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Prey Selection by Birds of Prey

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PREY SELECTION BY BIRDS OF PREY

by

Anisha Pokharel

A THESIS

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PREY SELECTION BY BIRDS OF PREY

Anisha Pokharel, M.S.

University of Nebraska, 2020

Advisor: John P. DeLong

Prey selection is key to determine predator prey interaction and understanding the complexity of food web structure. In this thesis, we used two different approaches to understanding prey selection by North American birds of prey. Using a conventional method, in Chapter 1 we compared pellet analysis and trapping data to assess patterns of prey selection of barn owls in western Nebraska. *Microtus* spp. comprised 55.8% of the prey items in the barn owl's diet. The proportion of several prey types in the diet were significantly different from the expected proportion based on trapping. This pattern may indicate barn owls actively select *Microtus spp.*, possibly because they are a relatively more energetically rewarding prey. In Chapter 2, we quantified the prey selection of migrating Sharp-shinned hawks and Cooper's hawks using DNA barcoding. Red-winged blackbirds (*Agelaius phoeniceus*) and rock pigeons (*Columba livia*) were the most common prey of Cooper's hawk, and American robins (*Turdus migratorius)* were the most common prey of sharp-shinned hawks. Our results indicate that these raptors tend to consume relatively common prey species, possibly reflecting an energy conserving migration strategy. Detailed understanding of raptor diet is essential to identify their potential vulnerabilities and to develop effective conservation strategies.

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INTRODUCTION

Predators play important roles in shaping ecological communities and provide important ecosystem services (Ives and Dobson, 1987). Not only can predators directly influence the population sizes of their prey, but they also can cause cascading effects on lower trophic levels (Beckerman et al., 1997; Estes et al., 2011; Fortin et al., 2005; Schmitz et al., 1997). Prey selection by predators help us understand the ecological interaction between predator and prey and their influence on food web structure.

Raptors are avian predators that are globally distributed, can structure biological communities (Brown et al., 1988; Herrera and Hiraldo, 1976; Sergio et al., 2008). Raptors are highly valued by humans as cultural symbols along with contributing in human wellbeing by scavenging vultures (Markandya et al., 2008; Martin, 1996) and indicators of overall environmental health (Bildstein, 2001; Donázar et al., 2016). Despite their important ecological functions, raptors worldwide are declining due to their ecology and life-history coupled with anthropogenic causes like habitat destruction and conversion to agricultural lands, poisoning, shooting (McClure et al., 2018). Understanding their diet is crucial to assess their vulnerability to changing prey availability and abundance (Rosenberg et al., 2003). In addition, food limitation can influence their life history traits and their population and community structure (Martin, 1987). The decision of what and where to forage may be guided by the strategy to maximize fitness, including maximizing their energy intake per unit time given the time and energy cost of searching, attacking and handling prey (Stephens and Krebs, 1986). Although energy demands of raptors on average must be met for individual and population persistence, those demands may vary through different phases of seasonal and

annual cycles including breeding and migration (Buehler and Piersma, 2008; Sillett and Holmes, 2002). Therefore, in this thesis I investigate raptor diets during the breeding and migratory seasons. In Chapter 1, I investigate the breeding-season prey selection of barn owls (*Tyto alba*) and, and in Chapter 2, I examine the *en route* prey selection of migrating sharp-shinned hawks (*Accipiter striatus*) and Cooper's hawks (*Accipiter cooperii*).

Barn owl diets have been studied worldwide (Bellocq, 1998; Bernard et al., 2010; Bull and Akenson, 1985; Clark and Bunck, 1991; Marti, 1973; Morton and Martin, 1979). They prey on wide range of prey items but mostly small mammals (Marti et al., 2005). However, there is not a clear consensus about what type of foraging strategy they employ. Some studies show that they take the prey in proportion to their availability (Hawbecker, 1945; Heisler et al., 2016; Rifai et al., 1998) indicating that they are good or random samplers of the prey community. In contrast, other studies show that they have preferences towards certain prey species and hence are selective (Askew et al., 2007; Marti, 2010). In Chapter 1, I evaluated prey selection in barn owls using pellet analysis and determined whether prey selection was proportional to the availability of prey in western Nebraska. I found that barn owls consumed more *Microtus* voles and fewer *Neotominae* rodents than expected by their availability in the mammal communities.

