Territorial Defense Strategies in the Northern Cardinal (*Cardinalis cardinalis*): Who is the Bigger Threat?

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TERRITORIAL DEFENSE STRATEGIES IN THE NORTHERN CARDINAL

(CARDINALIS CARDINALIS): WHO IS THE BIGGER THREAT?

by

Kaylee Michelle Gentry

A Thesis
Submitted to the Graduate School
and the Department of Biological Sciences
at The University of Southern Mississippi
in Partial Fulfillment of the Requirements
for the Degree of Master of Science

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December 2015
ABSTRACT

TERRITORIAL DEFENSE STRATEGIES IN THE NORTHERN CARDINAL

*(CARDINALIS CARDINALIS): WHO IS THE BIGGER THREAT?*

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This thesis examines the use of defensive strategies in relation to territories year round in the northern cardinal (*Cardinalis cardinalis*). Responses to recorded neighbor song and stranger song playback from the middle of a focal male’s territory were measured. This allowed for an estimation of aggression in both the winter and spring seasons. Each focal male was subjected to both treatments (stranger song and neighbor song). Males were more responsive over-all to neighbor song playback, however in the winter months, persistence of response to neighbor song playback increased. It was also shown that southeastern United States cardinals show year-round territory occupancy and more importantly the tendency to defend that territory during the entire year. Blood collected from a small number of birds during a neighbor STI trial shows that circulating testosterone does not significantly change from baselines or birds being challenged with a strange song playback.
DEDICATION

I would like to dedicate this work to my mother, ReAnne Gentry, my uncle, Shelby Gentry, my grandmother, Rose Gentry, and my younger brother, Kaleb Gentry, for their love and support. I would also like to acknowledge Joseph Keim for his encouragement and motivation, Aaron Holbrook, for his friendship and support, and Jessica Panella, for late night phone calls and words of encouragement.
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CHAPTER I
INTRODUCTION AND REVIEW

In its most simplistic form, a territory can be defined as any given area that is
defended and used by an animal for some distinct purpose at any given time (Noble
1939). In defending this area, any resources that may be within the territory are removed
from use by conspecific competitors. This, of course, demands a number of trade-offs for
the territory owner. For territoriality to occur, the costs of defense of the territory must
outweigh the costs of competing for free-ranged resources such as food resources and
nesting habitat (Brown 1964). Establishment of territories and the aggressive behaviors
associated with defending and maintaining a territory are well documented in a large
percentage of passerines (Nice 1941). Defense tactics can range from passive notification
of boundaries and owner presence, such as singing along territory boarders, to more
active defense such as fighting and ejection of an intruder (Brown and Orians 1970). In
this chapter, I will define the types of territories that have been observed in birds, the
benefits and costs of territoriality, and the strategies of defense that are used by
individuals as they are confronted with intruders/challengers on their defined territory
and emphasize potential territorial strategies in the Northern Cardinal (Cardinalis
cardinalis); the focal species of the fully described research.

Early literature puts emphasis on male defense of a territory (see extensive
reviews in Andersson 1994; Adkins Regan 2005), but in many species, females have
been shown to help maintain territory integrity and in some cases, hold territories on their
own. Nuttal’s White-Crowned Sparrow (Zonotrichia leucophrys nuttalli) females play a
role in defense of the territory (Blanchard 1936), and Northern Phalarope (Phalaropus
lobatus) females establish, maintain, and defend their territories without male intervention (Tinbergen 1935). Throughout this review, the discussed studies have focused on male territorial defense, the research to be described in full in this thesis also focuses on male defense of territories. The reader should be aware that many of the same hypotheses to be presented and discussed here can be advanced to explain female defense of territories and that there may be unique strategies that females display regarding territorial defense that are not yet fully explored.

Territory Types, Definitions, and Examples

Ernst Mayr (1935) first classified avian territories by setting parameters of usage by owners (for example, exclusive use of a defined area with resources needed for reproduction) which was then modified and expanded on by Margret Nice (1941) into six different types (called Types A-F). Territories defined as “Type A”, or all-purpose, are used annually or year-round for multiple or all aspects of the annual cycle (reproduction, molt, over-wintering, etc.), and species which are non-migratory and use the same territory throughout their lives would be likely to fall under this classification of territory. Next is “Type B” or territories used just for mating and nesting, annually these are defined and defended and used just for reproductive purposes (note, not in colonial species). “Type C” or lekking territories are small areas defended by a male of a lekking species where he will display and copulate with a female. Nesting territories, or “Type D”, are seen in colonial nesting birds where the defended area is only utilized for nesting; feeding and mating occur elsewhere. “Type E” territories are those that are established and maintained over the non-breeding season by either a single bird or a mated pair and the final type, “Type F” are territories used by individuals or large flocks as a roosting
territory. These territory types are not always discrete for every species, and in some cases, birds may use a blend of territory types over the course of their lives; however, there are numerous species that do indeed fit discretely in to one of these groups.

Classic, all-purpose territories (Type A territories) are used by their tenants for mating displays, mating, nesting, and foraging. All activities take place within the boundaries defended by the owners so that food resources for adults and offspring and ideal nesting habitat are included within the territories (Temeles 1994; Altum 1868; Howard 1920). These types of territories can also be broken down into the length of use: either breeding season specific or year-round. The majority of song bird territories like those of the Song Sparrow (*Melospiza melodia*), Snow Bunting (*Plectrophenax nivalis*), many warblers, and Eastern Towhees (*Pipilo erythrophtalmus*) fall into this category (Nice 1941). Either annually (breeding season) or throughout the entire calendar year, territory boundaries are announced and defended, and individuals use resources to move through multiple life-history stages (e.g., reproduction to molt to preparation for migration). Type B territories are described as territories where mating displays, copulation, and nesting occur within the defended territory boundaries, but foraging activities take place outside of the defended area. Lekking (Type C) and nesting-only (Type D) territories can both be fairly small areas with males defending just the lekking area for male displaying and mating, as seen in Swallow-Tailed Hummingbirds (*Eupetomena macroura*) (Pizo and Silva, 2001) and White-Throated Manakins (*Corapipo gutturalis*) (Théry and Vehrencamp 1995), or pairs defending a small area around the nest, as seen in South Polar Skuas (*Catharacta maccormicki*) (Trillmich 1978). Type C lekking territories are often aggregated and territory holders may be physically very close
to another displaying male. Lekking territories do not contain food or nesting resources and are abandoned by the male after the lekking event. Females establish nests away from the lekking ground and raise young without the help of the male (Höglund and Alatalo 1995). Nesting territories, Type D, most frequently occur in colonial species, the large majority of which are water fowl. The defended area usually includes a buffer zone immediately outside the nest, the size of which depends on the density of the nesting colony (Hotker 2000). Colonial nesters do not court or copulate on their territories, and leave their territories to forage. Indeed, a group of nesting birds is only considered a colony when members of said colony frequently leave the nesting area for foraging activities (Wittenberger and Hunt 1990). Wintering territories (Type E) are functionally all purpose territories that are in a different location than the breeding territory (usually become linked with a Type A or B territory). Species that have winter Type E territories may have all-purpose territories (Type A) or breeding territories (Type B) in the spring/summer and then move to a separate all-purpose winter territory outside of breeding. A distinction to be made is that the wintering territory is also behaviorally defined and defended from conspecifics. Defense of winter territories has been shown in a number of species including European Robins (*Erithacus rubecula*; Schwabl, 1992), Northern Mockingbirds (*Mimus polyglottus*; Laskey 1936; Michener and Michener 1935), and Herring Gulls (*Larus argentatus*; Goethe 1937). Mockingbirds in particular are shown to defend their over-wintering territories more aggressively than their summer territories with both the male and female participating in the defense (Michener and Michener 1935). Wintering territoriality may be linked to non-breeding resources being rarer in occurrence than breeding resources. Many long- and short-distance migrants
display Type E territoriality and have this linked with territoriality of some kind in the breeding season (Type A or B). Both male and female European Robins maintain and defend winter territories separate from one another with females showing aggression that includes singing against intruding conspecifics. In the breeding season, however, pairs live on a shared Type A territory with the male showing more aggression and defensive action against intruders than the female (Schwabl 1992). The final territory type in Nice’s (1941) scheme is the roosting territory which consists of an area used by a solitary member or even variously sized groups of a species only for shelter, as seen in the Eurasian Treecreeper (*Certhia familiaris*) (Rakin 1940). Treecreepers will return to several roosting holes excavated in trees like Giant Sequoias (*Sequoia gigantea*) to roost and have been shown to defend these preferred shelters from intruders, although some holes may be used by other species such as the Coal-Tit (*Periparus ater*). The roosting territory contains multiple holes spread out over several different trees which allow the treecreepers to preferentially move to different holes for better shelter from inclement weather (Rankin and Rankin 1939).

All of the described territory types are used by birds and as seen with many species that migrate (e.g., the Song Sparrow described above) either long or short distances, multiple territory types can be used by a single species and there can be fluid movement between territory types; what type of territoriality is used can be strongly driven by the costs and benefits of defending a specific resource (to be discussed in greater detail in the next section) and the resources under consideration. Long term, single locale territories (or year-round territories) would need to have all of the resources used for both over-winter survival (e.g., appropriate food type and abundance for
survival, protected sheltering sites) and for successful reproduction (appropriate nesting
habitat, foods appropriate for nestlings, abundant food for energetic demands of egg
production/offspring growth). This could make year-round occupancy of single
territories difficult, energetically expensive, rare, and a phenomenon only seen in a non-
migratory species like the Northern Cardinal (*Cardinalis cardinalis*), to be described in
greater detail below. Migratory birds may change territory focus between their wintering
and breeding grounds and may not be territorial in both seasons. When declaring a
breeding season territory, owners may be more concerned with suitable nesting habitat
than food availability. However, in the winter season, food and shelter may be the more
coveted resources. Regardless of the type of territory used, there are an important number
of costs and benefits associated with the acquisition and defense of the defined space.

Costs and Benefits of Territoriality

The costs and benefits of territoriality are tied together and can fluctuate with the
resource defended, resources required for reproduction/survival, the type of defensive
behavior shown, time of year, environment or resource quality, and together these can
impact the size of territory defended and the level of aggressive behavior that an
individual demonstrates. This becomes a complex pattern with many variables that need
to be taken into account and which can yield some very interesting, and occasionally
counter-intuitive, outcomes. For example, the ties between food availability and cost of
defending a territory have been studied in many song bird species (Marshall and Cooper
2004; Shank 1986; Pitelka 1955; Lyon 1976; Holmes 1970). A classic example
illustrating this association is in the Ovenbird (*Seiurus aurocapillus*) where the size of the
territory defended changes with fluctuating food availability; territories become smaller
during periods of high food yield (Stenger 1958). However, in some situations, food is not the limiting resource, it could be appropriate nesting environment, and focus on the resource defended changes (Raphael et al. 2002; Rands 1986; Jehl Jr. 1989). For example, some colonial nesters that use broadly-available and highly-abundant food resources are more limited with respects to appropriate nesting habitat. Most seabirds are able to forage in the oceans for food; however, nesting territories are located on islands in many cases, and can be specific microhabitats on the island, making this resource scarce and the main focus of a defended territory. High fidelity territory nesters like California Gulls (Larus californicus) will attempt to reestablish territories in the same area as previous breeding seasons which can lead to serious altercations when nesting availability becomes limited due to habitat loss or influx of new pairs (Jehl Jr. 1989).

