The foraging ecology of raptors migrating along the coast of California revealed with eDNA metabarcoding

By

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Abstract

Bird migration is one of the most complex natural phenomena to occur on a global scale. Every fall, billions of birds migrate thousands of kilometers from their breeding territories to wintering grounds. Within migration corridors around the world, avian communities coexist spatially and temporally, competing for resources at stopover sites, and even interacting as predators and prey. Migrations of raptors and songbirds often overlap in space and time, creating interactions that shape their behaviors and migration strategies. However, the wide-ranging movements of migrating avian communities make ecological interactions logistically challenging to study, especially when many migrating raptors hunt regularly along a migration route. Literature on the diet of raptors that feed en route is mainly based on opportunistic observations and correlations between peak movement activity between predator and prey migrants. Research about diets during migration requires innovative approaches to systematically document prey selection by migrating predators. This dissertation developed and demonstrated the utility of a research framework to investigate the foraging ecology and coevolution of migrating raptors and their prey. Firstly, this dissertation developed and tested a new method to obtain robust dietary datasets for migrating raptors. In Chapter 1, we tested a new technique to collect trace prey environmental DNA (eDNA) from the exterior of a raptor. When raptors feed, they grasp and tear their prey with their sharp talons and beak, leaving residual traces of prey material that can be retrieved by swabbing the exterior of the beak and talons. The residues of prey can be identified to species through application of DNA barcoding techniques. Our results indicated that DNA identified on the swab is directly linked to a previously consumed meal. In Chapter 2, we employed our swab method to study the diet of migrating Merlins (*Falco columbarius*). We swabbed migrating juvenile Merlins during two fall migration seasons at a long-term migration

monitoring station operated by the Golden Gate Raptor Observatory (GGRO) in Marin County, California, a non-profit organization powered by volunteer community scientists. Using eDNA metabarcoding techniques, we detected the presence of 40 distinct prey species derived from 210 individual prey detections on 63 of the 72 (87.5%) Merlins sampled. Our results supported the hypotheses that describe migratory prey as abundant food sources of bird-eating raptors and suggest raptors select abundant prey species during fall migration. In Chapter 3, we applied our swab method to migrating Sharp-shinned Hawks (*Accipiter striatus*). Using eDNA metabarcoding techniques at GGRO, we obtained prey species detections from 94.1% of the hawks sampled (n=525) comprised of 1396 prey items and 65 prey species. To gather prey availability data, we extracted weekly abundances within our study region from the publicly available eBird Status and Trends data. By combining hawk diet data and songbird abundance data collected through two community and citizen science organizations, we were able to use discrete choice models to test how prey traits influence the interactions between raptors and songbird communities during fall migrations. This dissertation demonstrates that the logistical challenges of documenting raptor diet and prey availability within a migration corridor can be overcome by combining eDNA metabarcoding and big data generated by citizen science platforms, like eBird. Taken together, this dissertation contributes a framework for revealing the ecological and evolutionary relationships between raptors and songbirds that have remained elusive in migratory systems around the world.

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validation of this research. I would like to thank Andrea Schreier and Alisha Goodbla from the Genomics Variation Laboratory (GVL) for allowing me to conduct my genetics work in the lab. Special thanks to Alisha for helping me develop my lab protocols, troubleshooting, and for being patient with me as a field biologist learning how to navigate a genetics lab. I would also like to thank Amanda Coen, Ann Holmes, Shannon Kieran, Grace Auringer, Emily Funk and others in the GVL for either assistance or guidance regarding library preparation and DNA sequencing. Special thanks to Megan Crane for giving me DNA extraction practice and getting me started in the genetics lab. Special thanks to Cody Aylward for collaborating on the DNA metabarcoding and bioinformatics methods so that Chapters 2 and 3 could reach their full potential. Special thanks to Chris Tyson for brainstorming and collaborating with me on statistical analyses in Chapter 2. Special thanks to Tim Meehan for brainstorming and collaborating on statistical analyses and eBird data extraction in Chapter 3. Thank you to Breanna Martinico for all the support and help assembling sampling kits and writing papers with me. Thank you to Diana Humple and Renée Cormier with Point Blue Conservation Science for support on this project. Thank you to everyone around the world who appreciates birds and submits eBird checklists, without your birding habits there would be no comparable data for this type of science. There are too many to list here, but I would also like to thank all my bird nerd lab mates from over the many years for all their support and shoppe talk. Throughout my Master's and PhD, I held a teaching assistant position each quarter, so I would like to thank Joel Ledford for hiring me as teaching assistant and Geoff Benn for allowing me to teach for over 20 quarters so that I could finish my degree at UC Davis. And thanks to the 1000+ students who were in my classes over the years, all those students who allowed me to continue growing as a teacher and science communicator. Thank you to Julie Phillips, Ryan Phillips, and everyone from the De Anza

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Introduction

Picture hiking along a trail on an autumn day in coastal California, accompanied by hundreds of migrating songbirds, hawks, and falcons. The influx of both avian predators and prey along the Pacific Coast happens every September and October because the coastal shoreline and mountains guide the migratory routes for many birds that migrate along the Pacific Flyway. On peak migration days, migrating raptors seem to swarm the hillsides and forest canopies of the Marin Headlands, ambushing their unsuspecting prey to refuel before crossing the San Francisco Bay and continuing onto the next leg of their long journey. At the same time, dispersing and migrating songbirds are also very active, balancing their energy between evading starvation and predators on the hunt.

The interactions between migrating raptors and songbird prey within migration corridors are a fascinating and integral component of migration systems around the world, yet studying them and documenting prey selection is challenging. My objectives for this dissertation were to develop a research approach to document the prey species that migrating raptors are consuming and to implement a new technique to investigate the foraging ecology of two bird-eating raptor species during their migration through coastal California. This dissertation consists of three independent papers:

In ***Chapter 1***, I describe the development of a novel molecular approach to investigate whether trace prey DNA (i.e., environmental DNA) can be obtained from migrating raptors and identified to species. This technique was important to validate before considering its use as a tool for answering larger ecological questions within migration corridors.

In ***Chapter 2***, we used the method developed in Chapter 1 to investigate the migration diet and foraging ecology of a cryptic migratory falcon in the Pacific Flyway, the Merlin (*Falco*

columbarius). Merlins are considered cryptic during fall migration because they occur in relatively low abundances and their movements along the Pacific Coast often go undetected. My goal for this chapter was to document which prey species juvenile Merlins consume and investigate the relative frequencies of migratory and sedentary songbird species in Merlin diet during fall migration.

In ***Chapter 3***, we used the method developed in Chapter 1 to investigate the migration diet and foraging ecology of a migratory *Accipiter* hawk in the Pacific Flyway, the Sharp-shinned Hawk (*Accipiter striatus*). My aim was to determine whether prey selection is correlated with the local abundances of avian prey species on the landscape. We also investigated songbird traits that influenced prey choice by migrating hawks. In this chapter, we introduce a framework for studying prey selection by collecting dietary data from a raptor migration monitoring station and extracting songbird abundance data from the publicly available eBird Status and Trends data.

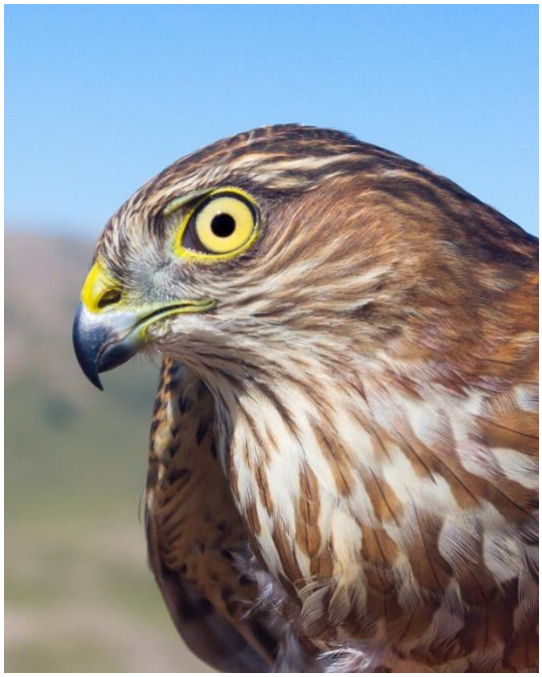

Photo caption: Portrait of a male Sharp-shinned Hawk (*Accipiter striatus*) during fall migration.

Chapter 1

Messy eaters: development of a method to collect and identify prey DNA from migrating raptors for which foraging is difficult to observe

Citation:
Bourbour, R. P., Martinico, B. L., Crane, M. M., Hull, A. C., & Hull, J. M. (2019). Messy eaters: Swabbing prey DNA from the exterior of inconspicuous predators when foraging cannot be observed. Ecology and Evolution, 9: 1452-1457.

Abstract

Complex coevolutionary relationships between predators and prey have shaped taxonomic diversity, life-history strategies, and even avian migratory patterns. Accurate documentation of prey selection is critical for understanding these ecological and evolutionary relationships. Conventional diet study methods lack the capacity to document the diet of inconspicuous or difficult-to-study predators where we cannot directly observe foraging, such as those with large home ranges, those occurring at low spatial densities, and those that rapidly move vast distances over short periods of time. Migratory raptors and their prey represent one such predator-prey interaction where detailed diet studies have been logistically challenging and, subsequently, there are gaps in our knowledge. To address knowledge gaps in the foraging ecology of migrant raptors and provide a broadly applicable tool for the study of enigmatic predators, we developed a minimally invasive method to collect dietary information by swabbing beaks and talons of raptors to collect trace prey DNA. Using previously published cytochrome oxidase subunit 1 (COI) primers, we were able to isolate and reference barcode sequences in an open access barcode database to identify prey to the level of species. This method creates a novel avenue to use trace molecular evidence to study prey selection of migrating raptors and could ultimately lead to a better understanding of raptor migration ecology. In addition, this technique

has broad applicability and can be used with any wildlife species where even trace amounts of prey debris remain on the exterior of the predator after feeding.

Introduction

Foraging ecology and predator-prey interactions have shaped the natural histories of species, including distribution and abundance as well as complex behaviors such as foraging strategies, interspecies competition, and timing and route of migration (Abrams, 2000; Alerstam et al. 2003). Even during migration, birds must feed along their migratory route, creating ephemeral dynamics between migratory predators and prey (Ydenberg et al. 2007). Diurnal birds of prey, for example, accipiters and falcons, use powered flight during migration and must continuously hunt en route to meet high energetic demands (Kerlinger 1989; DeLong & Hoffman 2004). To meet these energetic requirements, raptors are thought to time migration to track migratory avian prey species while some avian prey are thought to time migration to avoid the seasonal influx of predators (Aborn 1994; Ydenberg et al. 2007).

Determining the role predator-prey interactions play in shaping migration strategies is difficult without accurate dietary information. To date, our understanding of the diet of migrating raptors that feed en route is primarily based on opportunistic observations and correlations between peak movements of predator and prey migrants (Aborn 1994; Nicoletti 1996; Ydenberg et al. 2007). Currently, ecologists lack tools that can be utilized to study the diet of raptors while migrating, because traditional methods often fall short of providing reliable and relatively complete diet information when raptors on traveling quickly over vast distances (e.g., observations, nest cameras, prey remains, pellets, feces, and stable isotopes; Marti et al. 2007).

Limitations to diet studies of migrating raptors, and other enigmatic predators, may be alleviated by sampling molecular residues of prey remains from the exterior of beaks and talons. Prey DNA can then be referenced to cytochrome-oxidase-1 (COI) gene sequences, or other appropriate markers, that are unique to species (genetic barcodes) and have been previously catalogued in public barcode databases (Kerr et al. 2009). DNA metabarcoding has been a revolutionary tool in studying the diet of many wildlife species utilizing fecal or gut samples (Pompanon et al. 2012; Kress et al. 2015), but has yet to be implemented for raptor diet studies or by sampling the exterior of mouth and claws.

The benefit of DNA metabarcoding resides in the ability to document prey selection when traditional methods are not possible. DNA metabarcoding also has the potential to be utilized for any wildlife species where even trace amounts of prey debris remain on the exterior of the predator after feeding., such as, for vultures, piscivorous birds, insectivorous or predatory songbirds, and nectar-feeding bats and hummingbirds that are covered in traces of plant DNA from pollen (Nagarajan et al. 2018). Gathering DNA from the exterior of the body can be a viable alternative to fecal sampling, where prey DNA may be highly degraded or in low quantities compared to predator DNA (King et al. 2008; O'Rourke et al. 2012), or when fecal sampling is not possible. For example, exterior swabbing can minimize handling time and stress compared to fecal sampling, which is a critical consideration for raptor research (Heath 1997).

In North America, thousands of raptors are banded annually at monitoring stations situated along migration corridors, offering a valuable opportunity to study the diet of migrating raptors and test novel methods for identifying prey species through collection of trace DNA. For the first chapter of my dissertation, my objectives were to 1) to develop a minimally-invasive method for use in studying the diet of predators using raptors as a case study; 2) to verify that

prey DNA can be successfully obtained and identified from raptors with a known diet; and, 3) apply the method to wild migrating raptors to identify prey species.

Materials and Methods

Sampling method

For each raptor, we swabbed beaks and talons separately to 1) determine differences in DNA detectability and 2) as a precaution against the presence of PCR inhibitors that may be found on talons that come into contact with a variety of substrates. We first moistened nylon swab bristles (#25-2188 Puritan Medical Products Company) in 0.7 mL ultra-pure water. To sample beaks, we gently and thoroughly swabbed the entire exterior of the upper and lower mandible, targeting any visible prey blood or tissue that was present (Fig. 1). Precaution was taken to avoid contact between the swab and any interior mouth parts to minimize the risk of collecting predator DNA. To sample talons, we swabbed the entire surface of each talon, targeting any visible prey blood, tissue, or feathers (Fig. 2). Toe pads or scales were only swabbed if visible remains were present. For each sample collected, the nylon brush tip was removed and placed into individual 1.5 mL screw-top centrifuge tubes containing 0.7 mL of Longmire lysis buffer (100 mM Tris pH 8.0, 100 mM EDTA, 10 mM NaCl, 0.5% SDS, 0.2% sodium azide) stored at -20° C.