In comparison with breeding diets, the diets of actively migrating raptors are very poorly known, despite the extensive knowledge of their routes and timing of migration. Given this limited information on what migrating raptors eat during migration, I investigated the diets of two common migratory raptors migrating along the eastern Atlantic coastal flyway (Allen and Peterson, 1936; Niles et al., 1996). Cooper's hawk and sharp-shinned hawks are widespread across North America including and are regularly

observed at migratory concentration points from coast to coast. These hawks are partial migrants, and they are considered opportunistic hunters that target a wide range of birds and some mammal prey species (Storer, 1966; Kennedy and Johnson, 1986; Rosenfield and Bielefeldt, 1993; Joy et al., 1994; Bildstein and Meyer, 2000; Cartron et al., 2010). In Chapter 2, I assessed the prey selection by Cooper's and sharp-shinned hawks migrating through Cape May, New Jersey, USA, using DNA barcoding of prey remains. Although the initial plan was to compare the diet of these hawks with prey availability of songbirds using banding data, I did not have enough sample of the prey base to compare with. However, even with limited results, it appeared both of these species tended to focus their foraging effort on relatively common, medium-sized birds that are often found in semiurban settings.

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CHAPTER 1: ASSESSING PREY SELECTION OF BARN OWLS (*Tyto alba***) IN WESTERN NEBRASKA USING PELLET ANALYSIS**

ABSTRACT

Research on prey selection strategies are important for understanding the connections between predators and their prey communities. Studies on prey selection by barn owls show contradictory results; some showed that they are efficient samplers of the small mammal communities whereas others claimed that they take prey disproportionately indicating they have preference. Here we compared pellet analysis and trapping data to assess patterns of prey selection of barn owls in western Nebraska. *Microtus* species. comprised 55.8% of the 1,163 prey items. The proportion of several prey types in the diet was significantly different from the expected proportion based on trapping. *Microtus* occurred more frequently in the diet whereas *Peromyscus* occurred less frequently in diet than expected. This pattern may indicate barn owls actively select *Microtus spp.*, possibly because are twice as large as *Peromyscus* and are therefore relatively more energetically rewarding prey. Alternatively, it suggests that *Microtus* may be more available to barn owls than *Peromyscus*.

INTRODUCTION

Food web structure and the patterns of interactions among predator and prey play a key role in setting the stability of ecosystems and their ability to provide ecosystem services (Beckerman et al., 1997; Estes et al., 2011; Fortin et al., 2005). For example, McCann et al., (1998) found that food webs are characterized by many weak and few strong interactions, leading to community stability and persistence by dampening the fluctuations caused by strong interactions. Similarly, trophic cascades, such as that of Yellowstone's wolf-elk-aspen system, depend on the strength of predator-prey interactions and the particular patterns of interactions among predator and prey (Ripple et al., 2001; DeLong et al., 2015).

A predator's diet reflects its connections to the community in which it lives, so documenting diets provides information on food web structure. The actual pattern of prey selection by a predator could be a random sample of the prey in the community or an outcome of behaviors or strategies that have evolved to maximize fitness. Searching predators may make decisions about where to forage, when to forage, and what to attack when they encounter potential prey (Stephens and Krebs, 1986). There is an extensive literature assessing the degree to which a predator makes decisions that can increase or maximize fitness, including the questions of how long to hunt in a patch and what prey to include in the diet (Charnov, 1976). Although debate continues about whether predators forage optimally, it is also still unclear the degree to which many predators take prey disproportionate to their availability in the environment. Such patterns may or may not reflect adaptive strategies but are still important in understanding the connections between predators and their prey communities.

Among vertebrate predators globally, barn owls (*Tyto alba*) are widely studied for their prey selection. Interest in barn owl diets stems from their global distribution, conservation concern, susceptibility to rodenticides, and use as a biocontrol agent for rodents (Kross et al., 2016; Moore et al., 1998). Barn owls may be unbiased samplers of the small mammal community upon which they principally prey, with some studies having shown that prey in barn owl diets are proportional to their abundance in the community (Hawbecker, 1945; Rifai et al., 1998; Andrade et al., 2016; Bernard et al., 2010; Heisler et al., 2016; Hucks et al., 2016). Moreover, Avenant (2005) demonstrated that barn owls are able to sample the small mammal community better than humans are by trapping. Overall, some diet studies suggest that barn owls show little preference towards certain species and select a wide range of prey items including small mammals, birds, amphibians, arthropods, fish and bats, depending upon availability (Morton and Martin, 1979).