Territoriality hinges on a balance between costs incurred by the owner during territory defense and the benefits gained by maintaining resources for exclusive use. Costs usually arise from the behaviors associated with establishing and keeping a territory, mainly the restriction and defense of an area, but also advertisement of ownership (Hinde 1956). The establishment of territories can lead to intense fighting with conspecifics, particularly when available habitat is scarce (Jehl Jr. 1989), potentially leading to physical injury. Birds that cannot successfully establish a territory in the breeding season (or in time for the next breeding season in the case of non-migratory birds) may forfeit their fitness for the season, and birds who are unable to establish over-wintering territories face the possibility of starvation. Once a territory is established, the owner must constantly work to remove any intruders who if unchallenged, might capitalize on food resources, or try to annex part or all of the territory. Notification of
boundary lines by singing at points along the territory boarders sets the expectation of exclusion and alerts neighbors or strangers in the area that the resources are not available. Establishment, advertisement, and especially defense of a territory can be energetically expensive and require time that could be spent foraging to be reallocated to the expulsion of an intruder, or a song bout with a neighbor. To be feasible, the gains from the available resources must balance the energy devoted to defense (or simply cost to the defender). It must be more economical for the individual to defend a resource from others rather than a free-for-all model of foraging, shelter, or nesting habitat (Brown 1964).

Benefits of territory defense must outweigh the costs in order for territoriality to evolve and be maintained. Seasonal variations in resource abundance or perceived importance are the most likely roots for the variation in cost/benefit ratios and the development of the many different types of territoriality described earlier. Additionally, species ecology will factor into cost/benefit ratios and the development of territorial strategies. For example, species that are food specialists (Stiles and Wolf 1970; Dearborn 1998) are often migrates and territorial at both their wintering and breeding grounds due to the need to isolate their specialized food resource for individual use. Species that are diet generalist, like the Northern Cardinal (Halkin and Linville 1999), may be more easily able to defend a general use territory that does not fluctuate between summer and winter. Here the broad spectrum of acceptable food types allows for an individual to find adequate food throughout the year in a single location. Regardless of the type of territory defended, the benefits should outweigh the costs, and the specific costs of defensive behaviors displayed is a topic that has been well studied in birds and that has a history of strategic variability in the behavior shown.
Territorial Behavior: Defensive Actions and Strategies

Territories are defended through the display of a wide variety of behaviors in birds which can include song production, call production, physical displays on territory boundaries, and active patrolling along territory boundaries (reviewed in Andersson 1994). When intruders are discovered, territory defense responses can range from just appearing in the area of the intruder, song or call production directed towards the intruder, displays of body size or ornamentation to the intruder, or physical altercations with the intruder (displacement flights, actual physical attacks) (Andersson, 1994). What a territory defender does can be impacted by many potential variables. Territory type, resources, and seasonal presence of resources and potential competitors can act as indicators for strategies the owners may use to defend against potential intruders and usurpers (Temeles 1992). For instance, birds who maintain Type B territories would not be concerned with defending food resources because all foraging activities take place away from their nesting territory. Therefore, birds who may be foraging in the area may not be engaged aggressively, but birds who appear to be prospecting for available nesting space may present a bigger threat and require a behavioral response. Who the territory defender is faced with can also have an impact on defensive behavior (Fisher 1954). Birds have been shown to be able to distinguish between familiar and unfamiliar phenotypes (Colah 1983), which leads to two possible types of recognizable intruders: either a neighbor from a contiguous territory or a stranger bird. The level of aggressive action taken against the intruder also depends on the intruder type and the perceived threat posed by the intruder in question to the owner. There are multiple strategies that
have been described for how a territory owner responds to an intruder; here I focus on the “nasty neighbor” and the “dear enemy” strategies.

The “dear enemy” phenomena is that of lowered aggression toward familiar neighbors (Fisher, 1954). Ultimately, this lowers the expenditure of energy for both parties where territorial posturing is concerned (Fisher, 1954). This strategy suggests that intruding stranger birds, often termed ‘floater’ birds, will be treated as a greater threat than an intruding neighboring bird and thus elicit a more aggressive response from the territory owner. Multiple hypotheses regarding the reduced aggression during an encounter with neighboring exist in the literature, including the idea of degree of familiarity (Ydenberg 1988; Getty 1989), which suggests that the more familiar a bird is with its neighbor, the less aggressively it will respond to a neighbor’s presence. Resources and defensive abilities have been tested and are known between neighbors and association hierarchies have been defined, additional elevated aggression is not generally necessary. While familiarity is an important aspect of the dear enemy phenomena, in that it allows recognition of neighbors through song, local dialects, and phenotype (Falls and Brooks 1975; Wiley and Wiley 1977; McGregor and Avery 1986; Brindley 1991), it is the relative threat theory presented by Temeles (1990) that is the most widely accepted for the dear enemy phenomena. Following this theory, for a bird to implement the dear enemy strategy the potential to lose resources to a strange bird must be higher than the potential to lose out to a neighboring bird (Temeles 1990). By eliminating overly aggressive interactions between neighbors, the potential for injury to either party is lowered and allows more time for foraging, mating, and nestling care (Wilson 1975; Wingfield 1990). Therefore territory owners should be more likely to respond to stranger
males singing on their territories with aggressive singing and physical confrontation than to a singing neighbor in the same scenario (Falls 1982). For this strategy to be effective, both parties must show restraint in off-territory challenges, and respect the existing boundaries, thereby losing the opportunity to expand their individual territories (Godard 1993). Temeles (1992) showed that 39 of 39 species reviewed with multi-purpose breeding territories (e.g., Type A or B territories) displayed the dear enemy phenomena. Evidence for this model exists within insects, mammals, fish and birds including Carolina Wrens (*Thryothorus ludovicianus*), (Eason 1994; Price 1999; Ydenberg 1988; Temeles 1994). Wrens were subjected to playback of neighbor and stranger song before and after simulated territory intrusions and aggression to each treatment was measured by scoring specific behaviors such as how long it took the owner to investigate the song, how long they responded to playback, singing, and closeness to the hidden speaker (Hyman 2002).

The opposing “nasty neighbor” hypothesis states that territory owners will respond more aggressively towards neighboring birds than unknown birds (Muller, 2007). A study by Temeles (1989) showed that Northern Harriers (*Circus cyaneus*) defended their territory more aggressively against neighbors than stranger birds, exempting them from the ‘dear enemy’ hypothesis. European Robins have been shown to become more aggressive when mated, and mated males often attempt to steal territory from unmated neighbors as a result functioning as ‘nasty neighbors’ (Lack 1940). In a number of species, territory owners show greater aggression towards familiar neighbors who are constantly pushing territorial boundaries in an attempt to expand their own. This behavior has been documented in Carolina Wrens (*Thryothorus ludovicianus*; Hyman 2002), Hooded Warblers (*Setophaga citrina*; Godard 1993), and Red-Winged Blackbirds
(Agelaius phoeniceus; Oldendorf 2004). In many cases stranger birds may be less likely to be able to take over a territory due to their lack of familiarity with territory boundaries, territory resources, and the abilities of the territory owner. A known neighbor can continuously ebb away at territorial boundaries that they are aware of; they can intrude multiple times over the course of the season, and they will be more familiar with the physical abilities of their neighbors whom they are challenging (Temeles, 1990).

Ultimately, the type of territoriality that is being shown by a species, the resources under consideration, and the population structure all have a role to play in the development of territory strategies; also, there is evidence that birds do not use a single defensive strategy throughout their whole lives.

Birds may show both the nasty neighbor and dear enemy effects when there is a change in the population makeup or availability of resources, switching from one to the other as the situation calls for (Yoon 2012; Muller 2007; Briefer 2008; Newey 2008). Strategy switching can be related to changes in population make up, as has been documented in Wrentits (Chamaea fasciata); a non-migratory year round territorial species (Erickson 1938). When juvenile flocks of territorial-less floater birds move through the environment in the fall, territory availability is limited by how many of the long-term adult pairs have died off, leaving available space for territory acquisition by juveniles. In this scenario, reproductive success depends strongly on finding a territory in the area with space being the limiting factor (Erickson 1938). How a territory holding adult views its neighbors versus strangers may depend quite strongly on the concentration of juveniles and non-territorial adults in the population and may drive strong annual
variation in territory strategy, though little work has been done looking at change in strategy throughout a single year.

The Northern Cardinal: Focal Species

Northern Cardinals are socially monogamous, non-migratory passerines that range from Guatemala to southern Canada (Halkin and Linville 1999) that inhabit territories year-round (J.M. Jawor, unpubl. data). Year-round territorial passerines, like the cardinal, will use song to define and defend a territory, alerting others of their boundaries (Catchpole and Slater 1995). Song, in cardinals and other species, is also used to individually identify the bird singing and allows for recognition of stranger from neighboring birds (Falls 1982). Frequency and quality of song can be affected by time of season, overall individual health, and the newness of the focal bird to the territory (Tobias 1997; Vondrasek 2006) giving both neighbors and strangers some level of information on territory owners’ abilities. Song frequency can also be affected by aggressive actions with a neighboring territory owner or a stranger bird intruding on a territory. Typically, song frequency increases when aggressive interactions are common (Baker, Wilson, and Mennill 2012). Competing males can initiate potential boundary shifts by intruding inward on neighboring territories and singing. If the owner does not defend the area in question, the intruding bird may be able to expand his territory’s reach to gain extra resources, nesting habitat, and better mating opportunities (Brown, 1964). Because of the importance of song I focus on this as an indicator of territorial defense in this research.

Cardinals use song to define and maintain multi-purpose classical territories (Type A) that can range in size from 0.21 to 2.60 ha. Ideal territories include good nesting habitat in shrubby, thick areas with small trees such as hawthorn (Crataegus spp.)
and dogwood (*Cornus spp.*), as well as the availability of preferred food items such as wild grape (*Vitis spp.*), dogwood (*Cornus spp.*), mulberry (*Morus spp.*), and knotweed (*Polygonum spp.*) seeds and fruits (Halkin and Linville 1999; Wolfenbarger 1999). Annually population dynamics change with a fall/winter influx of juvenile individuals looking for a territory to occupy; successful future reproduction and survival depends on the acquisition of a territory soon after fledglings become independent of their parents (Halkin and Linville 1999; J.M. Jawor and R. Breitwisch, unpubl. data). During the winter, many floater males who were fledglings from the previous breeding season flock together in search of their own territories, changing the population dynamic and potentially the response of established territory owners to intruders.

In this work, I will investigate two main questions: (1) Do male Northern Cardinals display territorial defensive behaviors year-round? Halkin and Linville (1999) describe more northern populations of cardinals as not displaying year-round territorial behavior; observations on the south-eastern study site used here have found that cardinals are resident on territories year-round and that song is produced by territory holders in the winter as well as the spring/summer (Jawor et al. 2014; J.M. Jawor and M.S. DeVries, unpubl. data). Here I will specifically test the response of territory holders in both the early breeding season and the winter to determine if territorial defense is shown year-round in this population. Because there is a change in population structure through time in Northern Cardinal populations (many territory-less individuals in the fall and winter, few territory-less individuals in the spring but potential attempts made by established birds to acquire more territory area), I will investigate whether cardinals show evidence of switching between territory defense strategies, specifically whether they show
evidence of using a dear enemy strategy in the winter when there are many strangers in
the population and a nasty neighbor strategy in the breeding season when there are
potential opportunities to expand existing territory boundaries.

