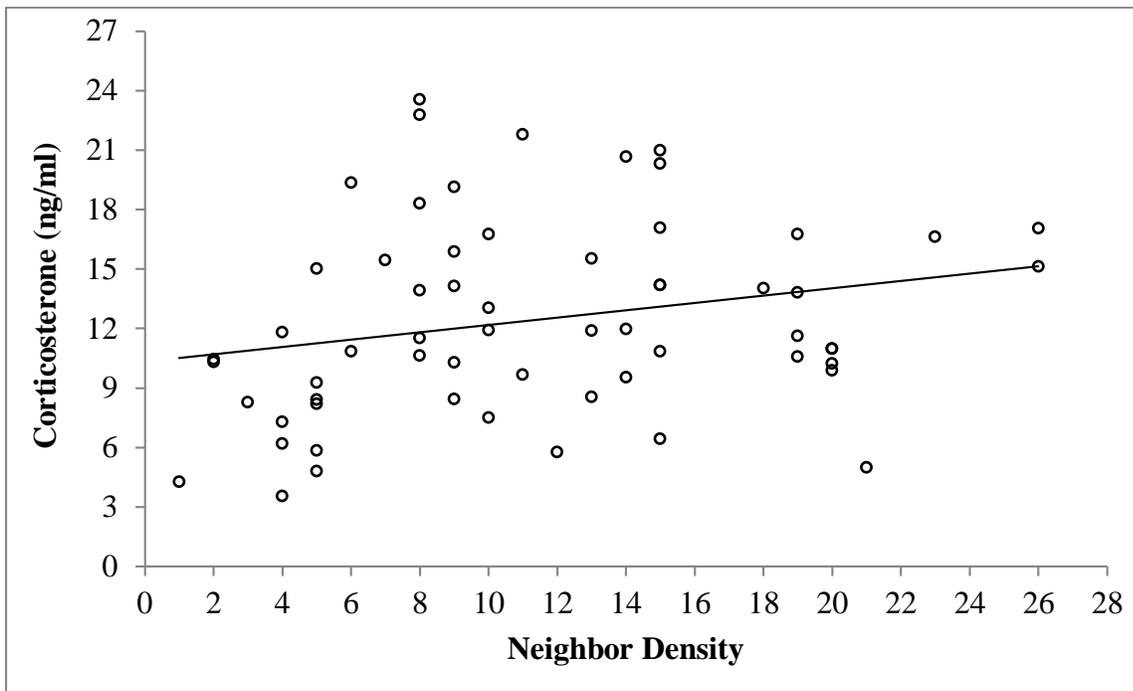


Figure 4.1. Baseline corticosterone concentrations (ng/ml) of Prothonotary Warbler (*Protonotaria citrea*) adults in relation to conspecific neighbor density in southern Illinois, 2008-2012.