In contrast, other studies suggest that barn owls take some prey species disproportionate to their abundance. Typically, voles (*Microtus* spp.) are the principle prey of barn owls in temperature regions (Kopij, 2013; Marti et al., 2005; Myers et al., 2009). This is consistent with the results of studies that showed that given a choice, barn owls preferred *Microtus* over other common rodents such as deer mice (*Peromyscus*), at least in a captive setting (Derting and Cranford, 1989; Fast and Ambrose, 1976). Taylor (2009) found that barn owls showed strong a preference for voles over mice in Scotland. Similarly, Gubanyi et al., (1992) showed that *Microtus* were taken as prey more than, and *Peromyscus* was taken less than, expected by their abundance. Several additional studies showed evidence of selective predation by barn owls on voles, irrespective of their

habitat and prey availability (Askew et al., 2007; Colvin, 1985; Hindmarch and Elliott, 2015; Marti, 2010). This higher prevalence of *Microtus* in the diet compared to other small rodents could result from them being relatively vulnerable or yielding relatively high energy intake per unit handling time (DeLong et al., 2013; Fast and Ambrose, 1976; Stephens and Krebs, 1986). Alternatively, barn owls hunt mainly using acoustic cues, so it is possible that voles are louder and therefore more detectable than smaller alternative prey (Derting and Cranford, 1989; Taylor, 2009). Barn owl prey selection also may be influenced by mass of the prey (Colvin, 1985; Marti, 2010; Taylor, 2009). Yom-Tov and Wool (1997) showed that although barn owls might choose prey randomly, they prefer larger prey individuals over smaller ones. In contrast, Dickman et al., (1991) and Trejo and Guthmann (2003) showed that barn owls have some preference towards smaller prey size.

Overall, then, past results are contradictory in presenting barn owls as having preferences or reflecting a foraging through a random sampling of the prey community. In this study, we evaluated prey selection in barn owls using pellet analysis and determined whether prey selection was proportional to the availability of prey. We focused on a site in western Nebraska for which small mammal community trapping data was available. Previously, Gubanyi et al., (1992) have recorded around 17 mammal species in the diet of barn owl in western Nebraska, with the majority of prey being species of *Microtus*, *Reithrodontomys* and *Peromyscus.* In addition, Geluso and Bonner (2010) recorded 12 species of mammals in the diet of barn owls at Crescent Lake National Wildlife Refuge in northwestern Nebraska, mostly comprising *Microtus* species*, Dipodymus* species, and *Peromyscus* species. Huebschman et al., (2000) found 11

mammal species in pellets collected from 24 counties in Nebraska from 1980-1998, and they found *Microtus* spp. and *Peromyscus* spp. constituted the principle prey items. We tested whether barn owl diets reflected prey availability by species, reflecting a good sampler strategy, or whether diets were disproportionate to prey relative abundance, suggesting either the existence of foraging strategies or differences in prey availability. We then tested whether the overall distribution of prey in the diet differed from the prey community by body mass, to determine whether barn owls could be selecting simply larger or smaller prey rather than targeting specific species. Our results contribute to an overall understanding of prey selection behaviors in barn owls.

STUDY AREA

The study area was located in western Nebraska (Keith county), at and in the areas surrounding the University of Nebraska – Lincoln's Cedar Point Biological Station (CPBS). The station lies at the nexus of mixed grass prairies, dry and irrigated agricultural fields, and the North Platte river valley and harbors a wide range of flora and fauna. Great Horned owls (*Bubo virginianus*), Eastern Screech owls (*Megascops asio*)*,* Burrowing owls (*Athene cunicularia*), Long eared owls (*Asio otus*), and barn owls all occur in the area.

METHODS

We searched for owl nests and collected pellets from May-July of 2016-2018 from five territories at CPBS and along State Highway 92. We autoclaved and cleaned the pellets, separating the skull and jaw bones. We identified individual prey by skulls and matched jaws to their skulls where possible, counting additional prey individuals for unmatched jaws (Marti, 1973). We measured skull length (maximum length) and width

(maximum distance between zygomatic arches) as well as the length of both jaws if available in mm.