Given that cardinals occupy their territories year-round in the study population to
be used here, I predict that year-round defense will be seen. Evidence of this already
exists through casual observations but specific assessment is necessary to confirm these
observations. The level of defense and the strategy or strategies pursued may vary
throughout the year. In identifying the strategy or strategies used during defense of
territories the strength of responses by territory holders will be the basis for determining
strategy type. Behaviorally stronger responses (described in later chapters) will be
indicative of response to a perceived stronger threat. I predict that neighbors will be a
stronger threat in the early breeding season. They are likely birds that have been on site
for at least one year and have had multiple interactions with their neighbors, and there are
few territory-less strangers in the population. I predict that this will change during
defense in the fall and winter when more territory-less strangers are to be found in the
population.
CHAPTER II
AN INVESTIGATION INTO THE OCCURANCE OF YEAR-ROUND TERRITORIAL BEHAVIOR IN THE NORTHERN CARDINAL (CARDINALIS CARDINALIS)

Year-Round Aggression and Territoriality

Territoriality, or the guarding of resources for specific use by the defender, is exhibited by most song birds at some point during their lives. While territoriality has costs associated with the maintenance and defense of borders from intruders, the benefits accrued by exclusive use of resources by an individual or pair are greater than if defenses were dissolved and resources available to all conspecifics (Temeles 1994). The length of time throughout the year that individuals are territorial and the resources that are preferentially guarded varies and territoriality can be quite complex in its expression.

Many songbird species defend a territory only during their breeding season. Resources such as good nesting habitat and nearby, plentiful food resources are important to successful breeding and for the feeding of nestlings after hatching. The quality of territories, or how well the territory fits the required needs of a species, can be used as an assessment of males by unpaired females during pre-breeding mate choice (Przybylo et. al. 2001; Zimmerman 1971). After the breeding season is over, the requirements for survival may change, and maintenance of a territory during the non-breeding season may no longer be beneficial. In non-territorial flocking species (i.e., no territoriality in the non-breeding season), grouping together while over-wintering may lower the risk of predation (Moynihan 1962), reduce aggression from other species (Barash, 1974), and increase foraging ability (Krebs et. al. 1972; Pulliam and Millikan 1982). However, winter flocking isn’t cost free. Birds of the flock share food resources among the group
and when hierarchies exist, birds in a submissive role may receive less food than more dominate birds (Hammerstrom 1942; Hartzler 1970).

In contrast, birds may migrate only to form a new, over-wintering territory that they defend similarly to their breeding territory; essentially showing year-round territorial behavior across multiple locations and territories. Defense of a breeding territory ensures nesting sites and food for both adults and dependent offspring, and may provide a safe, resource rich area to complete molt. Defense of a wintering territory likely provides safe roosting places and reliable food resources for the non-breeding season, those who are unable to establish wintering territories run the risk of starvation (Davies and Huston 1984; Winker 1990). However, costs of this type of territorial system may be high; migrant birds must establish new territories each breeding and non-breeding season (though they can show general site fidelity Faagborg and Arendt 1984; Loftin 1977) and then aggressively defend both territories, individually, through an entire year. While year round aggression in association with the defense of a territory has been noted in numerous species it can be quite complex in occurrence and how it is managed. Both Song Sparrows (Zontricha melodia) and European Robins (Erithacus rubecula) show defensive tactics concerning both breeding and non-breeding territories, though they are not year round residents of a single territory; breeding and non-breeding season territories are in separate locations (Wingfield 1992; Schwabl 1992). Song Sparrows in particular exhibit a wide variety of territoriality during the non-breeding season. Both males and females will defend winter territories on their own or they may make alliances with other birds to defend a single territory used by all in the group. Alliances can range from male-
female pairs, to male-male pairs, as well as coalitions of multiple birds (Wingfield and Monk 1992).

Year-round territory occupancy where no movement occurs between separate breeding and non-breeding territories does occur and this would be limited to non-migratory species. The benefits and costs for a non-migratory bird showing year-round territory occupancy are long term; the territory and resources defended in the winter season remain in the owner’s possession for the upcoming breeding season (Ekman, 1979; Smith, 1984) and they will still be there in the following non-breeding season. For example, Tropical Mockingbirds (Mimus gilvus) have been shown to display year-round aggression as well as maintaining and residing on the same territory year round (Langmore, 1998). Indeed, year round territoriality, or territories that are defended and lived on in both the breeding and non-breeding season with no change in locale, are typical of tropical environments where food availability is less variable and readily available throughout the year (Stutchbury 2001). Many tropical species such as Bay Wrens (Thyrothorus nigricapillus) and White-Browed Sparrow Weavers (Placepasser manli) maintain territories year round and show aggressive behaviors such as singing, song-matching, and attacking decoy intruders (Levin and Wingfield 1992; Wingfield 1991; Wingfield and Hahn 1994) regardless of season. In non-tropical areas (particularly the temperate zone), this type of tenancy is fairly uncommon. Significant seasonal changes in temperature and water availability in the temperate zone can drastically reduce food availability and harsh weather conditions can increase mortality rates. In most temperate zone bird species, individuals migrate away from the breeding area in the temperate zone, either setting up new, non-breeding territories with appropriate and
adequate food resources that are then defended or joining large flocks to facilitate ease in finding food (often in the tropics, but not always). The influence of food availability is of particular importance for the possibility of developing year-round territory occupancy and defense. Special cases where environments have provided natural barriers against the weather which leads to protected areas with a steady year-round food supply have been shown to select for year-round territoriality in populations of species that do not normally exhibit it. An example was found with the Lesser Sheathbill (*Chionis minor*) where individuals maintained year-round territories when a large intertidal zone lowered the seasonal variability of food resources, allowing for reliable amounts of food throughout the winter; typically this species does not exhibit this behavior (Bried and Joventin, 1998).

Because food resources can be an important variable in the development of territorial patterns, one would predict that among non-tropical species those that are food generalists would be more likely to show year-round territoriality. Among temperate-zone species, those that focus on a wider variety of food resources, might be more readily able to find all needed resources on a single territory even in inclement weather. Here I investigate whether Northern Cardinals (*Cardinalis cardinalis*), a food generalist found in the eastern United States (Halkin and Linville, 1999), shows evidence of year-round territoriality.

Current literature and observations concerning Northern Cardinals are conflicting with respects to territoriality during the non-breeding season. Published reviews state that the territories that are maintained and defended during the breeding season (March to September) collapse during the fall to winter seasons giving way to winter flocks of both
adults and juvenile birds that forage together (Halkin and Linville, 1999). It has been noted that some birds in the lower portion of the United States (Tennessee) remain on their breeding territory for a short while during the winter months but then join incoming flocks later on in the season following them for foraging opportunities (Laskey, 1944). However anecdotal observations of banded individuals and cardinal behavior in Dayton, Ohio (J. M. Jawor and R. Breitwisch, pers. obs.), Bloomington, Indiana (J.M. Jawor, pers. obs.) and Hattiesburg, Mississippi (J.M. Jawor and M.S. DeVries, pers. obs.) has recorded pairs occupying breeding season territories during the winter and responding to simulated territory intrusions (STIs) in November and December (taped calls and placement of taxidermy cardinal; J.M. Jawor, unpubl. data). Cardinals can and do sing during the non-breeding seasons, a behavior that is associated with announcement of territory ownership and defense of territory boundaries. Song production has been recorded as early as February in more northern populations of the cardinal (Jawor and MacDougall-Shackleton 2008) and to occur virtually year-round in more southern populations of cardinals (Hooker 2011). Non-breeding territoriality and defensive behaviors in this species have not yet been stringently assessed in the non-breeding season in any area of the extensive species range, here I will assess defensive behaviors of cardinals in a population in the southeastern United States during the non-breeding season. Previous work concerning cardinal territorial aggression has shown that using STIs will elicit an aggressive behavioral response from cardinal males during the early breeding season (counter singing, dives at speakers and taxidermy models, rapid chipping; DeVries, et al. 2012). My work aims to establish if cardinals are present on the same territories throughout the year and to expand on the work done by DeVries et al.
(2012), to assess if they defend these territories aggressively during the non-breeding season.

Methods

Simulated territory intrusions (STIs) were performed with neighbor and stranger song to assess aggressive behavioral responses from territory owning Northern Cardinals. Simulated territorial intrusions are a conventional method of testing aggression in response to an intruder on the focal bird’s territory and have been used successfully with cardinals in the past (Jawor et al. 2004, DeVries et al. 2012). Playbacks of song were conducted at the Lake Thoreau Environmental and Research Center, Hattiesburg, Mississippi (31° 20' 26.8224" N 89° 25' 11.5500" W). Data was collected from two successive breeding seasons (January to May 2014; January to May 2015) and one non-breeding season (September to December 2014). Territory boundaries of mated pairs are estimated through past breeding records by observations of singing males during the early breeding season of 2014, through observations of territorial behavior in the early breeding season of 2014, and nesting behavior (J.M. Jawor, unpubl. data) in 2013. Cardinals at Lake Thoreau have been observed and recorded for 7 years, allowing for confident identification of territories, of territory owners, and of their neighbors.

Here I describe the acquisition of song to be used in the STIs, but note that behavioral responses to the different songs types are combined in these analyses, variation in response based on type of song is described in Chapter 3. Focal males were exposed to song as a form of STI given that song is an important part of territory defense in this species. Males heard two categories of song; song from known neighbor birds and song from stranger birds. Song from male Northern Cardinals was collected on site from
identified song posting males using a Marantz PMD620 MKII 24-bit Handheld Digital Recorder and a Sennheiser MKH 416 shotgun microphone. Only songs that were clear and loud enough were used for playback. Song recorded on site constituted neighbor song and for a focal bird neighbors are considered to be birds on contiguous territories around the focal male. Male songs from The Cornell Lab of Ornithology’s Macaulay Library were used as stranger songs. These songs were recorded from outside the Lake Thoreau population and are from a number of different years. All stranger songs are recorded from birds in the Southern United States for dialect purposes.

STIs were performed using a portable Altec Lansing Music Speaker and a Sansa® Clip+ MP3 Player which were put in the approximate middle of the focal bird’s territory (as in DeVries et al., 2012; Harris and Lemon, 1974; Hyman, 2002). The same volume level was used throughout all tests to standardize playback (Yoon, 2012). Audacity ® 2.1.1 (Dominic Mazzoni, Creative Commons Attribution License, version 3.0) sound editing software was used to prepare captured song (e.g., remove background noises and amplify sound). Amplification was used to standardize loudness of all song used. During STIs an observer was concealed in camouflage amongst vegetation to record behavioral responses by the focal male territory owner. Two STI sessions were randomly conducted per territory: one using neighbor song and one using the stranger song (as in Yoon, 2012). The sessions were separated by 2 to 5 days to avoid habituation. Length variance between sessions was due to weather or interference on the study site. The initial song used for the first playback session was randomly chosen for each male and its opposite STI type occurring secondarily. Simulated territory intrusions that were interrupted by human activity were not used in analyses (Botero 2006).
To determine the level of response, several factors were recorded, including time to first visual identification of responding male, dives at the speaker, time spent singing in response to the recording, duration of time spent in area. To determine how close males approached the speaker, the ‘intruder’, flagging tape was used to mark 2 meters, 5 meters, and 10 meters away from the speaker (as in Botero 2006). Each STI lasted a total of 20 minutes with 5 minutes allotted for both pre and post intrusion observations and a 10 minute playback of song where behavioral response was recorded.