DNA extraction and quantification

We extracted DNA from each brush tip using the QIAamp DNA Mini Kit (QIAGEN Inc.) with a modified protocol. After 20 µL proteinase K and 600 µL buffer AL was added, vortexed, and incubated for 15 min, all liquid was transferred from the 1.5 mL screw-top

centrifuge tube to a 2.0 mL safe-lock centrifuge tube to allow space for 600 µL of 100% ethanol. Following buffer washes, DNA was eluted into 30 µL of molecular grade H_2O twice (60µl H_2O total). The purpose of using H_2O for DNA elution is to have the option to increase DNA concentrations via evaporation. We quantified DNA concentration of each sample using Qubit dsDNA BR Assay Kit and 2.0 µL of DNA.

Figure 1: Close-up images of migrating sharp-shinned hawk beaks and talons. Visible prey feathers and blood are good indicators that prey DNA remains from a previous meal. (Top right photo, Siobhan Ruck, others RPB)

Controlled study

To validate swabbing methods, we sampled three resident raptors at the California Raptor Center in Davis, California, USA. Each of the three raptors were fed different meals: 1) mice *Mus musculus* only, 2) hatchling chickens *Gallus gallus* only, or 3) both mice and hatching chickens. Feedings occurred at 0800-0900 every morning and sampling occurred at 1400-1600. Although the exact time of meal consumption was not documented, meals were completely or partially eaten prior to sampling. We sampled each raptor three times every other week. We tested for the presence of chicken DNA using a previously published chicken primer that

targeted a 133-bp amplicon (Dooley et al. 2004; Chicken forward: 5'–AGCAATTCCCTACATTGGACACA–3'; Chicken reverse: 5'-GATGATAGTAATACCTGCGATTGCA–3'). We did not test for mouse DNA because we could not control for mice entering enclosures where captive raptors have been documented eating pest rodents.

Field study

We swabbed migrating Sharp-shinned Hawks *Accipiter striatus* (n=285) and Merlin *Falco columbarius* (n=41) during fall of 2015 that were trapped by the Golden Gate Raptor Observatory in the Marin Headlands, CA, USA (37.8262° N, 122.4997° W). All individual raptors were trapped by dho-ghazzas, which are passive nets that collapse upon impact, making contact with trap bait unlikely. We checked for the presence of songbird prey DNA in a random subset of the wild samples with DNA quantities >2.0 µg/mL using a previously published bird primer (González-Varo et al. 2014; COI-fsdF: 5'–GCATGAGCCGGAATAGTRGG-3'; COI-fsdR: 5'–TGTGAKAGGGCAGGTGGTTT-3') and used DNA extracted from songbird tissue samples as controls. Primers were ordered with barcode sequences attached to both forward (ACTG) and reverse (ATGCTAA) COI-fsd primers consistent with the first round of PCR during library preparation for high-throughput sequencing for DNA metabarcoding (Vo & Jedlicka 2014).We prepared and sent DNA sequences to Quintara Biosciences, Inc. (Hayward, CA, USA) for Sanger-sequencing. We used a standard nucleotide BLAST search to reference all barcode sequences.

Results

Controlled study

Quantifiable (>1.0 µg/mL) DNA was detected on all swabs collected from captive raptors. DNA concentrations for the mouse-only raptor from talon swabs ranged from 5.0–16.0 µg/mL and beak swabs ranged from 1.31–4.24 µg/mL, with no chicken DNA detected on any swab. DNA concentrations for the 'chicken only' raptor from talon swabs ranged from 5.20–55.6 µg/mL, and beak swabs ranged from 1.65–2.37 µg/mL with all swabs testing positive for chicken DNA. DNA concentrations for the both-mice-and-chicken raptor from talon swabs were 39.3–171.0 µg/mL and from beak swabs they were 2.15–3.24 µg/mL, with chicken DNA detected on all talon swabs and only one (3.24µg/mL DNA) of three beak swabs.

Figure 2: A juvenile migrating sharp-shinned hawk having the beak swabbed for prey DNA collection after the banding process at a migration monitoring station. (Photo, Laura Young)

Field study

Out of 285 Sharp-shinned Hawks and 41 Merlins sampled, we obtained quantifiable (>1.0 µg/mL) DNA concentrations (potential dietary data) from 205 (71.4%) and 40 (97.6%)

individuals, respectively. Out of the 205 Sharp-shinned Hawks, 191 talon (92.7%) and 100 beak (48.5%) swabs had quantifiable DNA concentrations, and we detected songbird DNA from all swab samples that were sequenced: 9 talon swabs and 1 beak swab (Table 1). Out of the 41 Merlin individuals, 37 talon (90.2%) and 35 beak (85.4%) swabs had quantifiable DNA concentrations, and we detected songbird DNA on all swab samples that were sequenced: 4 talon swabs and 5 beak swabs (Table 1). The top match for all COI sequences were probable prey species in the sampling area (Table 1).

Figure 3: A captive raptor having its talons swabbed for prey DNA as part of our controlled study at the California Raptor Center.

Discussion

We successfully developed and tested a minimally invasive tool to document the diet of migrant raptors, and other enigmatic predators, by swabbing beaks and talons. We demonstrated that prey DNA can successfully be collected and identified from the exterior of a predator when a recent feeding was not evident and identifiable prey remains were not present. Importantly, this swabbing method can be used to study more than diet during raptor migration, a life-history stage where foraging ecology has never been systematically studied in raptors. It can also be applied to other wildlife species in studies with various objectives, such as those pertaining to food web dynamics, foraging ecology, predator-prey interactions (Pompanon et al. 2012; DeLong et al. 2013; Kress et al. 2015; Nagarajan et al. 2018), or even studies linking diet to microbiota (McFall-Ngai et al. 2013).

Molecular markers should be selected appropriately for prey species groups, and consequently previous knowledge of the probable prey is necessary; novel or rare prey species can still be detected if the DNA can be targeted with primers in situ and amplified with PCR (Pompanon et al. 2012). In this study, we targeted the COI gene because sequences are well represented and catalogued for songbirds and can resolve closely related species (Kerr et al. 2009; Patel et al. 2010). Prey DNA may be subject to differential degradation rates due to external environmental factors. To account for this, multiple primer sets may be used to reconstruct the marker region if DNA is found to be highly degraded (Patel et al. 2010). If prey DNA is not catalogued publicly, a reference library can be developed by sequencing potential prey DNA at the marker selected, but breadth of diet needs to be taken into account in order to create a thorough reference library (DeLong et al. 2013).

We only detected probable songbird prey on migrating raptors. However, two of the sequences matched bird species used to bait traps that are also common prey in the wild, which should be taken into account in dietary analyses when species are caught with baited traps. We determined that beaks and talons can be swabbed together to increase the likelihood of collecting prey DNA, as both sample types contained prey DNA that was able to be amplified and sequenced. Although, it may not necessary to sample both beaks and talons, especially if talon swabs of some predator species are likely to yield contaminant DNA or PCR inhibitors (e.g., an owl roosting on feces and regurgitated pellets from other individuals might only have its beak swabbed). We did not detect predator DNA on swabs, so blocking primers were not necessary (O'Rorke et al. 2012), even when using primers to detect prey from the same class (Aves). For studies including a higher quantity of samples with potentially multiple prey species per sample, DNA metabarcoding using high-throughput sequencing may be more appropriate and

economical than Sanger sequencing (Vo & Jedlicka et al. 2014). Finally, all biases associated with using DNA metabarcoding for dietary analyses should be considered, such as primer bias, contamination, and interpretation of relative read abundances (Deagle et al. 2019).

Species	Sample	µg/mL	Trap/Lure	Crop	Prey	E Value	% Match
Sharp-shinned Hawk	Talon	28.8	DG/ST	f	Fox Sparrow (*Passerella iliaca*)	0	95%
		18.7	DG/ST	f	Townsend's Warbler (*Setophaga townsendi*)	$5x10^{-179}$	95%
		13.8	DG/ST	f	Fox Sparrow (*Passerella iliaca*)	$2x10^{-95}$	94%
		14.6	DG/ST	f	American Goldfinch (*Spinus tristis)*	0	99%
		33.8	DG/ST	f	California Thrasher (*Toxostoma redivivum*)	0	95%
		4.61	DG/ST	f	Swainson's Thrush (*Catharus ustulatus*)	0	96%
		6.11	DG/ST	e	Red-breasted Nuthatch (*Sitta canadensis*)	0	98%
		7.21	DG/HS	f	Red-breasted Nuthatch (*Sitta canadensis*)	$3x10^{-127}$	88%
		24.5	DG/ST	f	Dark-eyed Junco (*Junco hyemalis*)	0	99%
	Beak	30.2	DG/ST	e	California Towhee (*Melozone crissalis*)	0	99%
Merlin	Talon	2.24	DG/HS	e	Red-breasted Nuthatch (Sitta canadensis)	$7x10^{-173}$	99%
		62.8	DG/HS	e	Red-breasted Nuthatch (Sitta canadensis)	0	99%
		8.07	DG/ST	e	Yellow Warbler (*Setophaga petechia*)	$6x10^{-153}$	95%
		3.44	DG/ST	e	European Starling (*Sturnus vulgaris*)	$4x10^{-175}$	95%
	Beak	13.26	DG/HS	e	Varied Thrush (*Ixoreus naevius*)	$4x10^{-160}$	95%
		2.04	DG/HS	e	House Sparrow (*Passer domesticus*)	0	99%
		4.14	DG/HS	e	House Finch (*Haemorhous mexicanus*)	$6x10^{-158}$	97%
		3.03	DG/ST	e	Yellow Warbler (*Setophaga petechia*)	$2x10^{-173}$	97%
		2.83	DG/ST	e	American Robin (*Turdus migratorius*)	0	99%
Control	SWTH	5.0	—	—	Swainson's Thrush (*Catharus ustulatus*)	0	98%
	OCWA	5.0	—	—	Orange-crowned Warbler (*Vermivora celata*)	0	98%

Table 1: Results from Sanger sequencing a random subset of samples collected from beaks and talons of migrating Sharp-shinned Hawks and Merlins. Presented are DNA concentrations (µg/mL) obtained from nylon brush tip, the trap (dho-ghazza=DG) and bait (European Starling=ST; House Sparrow=HS) used to catch raptor, the status of crop upon capture (empty=e; full=f), and the species that COI sequence most closely aligned with using BLAST search tool (E-Value=likelihood match is by chance; %Match=percentage of nucleotides aligned). Refer to Supplementary Table 1 for COI sequences used to match species with BLAST search.

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Chapter 2

Falcon fuel: DNA metabarcoding reveals songbird prey species in the diet of juvenile Merlins (*Falco columbarius*) migrating along the Pacific Coast of western North America

Citation:
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Abstract

During fall migration, bird-eating raptors are thought to rely on flocks of migrant songbirds (Passeriformes) as a critical resource to meet the energetic demands of long-distance migration. However, this hypothesis has been challenging to investigate, and the foraging ecology during migration of most migrant raptors remains unexplored. To address these knowledge gaps, our objective was to document the diet of a bird-eating falcon during active migration. We swabbed visible and trace prey residues from the exterior surface of beaks and talons of migrant juvenile Merlins *Falco columbarius* in the fall of 2015 and 2016 at the only raptor migration monitoring station positioned on the Pacific Coast of western North America. We used a DNA metabarcoding approach and detected the presence of 40 distinct prey species derived from 210 individual prey species detections on 63 of the 72 (87.5%) migrant juvenile Merlins sampled. We detected an average of 3.3 ± 1.6 prey species on individual Merlins (n=63). We found that juvenile males selected smaller prey on average compared to juvenile females. Of the prey species detected, over 80% were migratory songbird species that migrate within the Pacific Flyway. In 2015, we detected a greater proportion of irruptive migrants in juvenile Merlin diet compared to 2016. In 2016, we found that the proportion of annual migrants

consumed by Merlins corresponded to the timing of the annual peak of songbird migration in the Pacific Flyway. This study represents one of the first detailed descriptions of songbird prey species consumed by a migrating raptor and supports the hypothesis that migrating juvenile Merlins rely on migrant songbirds to support the energetic demands of migration.

Introduction

Migration is an energetically demanding and inherently dangerous life-history strategy presenting migrants with a constant risk of starvation and predation (Newton 2010, Dingle 2014). Research focusing on predator-prey interactions during migration has often been prey-centric, mainly revolving around predator-avoidance behaviors in relation to food and safety at refueling sites (Alerstam & Lindström 1990, Ydenberg et al. 2007, Newton 2010). Consequently, the foraging ecology of migrating predators remains relatively unexplored in the field of ornithology (Lima 1998, Ydenberg et al. 2007), resulting in essential life-history and ecological information (i.e. diet) that is missing from the literature for migratory birds of prey (raptors).

Many bird-eating raptor species are hypothesized to migrate with their prey (Lindström 1989, Aborn 1994, Nicoletti 1997, Ydenberg et al. 2007), a strategy that could increase hunting opportunities for smaller raptors that depend heavily on powered flight and store relatively little excess fat reserves during migration (Kerlinger 1989, DeLong & Hoffman 2004, Bildstein & Zalles 2005). For migrant bird-eating and insectivorous raptors, specifically accipiters (Accipitriformes) and falcons (Falconiformes), prey within a migration corridor are spatially and temporally heterogeneous throughout the migration season, and prey abundances shift continually along a route, and may vary among years (e.g., songbird irruption years). Our understanding of how migrant raptors are influenced by the spatial and ephemeral distributions

of prey within a migration corridor is limited, especially for raptors that hunt regularly to fuel long-distance journeys.