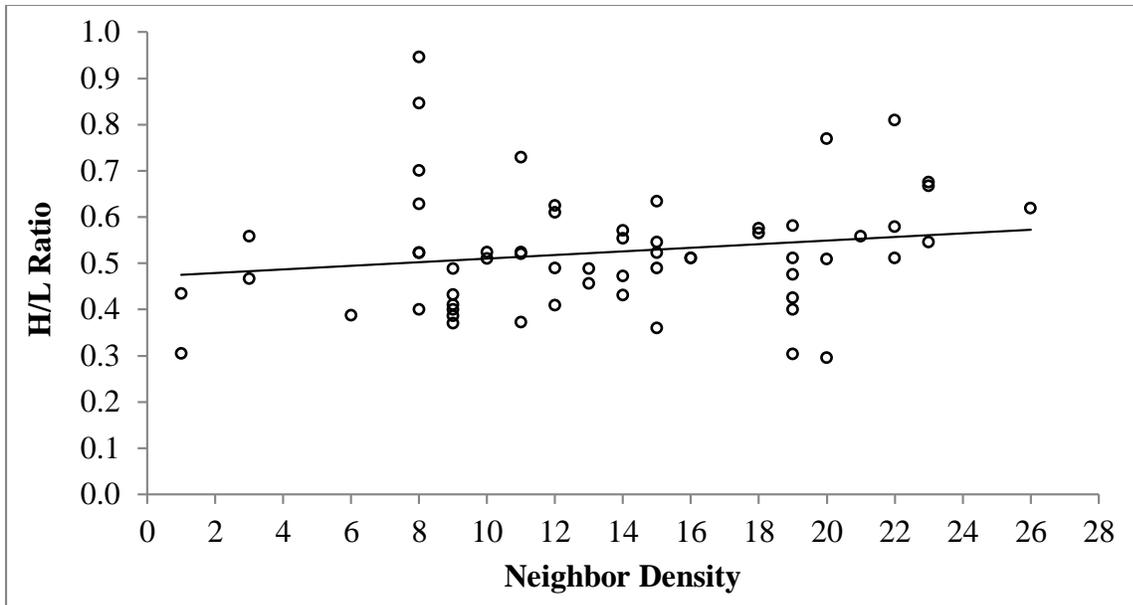


Figure 4.2. Heterophil/Lymphocyte (H/L) ratios of Prothonotary Warbler (*Protonotaria citrea*) adults in relation to conspecific neighbor density in southern Illinois, 2010-2011.



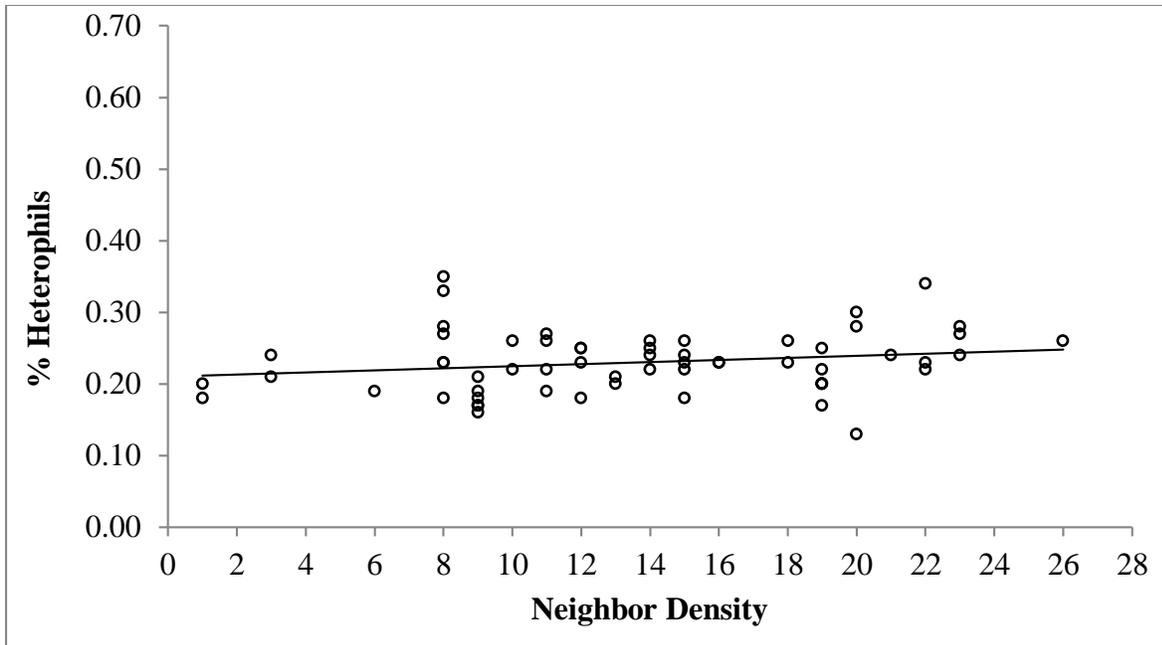


Figure 4.4. Proportion of heterophils in relation to conspecific neighbor density of Prothonotary Warblers (*Protonotaria citrea*) in southern Illinois, 2010-2011.