Statistical analyses included compression of data using a principle components analysis (PCA, see Results for complete description of resulting components) and PCA scores were then analyzed using T-tests as well as Pearson’s Correlation between the components. As individuals experienced both a neighbor and stranger STI in a single season one of the two types was randomly removed for analyses (e.g., for a male tested with both STI types in the winter either the neighbor or the stranger STI was randomly removed). Due to low sample sizes for the winter analyses there was no random removal of birds who experienced both a spring and winter STI. All tests were run using JMP®, Version 11. SAS Institute Inc., Cary, NC, 1989-2007. All work described here was completed under The University of Southern Mississippi’s Institutional Animal Care and Use Committee approval (#10081204), United States Fish and Wildlife Bird Banding Lab authorization (#23479, issued to J. M. Jawor) and Mississippi Administrative Scientific Collecting Permits (#0319131 and 0603142).
Results

Overall, 25 birds were subjected to STIs at some point in the spring or winter. Of those 25, 8 birds were exposed to playback in both the winter and the spring. Of the winter tested birds 87.5% showed some level of response to song playback on the territory (87.5% responded to neighbor song, 62.5% responded to stranger song) while 100% of birds responded to either neighbor or stranger playback during the spring season (92% responded to neighbor song, 80% responded to stranger song). Females were more likely to join the territory holding male in response to STIs during the spring (64% of the time females joined the response for spring, 25% of the time females joined the response for winter) and were more often involved in neighbor STIs than stranger (44% response for neighbor playback, 20% response for stranger playback). In the occurrences of female involvement, female behavior usually consisted of rapid, aggressive chipping though instances of female song were recorded. Reports here focus on the behavioral response of males.

A principle components analysis (PCA) was used to consolidate behavioral variables like time of first visual once playback of song had begun, dives at the speaker during the STI, time spent singing during the playback of song, duration of overall response (time spent in the area doing some sort of behavior) into a more manageable format for analyses. From the PCA, two principle components were relevant, the first of which included the time of first visual of the territory holder during the STI, number of times the owner dived at the speaker during the STI, and how long the territory owner sang in response to playback during the STI. This component, labeled “Responsiveness”, explained 40% of the variation seen in the data. An additional 27% of data variation was
explained by the second principle component, referred to here as “Persistence”. This axis was entirely comprised of the overall time of response of the territory holder regardless of behavioral action. Scores of Responsiveness and Persistence were used in further analyses of behavior in the early breeding and during the non-breeding seasons.

A T-test was performed to determine whether differences in response for each principle component existed across seasons. There was a significant difference between winter and spring for each component with Responsiveness dropping in the winter months (Responsiveness: $t=2.62$, df=60, $p=0.01$, Fig. 1). However, Persistence, or overall duration of response, was elevated during the winter months (Persistence: $t=1.79$, df=60, $p=0.07$, Fig. 1). Due to low sample for the winter season, individuals who were exposed to STIs in the winter and spring seasons (n=8) were not removed for this analysis. While males were willing to respond to intruders throughout the year the level to which they responded and the type of response (persistent attendance verses active movement and song) changed with time. There was no correlation between Responsiveness and Persistence in individuals (Pearson correlation: $\rho =0.036$, $p=0.78$, n=25).

Discussion

In the current literature cardinals are not described as staying on their territories year round (Halkin and Linville 1999) but observations suggest otherwise (J.M. Jawor, R. Breitwisch, M.S. DeVries pers. obs., Ritchison and Omer 1990). In addition to confusion related to whether cardinals are year-round territorial residents, non-breeding season song and anecdotal observations suggest defense of territories in the winter, but territorial defense during winter has never been truly assessed. This investigation shows that
cardinals in the southern region of Mississippi are present on territories year-round and will defend them during the winter, though less aggressively than in the breeding season. Males and pairs were found in the winter on the same territories they had occupied in the previous summer, and they were still found on the same territories in the following breeding season after winter territory assessment (if they were still alive) further supporting year-round territorial ownership (as discussed in Jawor 2007). Though responsiveness to playback stimuli did significantly change from spring to winter with behaviors dropping in intensity during the winter months, males still responded to STIs performed during the non-breeding season. While Responsiveness scores dropped during the non-breeding season, Persistence scores increased during the winter months. Males remained in the area longer in response to song playback than they did in the spring season.

This data is important for two reasons: firstly, it shows that populations of Northern Cardinals are maintaining and defending their territories year round, at least in southern Mississippi, contrary to reports in some literature (Halkin and Linville 1999) and secondly, that these territories are being defended aggressively, even though that aggression is less intense than is displayed in the breeding season. In the breeding season, defense of resources for reproduction is fairly common across species, however, the defense of territories during the winter in a non-migratory species is more unique. During the winter months, flocks of unpaired juveniles begin their search for a breeding territory for the upcoming breeding season and in this species this may be the only territory a male/pair occupies for their entire lives. Faced with large numbers of strange birds who may try to carve out bits of territory for themselves, tenant birds must respond
to intruders and if necessary eject intruding birds from their territories. Because these birds are staying year round on the same territory, the cost of losing has long term effects, particularly on the next breeding season. Loss of a territory to a new bird would force the spurned male to establish a new territory (either finding and defending an unoccupied territory or ejecting an established resident) and they would be in competition with the flocking young birds, and all of this in time for the next breeding season. The importance of retaining an incredibly important resource (for both reproduction and overwinter survival) has led to year-round occupancy and year-round defense of the territory; this defense is likely aided by cardinals being best described as a generalist in both breeding requirements and in foraging habits (Halkin and Linville 1999) thus allowing a single territory to provide all needed resources year-round.

Interestingly, the type of defensive behavior changed as individuals moved from one season to the next. While responsiveness drops in the non-breeding season, persistence increases. Several reasons may exist for this change, and future work may be able to tease these apart. First, population structure changes throughout the year with varying levels of juvenile birds appearing in the population; typically, there are more juveniles in the fall and winter than in the spring (Halkin and Linville 1999). For adult cardinals, even just making an appearance may be enough for a territory owner to scare a younger competitor away during the non-breeding season; age has been shown to be a strong factor in social dominance. Older Carolina Wrens (*Thryothorus ludovicianus*) have been shown to be dominate over new, incoming birds during a similar unpaired flock push in the winter seasons (Hyman 2001). Energetically being able to switch to a much lower intensity defensive strategy (appearance and persistence) would be highly
advantageous for adult cardinals. Additionally, being able to avoid extensive fights and potential injury by a young individual would be beneficial as well; just knowing that a territory is occupied may be the information needed to keep moving on in their search. The more active aggressive responses observed in the spring may be linked to fewer young birds in the population and more birds of similar age and fighting ability (e.g., territory holders from previous seasons) being forced to elevate aggressive actions in order to maintain specific territory boundaries.

Another possibility for changes in aggressive level may be just how valuable the defended resources are throughout the year. Cardinals are generalists in terms of foods consumed by both adults and offspring (Halkin and Linville 1999) and in the winter, the lack of specificity may make strict, highly aggressive territory defense unnecessary. It may still benefit an individual to guard and keep an eye on who might be using their food resources, but the general nature of what is consumed may not make that defense important and urgent in nature. In the spring, more actively and aggressively maintaining strict boundaries on territories for the retention of nest sites and guarding mates may select for increased aggressive behavior. Cardinals typically nest in edge habitat and suffer from high nest predation rates, in some cases a single pair can produce up to 10 nests in a single season and nest sites are not reused within a single season (Jawor 2002). Additionally, males may be guarding their mates against potential extra pair copulations which have been recorded in cardinals (Linville 1998). Together these changes in the importance of what is being defended may be a source of the variation in aggressive behavior shown and deserves further attention.
One last influence on changes in behavior may be changes in testosterone influencing behavioral responses to territorial intruders. While most bird species experience a drop in circulating testosterone during the winter months, prior work has shown that cardinals maintain measurable levels of testosterone year round (Jawor 2007; DeVries et al. 2011). Ample research has been completed that links testosterone with aggressive behaviors in a large number of species (Goymann et al. 2007). Classically testosterone is thought to increase significantly during territory challenges and defense (the ‘challenge hypothesis’), and the classic drop observed in territorial behavior with the onset of winter is linked to seasonal decreases in circulating testosterone levels (Wingfield, et al. 1991). For cardinals the continually circulating testosterone that has been observed may help facilitate the general aggressive winter response observed here (e.g., influence the willingness to show up and to produce song). However GnRH challenges have revealed that the ability of cardinals to elevate their testosterone above general baselines during the winter is limited (DeVries et al., 2011; Hooker 2011) which may help explain the qualitative switch of aggressive actions between seasons. Strong spring responsiveness is expected in territorial birds. During the breeding season, intruders not only represent a threat to resources such as nesting habitat and food, but they may also be a mating threat, particularly in birds that have been shown to participate in extra pair copulations. Minor elevations in baseline testosterone, or the increased ability to elevate testosterone above baseline, may facilitate the change in observed levels of aggressive behavior in cardinals (Jawor 2007; DeVries et al. 2011).

It is important to note that previous literature describing Northern Cardinals’ territoriality were from populations in the northern United States where seasonal climatic
variability is much higher than it is in the southern portion of the country. Species with substantial breeding ranges like the European Stonechat (*Saxicola torquata*) have been shown to have population differences in migration behaviors, reproduction, and molt (Helm 2009). Southern populations of the stonechat are non-migratory and defend the same territory for the entire year while populations in the northeast where the winters are harsher are recorded as short-distance migrants, relocating to southern Europe and North Africa for the winter (Urquhart, E. and Bowley, A. 2000). It is possible that the behavior displayed in this particular population may not appear in more northerly populations of the same species, or to a much reduced level. This flexibility may be aided by the lack of distinct seasonality in the more southern United States. The humid subtropical climate of southern Mississippi may provide a more substantial food resource, making the formation of flocking winter groups unnecessary for these populations. Specific assessment of non-breeding territoriality is needed in more northern populations of the Northern Cardinal before this pattern can be firmly defined, but the observed year-round occupancy noted in more northern populations suggests a high likelihood that territories will be defended year round in these populations as well and may be a hallmark of this species.

In summary, this work shows that in the southeastern United States cardinals show year-round territory occupancy and more importantly the tendency to defend that territory during the entire year. The level of aggressive behavior appears to change between breeding and non-breeding seasons and could be linked to changes in testosterone, changes in population structure (to be discussed in Chapter 3), or even changes in resource value (defense of fertile females and nesting sites versus defense of roost sites and non-breeding season foods). Future work in more populations of the
Northern Cardinal will allow the determination of year-round territoriality is a true hallmark of this species and assessment of what resources are most important to cardinals in each season may help determine why change in defensive strategies exists.

Figure 1. Responsiveness and Persistence shown during the spring and winter months. Data presented as mean PCA scores. Responsiveness and Persistence are demonstrated year-round, the frequency with which these behaviors are shown changes with time.

Figure 2. Scatterplot Spearman’s correlation between PC1 (Responsiveness) and PC2 (Persistence). Behaviors are not correlated within individuals.
CHAPTER III

TERRITORY DEFENSE STRATEGIES DISPLAYED BY NORTHERN CARDINALS

(CARDINALIS CARDINALIS): DOES SWITCHING OCCUR AND WHY?