We estimated the mass of prey items identified from pellets through a set of regressions linking bone measurements to wet mass of whole individuals. We estimated the mass (g) of *Microtus* spp. using the equation $M = 5.87 * BL - 101.06$, where BL is basilar length in mm (Pagels and Blem, 1984). For the samples missing skull length, we applied the regression equations using either of the cranial measurements: $y = 0.76x +$ 11.9 and $y = 2.9 + 1.5x$, where $y =$ skull length(mm) $x =$ jaw length and zygomatic width respectively. In the case of samples missing both skull and jaw length measurements (due to skull or jaw damage), we used the mean mass of the specimens of the appropriate species housed at Nebraska State Museum. We estimated the body mass (g) of *Peromyscus* spp. using the regression equation $\text{Log } M = \text{Log } a + b$ ($\text{log } ML$), where $a = -2.972$, $b = 4.146$, and ML is mandible length (mm) (Hamilton, 1980). In the case of samples that lacked the jaw length, we applied regression equation, $y =$ $0.485x + 7.80$, where x= zygomatic width (mm) and y = jaw length (mm), and then plugged this value into the mass/mandible length equation. Because the mandible length does not include the incisors and thus is shorter than the total jaw length, we corrected our jaw lengths to basilar lengths with the correction factor of 0.728, which is the average proportion of mandible length to jaw length in a random sample of ten jaws from our pellets. For *Reithrodontomys* spp*.,* we estimated mass using the same mass/mandible length equation, with $a = -1.769$ and $b = 2.958$ (Hamilton, 1980).

We categorized some prey in the family Neotominae if they could not be classified as either *Peromyscus* and *Reithrodontomys*, which was the case when prey items were only present only as jaws. We estimated the mass of these prey items using the same mass/mandible length equation, with average values of a and b across both *Peromyscus* spp. and the *Reithrodontomys* spp. We also calculated the average mass of the other identified prey, *Blarina* spp*., Sorex* spp*., Geomys* spp*.* and *Dipodymus* spp. as the average of locally collected specimen data provided by the Nebraska State Museum.

Small mammal sampling

We compiled mid-July – mid August trapping data collected at CPBS (2012-16) as a part of a long-term Field Parasitology class. Small mammals were trapped on CPBS grounds and four other different sites in Keith county. The sampled habitat types included various grassland, woodland, and riparian-wetland habitats. The class used Sherman traps, live and snap traps, and pitfall traps to capture small mammals. They set up traps in the late evening and checked them the next morning to collect the mammals. They kept the traps closed all day. A mix of vanilla and peanut butter and oatmeal was used to bait the traps. All trapped individuals were prepared as voucher specimens and deposited in the mammal collection of University of Nebraska State Museum (Gardner, 1996).

Statistical analyses

All statistical analyses were carried out using Matlab 2019. We ran Chi-square tests to compare the proportion of prey types in the diet to the relative abundance of prey types from the trapping sample at the level of genus. We only considered small mammals here as we did not have a community sample for other prey types nor identifications for the avian prey items. We also compared the distribution of body masses between the trapping and prey item samples using a two-sample Kolmogorov-Smirnov test. For the test, we selected the species under mass of 200 grams to exclude the larger species that were

trapped but never found in the barn owl diet (e.g., *Vulpes* spp., *Lepus* spp.).

RESULTS

We identified a total of 1,163 number of prey items from all pellet material (Table1). *Microtus* spp. made up the majority (649 prey items, 55.80%) of the diet, including both prairie voles (*M. ochrogaster*, 291 prey items) and meadow voles (*M. pennsylvanicus*, 336). In addition, the diet also included deer mouse (*Peromyscus* spp., 8.77%), harvest mouse (*Reithrodontomys* spp*.,* 13.15%)*,* short tailed shrew (*Blarina* sp*,* 3.3%), common shrew (*Sorex* sp*,* 3.09%), kangaroo rat (*Dipodymus* sp,2.8%), plains pocket gophers (*Geomys sp*, 1.7%), and unidentified birds (1.1%). Unidentified neotominae constituted 13.06% of the prey items. We also found evidence of northern crayfish (*Orconectus virilis*) from remains below one barn owl nest**.** 592 individuals of 14 species were identified through trapping (Table 1).