Studying the diet and foraging ecology of migrant raptors within a migration corridor is logistically challenging. Much of what we currently understand is based on opportunistic observations and correlations of peak movement activity between migrant raptors and probable migrant prey at individual locations along a migration route (Aborn 1994, Nicoletti 1997). While DNA metabarcoding methods are increasingly used to investigate foraging ecology of various wildlife species when direct observations are not feasible (Pompanon et al. 2012), few studies have focused on raptor diet using prey DNA (DeLong et al. 2013, Han & Oh 2018, Nota et al. 2019, Pokharel 2020, Tobe et al. 2020). To date, DNA metabarcoding remains an underutilized technique for advancing the field of avian migration ecology. Recent efforts have demonstrated that prey DNA can be collected from the exterior of migrant raptor beaks and talons using swabs, a sampling method that can be implemented at raptor migration monitoring stations to provide dietary data at a resolution that exceeds the capabilities of direct field observations (Bourbour et al. 2019a).

In this study, we utilized a DNA metabarcoding approach to investigate the migration diet of juvenile Merlins *Falco columbarius* in the American Pacific Flyway. Merlins are a small, compact, and dashing cosmopolitan bird-specialist (Cade 1982, Warkentin et al. 2005), with an inconspicuous migration along the Pacific Coast of western North America (Wade 1990, Goodrich & Smith 2008). During autumn migration, Merlins rely on powered flight and continuously hunt to fuel their high energetic demands (Bildstein & Zalles 2005), making them ideal candidates for studying how bird-eating raptors respond to the dynamic prey landscape during fall migration. Our objectives were to collect dietary information from migrant juvenile

Merlins, with four aims: 1) to describe the composition of avian prey species consumed; 2) test the long-standing hypothesis that migratory avian prey are a substantial energetic resource, 3) test whether reverse sexual size dimorphism results in differential prey size selection between males and females, and 4) assess temporal variation in the occurrence of different prey species in the diet. We discuss these results in the context of possible functional responses to changes in migrant songbird species composition.

Methods

Study Site and Sample Collection

We sampled Merlins (n = 72) at a raptor migration monitoring station situated along the Pacific Flyway in the Marin Headlands, California, USA, during fall migration in 2015 (n = 44) and 2016 (n = 28) from September 1 – December 15. The study site represents the only long-term raptor migration monitoring station on the Pacific Coast of western North America and is operated by the Golden Gate Raptor Observatory (GGRO; 37.8262° N, 122.4997° W), a non-profit, community science organization of the Golden Gate National Parks Conservancy in cooperation with the United States National Park Service. The Marin Headlands create the largest known migration bottleneck along the Pacific Coast of North America where migrating raptors converge and gain altitude before crossing San Francisco Bay (Goodrich & Smith 2008). As with most coastal migration sites in North America, the vast majority of individuals observed are juveniles; consequently, this study examines the diet of juvenile migratory Merlins.

Merlins were trapped using lure animals (Rock Doves *Columba livia*, European Starlings *Sturnus vulgarus*, and House Sparrows *Passer domesticus*) in dho-ghazzas, mist-nets, or bownets (GGRO 2018). All Merlins trapped and sampled in this study were aged as hatch-year (juvenile)

and sexed by wing chord and weight (GGRO 2018). To collect prey DNA, we swabbed the entire exterior surface of an individual's beak (upper and lower) and talons separately. We targeted visible prey blood, flesh, or feathers if present (Fig. 1), but swabbing took place even if beaks and talons appeared to be clean (see Bourbour *et al.* 2019a for details on the sampling protocol). We conducted all aspects of this research in accordance with strict Institutional Animal Care and Use Committee (IACUC; permit #: CA_GOGA_Ely_Raptors_2020.A3), California Department of Fish & Wildlife (California State Permit #: SCP 13739), and United States Geological Service guidelines (federal bird banding permit #: 21827).

Figure 1: A migrant juvenile Merlin with visible prey tissue leftover from a previous meal. Swabs were used to collect visible and trace prey DNA from the exterior of beaks and talons. (Photo: Robyn Boothby)

DNA Extraction, Amplification, and Sequencing

We processed all swab samples in the Genomic Variation Laboratory at the University of California, Davis (UC Davis), a lab that had not processed songbird DNA previously. We extracted DNA from each swab tip using the QIAamp DNA Mini Kit (QIAGEN Inc.) with a modified protocol (Bourbour *et al.* 2019a). Because prey DNA could be successfully amplified from both beak and talon swabs (i.e. no PCR inhibitors; Bourbour *et al.* 2019a), we pooled 20 µL of DNA extracted from both beak and talon swabs from each individual (combined 40 µL DNA) into a 96-well plate.

We targeted a 464 base pair (bp) amplicon region of the cytochrome c oxidase subunit I (COI) gene using primers COI-fsdF and COI-fsdR (González-Varo *et al.* 2014) modified with an overhang sequence to allow annealing to indexed Illumina adapters (Illumina 2013, 2018; see Supplementary Material Table 1 for primer and adapter sequences). We chose this specific region because COI barcodes are well represented in the public barcode database for avian taxa. To test primers, we extracted DNA from Orange-crowned Warbler *Vermivora celata*, Swainson's Thrush *Catharus ustulatus*, Yellow Warbler *Setophaga petechia*, Northern Flicker *Colaptes auratus*, White-crowned Sparrow *Zonotrichia leucophrys* and Least Sandpiper *Calidris minutilla* tissue samples as potential avian prey species, courtesy of the Museum of Wildlife & Fish Biology at UC Davis. We used Orange-crowned Warbler and Swainson's Thrush DNA as positive controls during library preparation alongside negative controls. We used PCR-grade water for negative controls, which were used in filtering out false positives that may arise during library preparation and sequencing.

We followed the two-step PCR amplification protocol outlined in Illumina (2013). First, we conducted an amplicon PCR using the COI primers followed by an index PCR to provide a

unique identifier for each sample. Amplicon PCR reactions were performed in 25 µl with the following components: 12.5 µl of 2X KAPA HiFi HotStart ReadyMix, 5 µl of 1.0 µM of forward and reverse primer, and 2.5 µl of template DNA. Amplicon PCR consisted of initial denaturation at 95° C for 4 min, 30 cycles of 95° C for 45 s, 58° C for 45 s, and 72° C 45 s, followed by a final extension of 5 min at 72° C. A subset of PCR amplicons were visualized with 2% agarose electrophoresis to ensure amplification and then all samples were purified using Ampure beads (following manufactures guidelines, Agencourt). For the Index PCR, we used 18 (8 forward, 10 reverse) barcoded primers (Illumina 2013; see Supplementary Table 1). Index PCR reactions were performed in 50 µl with the following components: 25 µl of 2X KAPA HiFi HotStart ReadyMix, 10 µl water, 5 µl of 1.0 µM of forward and reverse primer, and 5 µl of template DNA. Index PCR conditions were as follows: An initial denaturation at 95° C for 3 min, followed by 8 cycles at 95° C for 30 s, 55° C for 30 s, and 72° C for 45 s, with a final extension of 5 min at 72° C. Amplicons were again purified using Ampure beads. We ran a random subset of paired samples from Amplicon PCR and Index PCR on an Agilent Bioanalyzer 2100 to confirm that indexed adapters had been successfully attached in the Index PCR. After library preparation, we quantified DNA using Quant-iT PicoGreen dsDNA Reagent (Thermo Fisher Scientific) with an FLx800 Fluorescence Reader (BioTek Instruments), and normalized each sample individually following Illumina (2013) protocols. We then sequenced the pooled library on half a lane using Illumina's MiSeq PE300 (v3) platform.

Reference Library and Bioinformatics

We compiled a custom reference library of probable and improbable (e.g., Ardeidae) Merlin prey (n = 205) that broadly range in the Pacific Flyway according to range maps based on

species accounts (Rodewald 2015; see Supplementary Table 2). We used the R package *PrimerMiner-0.11* (Elbrecht & Leese 2017) to batch download all publicly available COI barcode sequences from NCBI and BOLD databases for each potential prey species and manually reformatted the datafiles to be compatible with the R package *dada2* (Callahan et al. 2016) reference database format.

We filtered out low quality scores (<30) and reads below 250 bp using the program *Cutadapt* (Martin 2011). We used the R package *dada2* to filter out samples with >2 erroneous base calls, remove chimeras, and merge forward and reverse reads. We then matched all recorded barcode sequences to our custom reference library with >99% bootstrap support using the 'assignTaxonomy' command in *dada2*. We removed samples with <100 total assigned reads and used 1% as a conservative cutoff for rare sequences to account for false positives within a sample.

Statistical analysis

We performed all statistical analyses using R Studio v 3.5.1 (RStudio Team 2016). We excluded European Starling and House Sparrow detections from statistical analyses because we could not confidently rule out contamination from the presence of lure animals at the sampling site as the cause of their detection. To evaluate to what extent our sampling method represented Merlin diet composition during fall migration, we calculated rarefaction and extrapolation curves with a 95% confidence interval using the R package *iNEXT* (Chao et al. 2014) for both sampling years combined. We used estimated average mass of prey and migratory tendency using species accounts published in the *Birds of North America* online database (Rodewald 2015; see Supplementary Table 3). To investigate differences in prey species detections between irruptive

migrant, regular migrant (partial or complete), and resident prey between 2015 and 2016, we used a *z*-test for proportions (Newcombe 1998) with a Bonferroni correction ($\alpha/3 = 0.017$). We considered 'regular migrants' to be species that exhibit predictable seasonal migratory behavior and we considered 'irruptive migrants' to be species that exhibit unpredictable seasonal movements in relation to resource availability (Newton 2010). Complete and partial migrants were both included in the 'regular migrant' category because many partial migrants actively migrate through the sampling site (Rodewald 2015).

Because Merlins exhibit reverse-sexual size dimorphism, we tested for differential prey size selection between females and males. We constructed a linear mixed-effects model of prey weight as a function of sex with individual identity as a random intercept term to account for intra-individual variation of prey size selection. We also constructed a simplified linear model that did not include individual identity as a random effect and then compared the two models to evaluate the importance of the random effect term. The linear mixed-effect model with individual identity as a random effect did not explain significantly more of the variation within the data than the simplified linear model; therefore, individual identity was not included as a random effect in subsequent analyses (Likelihood ratio test, $\chi^2_1 = 0$, $P = 1.00$).

Because songbird prey diversity and abundance fluctuate temporally throughout fall migration within a migration corridor (MacMynowski & Root 2007), we tested for changes in proportions of prey species detections in the diet of juvenile Merlins over the fall migration season using a generalized additive model (GAM; Hastie 2017) with the R package *mgcv* (Wood & Wood 2015). For the GAM, we analyzed 2015 and 2016 separately to account for interannual variation. We used daily proportion of prey species detected as the response variable with

sampling date and migratory status (regular migrant, irruptive migrant, or resident) as fixed effects. Sampling date was used as the smoothed term with k = 10 and gamma = 1.

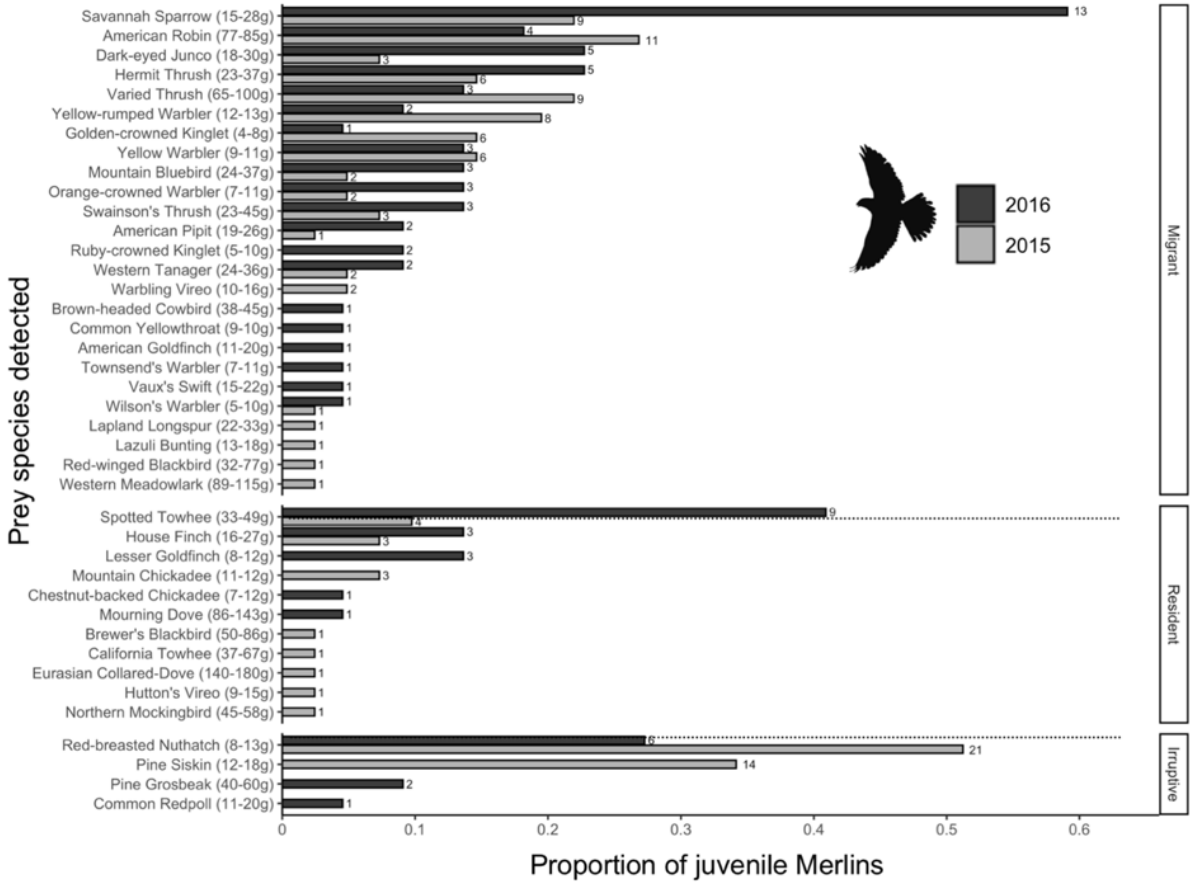

Figure 2: Proportion of migrant juvenile Merlins with prey species detections in 2015 and 2016. We detected 134 prey items on 43 individual Merlins in 2015 and 81 prey items on 22 individual Merlins in 2016. Prey species are grouped by migratory tendency in the Pacific Flyway: Resident, regular migrant (complete and partial), and irruptive migrant. The average prey mass ranges are displayed to the right of prey common names and the number of Merlins with detections of each species is displayed at the tip of each bar. We detected an average of 3.3 ± 1.6 sd prey species per individual Merlin sampled. Lure bird species (European Starlings and House Sparrows) are not included in statistical analyses due to possible contamination at the sampling site.