Nasty Neighbor and Dear Enemy

Territoriality comes with a host of its own challenges, costs, and benefits for a territory owner. For a bird to hold a territory, it must, by definition, exclude other conspecifics from utilizing any resources within the boundaries of that territory. By doing so, the territory owner insures predictable and, in some cases, premium resources for preferential use by the defending individual and/or its mate and offspring. Intruders will seek to usurp defended resources by either annexing resources into an existing territory (intrusion by a neighbor), potentially etching or carving out a new territory (intrusion by a stranger or floater individual) or even eject an established territory owner and take over the now vacant territory (potentially a neighbor or a stranger individual). Intruders must be dealt with by assessing the threat value of both (either a neighbor or stranger) and making an energetically responsible choice as to the level of effort and aggressive behavior to be displayed. When territory neighbors, or birds whose territories are contiguous and share a boundary, act less aggressively towards one another in the event of intrusion this is described as the ‘Dear Enemy’ effect. However, the case may arise when neighbors are gauged as more of a threat to resources and territory ownership, therefore eliciting a stronger aggressive response than a neighbor; described as a ‘Nasty Neighbor’ effect. Research has investigated both of these defensive strategies and there is interesting support for both as a part of territorial behavior.
The ‘Dear Enemy’ effect (or dear enemy hypothesis) is well documented not only in birds such as the European Robin (Erithacus rubecula, Brindly 1991), Eurasian Skylark (Alauda arvensis, Briefer, 2008), and Ovenbird (Seiurus aurocapillus, Weeden and Falls 1959), but also in other species like the Ring-tailed Lemur (Lemur catta), American Pika (Ochotona princeps), and Carolina Anole (Anolis carolinensis) (reviewed in Temeles 1994). When operating under the dear enemy hypothesis individuals will be overall less aggressive towards known neighbors than to complete strangers. Aggression may be shown to both neighbors and strangers, but it is significantly less intense aggression than is directed towards known neighbors. Under the dear enemy hypothesis, competitive abilities of neighbors are known and have been behaviorally tested and a stable association has likely been formed; intruders who are unknown are a greater threat to all individuals in a population and territory holders may work in concert to induce strangers to leave the general area. Lessened aggression towards neighbors allows the participants to focus their time and energy in other endeavors rather than constantly defending a boundary from an ‘enemy’ who is always there (e.g., always on the territory next door). This lessened aggression is conditional upon both parties adhering to the mutual non-aggression pact which would break down if pushed by constant aggression (Godard 1993). Eurasian Skylarks, tested with known neighbor song that shared song syllables and patterns with the focal bird, were shown to respond with less behavioral aggression to known neighbor song than to stranger song (e.g., songs that contained very few shared elements; Briefer et al. 2008). Findings supporting the dear enemy hypothesis indicate that focal individuals can recognize others known or not known and are able to
determine what level of threat they represent and act accordingly; here, the unknown individual is the bigger threat and must be responded to more aggressively.

Conversely, the ‘Nasty Neighbor’ effect (or nasty neighbor hypothesis) is a situation where known neighbors comprise a higher level of threat than a total stranger (Muller and Manser 2007). Again, all intruders may be responded to with aggression, but the intensity and even length of aggressive response is stronger to one type and now that is a known individual. One suggestion for why neighbors might be a larger threat is that by always being close by neighbors have intimate knowledge of a territory holders resources and the territory holder’s ability to defend said resources. Neighbors are also present all the time and may have the ability to determine when a territory holder is occupied with offspring, dealing with threats to their territory ownership by others, or if the territory owner is not at peak physical form and might be bested during a confrontation. Overall, the nasty neighbor hypothesis has been less studied, but it has been shown to occur situationally in Orange-crowned Warblers (*Vermivora celata*) among whom high population densities along with lower resource availability and smaller territories lead to neighbors constantly challenging boarders (attempts to gain more nesting resources), breaking the truce needed for the dear enemy strategy to be cost effective (Yoon 2012). An interesting set of questions do arise, why do some species show stronger defensive behaviors towards strangers than to neighbors (or the opposite pattern in the nasty neighbor hypothesis) and can individuals be situational and flexible in showing these behaviors.

As mentioned before Orange-crowned Warblers exhibit nasty neighbor behavioral tendencies when the environment calls for such (e.g., high population density), however
other populations of this warbler were shown to utilize a dear enemy stratagem when population density was lower. This shows that all individuals of a given species do not have to be set with respects to what type of territorial strategy they pursue. In this case, this situational switching is happening between populations, but perhaps this level of flexibility can be shown within a population when environmental circumstances change.

A study of the territorial strategies of Carolina Wrens (*Thryothorus ludovicianus*) showed that while they adhered to the dear enemy strategy during the spring (neighbors responded to less aggressively), aggression towards stranger birds dropped in the winter months causing a lapse in the dear enemy effect (Hyman 2005). This study also showed a trend of greater behavioral response to neighbor song playback than to stranger song playback during the fall, though the difference was not significant. Carolina Wrens are year-round territory holders who experience an influx of strange floater birds during the fall seasons. Could situational behavior change in terms of the type of territory defense displayed be a hallmark of species that occupy and defend territories year-round? This is the main objective to be investigated here.

In the previous chapter, I showed that Northern Cardinals do occupy the same territory year-round and they display year-round aggression in response to simulated territory intrusions (STIs) performed during the early spring and fall/winter seasons. Annually territory holding cardinals also find themselves amid an influx of first-year birds trying to establish their own territories during the fall (Halkin and Linville 1999), much like the Carolina Wren. Because cardinals hold the same territory for most if not all of their lives (Halkin and Linville 1999; Jawor 2002) they typically have the same neighbors for their entire lives as well; each spring after the flocks of juveniles are mostly
gone from the population, territory holders are faced with the reaffirmation of territory boundaries with neighbors and defense of resources from neighbors. This change in population structure or dynamics suggests that different territorial strategies could be employed by cardinals throughout the year; that a benefit might exist if individuals can tailor their behavioral responses to the most pertinent threats each season. Territorial strategies have never been assessed in the cardinal, for either the spring or winter seasons. This study’s aim was to uncover what, if any, strategy was being used and if the birds showed flexibility in tactics in the face of the changing population from spring to fall.

Methods

Simulated territory intrusion (STI) playbacks were conducted at the Lake Thoreau Environmental and Research Center, Hattiesburg, Mississippi (31° 20' 26.8224" N 89° 25' 11.5500" W) to assess the aggressive response of territory owners. Playbacks took place during two successive breeding seasons (January to May 2014; January to May 2015) and one non-breeding season (September to December 2014). Territory boundaries of mated pairs are estimated through past breeding records (this population has been monitored for the past 7 years), by observations of singing males during the early breeding season of 2014, through observations of territorial behavior in the early breeding season of 2014, and nesting behavior (J.M. Jawor, unpubl. data) in 2013.

Focal males were exposed to song as a form of STI given that song is an important part of territory defense in this species (Halkin and Linville 1999; Yamaguchi 1998). Males heard two categories of song; song from neighbor birds recorded on site and song from stranger birds. Neighbor song was collected from song posting male Northern Cardinals using a Marantz PMD620 MKII 24-bit Handheld Digital Recorder
and a Sennheiser MKH 416 shotgun microphone. Only songs that were clear and loud enough without substantial digital manipulation were used for playback. Song recorded on site constituted known neighbor song and for a focal bird neighbors are considered to be birds on contiguous territories around the focal male. Male songs from The Cornell Lab of Ornithology’s Macaulay Library were used as stranger songs. These songs were recorded from outside the Lake Thoreau population and are from a number of different years. All stranger songs are recorded from birds in the Southern United States for dialect purposes (Marler and Tamura 1962; Baptista 1975; Emlen 1971).

To perform the STIs, portable Altec Lansing Music Speakers and a Sansa® Clip+ MP3 Player used for playback were put in the approximate middle of the focal bird’s territory (DeVries et al. 2012). The same volume level was used throughout all tests to standardize playback (Yoon 2012). Audacity ® 2.1.1 (Dominic Mazzoni, Creative Commons Attribution License, version 3.0) sound editing software was used to prepare captured song (e.g., remove background noises and amplify sound). Amplification was used to standardize loudness of all song used. During STIs, an observer was concealed in camouflage amongst vegetation to record behavioral responses of the focal male territory owner. Two STI sessions were randomly conducted per territory: one using neighbor song and the other using the stranger song (as in Yoon 2012). The sessions were separated by 2 to 5 days with the initial song session being randomly chosen for each male and its opposite STI type occurring secondarily. Simulated territory intrusions that were interrupted by human activity were not used in analyses (Botero 2006).

To determine the level of response, several factors were recorded, including time to first visual identification of responding male, dives at the speaker, time spent singing
in response to the recording, and duration of time spent in area. To determine how close males approached the speaker, the ‘intruder,’ flagging tape was used to mark 2 meters, 5 meters, and 10 meters away from the speaker (Botero 2006). Each STI lasted a total of 20 minutes with 5 minutes allotted for both pre and post intrusion observations and a 10 minute playback of song where behavioral response was recorded.

Statistical analyses included compression of data using a principle components analysis (PCA, see Results for complete description of resulting components) and PCA scores were then analyzed using One-Way ANOVAs as well as T-tests between individual seasons and STI types. All tests were run using JMP®, Version 11. SAS Institute Inc., Cary, NC, 1989-2007.

Results

All birds received at least 2 STI treatments, a neighbor treatment and a stranger treatment (n=25). A total of 8 birds received treatments in both the winter and the spring. During the spring season, 84% of birds exposed to stranger showed behavioral response of some kind while 100% of birds responded to neighbor song. During the winter months, 63% of birds tested responded to stranger STIs while 88% responded to neighbor playback.

A principle components analysis (PCA) was used to consolidate behavioral variables like time of first visual once playback of song had begun, dives at the speaker during the STI, time spent singing during the playback of song, and duration of overall response (time spent in the area doing some sort of behavior) into a more manageable format for analyses. From the PCA, two principle components were relevant, the first of which included the time of first visual of the territory holder during the STI, number of
times the owner dived at the speaker during the STI, and how long the territory owner sang in response to playback during the STI. This component, labeled “Responsiveness”, explained 40% of the variation seen in the data. An additional 27% of data variation was explained by the second principle component, referred to here as “Persistence”. The second principle component was entirely comprised of the overall time of response of the territory holder regardless of behavioral action.

For each principal component, a One-Way ANOVA was used to analyze differences in season as well as STI type (which song was being used). Further T-tests were used to explore each combination from the total winter and spring data sets (neighbor/stranger song response from the winter was tested against neighbor/stranger song response from the spring for each principle component). Comparing the response of playback from season as well as STI type showed significant difference for PC1 (Responsiveness) though not for PC2 (Persistence) (PC1 ANOVA: Season= F(1)=5.72, p=0.0204; STI type= F(1)=24.10, p=<.0001; PC2 ANOVA: Season= F(1,62)=1.62, p=0.208; STI type= F(1,62)=1.49, p=0.231). These results are shown in Figures 2 and 3. T-tests comparing the differences between responses to neighbor song from winter to spring showed a significant difference with the stronger response shown in the spring (neighbor winter/spring, t=2.56, df=29, p=0.016). There was no significant difference between the responsiveness to stranger song (stranger winter/spring, t=1.82, df=29, p=0.07). T-tests were also used to look at the seasonal differences in PC2 (Persistence). There was no significant difference for neighbor song in persistence between seasons (neighbor winter/spring, t=0.507, df=29, p=0.61); However, there was a significant
difference between seasonal persistence in response to stranger song (stranger winter/spring, t=1.82, df=29, p=0.02).