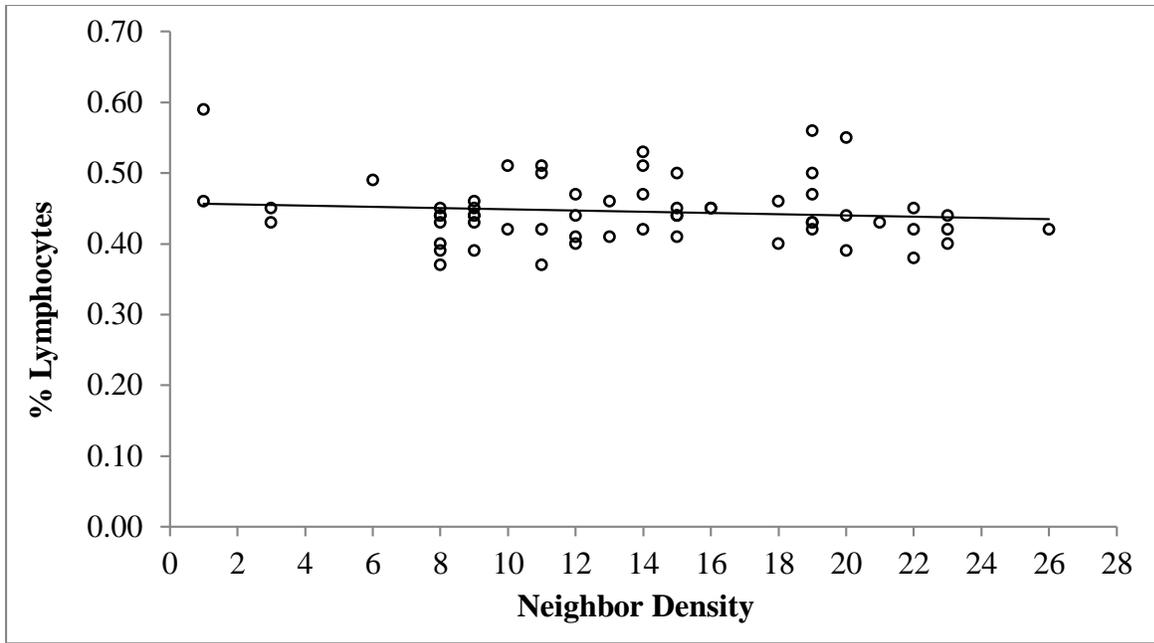


Figure 4.5. Proportion of lymphocytes in relation to conspecific neighbor density of Prothonotary Warblers (*Protonotaria citrea*) in southern Illinois, 2010-2011.

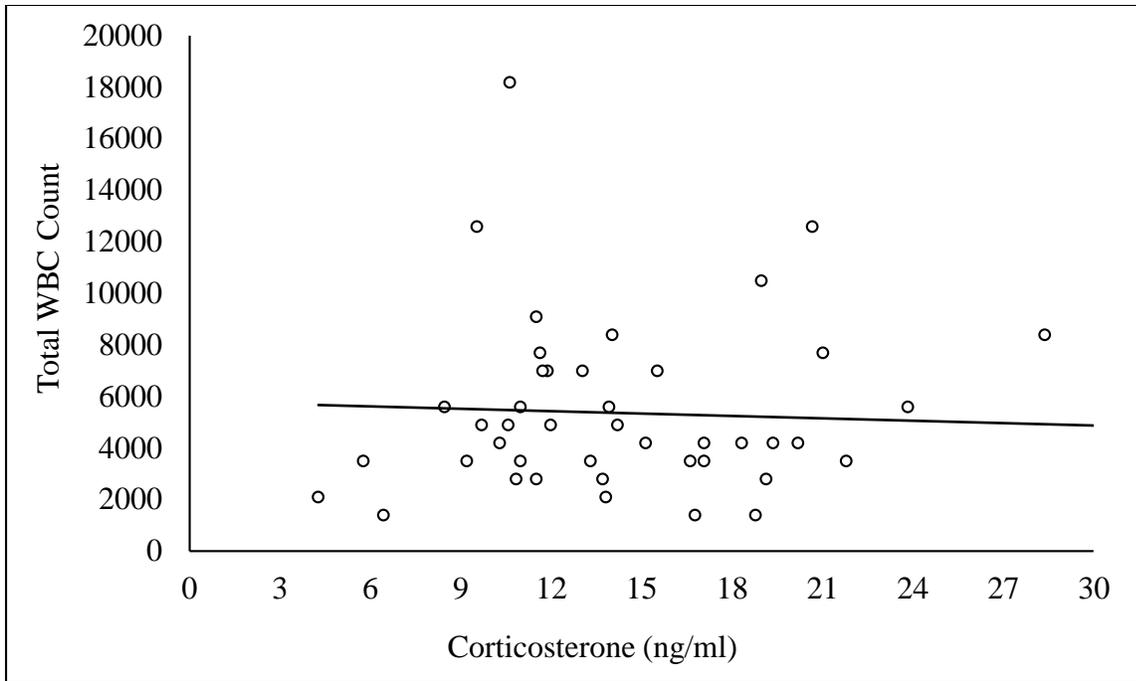


Figure 4.6. The relationship between baseline plasma CORT and estimated total WBC counts for Prothonotary Warblers (*Protonotaria citrea*) during 2010-2011 in southern Illinois.