Results

We obtained 13 million total raw reads with an average of 169k ± 175k sd per sample (see Supplementary Table 4 for summary of reads per sample). Reference sequences were available for 199 of the 205 (97%) species on our potential prey list. There were no published sequences available for *Ammospiza nelsoni*, *Cypseloides niger*, *Dryobates albolarvatus*, *Lanius borealis*, *Oreotyx pictus* and *Troglodytes pacificus* (see Supplementary Table 2 for reference library summary). After matching sequences to our custom reference library, the average sample had approximately 7k reads. The maximum abundance of reads in our negative controls possibly due to index hopping or low-level contamination was 0.6% of the number of reads in the average sample. After filtering using a 1% cutoff for rare sequences within a sample, nine possible prey species were removed: Band-tailed Pigeon *Patagioenas fasciata*, Loggerhead Shrike *Lanius ludovicianus*, Cliff Swallow *Petrochelidon pyrrhonota*, Blue-gray Gnatcatcher *Polioptila caerulea*, Western Bluebird *Sialia mexicanus*, Purple Finch *Haemorhous purpureus*, and Red Crossbill *Loxia curvirostra*. There were only five samples with <100 reads that were filtered out and each had single prey species assignment: European Starling, House Sparrow, Spotted Towhee *Pipilo maculatus*, Hermit Thrush *Catharus guttatus*, or Yellow Warbler. We did not detect Merlin DNA in our samples, possibly due to careful sampling or primer bias.

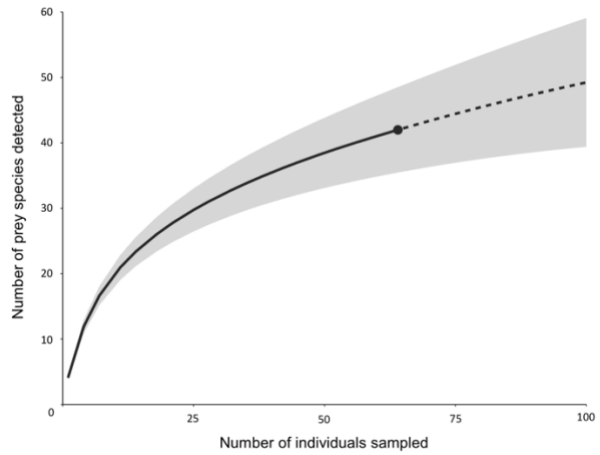

Figure 3: Rarefaction (solid line) and extrapolation (dashed line) sampling curves with a 95% confidence interval (shaded area) based on the prey DNA detected on 63 migrant Merlins in the Pacific Flyway.

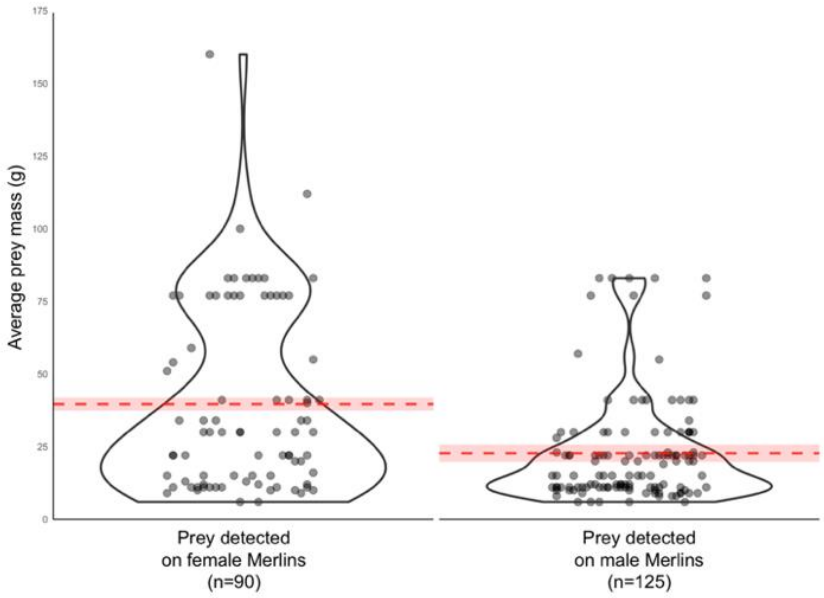

Figure 4: Prey size selection of female and male juvenile Merlins during 2015 and 2016 fall migration seasons. Over both seasons, 28 females yielded 90 prey detections and 37 males yielded 125 prey detections. The shaded dotted line represents modeled mean prey weight ± se.

We detected the presence of 42 prey species with 251 prey species detections (Supplementary Table 3) from 87.5% (63/72) of the migrant Merlins we sampled in 2015 (44 sampled; n = 41 Merlins with prey detections) and 2016 (28 sampled; n = 22 Merlins with prey detections); four of the 72 swab samples yielded no prey DNA detections. We detected European Starling and House Sparrow (lure animal species) DNA on 26 and 15 individual Merlins, respectively. Including these lure bird species, the average (mean ± sd) number of prey species detections was 3.98 ± 1.8 per Merlin (*n* = 63). Rock Dove DNA was not detected and no Merlins in this study were captured in a net positioned near Rock Doves. Excluding the lure species, we detected the presence of 40 prey species with 210 prey detections (Fig. 2) on 87.5% (63/72) of the migrant Merlins sampled in 2015 (*n* = 41) and 2016 (*n* = 22), and the average number of prey detections per individual Merlin was 3.3 ± 1.6 (*n* = 63). The rarefaction and extrapolation

sampling curve showed our samples were sufficient in detecting the majority of the avian prey species migrant Merlins were consuming along the Pacific Coast in western North America (Fig. 3).

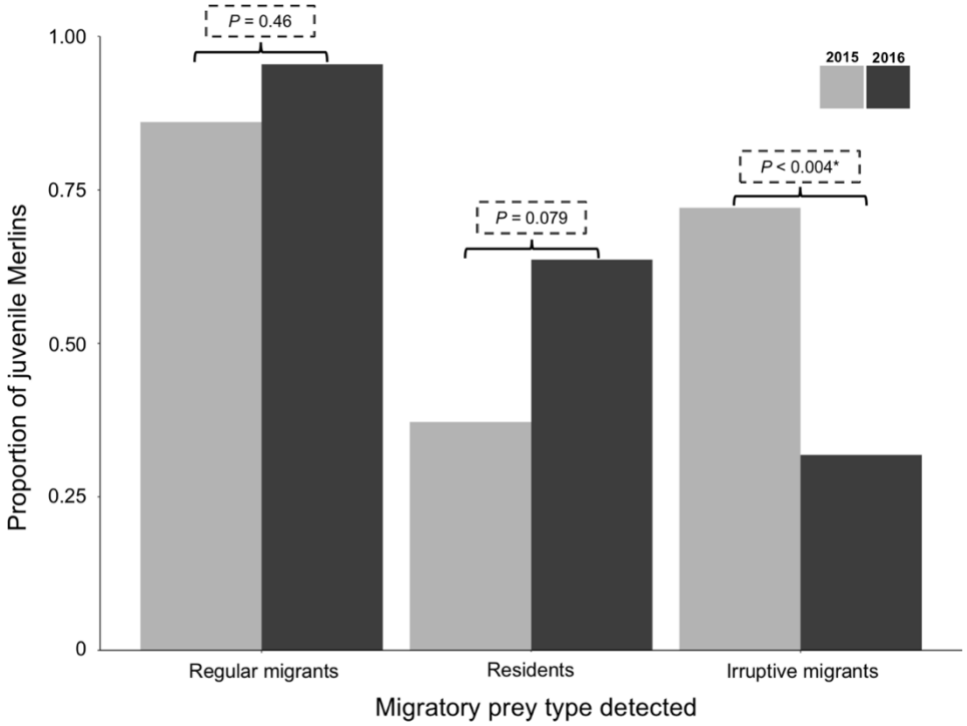

Figure 5: Proportion of Merlins with each of the migratory prey types detected. In 2015, 43 Merlins yielded detections for 37 regular migrants (86.0% of samples), 16 residents (37.2%), and 31 irruptive migrants (72.1%). In 2016, 22 Merlins yielded detections for 21 regular migrants (95.5%), 14 residents (63.6%), and 7 irruptive migrants (31.8%). P-values reported are the results of z-test for proportions and * represents statistical significance at the 0.017 α level. We detected a difference between the proportion of irruptive migrants detected in migrant Merlin diet in 2015 compared to 2016. We detected an average of 3.3 ± 1.6 sd prey species per individual Merlin sampled. Lure bird species (European Starlings and House Sparrows) are not included in the analysis.

Of the 63 juvenile Merlin individuals analyzed, males weighed (mean ± sd) an average of 150.6 ± 8.8g (n = 37) and females weighed 210.7 ± 12.7g (n = 26). Our model compared 127 prey detections for male Merlins and 83 prey detections for females. We found a statistically

significant relationship between prey size selection and sex (LM, $F_{1, 208} = 20.4$, $P < 0.001$), with male Merlins on average (mean ± se) selecting smaller prey species (23.7 ± 3.5g) compared to females (39.6 ± 2.7g; Fig. 4).

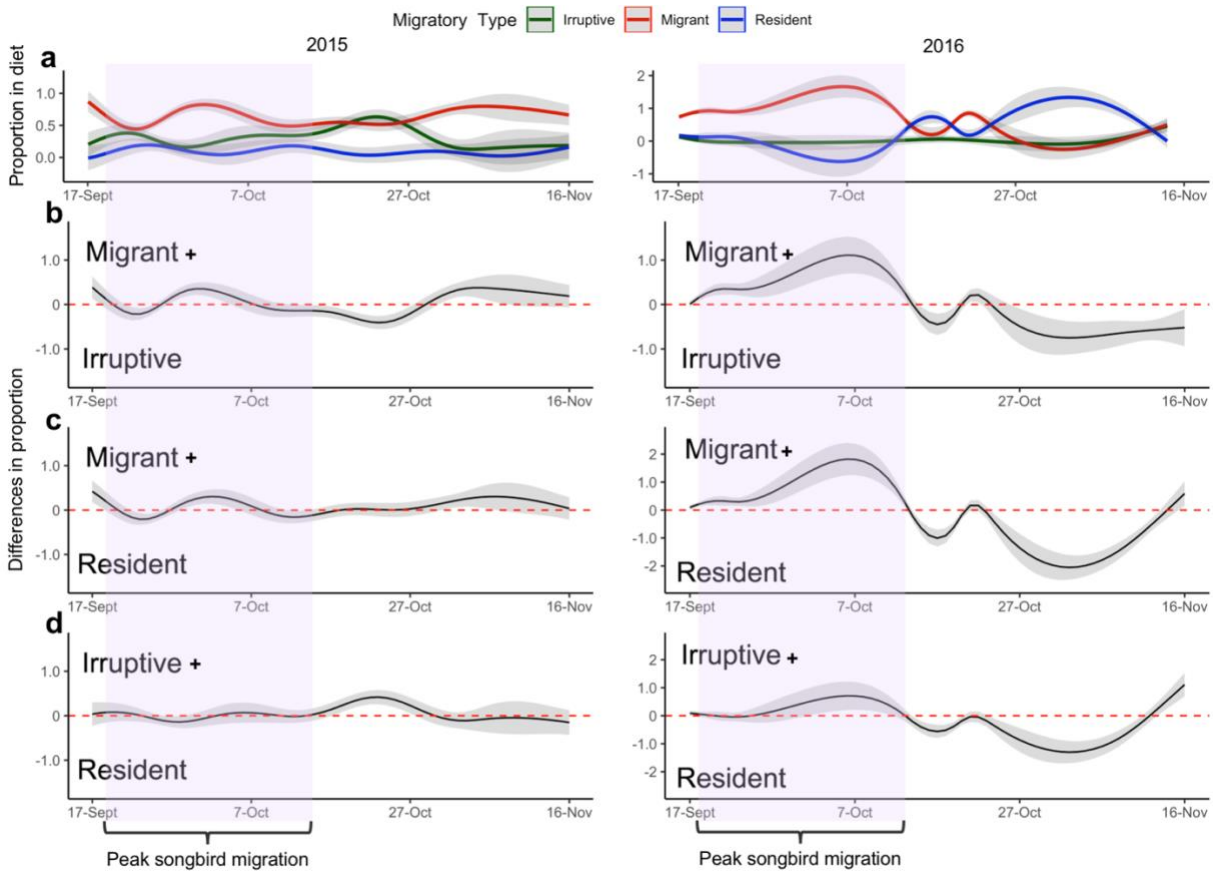

Figure 6: GAM model visualizations for 2015 and 2016 migration seasons. **Row a:** Variation in proportion of prey by migratory type consumed by all Merlins sampled in each year. **Rows b-d:** Differences in proportions between migratory prey types. + indicates that when the black line is above the red dotted line, the proportion of that prey type is greater than the prey type compared below. Where 95% CI does not overlap with the red dotted line, the proportion differences between the two migratory prey types is statistically significant. Highlighted in purple is the estimated time period when peak songbird migration occurs in the Pacific Flyway. Lure bird species (European Starlings and House Sparrows) are not included in the analysis.