Discussion

Findings here suggest that cardinals do change in the level of aggression they show throughout the year as they defend territories and that there is some alteration in response to the type of territory threat being responded to. The results indicate that in the spring seasons, cardinals show a neighbor bias for aggressive response as compared to how they responded to stranger STIs (i.e., fall under the nasty neighbor hypothesis). Latency to respond was lower, birds sang more, and they came closer to the speaker when neighbor song was used for playback (all accounted for in PC1). This may be due to the significant reduction of stranger birds in the area during the breeding season when territories are solidified and most juveniles have already moved through the area and have found a breeding territory elsewhere. Exact locations of territory boundaries may need to be redefined each spring even if overall area of the territory and general location does not; this will comprise some confrontation with known neighbors. In the winter, territory holders responded aggressively to intruders, but while response to known neighbors decreased, response to strangers increased and the preferred aggressive behavior shown was persistence (remaining in the area regardless of what activity was shown). While not cleanly falling under either the nasty neighbor or dear enemy hypotheses, winter aggression did lean more heavily towards stronger responses directed at strangers. Juvenile birds are more prevalent in the population in the fall and winter, and just by appearing an adult territory owner may be able to indicate that the territory is occupied and dominate the juvenile intruder through age alone (as in Chaves-Campos
However other species, particularly the Carolina Wren, also face a breeding season with low stranger bird activity and presence. Surprisingly, wrens demonstrate a dear enemy strategy in the spring, even in the face of low stranger numbers in the spring and gravitate towards seeing neighbors as a stronger threat in the non-breeding season (Hyman 2005). In this case, it is plausible that the ‘dear enemy’ effect is in place to cease all aggressive actions during breeding, not to redirect aggression to a bigger threat (i.e. stranger birds). But why then, do we not see the same in this population of Northern Cardinals? Mating concerns may have a part to play here. Cardinals have multiple extra pair copulations throughout the season and usually with neighboring birds (Linville 1998) while Carolina Wrens do not partake in extra pair copulations throughout the breeding season (Haggerty 2001).

In this work cardinals seem to be displaying flexible seasonal defense strategies. During the winter months, stranger importance seems to be increased while neighbor importance lowers. Ultimately this brought the response level of both types of STIs to nearly even levels. This could be explained by the floater populations moving through the area during the non-breeding season in search of their own territories. Carolina Wrens were also shown to change the level of importance of intruders on their territories during the winter; however, while there was a change in aggression towards strangers, there was actually a decrease in aggression rather than the increase we see here. Cardinals’ increased aggression towards a neighbor intruder during the breeding season and their less aggressive response to neighbors in the winter are contrary to what Hyman (2001) noted. In his study, Carolina Wrens responded less aggressively to neighboring birds during the spring (the dear enemy effect) and while there was a shift in aggression
(lessened aggression towards strangers in the winter) the level of aggression in the winter towards neighbors did not change. That we see an increased importance of response to stranger song in Northern Cardinals may be a consequence of using an alternate strategy where the aggressive parties are a constant presence, like a neighbor, rather than a fleeting irritant.

There are also winter/spring aggressive behavioral changes to be noted. While birds are more “Responsive” towards neighbors they are more “Persistent” towards strangers. This may be a side effect of familiarity with neighboring birds and their resource holding potential and a lack of information on competitive abilities for both the intruder and defender during stranger intrusions. Prior aggressive interactions between neighbors may set the stage for immediate action when the neighbor is caught intruding and repeated interactions may force neighbors to continually increase the level of aggressive response. Conversely, stranger birds are a veritable black box with unknown potential; defenders do not know the level of aggression or strength the intruder might show and the intruders do not know the value of the resource under contest or the strength or behaviors of the defender. Persistence, or simply being in the area and responding in some way, may err on the side of caution for the defender and allow first impressions to be made prior to behavioral actions being initiated. Alternatively, many of the fall intruders are juveniles who are frequently subordinate to adults, behavioral outcomes might be different if the intruding winter-time individual is an older adult.

While the song used here for stranger song was from unknown aged individuals, it is not clear that song quality changes with age in cardinals (J.M. Jawor, pers. obs.) so song
itself might not be enough of a cue regarding age. In the winter, coming in to observe the individual intruding may be necessary prior to any behavioral response.

In conclusion, during the spring breeding season, Northern Cardinals seem to be exhibiting the nasty neighbor effect rather than the dear enemy phenomena. This could be because of prolonged aggressive interactions between neighboring birds as well as the rarity of stranger birds in the population during the breeding season. Birds tested had an overall greater ‘responsiveness’ to neighbor song, meaning they sang more, came closer to the speaker, preformed more dives, and had a shorter latency to respond than when exposed to stranger song. However, when the population structure changes in the winter months and strange, floater birds move through the area, we see Northern Cardinals changing their responses to stranger playback by being more ‘persistent’ in their behavioral response to the stranger who is intruding. Clearly cardinals show the ability to display dynamic behavior and tailor their response to the type of intruder they are faced with. Variation in aggressive behavior may be selectively advantageous as it may guard against injury and elevated energy expenditure in the winter (e.g., most intruders are young and easily dominated by the presence of an older individual) while ensuring defense against reproductive losses in the spring (e.g., intruding neighbors may copulate with a territory holders mate and require a more intense level of aggression in order to be removed). Additional work with other year-round territory holding species is needed to determine if flexibility in strategy is a common occurrence in these types of species and whether there is congruence in how patterns of aggression are displayed.
Figure 3. Responsiveness (e.g., time to arrival, number of dives at ‘intruder’, etc.) to STIs (stranger or neighbor) over the winter and spring seasons.

Figure 4. Persistence (e.g., time spent in area of intruder regardless of behavior) duration in response to STIs (neighbors or strangers) over winter and spring seasons.
APPENDIX A

CIRCULATING TESTOSTERONE IN RESPONSE TO NEIGHBOR STIS

Testosterone its links to aggression

There are many studies concerning testosterone and its effects on aggression in a variety of vertebrate species. In songbirds, testosterone has been shown to activate aggression (Balthazart 1983; Harding 1983) as well as play a role in the acquisition of a mate, mate guarding, and territory establishment (Wingfield et al. 1987; Wingfield et al. 1990). Typically, testosterone-linked aggression studies have focused on males of a species, however there is some evidence that testosterone levels may play a part in female aggression as well (Sandell 2007). Here, work will focus on males in a species showing prolonged territoriality; however, in the study, species (described below) females have measurable levels of testosterone that could play an impact on their behavior as well (Jawor 2007).

Constantly high levels of testosterone, although impactful to behavior, are suggested to be physiologically detrimental and this has shaped the annual expression of testosterone and many of its dependent/influenced behaviors (Flostad and Karter 1992, Wingfield et al. 1997; but see Peters 2000). In the majority of temperate zone species testosterone fluctuates annually with high levels observed at the beginning of the breeding season, lower levels during offspring care (in biparental species, this differs in polygynous species) and unmeasurable levels in the non-breeding season (Wingfield and Silverin, 2002; Wingfield et al. 1990; Lynn et al. 2002). This annual variation in testosterone can be matched nicely to annual changes in testosterone; during times of high testosterone individuals are defining and defending territories and acquiring mates,
when testosterone is low typically no territorial or mating behaviors are observed (Wingfield et al. 1999).

This hormone-behavior pattern differs in most tropical species where continually measurable but low testosterone levels are seen year round (Levin and Wingfield 1992; Goymann et al. 2004). In many cases, year-round aggression or territoriality is observed as well (Stuchbury 2001) To mitigate the potential physiological costs of constantly circulating testosterone, tropical birds who exhibit year round territorial aggression and defense generally have lower circulating testosterone than temperate birds whose testosterone peaks during the breeding season; perhaps levels that keep them below some damaging hormone threshold (Bicudo 2010; Levin and Wingfield 1992; Hau et al. 2000; Goymann et al. 2004). Interestingly, tropical zone birds that breed at high altitudes and experience much more pronounced seasonality in weather (patterns similar to temperate zone species) also exhibit behavior and annual testosterone profiles similar to temperate zone birds (Moore et al. 2002), suggesting a strong impact of environment on the selective forces of hormone secretion and behavioral expression. Maintaining a behavioral and physiological response to low testosterone may be accomplished by tropical birds being more sensitive to circulating testosterone than birds found in the temperate zones (Canoine et al. 2006; Hau et al. 2000) although comparisons of receptor densities and sensitivities across geographic areas has not been done.

Aggression and establishment of a territory puts the territory holder in direct competition with other males of the same species and winning interactions over territory ownership and area can be quite important. Because aggression can be activated by testosterone, it is reasonable that competition for territory (area, boundaries) between
males can lead to an increase in testosterone to facilitate a more aggressive response. Wingfield’s Challenge Hypothesis (1990) states that when a male songbird is challenged by a male conspecific during the breeding season that circulating testosterone increases, leading to an increase in, and prolonging of, aggression. While many species have shown to follow this motif, others do not respond to simulated territory intrusions with higher testosterone (Wingfield et al. 1992; Hau et al. 2004; Moore et al. 2004). Most likely testosterone is influential to aggression in the greater majority of species, how it facilitates that aggressive response may be quite different across species; some species may experience an obvious increase in circulating testosterone while others experience a less obvious increase in testosterone but perhaps a change in receptor action.

**Endocrine Profile of the Northern Cardinal**

Northern Cardinals (*Cardinalis cardinalis*) are a temperate zone, non-migratory, year-round territorial bird (Halkin and Linville 1999; Jawor 2007). Typically, temperate zone birds have high testosterone in the breeding seasons and low, if not immeasurable testosterone during the winter months. In contrast, tropical species show year round territoriality and have low levels of testosterone throughout the year. Cardinals, a species in the temperate zone, have year round measurable testosterone in both males and females (Jawor 2007). Female cardinals have relatively stable, high levels (or high for a female bird, see Ketterson et al. 2005) of circulating testosterone compared to other female temperate birds. Male cardinals’ levels vary minimally throughout the year, but remain measurably high during the winter months (Jawor, 2007; DeVries, et al. 2011; Duckworth, 2015; see Fig. 5). Previous work by DeVries et al. (2011, 2013) and DeVries and Jawor (2014) has shown through gonadotropin releasing hormone (GnRH)
challenges (GnRH will ultimately induce the production of testosterone if the body is able to produce it) that while cardinals are capable of raising their testosterone levels at any time of the year, simulated territory intrusions typically do not elicit a measurable changes (DeVries, et al. 2012). There is a caveat to the findings of DeVries et al. (2012) and Jawor (2007), occasionally Northern Cardinal males are captured who do have greatly elevated testosterone during aggressive interactions and they are able to elevate testosterone following (via GnRH stimulation) following aggressive behavior. This suggests that cardinals can and do have testosterone elevations but the exact behavioral stimuli needed to facilitate this is not known.