Microtus spp. and *Reithrodontomys* spp. occurred significantly more in the diet, whereas *Peromyscus* spp. *and Dipodymus* spp. occurred less frequently in the diet than expected from their frequencies in the trapping sample (Table 1). We also found a significant difference between the distributions of the estimated mass of the prey items in the diet and the mass of individuals collected by trapping (kstest $= 0.24$, $p < 0.001$; Figure 1).

DISCUSSION

Barn owl diets have been extensively studied throughout the world (Bernard et al., 2010; Glue, 1972; Heisler et al., 2016; Marti, 1973). However, there seems to be two schools of thoughts regarding barn owl patterns of prey selection. Some studies have shown that barn owl diets reflect the abundance of small mammal communities,

concluding that they are unbiased samplers (Andrade et al., 2016; Avenant, 2005; Bernard et al., 2010; Heisler et al., 2016; Hucks et al., 2016; Terry, 2010). In contrast, other studies have claimed that barn owl diets contained prey types disproportionate to their availability in the community (Perrin, 1982), suggesting that their feeding strategy is selective (Bellocq, 1998; Hindmarch and Elliott, 2015; Jaksić and Yáñez, 1979).

In this study, we assessed the prey selection by barn owls in western Nebraska, comparing diets determined by pellet analysis with the potential prey revealed by trapping data (Table 1). Most of the prey species we documented have been reported as barn owl prey in previous studies (Marti et al., 2005; Maser et al., 1980). Although a broad range of prey species was observed in the diet, *Microtus* spp. constituted the majority of the diet, similar to many studies across North America including those from western Nebraska (Adams et al., 1986; Bull and Akenson, 1985; Gubanyi and Joseph, 1989; Huebschman et al., 2000; Marti et al., 2005; Wallick and Barrett, 1976). However, the proportion of some species differed significantly between the trapping and diet. For example, *Microtus* spp*.* and *Reithrodontomys* spp. occurred more frequently in the diet, whereas *Peromyscus* spp*. and Dipodymus* spp. occurred less frequently in the diet than expected from trapping results, which also is similar to some previous findings (Gubanyi et al., 1992; Pearson and Pearson, 1947). Although covering a wide range of habitats and using multiple trapping techniques, the trapping sample itself may not represent exactly the foraging habitats used by barn owls in our study area or be a perfectly random sample of the prey base. Nonetheless, the dramatic differences between prey and trapping frequencies for *Microtus* spp. (56.3% in diet versus 8.9 % in traps) and *Peromyscus* spp.

(14.1% versus 47.7%) suggests that, even given some error in sampling, barn owls show some selective foraging among potential prey.

Beyond prey identity, prey size may be an influential factor in prey selection by barn owls. Marti (1973) showed that barn owls prefer larger prey species over smaller ones, whereas Dickman et al., (1991) found the opposite. Our genus-level differences between prey items and potential prey also reflect that the body mass distribution of the diet is significantly shifted to right of the body mass distribution of trapped mammals, indicating selection for larger prey than are available overall (Figure 1). This result stems from the fact that the most abundant prey, *Microtus* spp., are twice the size of *Peromyscus* spp. This bias toward the larger voles in the diet might reflect the possibility that voles are a relatively more energetically profitable option, suggesting that barn owls in western Nebraska forage in a way that is consistent with an optimal foraging strategy (DeLong et al., 2013; Derting and Cranford, 1989). Alternatively, barn owls might be detecting *Microtus* more as they might be louder or more vulnerable, and hence more available to barn owl than *Peromyscus.*

Raptor diets are likely to be influenced by prey abundance, seasonal vegetation changes, habitat modification, prey behavior, and reproductive patterns (Rosenblatt et al., 2015; Taylor, 2009), which should be taken into consideration to understand the whole picture of prey selection. Overall, our results support the idea that barn owls select a wide range of prey items but are biased towards certain species (here, *Microtus*) despite the availability of other prey types. We suggest that, given the equivocal evidence for barn owls either being random samplers of the prey community or displaying selective

foraging behaviors, that future work consider the conditions under which selectivity or not would arise for barn owls, or raptors more generally.