Out of the 210 prey species detections in 2015 ($n = 127$) and 2016 ($n = 83$), 63.8% were regular annual migrants (25 species; 134 species detections), 21.0% were irruptive migrants (4

species; 44 species detections), and 15.2% were residents (11 species; 32 species detections). Out of 41 Merlins sampled in 2015, 36 (87.8%) yielded detections for regular migrants, 14 (34.1%) for residents, and 28 (68.3%) for irruptive migrants. Out of 22 Merlins sampled in 2016, 21 (95.5%) yielded detections for regular migrants, 13 (59.1%) for residents, and 7 (31.8%) for irruptive migrants. We found that irruptive migrants made up a greater proportion of juvenile Merlin diet in 2015 compared to 2016 ($z = 2.51$, df = 1, $P = 0.01$) and did not detect differences between years for regular migrant ($z = 0.54$, df = 1, $P = 0.59$) or resident ($z = 1.64$, df = 1, $P = 0.10$) prey species (Fig. 5). We found that date was a statistically significant predictor (Table 1; Fig. 6) of the proportion of migratory types detected in the diet of migrant juvenile Merlins in the Pacific Flyway for both 2015 (adjusted $R^2 = 0.72$, GCV = 0.031, deviance explained = 75.8%) and 2016 (adjusted $R^2 = 0.91$, GCV = 0.015, deviance explained = 92.9%).

Discussion

In this study, we collected trace prey DNA from the beaks and talons of migrating juvenile Merlins and used DNA metabarcoding to reveal songbird prey consumed to fuel fall migration. Our results indicate that migrant songbirds are an important ephemeral resource for migrant juvenile Merlins during migration, and that ecological processes independent of raptor migration likely influence predator-prey interactions within a migration corridor. These findings highlight the relationship between migrant songbirds and a migrant bird-eating raptor hypothesized to follow migrant prey during fall migration (Cade 1982, Kerlinger 1989, Aborn 1994, Bildstein & Zalles 2005, Ydenberg et al. 2007).

Understanding the composition of a migrant raptor's diet is important because it can reveal cryptic dietary trends when direct field observations of prey captures are not possible.

Previous studies have highlighted the correlation in migration timing between migrant prey and Merlins in North America (Dekker 1988, Raim et al. 1989, Aborn 1994, McCabe & Olsen 2015), as well as other bird-eating raptors (Sharp-shinned Hawk *Accipiter striatus*, Kerlinger 1989, DeLong et al. 2013; Peregrine Falcon *Falco peregrinus*, Aborn 1994), and hypothesized that these bird specialists utilize migrant songbirds as a primary energetic resource. Our study demonstrates that these correlations in migration timing are related to the composition of juvenile Merlin diet during fall migration and provide support for a migrating food-web hypothesis (i.e. raptors migrating with migratory prey).

With the amplicon primers we used in this study, over 95% of avian prey species detected were songbirds, and over 80% have a migratory life-history stage (complete, partial, or irruptive migrants) within the Pacific Flyway. The only non-songbird prey species detected were Mourning Dove *Zenaida macroura* and Eurasian Collared-Dove *Streptopelia decaocto*. The prey species we detected on a relatively high proportion of Merlins in this study, such as Savannah Sparrow *Passerculus sandwhichensis*, Hermit Thrush, and Spotted Towhee, could provide future avenues of targeted research to further understand whether migrating Merlins focus on certain migrant species or energetically rewarding prey (DeLong et al. 2013).

In addition to providing support for a migrant songbird diet hypothesis, we found evidence that juvenile Merlins respond to the interannual changes in songbird prey abundance within a migration corridor. In 2016, we found that the proportion of juvenile Merlins with regular migrant songbird prey detections was higher compared to resident or irruptive songbird detections during the end of September through early October, which is a time of peak songbird migration activity in the Pacific Flyway (MacMynowski & Root 2007, Hampton 2010, Shipley et al. 2018). In 2015, our sampling season coincided with an irruptive year for cone-crop

dependent songbird species in California (Hampton 2019, National Audubon Society 2020), and we found that the proportion of juvenile Merlins with irruptive songbird migrant prey DNA detected was greater than in 2016. In contrast to the predictable seasonal movements of regular annual migrations, irruptive migrations are highly unpredictable from year-to-year and variable in magnitude due to the interaction of complex interannual climate variables, forest ecology, and songbird biology (Newton 2010, Strong et al. 2015). Ultimately, songbird irruptions cause a large pulse of seed-eating songbirds to move outside of their typical range in numbers often greater than the occurrence of typical migrants within a migration corridor (Newton 2010), and this connection to migrant raptor foraging ecology has yet to be explored.

In fall and winter, Merlins are known to be common predators of shorebirds (Charadriiformes; Cade 1982; Dekker 1988; Warkentin et al. 2005). Surprisingly, however, shorebird prey species were not detected in this study. We did not include shorebird DNA as controls in library preparation; however, we did successfully amplify shorebird DNA from museum tissue samples with the primers used during primer trials. Despite our findings, shorebirds are still an important prey species for migrant Merlins along the Pacific Flyway (Page and Whitacre 1975). The lack of shorebird detections in our study could be due to sampling almost exclusively juvenile Merlins that traverse through interior forests and woodlands more than experienced adults. This might be explained by juvenile Merlins being excluded from coastal estuaries and beaches by territorial adult Merlins and Peregrine Falcons. For example, Page and Whitacre (1975) described the hunting habitats of an adult female Merlin (with a diet specializing on shorebirds) while holding territory during fall and winter along the Pacific Coast and made note of active aggression towards other Merlins that entered her territory on the beach. It may also be possible that shorebirds are more difficult to capture compared to songbirds for

inexperienced juveniles, and young Merlins may be more efficient at hunting passerines (Dekker 1988; Cresswell 1996). Additionally, songbirds and dragonflies are more familiar prey items to juvenile Merlins, as this is largely the diet on the breeding grounds (Laing 1985; Warkentin et al. 2005) and could be a driver of our findings.

Like most raptors, Merlins exhibit reverse sexual size dimorphism (Warkentin et al. 2005). We found evidence of differential prey size selection between juvenile male and female Merlins sampled on migration. We detected the DNA of smaller prey species more frequently on male Merlins, and larger prey species, such as American Robins, more frequently on females. Only females were found to have prey DNA from the top three largest prey detected: Western Meadowlark *Sturnella neglecta*, Mourning Dove and Eurasian Collared-Dove. The adaptive advantages of prey partitioning during migration are not clear; established hypotheses regarding reverse-sexual size dimorphism in raptors focus on nest defense and sexual size partitioning of prey between mated pairs (Temeles 1985, Slagsvold & Sonerud 2007).

There are some important considerations for applying this diet study technique to migrating raptors. First, it is impossible to know the precise time and location a prey species was consumed, i.e. detections on a migrant raptor may not represent prey captured in the immediate vicinity of the sampling location. For example, we detected four species that do not typically range in the general region of sample collection (Rodewald 2015): Mountain Chickadee *Poecile gambeli* (n = 3), Mountain Bluebird *Sialia currucoides* (n = 5), Pine Grosbeak *Pinacola enucleator* (n = 3), and most notably Common Redpoll *Acanthis flammea* n = 1) with a closest occurrence of over 1400 km north of the sampling site at the time of sample collection (eBird 2017). One explanation is that eBird reports for Common Redpoll in fall of 2016 may have been under-reported in various regions along the migration corridor (Kosmala et al. 2016); however,

raptors migrating along the Pacific Coast of western North America reportedly travel upwards of 265 km/day (e.g. Broad-winged Hawk *Buteo platypterus*; Capitolo et al. 2020). Our results indicate that prey DNA may be detectable on raptors for several days *en route*, despite DNA degradation and removal due to individual behavior, UV degradation, or precipitation. It is currently unknown how long DNA can last on the exterior of raptor beaks and talons, only that DNA on these surfaces are related to prey consumed (Bourbour et al. 2019a). Second, it is impossible to know how many individuals of a single species were consumed by an individual raptor because we can only determine the frequency at which a species was detected among samples. This is because amplicon read counts are not correlated with number of prey items in a sample using this methodology (Deagle et al. 2013). This second consideration is especially important when sampling for dietary DNA from the exterior of beaks and talons, because the concentration of DNA is reliant on how recent and messy the feeding was and the unknown degree of DNA degradation. Third, prey detections and non-detections are limited by the target amplicon primers used and are an important consideration in study design (i.e. non-detections or false negatives should be interpreted with caution). For example, due to limited resources we did not use additional amplicon primers that would detect invertebrate prey DNA, despite dragonflies (Odonata) being an important resource for Merlins during migration (Nicoletti 1997, Warkentin et al. 2005).

Raptor migration monitoring has historically contributed to our understanding of large-scale ecological processes and population dynamics of North American raptors (Bildstein 1998, Bildstein et al. 2008). However, research that quantifies the full range of prey species that raptors rely on to fuel migration has been difficult to implement. In this study, samples collected from a raptor migration monitoring station combined with modern genetic techniques provided the

opportunity and ability to empirically study raptor diet during migration when birds are moving quickly over vast distances, across broad geographic areas, and when foraging cannot be observed (Bourbour et al. 2019a). An understanding of migrant raptor diet and prey selection can better inform full-life-cycle conservation (Gorney & Yom-Tov 1994, Yosef 1996, Klaassen et al. 2014, Marra et al. 2015). For top predators, and especially Merlins, diet is directly related to bioaccumulation of environmental toxins, such as organochlorines (Schick et al. 1987), lead (Chandler et al. 2004), and mercury (Bourbour et al. 2019b, Keyel et al. 2020). Detailed diet descriptions during migration can provide missing data that can help delineate the potential exposure pathways of anthropogenic environmental toxins across a migratory species' entire annual cycle. Application of the methods presented in this study has the potential to strengthen our understanding of the basic life history strategies in a migratory raptor's annual cycle and reveal complex species interactions that have previously remained enigmatic in migration ecology.

	Prey proportion ~ s(date) + migratory prey type		
September - December 2015	est. *df*	*F*-value	*P*-value
Date:Migrant (complete & partial)	8.778	8.334	<0.001
Date:Resident	7.856	4.179	<0.001
Date:Irruptive migrant	5.696	6.791	<0.05
September - December 2016	est. *df*	*F*-value	*P*-value
Date:Migrant (complete & partial)	8.842	16.950	<0.001
Date:Resident	8.519	11.633	<0.001
Date:Irruptive migrant	5.106	4.276	<0.001

Table 1: GAM model output with approximate statistical significance of smooth terms for 2015 and 2016 migration seasons analyzed separately. In this model, prey proportion for each prey species detected was the response variable, and sampling date and migratory tendency of prey type were explanatory variables. The smoothing term was applied to sampling date with k=10 and gamma=1. Across both migration seasons, there were differences detected between the proportions of each migratory prey type.

Photo caption: A Merlin (*Falco columbarius*) perched on a fence post, overlooking a flock of Savannah Sparrows (*Passerculus sandwichensis*) on the east side of the Coast Range of California, USA.

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Chapter 3

Combining eDNA and eBird data reveals predator-prey interactions occurring among *Accipiter* hawks and songbird communities during fall migration in the Pacific Flyway

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Abstract

During fall migration, communities of songbirds and raptors traverse thousands of kilometers together across the globe. While predator-prey relationships among bird-eating raptors and songbird communities are expected to influence survival and drive the evolution of migration strategies, these interactions are difficult to study in real-time and require novel approaches. We aimed to investigate the foraging ecology and strategies of migrating *Accipiter* hawks and test for associations of songbird traits with selection of prey species by a songbird predator specialist during fall migration. We obtained trace prey DNA by swabbing beaks and talons of migrating Sharp-shinned Hawks (*Accipiter striatus*; n=588) at a migration monitoring station along the Pacific Coast of California, USA during fall 2015 and 2016. Using eDNA metabarcoding, we obtained prey species detections from 94.1% of the hawks sampled (n=525) comprising 1396 prey items and 65 unique prey species. We used maximum likelihood discrete choice logistic models to test for prey choice during peak fall migration. The dataset consisted of weekly relative abundances of prey species within our study region obtained from eBird and categorized avian species traits. We revealed that relative size, flocking behavior, non-breeding habitat association, and migratory tendency influenced prey choice by migrating Sharp-shinned Hawks. Sharp-shinned Hawks have a high degree of reverse sexual size dimorphism. Male Sharp-shinned Hawks selected smaller prey species, migratory species, and species associated

with woodland and shrubland habitats more often than females. Theories about the evolution of reverse sexual size dimorphism revolve around productivity on the breeding grounds; though partitioning prey during migration does not directly influence productivity, it may influence survival along a migration route. We found correlations between diet composition and eBird relative abundance of these prey species over the migration season, which is consistent with the hypothesis that prey availability is an important predictor of *Accipiter* diet composition. Understanding prey selection by *Accipiter* hawks during migration is the first step in answering questions about the co-evolution of migration strategies and Sharp-shinned Hawk tracking of certain prey species as a plentiful resource en route.

Introduction

Every fall, billions of birds migrate thousands of kilometers from their breeding grounds to more favorable environments (Dokter et al. 2018). The wide-ranging movements of avian communities during fall make ecological interactions within migration corridors logistically difficult to study with any specificity. Despite this challenge, understanding how communities of predators and prey interact within migration systems is important because predator-prey interactions shape behaviors and migration strategies (Lank et al. 2003; Ydenberg et al. 2004; Hope et al. 2020; Sabal et al. 2021). For example, interactions between songbirds and *Accipiter* hawks drive co-evolution of anti-predator defenses in songbirds and daily hunting patterns in hawks (Roth and Lima 2003, 2006; Cimprich et al. 2005; Roth et al. 2006; Cresswell 2008; Lang et al. 2021). The challenge of collecting both raptor and songbird data has resulted in a lack of empirical studies that consider avian predators and prey in migration and behavioral ecology research (Lima 2002; Ydenberg et al. 2007).