In the previous chapter, I outlined a marked behavioral difference in type of aggression shown by cardinals in the breeding season when responding to a neighbor or stranger simulated territory intrusion (STI) playback with birds responding more aggressively to neighbor song than to stranger (e.g., a more active response including dives at the ‘intruder’). Previous STIs were performed using stranger song (DeVries, et al 2012) and while individual cardinals responded aggressively they did not experience an increase in testosterone, but perhaps they were not experiencing the correct stimulus. The work I outline here addresses the possibility that the behavioral differences seen in response to neighbor song or stranger
might be related to testosterone levels, that responding behaviorally to a neighbor requires more behavioral aggression which is supported by increases in testosterone. In this work I utilize neighbor song playback for STI capture, a technique not used with cardinals in the past.

**Methods**

Simulated territory intrusions (STIs) were performed with neighbor song on territories of known birds. Simulated territorial intrusions are a conventional method of testing aggression in response to an intruder on the focal bird’s territory and have been used successfully with cardinals in the past (Jawor et al. 2004, DeVries et al. 2012). Playbacks of song were conducted at the Lake Thoreau Environmental and Research
Center, Hattiesburg, Mississippi (31° 20' 26.8224" N 89° 25' 11.5500" W). Data was collected from May to June 2015. Territory boundaries of mated pairs are estimated through past breeding records, by observations of singing males during the early breeding season of 2014, through observations of territorial behavior in the early breeding season of 2014, and nesting behavior (J.M. Jawor, unpubl. data) in 2013. Cardinals at Lake Thoreau have been observed and recorded for 7 years, allowing for confident identification of territories, of territory owners, and of their neighbors. All work was completed under The University of Southern Mississippi’s Institutional Animal Care and Use Committee approval (#10081204), United States Fish and Wildlife Bird Banding Lab authorization (#23479, issued to J. M. Jawor) and Mississippi Administrative Scientific Collecting Permits (#0319131 and 0603142).

Song from male Northern Cardinals was collected on site from identified song posting males using a Marantz PMD620 MKII 24-bit Handheld Digital Recorder and a Sennheiser MKH 416 shotgun microphone. Only songs that were clear and loud enough were used for playback. Song recorded on site constituted neighbor song, and for a focal bird, neighbors are considered to be birds on contiguous territories around the focal male. STIs were performed using a portable Altec Lansing Music Speaker and a Sansa® Clip+ MP3 Player which were put in the approximate middle of the focal bird’s territory (as in DeVries et al., 2012; Harris and Lemon, 1974; Hyman, 2002). The same volume level was used throughout all tests to standardize playback (Yoon, 2012). Audacity ® 2.1.1 (Dominic Mazzoni, Creative Commons Attribution License, version 3.0) sound editing software was used to prepare captured song (e.g., remove background noises and amplify sound). Amplification was used to standardize loudness of all song used. During STIs an
observer was concealed in camouflage amongst vegetation. A mist-net was set up before the STI began with a decoy male cardinal placed at the approximate center of the net and the decoy was covered with vegetation (to avoid confusion between a visual and sound cue not matching). The STI was run until the male was captured or a mark of 20 minutes had passed. Birds who were captured during the STI were removed from the net and blood samples were collected within 3 minutes of capture.

Plasma was analyzed using ELISA immunoassay kits (Enzo Life Sciences, Ann Arbor, Michigan, USA, #ADI-900-065) using techniques previously described for cardinals (Jawor, 2007; DeVries et al., 2011). Adding approximately 2000 cpm of H3-T (PerkinElmer) to each sample (30 μl of plasma) allowed for the calculation of recoveries after triple extraction with diethyl ether. Extracts were re-suspended with 50 μl of ethanol and diluted to 350 μl with assay buffer from the EIA kit. 100 μl from each reconstituted sample was used to determine recoveries. Levels of T were calculated using a 4-parameter logistic curve-fitting program (Microplate Manager; Bio-Rad Laboratories, Inc.) and corrected for incomplete recoveries. Plasma samples were analyzed on the same plate. Location of all samples on the plate were randomly assigned. Standard samples of known T concentration were also placed in three random locations within each plate for calculation of intra-assay and inter-assay variation. Intra-assay variation for T analyses was less than 10%. Captures of birds were only attempted during known neighbor STIs (n=10) but to compare testosterone levels plasma remaining from other researchers in the lab were used to provide data from stranger STIs and non-aggressive situations. Plasma samples from B. Matthew Duckworth (Duckworth 2015) were used as non-aggressive samples as these were collected while birds were feeding and not interacting with any
other cardinal. Plasma samples from M. Susan DeVries (DeVries 2012) were used for the stranger STI response (all birds lured in with outside population song in this work). Neighbor STI, stranger STI, and non-aggressive samples all came from the same time span (March-April) and all samples from MSD and BMD were reanalyzed in the same kit as the neighbor STI samples collected here. All tests were run using JMP®, Version 11. SAS Institute Inc., Cary, NC, 1989-2007.

Results

Plasma samples obtained during neighbor STIs were compared to plasma samples from previous performed stranger STIs as well as non-aggressive samples collected from birds using a joint-use feeder. A one-way ANOVA showed that there was no significant difference between STIs conducted with either song type (known neighbor versus outside population stranger) and the non-aggressive testosterone levels (F(2)=0.66, p=0.52).

![Testosterone Levels](image)

*Figure 6. Circulating testosterone for STI captures using neighbor and stranger playback.*
**Discussion**

Here preliminary results with a small sample of birds captured during STIs using known neighbor song did not show significant difference from those individuals captured using stranger song STIs or non-aggressive contexts. Neighbor STI response was fast with time until capture ranging from 1-20 minutes (mean = 8); DeVries et al. (2012) reports a similar span of times ranging from 1-26 minutes (mean=11). Type of behavior shown was not quantified either here or in DeVries et al. (2012) and it remains an open question whether level of aggressive behavior varies with testosterone during either STI type. Levels of testosterone here for known neighbor STIs were slightly higher, but not significantly so compared to both non-aggressive and stranger STI levels. But Northern Cardinals can have very elevated levels of testosterone during aggressive responses (Jawor 2007) so it is unclear what behavioral or environmental cue initiates this physiological change within cardinals who are being aggressive.

Tentatively, these results seem to suggest that testosterone levels do not change from neighbor to stranger STIs though behavior does. Many things can be at work here. It has been shown that baselines of circulating testosterone do not always correlate with expression of aggression (Wingfield and Soma, 2002); however, previous work shows that cardinals can indeed raise their testosterone levels in response to GnRH challenges following aggressive behavior (DeVries, et al. 2011, 2012; DeVries and Jawor 2013). Testosterone may still play a part in the aggressive behaviors shown by cardinals in the spring, but the control may be at the level of the target tissue via receptor loads on brain tissues or increased receptor affinity that would allow for relatively stable testosterone during an encounter but have behavioral consequences. Genetic investigations of the
Dark-eyed Junco (*Junco hyemalis*) revealed differential gene expression in the medial amygdala and hypothalamus between subjects treated with testosterone and the control individuals (no testosterone in implants, Peterson et al. 2013). Furthermore, elevated levels of mRNA that code for androgen and estrogen receptors as well as enzyme aromatase were noted in key brain areas during the non-breeding season in Spotted Antbirds (*Hylophylax n. naevioides*) which may allow these birds to respond to low levels of androgen in circulation (Canoine et al. 2007). However it should be noted that while Spotted Antbirds have low levels of T during the non-breeding season, cardinals maintain fairly high levels throughout the winter. Other hormones, such as estrogen and dehydroepiandrosterone (DHEA), have been investigated for their role in winter aggression in some species due to lack of appreciable levels of available testosterone in temperate winter territorial birds (Soma et al. 2000; Soma and Wingfield, 2001; Hau et al. 2004). Soma et al. (2000) investigated DHEA in wintering Song Sparrows (*Melospiza melodia*) and its potential links with aggression in a species that displays high levels of non-breeding season aggression yet unmeasurable levels of testosterone at this time. DHEA circulates all year round in Song Sparrows and in all species it can be converted to other steroids enzymatically. In particular, it is the conversion of DHEA in the brain to testosterone and then estrogen that was impactful to aggression in Song Sparrows and may be a potential pathway in which hormone dependent behaviors can be shown when levels of their normal hormone are low or absent. Target tissues in the brain may be responding to short term stimuli by either making localized testosterone for either use or conversion, or tissues may be converting existing circulating levels of testosterone into
estrogen. Further work concerning the brain tissues during perceived challenges must be pursued in cardinals and in other year-round territorial species.

The suggestion that more prolonged aggressive interactions with conspecific males may lead to an overall increase of testosterone has been made in the literature (Wingfield, ). Perhaps short term STIs, as used in this study, simply did not create the length of challenge needed for this species to raise current levels. Anecdotally, Jawor (2007) does mention in findings of males with higher testosterone in the early breeding season that these individuals were captured during prolonged fights. Additionally, observations described in Halkin and Linville (1999) describe territorial disputes going on for hours and even over multiple days in cardinals. Further work investigating the length of challenges using prolonged STIs and further considering known neighbor and stranger intruders should be considered.
APPENDIX B

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE ACTION

The proposal noted below was reviewed and approved by The University of Southern Mississippi Institutional Animal Care and Use Committee (IACUC) in accordance with regulations by the United States Department of Agriculture and the Public Health Service Office of Laboratory Animal Welfare. The project expiration date is noted below. If for some reason the project is not completed by the end of the three year approval period, your protocol must be reactivated (a new protocol must be submitted and approved) before further work involving the use of animals can be done.

Any significant changes (see attached) should be brought to the attention of the committee at the earliest possible time. If you should have any questions, please contact me.

PROTOCOL NUMBER: 10081204
PROJECT TITLE: GnRH challenges in northern cardinals
PROPOSED PROJECT DATES: 9/2014 - 9/2017
RENEWAL: No
PRINCIPAL INVESTIGATOR(S): Jodie Jawor
DEPARTMENT: Biological Sciences
FUNDING AGENCY/SPONSOR: NA
IACUC/COMMITTEE ACTION: Full Committee Approval
PROTOCOL EXPIRATION DATE: September 30, 2017

Frank Moore, Ph.D.
IACUC Chair
APPENDIX C

BIRD BANDING PERMITS

United States Department of the Interior
U.S. GEOLOGICAL SURVEY
PATUXENT WILDLIFE RESEARCH CENTER
BIRD BANDING LABORATORY
12100 BEECH FOREST ROAD STE-4037
LAUREL, MD 20708-4037
301-497-5790

FEDERAL BIRD BANDING PERMIT

Permittee: Personal
DR. JODIE M. JAWOR
DEPT. OF BIO SCI. UNIV. S MS
118 COLLEGE DR. #5018
HATTIESBURG, MS 39406-0001

Permit Number: 23479
Action: Renew
Action Date: 04/09/13
Issue Date: 03/2007
Valid Until: 05/31/16

Signature of Issuing Official,
Chief, Bird Banding Laboratory

Signature of Permittee

Permittee agrees to band in accordance with the general conditions of this permit and with the specific authorization/s listed below:

Permittee is Authorized To Band:

EASTERN BLUEBIRD
NORTHERN CARDINAL
SLATE-COLORED JUNCO

In the States of:

MS *

With Special Authorization to:

Band
Take, possess and transport blood samples-not to exceed 1% body mass
Trap at Cavity
Use Mist nets
Use Walk-in traps
Trap

And Additionally Authorized to Use The Following
Auxiliary Marking Authorizations:

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The following Subpermittee/s are authorized to band under the direction of the above permittee, in accordance with the same general conditions, and the subpermittee specific authorizations listed below:

(Number of Active Sub Permits 1)

23479 - A
MARGARET S DREVIRS
DEPT. OF BIO SCIENCES - U.S. M. 118 COLLEGE DR. #5018 HATTIESBURG, MS 39406-0001

Is Authorized To Band:

NORTHERN CARDINAL

In the States Of:

MS *

With Special Authorization to:

Band
Auxiliary mark
Take, possess and transport blood samples-not to exceed 1% body mass
Use Mist nets
Trap
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Permittee agrees to band in accordance with the general conditions of this permit and with the specific authorization(s) listed below:

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19 March 2013

ADMINISTRATIVE SCIENTIFIC COLLECTING PERMIT NUMBER 0319131

TO WHOM IT MAY CONCERN:

Permission is hereby granted to:

Jodie M. Jawor, Ph.D.
University of Southern Mississippi
Dept. of Biological Sciences
118 College Drive #5018
Hattiesburg, MS 39406-0001,

assisted by Susan DeVries, Benjamin M. Duckworth, and Lauren Gillespie, to capture and band northern cardinals (Cardinalis cardinalis) and collect cardinal eggs for research at the University of Southern Mississippi’s Eubanks/Lake Thoreau property (ELT-USM) in Forrest and Lamar counties. Permission is also granted to capture and band up to a total of 200 eastern bluebirds (Sialia sialis) at two sites, Hattiesburg Country Club, Forrest County, and private property in Lamar County, Mississippi.