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Table 1. Total number of individuals and percentage of small mammals in trapping samples and pellet analysis. Differences in the frequency of prey types between potential prey and actual prey were analyzed at the genus level through chi-squared tests.

Figure 1. Mass distribution of prey items using trapping data versus estimated mass distribution of prey items in the diet of Barn owl in western Nebraska. The diet distribution is significantly shifted towards the right of the trapping sample (kstest $= 0.25$, $p < 0.001$).

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CHAPTER 2: IDENTIFICATION OF COOPER'S HAWK (*Accipiter cooperii***) AND SHARP-SHINNED HAWK (***Accipiter striatus***) PREY DURING FALL MIGRATION USING DNA BARCODING**

ABSTRACT

Almost 66% of raptors migrate every year, however there is limited information on prey selection by migrating raptors. This is due in part to the logistical challenges of observing prey capture or hunting while raptors are *en route*. In this study, we quantified the prey selection of migrating Sharp-shinned hawks *(Accipiter striatus*) and Cooper's hawks (*Accipiter Cooperii*) using DNA barcoding. We compared 16S mitochondrial gene sequences of prey feathers or remains from the talons or beaks of hawks captured at Cape May, New Jersey, to reference samples at GenBank to identify prey. Red-winged blackbirds (*Agelaius phoeniceus*) and rock pigeons (*Columba livia*) were the most common prey of Cooper's hawk, and American robins (*Turdus migratorius)* were the most common prey of Sharp-shinned hawks. Our results indicate that these hawks select common and abundant prey species during migration, possibly reflecting an opportunistic, energy conserving migration strategy.

INTRODUCTION

Quantifying prey selection is key to determining predator-prey interactions and understanding the complexity of food web structures (Mittelbach and Osenberg, 1994). Prey selection influences how predators drive the pathways of energy flow within and between ecosystems (Fortin et al., 2005; Pompanon et al., 2012) and, as such, predator populations are sometimes considered to be biological indicators of overall ecological health (Bildstein, 2001; Carson, 1962). Predators may also perform ecosystem functions like controlling prey or pest populations (Derting and Cranford, 1982; Sekercioglu et al., 2004; Sergio et al., 2008; Estes et al., 2011; Wenny et al., 2011; Kross et al., 2016).

For some predators, life history stages or annual events generate different energetic demands (McEwen and Wingfield, 2003; Pagano et al., 2018). For example, migration and breeding are regarded as the most energetically demanding stages, particularly for predatory birds (Sillett and Holmes, 2002; Buehler and Piersma, 2008). Globally, about 66% of raptors travel across geographical regions in search of food, breeding territories, and wintering grounds (Bildstein, 2006). Unlike non-migratory species, migratory birds must meet the added energetic costs of the journey itself (Buehler and Piersma, 2008). To minimize the time and energy costs of migration, raptors may take advantage of lift generated by thermals and updrafts (Alerstam and Lindström, 1990; Kerlinger and Moore, 1989). Fat reserves are an important source of energy for migrating birds (Blem, 1980). Some raptors like Flammulated owls (*Psiloscops flammeolus)* and American kestrels (*Falco sparverius)* build up fat stores prior to migration (DeLong, 2006; Gessaman, 1979), while others like *Accipiter* spp. hunt *en route* to replenish during their journey (DeLong and Hoffman, 2004; Schaub et al.,

2008). Some raptors may selectively forage on the energetically most rewarding food to meet the additional energy demands during migration (Alerstam and Lindström, 1990; DeLong et al., 2013; Stephens and Krebs, 1986). Nonetheless, actively migrating raptors may have sufficient fat stores to survive without hunting for only one or two days (Delong and Hoffman, 2004), indicating a pressing need to hunt regularly while migrating.

Given the importance of foraging for fueling migratory journeys, food availability may influence raptor migratory patterns and strategies (Kerlinger, 1989; Newton, 2010). For example, some raptor species like the Merlin (*Falco columbarius*) may time their migration with the migration of their prey, presumably to improve prey capture conditions (Aborn, 1994). DeLong et al., (2013) found co-occurrence of migratory songbirds and bird-eating hawks like Sharp-shinned hawks (*Accipiter striatus*) in central New Mexico. In addition to supporting the continuation of their migratory journey, the ability of migrants to fulfill their energetic needs influences their survival not just during migration but also their productivity during the subsequent breeding season (Kerlinger and Moore, 1989). However, there is limited information on prey selection by raptors during migration due to the logistical challenges of observing and collect information on prey selection while they are migrating (Dekker, 1980; Moore et al., 1990; Ydenberg et al., 2007; Yosef, 1996).