Songbird species have evolved numerous foraging and migration strategies in response to avian predators that offer tradeoffs among feeding efficiency, vigilance, and habitat quality (Lindström 1989; Lind and Cresswell 2006; McCabe and Olsen 2015). Raptor migration is closely aligned with the movements of their avian prey in space and time (Lindström 1989; Aborn 1994). Synchronous migration with prey is a strategy that increases hunting opportunities for raptors along migratory routes (Duncan 1982; Kerlinger 1989; Culliney and Gardali 2011; DeLong et al. 2013), especially for *Accipiter* hawks that rely on powered flight and consistently hunt avian prey to meet energetic demands (Kerlinger 1989; Bildstein and Zalles 2005; Bildstein et al. 2020). The strong link between raptors and avian prey within migration systems creates opportunities to investigate enigmatic predator-prey interactions and describe the migrating food webs that exist around the world.

Understanding the co-evolution of migration strategies between songbirds and raptors requires novel approaches that extend beyond field observations and prey behavior data (Lima 2002). While documenting correlations between raptor and migratory prey abundances can guide hypotheses revolving around predator-prey interactions during migration (Aborn 1994; Nicoletti 1997), utilizing molecular and forensic techniques, specifically DNA barcodes (Kress et al. 2015), to study the foraging ecology of migrating raptors at migration monitoring stations has proven to be an effective method for collection of dietary data from predators on the move (DeLong et al. 2013; Bourbour et al. 2019, 2021; Pokharel 2020; Brouellette 2021). Despite the potential, DNA metabarcoding methods have remained underutilized in raptor ecology relative to other predator taxa and have only been employed in a few studies (e.g., Hopkins 2019; Nota et al. 2019; Bourbour et al. 2021; Brouellette 2021; Hacker et al. 2021; Kryshak et al. 2022). To date, using an environmental DNA (eDNA) metabarcoding approach at raptor migration banding

stations has proven to be the most effective method for obtaining robust dietary datasets during fall migration, with >85% of samples successfully capturing raptor diet information from migrating individuals (Bourbour et al. 2021).

To quantify prey selection by migrating *Accipiter* hawks, data on songbird prey availability must also be obtained and evaluated. Collecting substantial prey availability data from large geographic and temporal scales is another hurdle that requires a novel approach. Fortunately, citizen and community science interfaces, such as eBird and iNaturalist, have created opportunities to access public wildlife observation data collected over large spatial scales (Sullivan et al. 2009; Unger et al. 2021). The eBird database has proven to be a valuable and informative tool for research addressing biodiversity (Callaghan et al. 2017), conservation (Callaghan and Gawlik 2015; DeLuca et al. 2021; Michel et al. 2021), and population statuses (Clark 2017; Walker and Taylor 2017; Robinson et al. 2018; Neate-Clegg et al. 2020). Therefore, observations submitted to the eBird database within migration corridors provide an opportunity to harness relative abundance indices of avian species that are available to their co-occurring predators.

We investigated interactions between Sharp-shinned Hawks (*Accipiter striatus*) and a songbird community during fall migration along the Pacific Coast of North America by combining eDNA data collected from a long-term raptor migration monitoring station operated by community scientists in combination with eBird citizen science data. Our objectives were as follows: 1) Assess the composition of avian prey species in the diet of migrating Sharp-shinned Hawks. 2) Relate Sharp-shinned Hawk diet and avian prey abundance over several counties on the migration route to investigate prey choice. 3) Determine songbird traits and life-history

characteristics that influence predation during migration. 4) To examine temporal abundances of top songbird prey in relation to Sharp-shinned Hawk diet across the fall migration season.

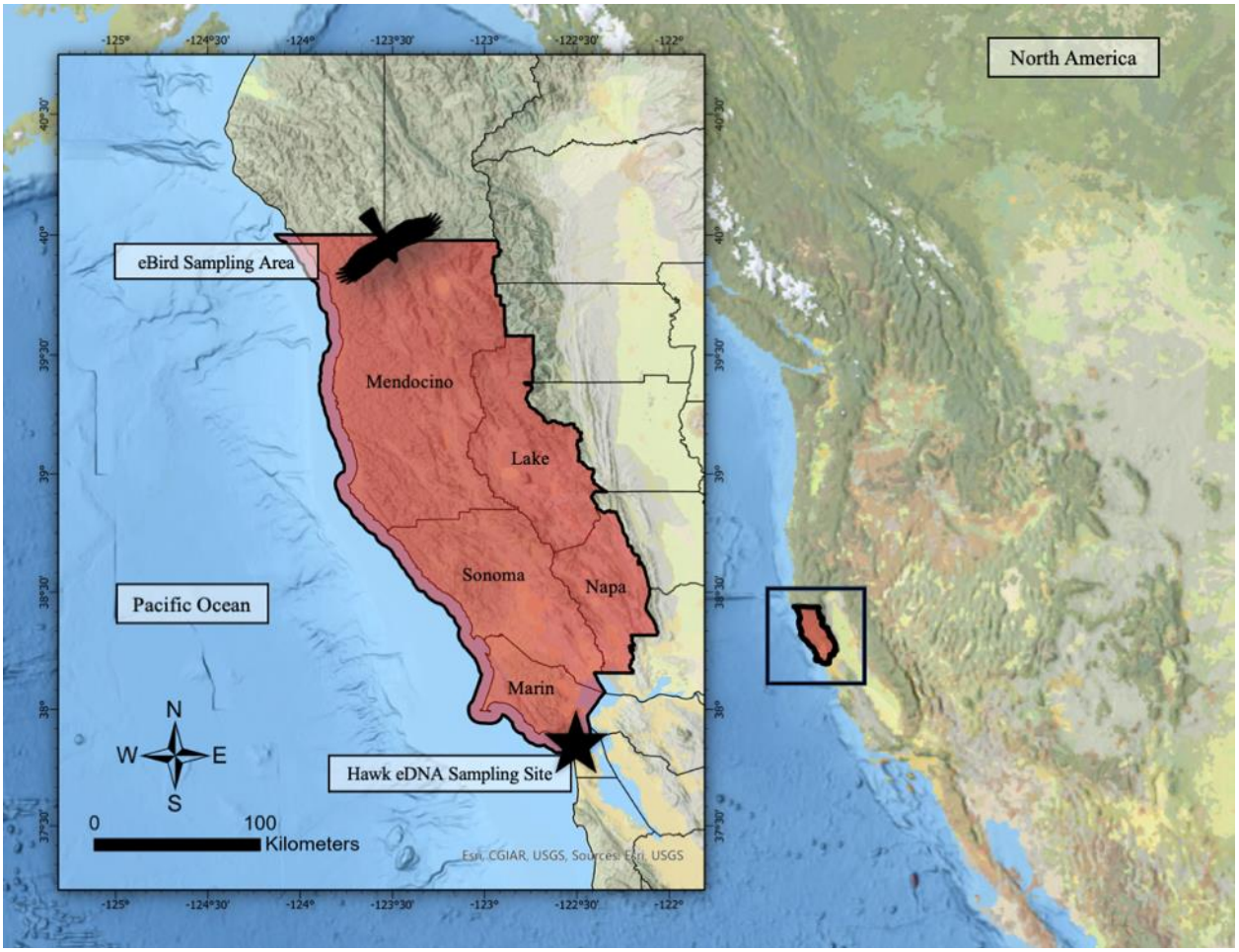

Figure 1: Study area map showing the eBird sampling area (outlined in black and shaded). We extracted eBird data from Marin, Sonoma, Napa, Lake, and Mendocino counties. The eDNA sampling site is where migrating Sharp-shinned Hawks had eDNA collected from beaks and talons. The sampling site is a long-term raptor migration monitoring site in the Marin Headlands operated by the Golden Gate Raptor Observatory. This map was made using ArcGIS Pro version 2.8.0 (Esri, Redlands, CA, USA).

Methods

Study Region

Our study region is along the northern coast of California, USA, comprising Marin, Sonoma, Napa, Lake, and Mendocino counties. This region is part of the Pacific Flyway and is

an important migration corridor for migrating hawks (Accipitriformes; Goodrich and Smith 2008) and songbirds (Passeriformes; MacMynowski and Root 2007). At the southern end of this region, the Marin Headlands facilitates the largest known raptor migration bottleneck along the Pacific Coast of North America, where migrating raptors converge and gain altitude before crossing the San Francisco Bay (Goodrich & Smith 2008).

Figure 2: A Sharp-shinned Hawk being sampled during the banding process. Predator sampling involved swabbing the exterior of beaks and talons, where prey eDNA would be collected on the swab tip.

We collected raptor diet data from a raptor migration monitoring station located in the Marin Headlands and operated by the Golden Gate National Parks Conservancy/Golden Gate Raptor Observatory in cooperation with the United States National Park Service (37.8262° N, 122.4997° W; Figure 1). We extracted eBird relative abundance data (Fink et al. 2020) from the defined study region to represent prey availability for hawks migrating south prior to reaching the sampling point; migrating hawks in California may migrate distances over 100 km in a day (Capitolo et al. 2020).

Predator Diet Sampling

We collected eDNA from the exterior surfaces of the beaks and talons of Sharp-shinned Hawks (n = 558) during fall migration in 2015 (n = 282) and 2016 (n = 276). Sampling effort was consistent from September 1 – December 15; however, Sharp-shinned Hawk migration

activity peaks at the end of September and abundance steadily decreases over October and November (Hull et al. 2012; Figure 3). We used sterile histobrushes (#25-2188 Puritan Medical Products Company, Maine, USA) to target visible prey blood, flesh, or feathers on the external surfaces of beaks and talons, and swabbing occurred even if beaks and talons appeared clean (Bourbour *et al.* 2019, 2021; Figure 2). Sharp-shinned Hawks were trapped in dho-ghazzas, mist-nets, or bownets using lure animals (Rock Doves *Columba livia*, European Starlings *Sturnus vulgarus*, and House Sparrows *Passer domesticus*; GGRO 2018). We categorized Sharp-shinned Hawk age as juvenile (hatch-year) or adult (after-hatch-year) by plumage, and sex as female or male according to wing chord (GGRO 2018). We conducted all aspects of this research in accordance with Institutional Animal Care and Use Committee (IACUC; permit #: CA_GOGA_Ely_Raptors_2020.A3), California Department of Fish & Wildlife (California State Permit #: SCP 13739), and United States Geological Service guidelines (federal bird banding permit #: 21827).

Prey DNA Extraction, Amplification, and Sequencing

We used QIAamp DNA Mini Kit (QIAGEN Inc.) to extract prey DNA from swab tips. We conducted lab work in a genetics lab that had not previously processed songbird DNA to minimize risk of contamination. We used primers COI-fsdF and COI-fsdR (González-Varo *et al.* 2014) to target a 464 base pair (bp) amplicon region of the cytochrome c oxidase subunit I (COI) gene. We modified the primers to have an overhang sequence that would anneal to indexed Illumina adapters (Illumina 2013, 2018; Chapter 3 Supplementary). We tested primers using avian tissue samples from the Museum of Wildlife & Fish Biology at UC Davis. We used Orange-crowned Warbler (*Vermivora celata*) and Swainson's Thrush (*Catharus ustulatus*) DNA

as positive controls alongside negative controls during library preparation to confirm detection of probable prey species. For negative controls in DNA extractions and library preparation, we used PCR-grade water to be used in the process of filtering out false positives that may arise during library preparation and sequencing.

We followed the two-step PCR amplification protocol outlined in Illumina (2013). We assessed a random subset of paired samples from Amplicon PCR and Index PCR on an Agilent Bioanalyzer 2100 to confirm that indexed adapters had been successfully attached in the Index PCR. After library preparation, we quantified DNA using Quant-iT PicoGreen dsDNA Reagent (Thermo Fisher Scientific) with an FLx800 Fluorescence Reader (BioTek Instruments). We then normalized each sample individually following Illumina (2013) protocols. We then sequenced the pooled library on two lanes using Illumina's MiSeq PE300 (v3) platform.

Reference Library and Bioinformatics

We compiled a custom reference library of birds (n = 205) that broadly range in the Pacific Flyway according to species account range maps (Billerman et al. 2020; see Supplementary Table 2). We used the R package *PrimerMiner-0.11* (Elbrecht & Leese 2017) to download all publicly available COI barcode sequences from NCBI and BOLD databases for each species and manually reformatted the datafiles to be compatible with the reference database format used by the R package *dada2* (Callahan et al. 2016).

We filtered out low quality scores (<30) and reads below 250 bp using the program *Cutadapt* (Martin 2011) and used the R package *dada2* to filter out samples with >2 erroneous base calls, remove chimeras, and merge forward and reverse reads. We matched all barcode sequences to our custom reference library with >99% bootstrap support using the

'assignTaxonomy' command in *dada2*. We removed prey species detections with <60 total assigned reads and used 0.5% as a conservative cutoff for rare sequences to account for false positives within a sample.

Prey abundance

To obtain an index of Sharp-shinned Hawk prey abundance, we extracted abundances of prey species detected in the diet from the eBird Status and Trends data from August – November using the R package *ebirdst* (Fink et al. 2020; Strimas-Mackey et al. 2021). Because prey DNA on beaks and talons represents diet from previous meals and may be detectable for multiple days for a migrating raptor (Bourbour et al. 2021), prey abundance data was extracted along the northern California Coast, including the counties of Marin, Napa, Sonoma, Lake, and Mendocino (Figure 1). We extracted weekly spatial concentrations of prey species occurring in the defined study area and summed weekly relative abundance values across pixel cells (2.96 km^2) where Sharp-shinned Hawks occurred within the defined study area.