This permit is valid from 19 March 2013 to 18 March 2014.

SPECIFIC CONDITIONS AND RESTRICTIONS

1.) Copies of valid federal banding and scientific collecting permits must be on file with MDWFP.

2.) A copy of this permit and 1) a copy of a valid Federal Bird Banding Permit (bird banding activities) or 2) US Fish and Wildlife Service Migratory Bird Scientific Collecting Permit (egg collecting, capture and holding of birds for experiments) must be in the possession of the collectors during all collecting or banding activities.

3.) Up to 200 northern cardinals at the MCMWA, up to 150 northern cardinals at the ELT-USM, and 200 eastern bluebirds (adults and nestlings) may be captured and banded. In addition, blood samples may be taken from these birds for periodic assay of hormone levels. These birds will be released immediately at the capture site.
4.) Up to 300 northern cardinal eggs may be collected for evaluation of hormones and carotenoids.

5.) Birds may be captured with mist nets, walk-in traps, and nest-box traps. All collecting gear left unattended must be labeled with the collector’s name, institution, and permit number.

6.) At the conclusion of breeding and just prior to onset of molt, an additional 20 adult female northern cardinals from the ELT-USM site may be captured and held through the molt at the University of Southern Mississippi’s Animal Research Facility. These birds will be released at the capture site following the molt period.

GENERAL CONDITIONS AND RESTRICTIONS:

1) Specimens retained after collection must be placed in a public museum or collection where they will be available for examination by the scientific community. The Mississippi Museum of Natural Science (MMNS), 2148 Riverside Drive, Jackson, MS 39202-1353, ph: (601) 354-7303, is the principal repository of terrestrial and freshwater vertebrates, freshwater mollusks, and crayfish collected in Mississippi, and welcomes additional specimens. **Unless alternative arrangements are made with the MMNS Collections manager (Scott Peyton, 601-354-7303) or curatorial staff at the MMNS, all collections of federally listed and state listed species will be deposited at the Mississippi Museum of Natural Science.**

2) This permit does not authorize the taking of any federally threatened or endangered species or any state endangered species (list attached), unless otherwise specified in this permit.

3) All wildlife, including fish and invertebrates, collected under the permit are considered to be a natural resource of the State of Mississippi. Collected specimens should be handled humanely, and live, uninjured specimens not needed for permanent collections should be returned to appropriate habitat at the capture locality when no longer required. Specimens that die incidental to collection activities or which are intentionally preserved must be maintained in a scientifically acceptable fashion in a study/research collection where they will be available for examination by the general scientific community, or should be offered to a museum. The intent of the scientific collecting permit is to encourage meaningful study and to discourage the loss of specimens and information.

4) The issuance of a permit does not authorize trespass by the permittee. Permit is also void if permittee has not obtained other necessary permissions/permits for collection activities on public lands.

5) Collection of migratory birds, their nests, or eggs, collection of federally listed endangered species, and collection of federally listed threatened species (when the
collector is not an agent of the State of Mississippi) requires a federal permit in addition to a state permit.

6) Copies of publications, survey reports, and other printed materials produced as a result of this collection should be sent to the Mississippi Museum of Natural Science (Attn: Scientific Collection Permit Review Committee.) 2148 Riverside Dr., Jackson, MS 39202.

REQUIRED COLLECTING PERMIT REPORTS

1) A collecting permit report using format described below must be filed within 15 days of the expiration of the permit. A new permit will not be issued until the report has been received. Collection reports should list taxa collected, number of individuals of each, exact collection locality and date of collection. Locality information must include the county of collection, and it is preferred that precise locality information be provided in latitude/longitude (GPS) or in the township, range, and section (TRS) system. If the TRS system is used, precise location within a section should be indicated (e.g.: NW4 of SE4 of Sec 11), if possible. If GPS or TRS information is not provided, include instead a clear and precise description of the location of the collection site relative to the nearest named or numbered road, town, intersection, and/or other feature(s) likely to be mapped on a USGS quad map. For aquatic species, provide the name of the stream in which collections were made.

Instructions for completing Scientific Collections Report

Below is a list of information that should be included in scientific collecting reports, if it applies to the activities covered by the collecting permit. Because of the broad spectrum of activities covered by collecting permits, individual reports may require an altered format or other information not described below. If possible, reports should be submitted electronically in a spreadsheet format (preferably in Excel or Access). A blank spreadsheet with the requested fields can be provided to you by Email. Please include the following fields in the spreadsheet, if they apply to the work conducted under the permit. If you cannot provide an electronic version of the collections report, a blank hard copy of a collections report form can be provided to you. If you have any questions, please contact Scott Peyton at 601-354-7303 or collections.manager@mmns.state.ms.us.

A. SPECIES - species name (scientific name), or lowest taxonomic description possible, for each collected taxon.
B. SACRIFICED - If specimens were killed for vouchers or other scientific purposes, indicate the number taken.
C. NUMBER – total number of each species collected or handled. Include both the number taken and the number released in this total.
D. DATE – specific date of each collection.
E. COUNTY – county where each collection occurred.
F. COORDINATES (X) - latitude/longitude, UTM coordinates
G. COORDINATES (Y) - latitude/longitude, UTM coordinates
H. UTM ZONE - UTM coordinates only
I. TRS - Township, Range and Section (optional, but please include if possible)
J. LOCALITY - brief description of locality, e.g. Chickasawhay River 100m upstream from HWY 84 bridge.
K. COLLECTOR(S) - person or persons who made the collection.
L. TISSUE - Indicate the number of specimens from which tissue samples were taken for genetic analysis or other purposes. If no tissue samples were taken, this column can be omitted.
M. DISPOSITION - For sacrificed specimens or tissue samples, list institution(s) where specimens/samples were deposited. For specimens released, indicate where the specimens were released.
N. TEMP EXP or TEMP PROP - If specimens are held in captivity temporarily for experimental purposes or for propagation and later released, a field should be included to capture this information.
O. TAGGED - If specimens are marked or tagged and released, a field should be included to capture this information.

2) Those collecting federally listed species specified in this permit must submit an additional report to the state, due the first week of October, detailing collections of listed species made between 1 October of the previous year and 30 September of the current year.

Charles Knight, Director
Mississippi Museum of Natural Science
Mississippi Department of Wildlife, Fisheries, & Parks

CK: vm, conservation biology section

Enclosure
MISSISSIPPI
DEPARTMENT OF WILDLIFE, FISHERIES, AND PARKS
Sam Polles, Ph.D.
Executive Director

3 June 2014

ADMINISTRATIVE SCIENTIFIC COLLECTING PERMIT NUMBER 0603142

TO WHOM IT MAY CONCERN:

Permission is hereby granted to:

Jodie M. Jawor, Ph.D.
University of Southern Mississippi
Dept. of Biological Sciences
118 College Drive #5018
Hattiesburg, MS 39406-0001,

assisted by Benjamin M. Duckworth, Kaylee Gentry, and Lauren Gillespie, to capture and band northern cardinals (Cardinalis cardinalis) and collect cardinal eggs for research at the University of Southern Mississippi’s Eubanks/Lake Thoreau property (ELT-USM) in Forrest and Lamar counties. Permission is also granted to capture and band up to a total of 200 eastern bluebirds (Sialia sialis) at two sites, Hattiesburg Country Club, Forrest County, and private property in Lamar County, Mississippi.

This permit is valid from 3 June 2014 to 2 June 2015.

SPECIFIC CONDITIONS AND RESTRICTIONS

1.) Copies of valid federal banding and scientific collecting permits must be on file with MDWFP.

2.) A copy of this permit and 1) a copy of a valid Federal Bird Banding Permit (bird banding activities) or 2) US Fish and Wildlife Service Migratory Bird Scientific Collecting Permit (egg collecting, capture and holding of birds for experiments) must be in the possession of the collectors during all collecting or banding activities.

3.) Up to 200 northern cardinals at the MCWMA, up to 150 northern cardinals at the ELT-USM, and 200 eastern bluebirds (adults and nestlings) may be captured and banded. In addition, blood samples may be taken from these birds for periodic assay of hormone
levels. These birds will be released immediately at the capture site.

4.) Up to 300 northern cardinal eggs may be collected for evaluation of hormones and carotenoids.

5.) Birds may be captured with mist nets, walk-in traps, and nest-box traps. All collecting gear left unattended must be labeled with the collector’s name, institution, and permit number.

6.) At the conclusion of breeding and just prior to onset of molt, an additional 20 adult female northern cardinals from the ELT-USM site may be captured and held through the molt at the University of Southern Mississippi’s Animal Research Facility. These birds will be released at the capture site following the molt period.

GENERAL CONDITIONS AND RESTRICTIONS:

1) Specimens retained after collection must be placed in a public museum or collection where they will be available for examination by the scientific community. The Mississippi Museum of Natural Science (MMNS), 2148 Riverside Drive, Jackson, MS 39202-1333, ph: (601) 576-6000, is the principal repository of terrestrial and freshwater vertebrates, freshwater mussels, and crayfish collected in Mississippi, and welcomes additional specimens. **Unless alternative arrangements are made with the MMNS Collections manager (Scott Peyton, 601-576-6000) or curatorial staff at the MMNS, all collections of federally listed and state listed species will be deposited at the Mississippi Museum of Natural Science.**

2) **This permit does not authorize the taking of any federally threatened or endangered species or any state endangered species (list attached), unless otherwise specified in this permit.**

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I. TRS - Township, Range and Section (optional, but please include if possible)
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[Signature]
Charles Knight, Director
Mississippi Museum of Natural Science
Mississippi Department of Wildlife, Fisheries, & Parks

CK:as, conservation biology section

Enclosure
REFERENCES


DeVries, M.S. (2013). Interrelationships between testosterone, aggression, and parental care of a temperate-zone, resident songbird, the northern cardinal (Cardinalis cardinalis) (Doctoral dissertation). Hattiesburg, Mississippi: University of Southern Mississippi.