## CHAPTER 5

### GENERAL CONCLUSIONS

A firm understanding of the mechanisms regulating populations has been difficult to obtain, yet it is considered to be one of the most important tasks still facing ecologists (May 1999). The goal of my dissertation was to test prevailing theories about the effects and mechanisms of conspecific density on a previously untested songbird species, the Prothonotary Warbler (*Protonotaria citrea*). By manipulating the availability of nest boxes and controlling nest predator access to and Brown-headed Cowbird (*Molothrus ater*) brood parasitism on warbler nests, I was able to experimentally more than double warbler densities on my site and achieve local densities that ranged from 1-27 neighbors/breeding pair. My results from Chapter 2 indicate that even at extreme local conspecific densities, warbler pairs were able to raise and provision a similar number of young in similar body condition compared with less-crowded warbler pairs. In Chapter 3, I found weak evidence that food was becoming less available for males with more neighbors. Nonetheless, the slight decrease in food availability did not translate to reduced reproductive output. Assuming that a physiological mechanism of density dependence may mediate a trade-off between reproductive output and self-maintenance, I looked for but failed to find evidence for chronic stress or immunosuppression in Chapter 4.

Neither an interference mechanism (i.e., competition for food) nor a physiological mechanism of density dependence seems to be at work on this warbler population during the breeding season. Some of the most productive habitat supporting a high diversity of flora and fauna in North America can be found in the bottomland hardwood forests of southern Illinois (Kellison and Young 1997, Kozlowski 2002). Although competition for resources, especially

food, is considered to be one of the most prominent processes through which density-dependent reproduction occurs in songbirds (Lack 1954, Newton 1998), I did not find evidence for this in the population of Prothonotary Warblers that I studied in southern Illinois. Increased conspecific density can influence male territory size (Petit and Petit 1996, Both and Visser 2000, Sillett et al. 2004), but warbler males may have prevented their territories from becoming so contracted that it affected their food resources. Alternatively, a site-dependent mechanism of regulation based on habitat heterogeneity across the larger landscape may preclude any within-habitat interference mechanisms (Rodenhouse et al. 1997). Ultimately, multiple mechanisms likely interact to regulate populations (Krebs 2002, Rodenhouse et al. 2003) and long-term and experimental studies are still needed to elucidate the importance of biotic and abiotic factors on avian populations across landscapes and throughout their annual cycles.

In addition to the theoretical aspects of my dissertation, I also see the applied value in my work. Bottomland hardwood forests represent one of the most imperiled forest habitat types in North America (Abernethy and Turner 1987, Gosselink and Lee 1989). Restoration efforts in southern Illinois have been aimed at reconnecting forest fragments and restoring the hydrological function of off-channel wetlands (Hoover 2009). Prior work in this system has shown that wetland restoration efforts can lead to increased standing water depth of treatment wetlands and a concomitant increase in the natural densities of Prothonotary Warblers (*Protonotaria citrea*) through a chain of events (Hoover 2009). Whereas raccoons (*Procyon lotor*) are a primary nest predator of Prothonotary Warblers and are likely the major factor limiting warbler reproductive output on some sites, raccoon access to warbler nests is diminished when standing water depths increase in response to wetland restoration. The diminished impact of nest predation and subsequent increase in warbler densities on restored wetland sites may change the interplay of

density-dependent factors affecting this warbler population. Thus, understanding the impact of forest fragmentation, stream channelization, and habitat restoration on wildlife populations in this system is necessary for informing management decisions.

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## APPENDIX A

### SONG RATES OF MALE PROTHONOTARY WARBLERS

Song rates of male Prothonotary Warblers in relation to neighbor density, nest stage, male age, and the density x stage interaction during first broods in 2010 in southern Illinois. Results are from a GLMM (Poisson distribution and log link function) with observation time specified as an offset variable. Estimates are on the log scale.

*P*-values in bold are significant at the  $p \leq 0.10$  level.