Statistical analyses

We performed all statistical analyses using R version 4.1.0 (R Core Team 2021) in RStudio version 2022.2.3 (RStudio Team 2022). We excluded European Starling and House Sparrow detections from statistical analyses because we cannot confidently rule out contamination from the presence of lure animals at the sampling site as the cause of their detection. We calculated species accumulation curves with a 95% confidence interval using the R package *vegan* (Oksanen et al. 2020) to assess sampling effort (Figure 4). Because Sharp-shinned Hawks exhibit reverse sexual size dimorphism, we used a linear mixed effects model

with average prey mass (extracted from Tobias et al. 2022) as the dependent variable, sex as a categorical explanatory variable, and sample ID (individual hawk) as a random effect using the R packages *lme4* (Bates et al. 2015), *lmerTest* (Kuznetsova et al. 2017), and *afex* (Singmann et al. 2022), and visualized the model using the R packages *effects* (Fox and Weisberg 2019) and *ggplot2* (Wickham 2016).

Prey traits – We categorized each species detected according to relative size, non-breeding habitat association, trophic niche, and behavior. For prey size, we used average mass (Tobias et al. 2022) to classify each species as small (<30 g), medium (between 30 and 60 g), or large (>60 g). For non-breeding habitat, we used terminology from Soykan et al. (2016) along with non-breeding (migration and wintering) habitat information from Billerman et al. (2022) to classify habitat: Woodlands refer to species that are commonly associated with coniferous forest, deciduous forests, oak woodlands, or riparian forests. Shrublands refer to species commonly associated with scrub habitats or dense understory habitats. Grasslands refer to species that are commonly associated with savannahs and open habitats, such as agricultural fields and sandy habitats. Wetlands refers to species commonly associated with wetland and marsh habitats. Various refers to species that are commonly found in a variety of habitats from forested, urban, to open habitats. We grouped woodland and shrubland associated species because both habitat types may be utilized during fall migration in the study region by species associated with either habitat (CDFW 2014; Billerman et al. 2022). For non-breeding flocking behavior, we used Billerman et al. (2022) to classify species as gregarious if they were described as being highly social year-round with conspecifics, or as mixed-species flocking if the species are described as commonly joining and foraging with mixed-species-flocks during the non-breeding season. For

trophic niche, we used Tobias et al. (2022) to classify species as invertivores or non-invertivore (including nectarivore, frugivore, granivore, or omnivore).

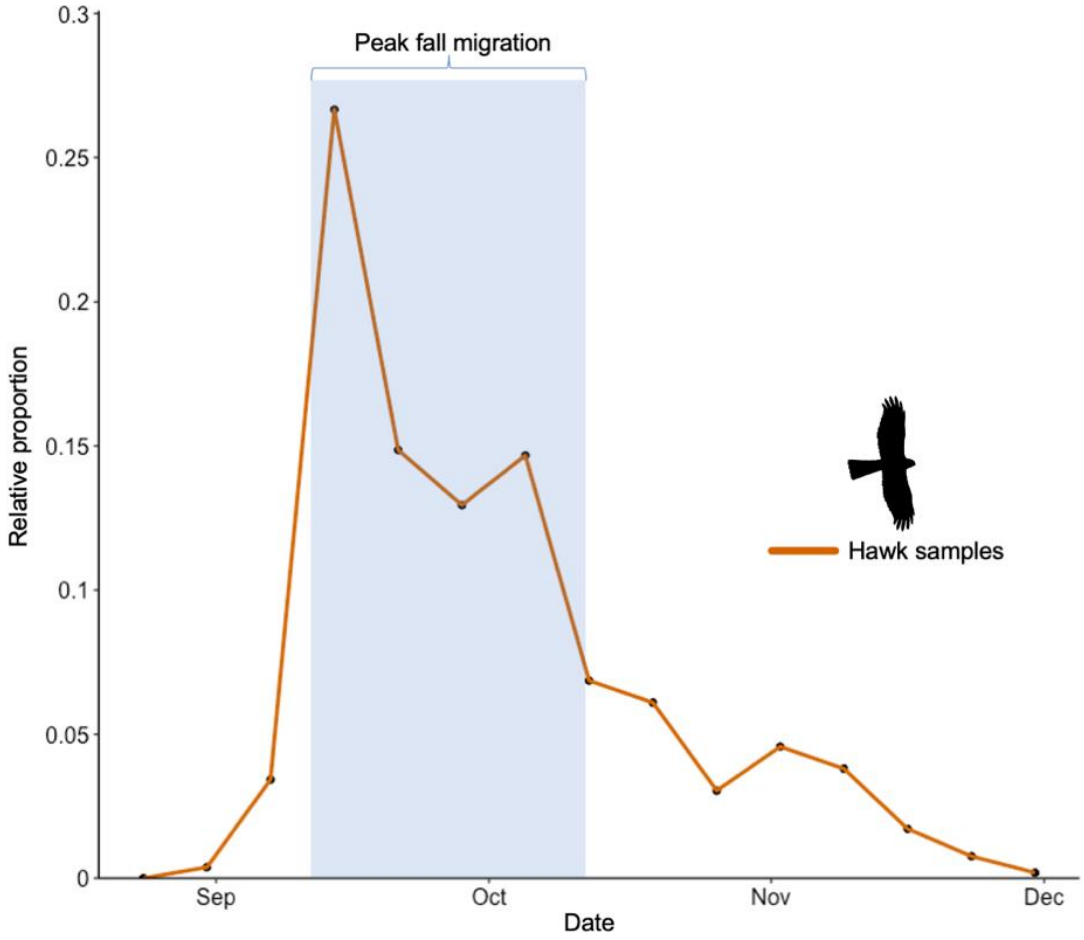

Figure 3: Weekly sample distribution of Sharp-shinned Hawks during fall migration. The peak movement along the California Coast of this migratory raptor species is highlighted in blue (September 14 – October 11).

Prey choice – We focused our analysis on peak migration. We modeled data between September 14 and October 11 (week 38 – week 41; Figure 3). This time window is ecologically significant as it captures peak migratory movement activity in the study region (Goodrich and Smith 2008; Hull et al. 2012). We fitted separate models for male and female hawks given distinct body size differences. We calculated multiple prey choices and alternate choices per individual hawk and

performed maximum likelihood multinomial logistic regressions (i.e., discrete choice models) using the R package *mlogit* (Croissant 2013). For both models, we computed the variance-covariance matrix of the parameters to account for repeated measures using the R package *sandwich* (Zeileis 2006; Zeileis et al. 2020). These models were used to predict the probability that a species was detected on the beaks and talons of the migrating predator as a function of species abundance and traits. We used prey choice as the dependent variable and included the following as explanatory variables: prey abundance (extracted from eBird Status and Trends Data), size (small, medium, large), non-breeding habitat association (woodlands/shrublands, other habitat), trophic niche (invertivore, non-invertivore), non-breeding behavior (mixed-flocking, gregarious).

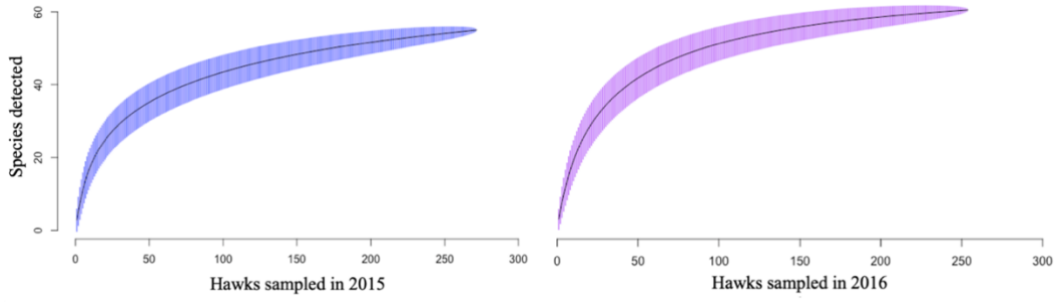

Figure 4: Prey species accumulation curves representing sampling effort in fall 2015 and 2016

To visualize the spatial and temporal co-occurrence of Sharp-shinned Hawks and their most preferred prey, we plotted the weekly relative abundances of the top five prey species selected by both males and females over time along with the weekly proportion detected in the diet. We used the scale function in R to normalize (*z*-score) both eBird relative abundances and proportions in diet.

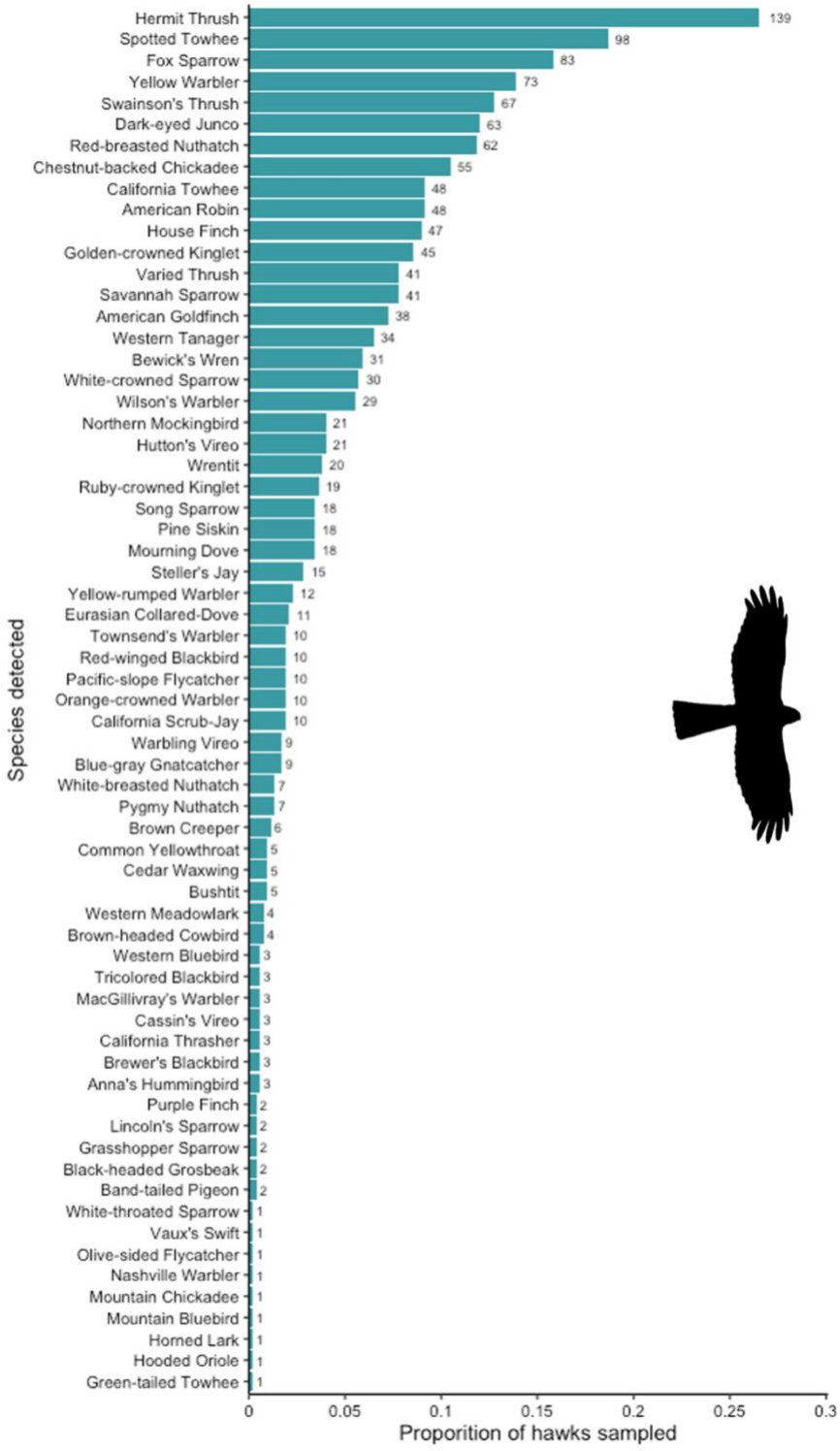

Figure 5: Summary of prey species detected (n=1396) on the beaks and talons of Sharp-shinned Hawks over two fall migration seasons. Bar chart represents the proportion of hawks (n=525) sampled with each prey species. Numbers at the terminal ends of each bar indicate the number of hawks with the prey DNA detected.

Results

We obtained 8.5 million raw reads with an average of 30,000 ± 28,000 SD per sample in the first lane, and 12 million total raw reads with an average of 45,000 ± 29,000 per sample in the second lane (Chapter 3 Supplementary). We found reference sequences available for 199 of the 205 (97%) species on our potential prey list. There are no published sequences available for *Ammospiza nelsoni*, *Cypseloides niger*, *Dryobates albolarvatus*, *Lanius borealis*, *Oreotyx pictus* or *Troglodytes pacificus*. After matching sequences to our custom reference library, the average sample had approximately 7700 reads in lane 1 and approximately 15000 reads in lane 2. The maximum abundance of reads in our negative controls was <0.5% of the number of reads in the average sample.

We obtained dietary data from 94.1% of the individuals we swabbed (n=525) in 2015 (n=272) and 2016 (n=253). Prey species accumulation curves for both sampling years showed that we obtained a good representation of species detections with our sampling method (Figure 4). Out of these samples, we detected 65 unique prey species, a total of 1396 prey species detections (754 prey in 2015 and 641 prey in 2016), and an average (mean ± sd) of 2.66 ± 1.4 prey species detections per individual hawk (Figure 5). Of the 525 individuals analyzed, males weighed an average (mean ± sd) of 99.7 ± 7.0 g (n=207) and females averaged 166.7 ± 13.0 g (n=318). Prey mass ranged from ~4 grams (Anna's Hummingbird, *Calypte anna*; n=2) to ~366 grams (Band-tailed Pigeon, *Patagioenas fasciata*; n=2). There was a significant relationship between prey mass and predator sex (LMM, $F_{1, 269.7} = 107.2$, $p < 0.001$), with males selecting smaller prey on average (mean ± se = 22.0 ± 1.5 g) compared to females (38.0 ± 1.0 g; Figure 6).