Cooper's hawks (*A. cooperii*) and Sharp-shinned hawks (*A. striatus*) are widely distributed across North America (Rosenfield and Bielefeldt, 1993; Bildstein and Meyer, 2000). They are partial migrants and are considered opportunistic hunters that target a wide range of birds and some mammal prey species (Storer, 1966; Kennedy and Johnson, 1986; Rosenfield and Bielefeldt, 1993; Joy et al., 1994; Bildstein and Meyer, 2000; Cartron et al., 2010). Both species of hawk can frequently be found migrating along migration corridors across North America, including along the eastern Atlantic coast (Allen and Peterson, 1936; Niles et al., 1996; Goodrich, 2010). Little is known about their prey selection during migration.

In this study, we assessed the prey selection by Cooper's and sharp-shinned hawks migrating through Cape May, New Jersey, USA, using DNA barcoding of prey remains. This method has greatly improved our ability to study diet of various wildlife species including raptors, particularly during migration when few other techniques are available (Bourbour et al., 2019; DeLong et al., 2013).

METHODS

Cape May peninsula is located at the southern tip of New Jersey, bordered by Delaware Bay on the west and Atlantic Ocean on the east. The fall migration of raptors here usually stretches from September to December (Allen and Peterson, 1936; Niles et al., 1996). During the fall of 2013-2017, Sharp-shinned and Cooper's hawks were trapped using bow nets, mist nets and dho-gaza nets at the Cape May Raptor Banding station as part of an ongoing, long-term monitoring program. Rock pigeons, European starlings and house sparrows were used as lures. When feathers were found stuck to the talons or beaks of captured hawks, they were collected and saved for later analysis.

We extracted DNA from these feather samples. We extracted DNA using QIAGEN DNEASY blood and tissues protocol. We lysed the feathers in a mixture of 20µl proteinase K, 500 µl ATL buffer, and 40 µl DTT. We targeted the 16S region, as it is the least variable mitochondrial gene (Vences et al., 2005). We used the following

customized 16S primers: 16sAN1_L 5'-CCCGACTGTTTACCAAAAACATA-3' and 16sBN1_H 5'-AGACGAGAAGACCCTGTGGA-3' and rev 5'-

TCCACAGGGTCTTCTCGTCT-3'. We prepared and sent DNA sequences to GenWiz Inc. (New Jersey) for Sanger sequencing. We used Geneious to annotate the sequences and then a standard nucleotide BLAST search to identify matching barcode sequences. We also used feathers from locally collected, known species (American robin, *Turdus migratorius*; American goldfinch, *Spinus tristis;* and white-breasted nuthatch, *Sitta carolinensis*) to validate our species identification approach. Our approach correctly identified all three control feathers.

RESULTS

We obtained 25 Sharp-shinned hawk and 50 Cooper's hawk prey feathers. Of these, we identified 19 Sharp-shinned prey items and 44 Cooper's hawk prey items (Table 1). Not all samples could be identified to the species level; some could be identified only to family level or to the order Passeriformes. A few additional samples yielded DNA sequences that were of too poor quality to be matched to any taxa. Results from the DNA barcoding shows that both Cooper's hawks and sharp-shinned hawks hunted wide range of prey species. The most commonly observed prey items in Cooper's hawk were rock pigeon (*Columba livia*, 5), European starling (*Sturnus vulgaris*, 5), redwinged blackbird (*Agelaius phoeniceus*, 4). American robin (*Turdus migratorius*, 9) was the most common prey of Sharp-shinned hawks. We also matched the prey identified with the lures used while trapping the hawks to correct for potential false positives arising from the interactions with lures. We found that five starling and 2 rock pigeons' feathers we identified from Cooper's hawks possibly originated from lures (Table 1). Based on

the data, we also found that male Cooper's hawks were preying on more *en route* than female (Table 1). In contrast, female sharp-shinned hawks were preying on more than the males while passing through Cape May (Table 1). Additionally, we looked at the capture date of hawks with prey feathers over the duration of fall migration and it seems October is the peak month of sample collection (Figure 1).