Explanatory variables	F-value	<i>df</i>	Estimate $\pm$ SE	<i>p</i> -value
Intercept			0.563 $\pm$ 0.381	0.148
Neighbor density	1.25	1, 39	0.026 $\pm$ 0.022	0.293
Nest stage	1.27	2, 39		0.327
Nestling			0.609 $\pm$ 0.533	0.283
Incubation			-0.043 $\pm$ 0.499	0.933
Pre-incubation			0	
Male age	3.58	1, 39		<b>0.091</b>
ASY			-0.323 $\pm$ 0.171	0.091
SY			0	
Neighbor density x stage	2.28	2, 39		0.158
Nestling			-0.047 $\pm$ 0.033	0.189
Incubation			0.016 $\pm$ 0.032	0.615
Pre-incubation			0	

## APPENDIX B

### FOOD SEARCH RATES OF MALE PROTHONOTARY WARBLERS

Food search rates of male Prothonotary Warblers in relation to neighbor density, nest stage, male age, and the density x stage interaction during first broods in 2010 in southern Illinois. Results are from a GLMM (Poisson distribution and log link function) with observation time specified as an offset variable. Estimates are on the log scale. *P*-values in bold are significant at the  $p \leq 0.10$  level.

Explanatory variables	F-value	<i>df</i>	Estimate $\pm$ SE	<i>p</i> -value
Intercept			0.723 $\pm$ 0.511	0.154
Neighbor density	2.87	1, 39	0.032 $\pm$ 0.028	0.125
Nest stage	11.14	2, 39		<b>0.004</b>
Nestling			0.657 $\pm$ 0.584	0.289
Incubation			1.691 $\pm$ 0.551	0.013
Pre-incubation			0	
Male age	1.32	1, 39		0.281
ASY			-0.347 $\pm$ 0.302	0.281
SY			0	
Neighbor density x stage	6.09	2, 39		<b>0.021</b>
Nestling			0.034 $\pm$ 0.036	0.375
Incubation			-0.030 $\pm$ 0.036	0.421
Pre-incubation			0	

## APPENDIX C

### FLIGHT RATES OF MALE PROTHONOTARY WARBLERS

Flight rates of male Prothonotary Warblers in relation to neighbor density, nest stage, male age, and the density x stage interaction during first broods in 2010 in southern Illinois. Results are from a GLMM (Poisson distribution and log link function) with observation time specified as an offset variable. Estimates are on the log scale.

Explanatory variables	F-value	<i>df</i>	Estimate $\pm$ SE	<i>p</i> -value
Intercept			0.907 $\pm$ 0.436	0.044
Neighbor density	0.75	1, 39	-0.030 $\pm$ 0.025	0.409
Nest stage	1.47	2, 39		0.280
Nestling			0.675 $\pm$ 0.543	0.245
Incubation			0.203 $\pm$ 0.524	0.708
Pre-incubation			0	
Male age	0.30	1, 39		0.596
ASY			-0.101 $\pm$ 0.184	0.596
SY			0	
Neighbor density x stage	0.77	2, 39		0.490
Nestling			0.016 $\pm$ 0.032	0.632
Incubation			0.035 $\pm$ 0.031	0.296
Pre-incubation			0	

## APPENDIX D

### PREY ATTACK RATES OF MALE PROTHONOTARY WARBLERS

Prey attack rates of male Prothonotary Warblers in relation to neighbor density, nest stage, male age, and the density x stage interaction during first broods in 2010 in southern Illinois. Results are from a GLMM (Poisson distribution and log link function) with observation time specified as an offset variable. Estimates are on the log scale.

Explanatory variables	F-value	<i>df</i>	Estimate ± SE	<i>p</i> -value
Intercept			-1.418 ± 0.778	0.076
Neighbor density	0.23	1, 39	0.090 ± 0.044	0.644
Nest stage	2.30	2, 39		0.156
Nestling			1.188 ± 1.017	0.273
Incubation			2.000 ± 0.960	0.067
Pre-incubation			0	
Male age	1.81	1, 39		0.212
ASY			-0.507 ± 0.377	0.212
SY			0	
Neighbor density x stage	2.64	2, 39		0.125
Nestling			-0.091 ± 0.057	0.145
Incubation			-0.136 ± 0.059	0.048
Pre-incubation				