DISCUSSION

Many raptors migrate across large geographical areas, and to meet the high energetic demands of migrating, some must continuously hunt *en route*. There is very little systematic information about the diet of migrating birds of prey, but here we successfully identified the prey items of both Sharp-shinned and Cooper's hawks using DNA barcoding techniques. Most of the prey items we identified have already been documented in the diet of these species (Table 1). We found that American robins constituted the majority of prey in the diet of migrating Sharp-shinned hawks at Cape May, which is consistent with the results of DeLong et al., (2013) for Sharp-shinned hawks migrating through central New Mexico. Our results show that migrating Cooper's hawks are frequently selecting species like rock pigeons, starlings and red-winged blackbirds that are typically common in urban and semi-urban habitats (Roth and Lima, 2003). In addition,

Although we did not have enough samples to make comparisons with the available prey in the community, the results suggest that these hawks are opportunistically selecting prey that are abundant in urban setting like Cape May. Both sharp-shinned hawks and Cooper's hawks are adept at hunting in urban areas and have been observed preying upon non-native species and relatively large prey (Estes and

Mannan, 2003; Cava et al., 2012; DeLong et al., 2013). This might also indicate that choosing these species might be energetically beneficial, as they would require minimal time and energy investment relative to searching for other smaller potential prey species such as Neotropical migratory passerines that might be more abundant in woodlands.

A few of the most common prey items that we detected, like rock pigeons, starlings, and house sparrows, were used as lures in the banding stations at Cape May. They therefore could be false positives resulting from the trapping process itself. Checking these hits against trapping methods suggests that most of these hits would not have come from trapping events, but several of the starling hits for Cooper's hawks could have, indicating a need to take trapping method into account when using barcoding techniques. It may be possible to improve barcoding approaches by collecting the samples from blood on beaks and talons, which may be more indicative of predation events and not trapping lure attacks (Bourbour et al., 2019). Overall, genetic approaches are proving useful for filling the knowledge gaps about prey selection by migrating birds of prey.

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Table 1. Frequency of prey identified with DNA barcoding for males and females Cooper's hawk (*A. cooperii)* and sharp-shinned hawks (*A. striatus*) migrating through Cape May, New Jersey. For Cooper's hawks, we also list the prey after removing potential false positives from trapping.

Figure 1. Distribution of day of captures of Cooper's hawk and Sharp-shinned hawks with prey feathers during fall migration in Cape May. The distribution indicates the peak of the captures on October.

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CONCLUSION

Prey selection by birds of prey provides insights into their functional connections in food webs and the potential role they play in driving ecosystem processes as top predators (Donázar et al., 2016; Johnston and Hill, 1987). The actual pattern of prey selection by any predator could be a random sample of the prey in the community or an outcome of behaviors or strategies that have evolved to maximize fitness.

In this thesis, I assessed the prey selection by barn owls in western Nebraska, comparing diets determined by pellet analysis with the potential prey revealed by trapping data. Based on the results, barn owls in western Nebraska show some selective foraging on *Microtus* species among potential prey. The proportion of some species differed significantly between the trapping and diet similar to earlier studies (Gubanyi et al., 1992). I then identified prey selection by migrating Cooper's hawk and sharp-shinned hawks using DNA barcoding techniques. Although I did not have enough sample size to make comparisons with the available prey in the community, the results suggest that these hawks are opportunistically selecting prey species that are abundant in urban setting like Cape May. Together, these studies improved our knowledge of prey selection by raptors using both conventional and novel techniques. Results of both studies are consistent with the hypothesis that raptors choose prey that might be energetically profitable or relatively easy to encounter. However, raptor diet is also likely to be influenced by prey abundance and behavior, seasonal vegetation changes, habitat modification, reproductive patterns (Comay and Dayan, 2018; Rosenblatt et al., 2015; Taylor, 2009), which should be considered in future research of raptor prey selection at a

finer scale. Further research on raptor diet is crucial in assessing their vulnerabilities with

changing climate and to develop effective conservation strategies.

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