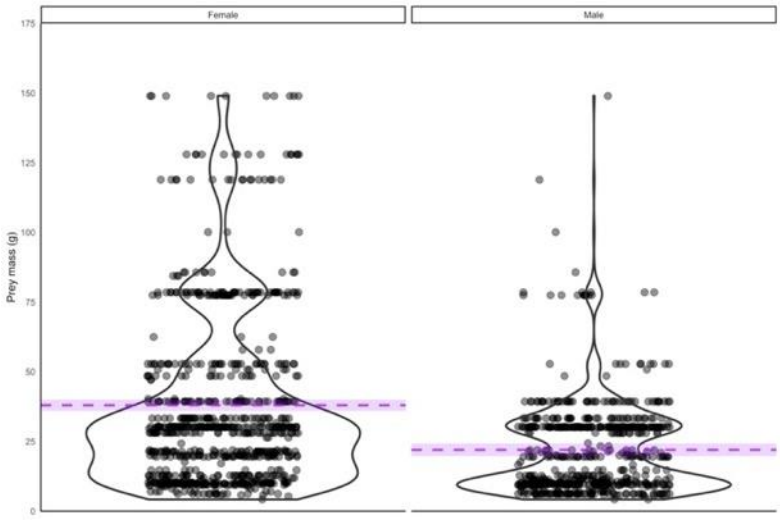

Figure 6: Prey size selection of female (left) and male (right) Sharp-shinned Hawks. The shaded purple dotted line represents the modeled mean with 95% confidence intervals. Band-tailed Pigeon (~366 g) detected on females hawks (n=2) are excluded from graph.

Discrete choice models revealed that eBird relative abundance was positively correlated with prey choice ($p < 0.001$; Table 1). Our results also indicate that medium-sized prey, mixed-species flocks, and non-aerial lifestyles significantly ($\alpha < 0.05$) influenced Sharp-shinned Hawk prey choice during peak fall migration (Table 1; Figure 7). Females were significantly less likely to choose invertivore prey, and males were more likely to choose migratory prey and species associated with woodland and shrubland habitats during the non-breeding season (Table 1; Figure 7).

After accounting for relative abundance and species traits in the discrete choice models, the top five selected prey species shared by both males and females were Spotted Towhee (*Pipilo maculatus*), Yellow Warbler (*Setophaga petechia*), Hermit Thrush (*Catharus guttatus*), Swainson's Thrush (*Catharus ustulatus*), and Fox Sparrow (*Passerella iliaca*; Figure 8). Relative abundance and proportion in diet were closely aligned over the migration season for each species (Figure 9).

Discussion

We combined eDNA metabarcoding techniques and eBird citizen science data in an effort to explore the predator-prey relationships in an avian community during fall, a novel approach for studying migratory predator-prey systems. We demonstrated that migrating Sharp-shinned Hawks interacted with a community of songbirds within a migration corridor, and found support for hypotheses predicting that Sharp-shinned Hawks focus hunting efforts on highly available prey to increase successful capture rates during active migration. Discrete choice models revealed that relative size, habitat association, flocking behavior, lifestyle, and trophic niche of songbirds influence prey selection during fall migration. Our results highlight differential prey preferences among the sexes, demonstrating that female and male Sharp-shinned Hawks are functionally different avian predators on the landscape during fall migration.

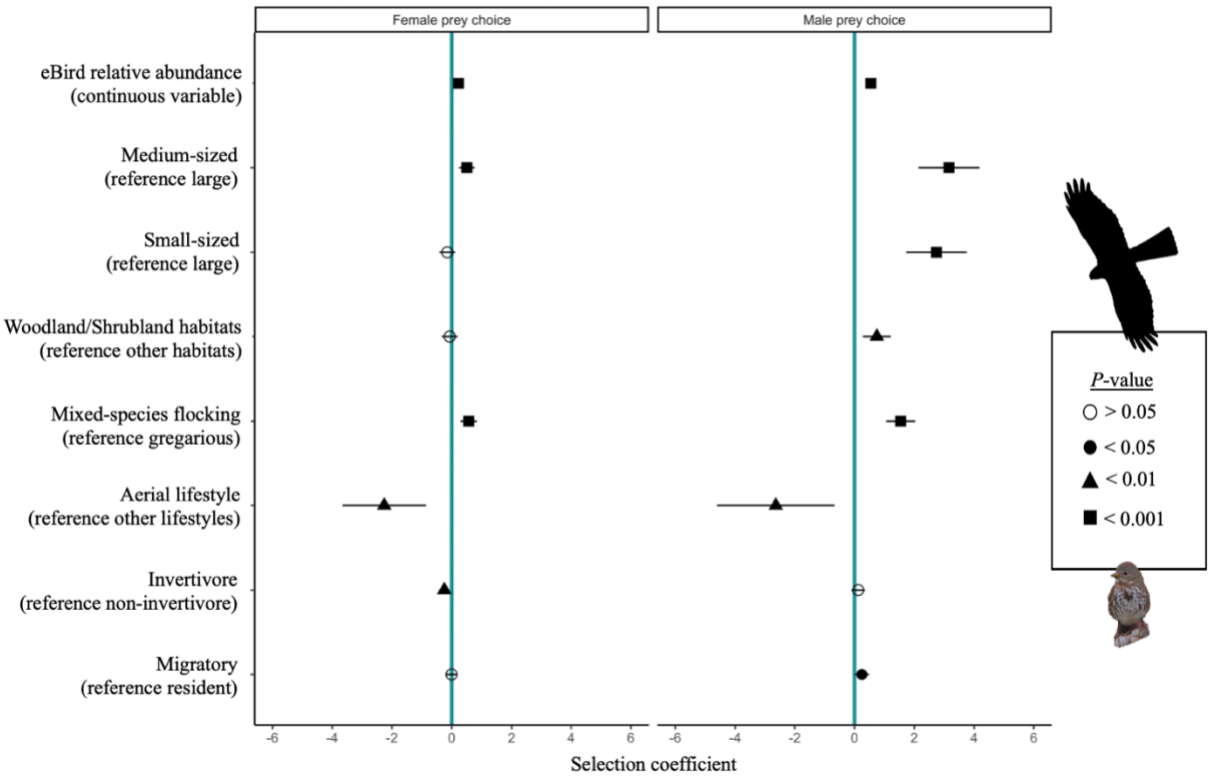

Figure 7: Discrete choice model results for prey selection. Points to the right of the solid line (0) represent choice of the trait, and points to the left represent choice of the reference trait.

We found that prey species relative abundance was positively correlated with selection by Sharp-shinned Hawks during fall migration. For the top selected prey species, songbird relative abundance and proportion in Sharp-shinned Hawk diet were correlated over the migration season. The feeding efficiency hypothesis suggests that Sharp-shinned Hawks, especially juvenile hawks on their first journey, likely follow abundant prey to increase hunting opportunities during migration (Rosenfield and Evans 1980; Kerlinger 1989). Migration coupling or tracking of migrant prey (Furey et al. 2018) is a conceivable strategy for an ambush predator that stores relatively little fat (DeLong and Hoffman 2004), relies more on powered flight than soaring (Bildstein et al. 2020), and for inexperienced juveniles (Rosenfield and Evans 1980). Whether Sharp-shinned Hawks time their migration or select routes based on the migratory activities of specific avian taxa requires further investigation.

Many of the most frequently selected species in this study display solitary behaviors at certain stages in their annual cycle, but are described as short-term followers of mixed-species flocks during migration (Billerman et al. 2020). Joining mixed-species flocks during migration is a foraging strategy that allows an individual to reduce the energy needed for vigilance while increasing foraging efficiency (Morse 1977; Herrera 1979). Hermit Thrushes, Swainson's Thrushes, Spotted Towhees, and Yellow Warblers are often found foraging in mixed-species flocks during fall migration (Dellinger et al. 2020; Mack and Yong 2020; Bartos Smith and Greenlaw 2020; Lowther et al. 2020), a strategy that facilitates refueling at stopover sites but may increase the chances of attracting Sharp-shinned Hawks hunting to refuel for the next leg of their migration. *Accipiter* hawks are considered a major evolutionary driver of songbird mixed-species flocking behavior and foraging during the non-breeding season (Gaddis 1980; Lindström 1989; Roth et al. 2006; Roth and Lima 2003, 2007). Most behavioral ecology research has

focused on songbird anti-predator defenses and responses to the activity of *Accipiter* hawks in a single location (Lima 2002; Roth et al. 2006). Our study contributes to the predators' perspective by providing data on which songbirds are likely selected as prey from among those in mixed-species flock under attack.

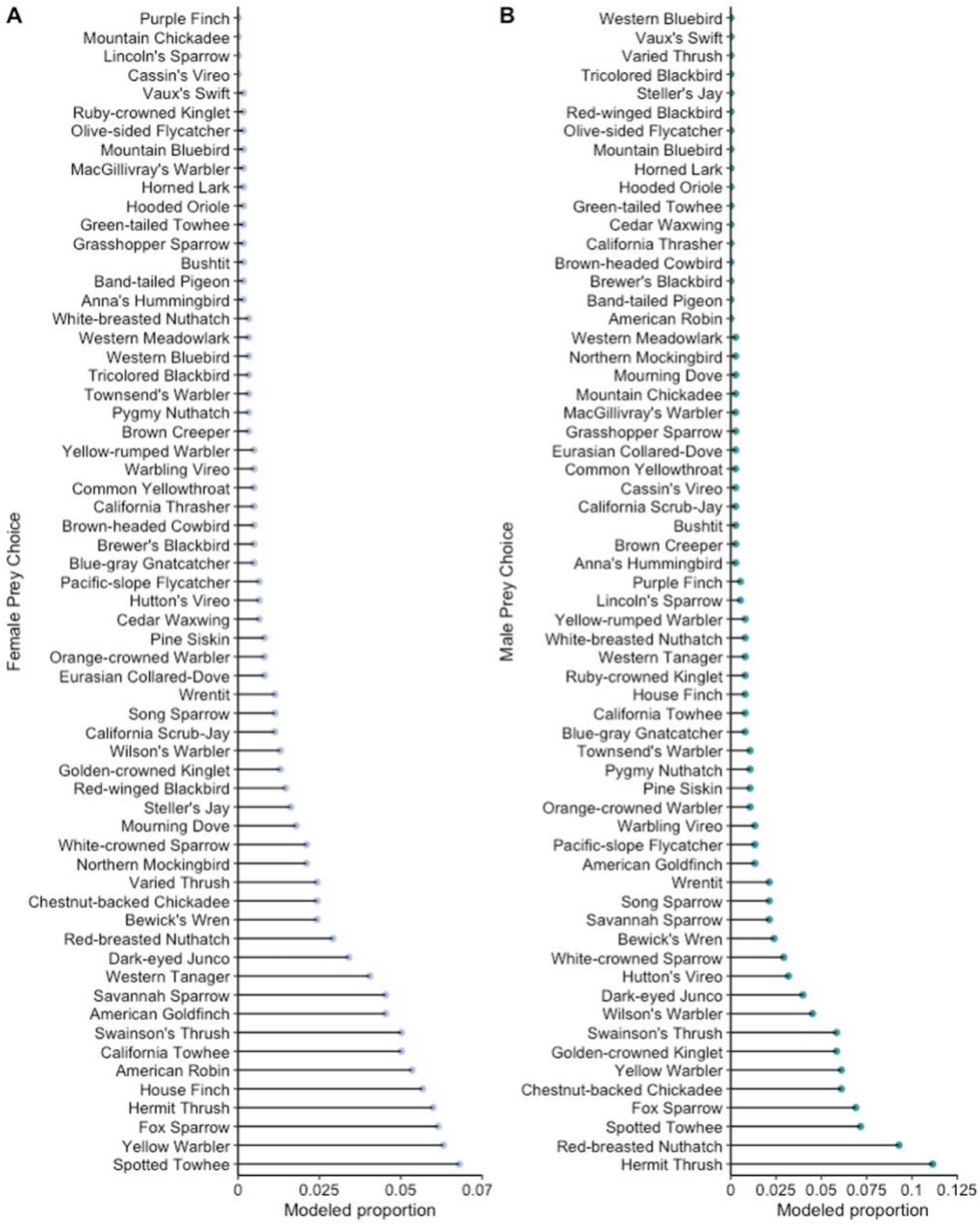

Figure 8: Modeled proportions from multinomial discrete choice models for females (A) and males (B).

Female and male Sharp-shinned Hawks exhibit differential migration in California and western North America (DeLong and Hoffman 1999; Hull et al. 2012). There are various theories discussing the evolutionary causes that may lead to differential migration between the sexes, from males leaving later to occupy breeding territories longer, to females leaving earlier because they are inefficient hunters that need to migrate with more available prey towards the end of summer (Rosenfield and Evans 1980; Kerlinger 1989; Mueller et al. 2000). In western North America, female hawks migrate earlier in fall (Hull et al. 2012). In our study, we found females had a top preference for Yellow Warblers, which are considered early migrants with peak migratory movements in August through mid-September (Witynski and Bonter 2018; Lowther et al. 2020). Male hawks migrate later than females, and had a preference for Hermit Thrushes, which are considered late fall migrants with an extended migration season that peaks in October (Mills 2005; Dellinger et al. 2020). These differences in prey preference and correlations with the movement patterns of certain migrant songbirds highlight that the evolution of differential migration in *Accipiter* hawks has cascading effects in the food web.

In this study, both males and females selected small and medium sized prey, but males selected small prey more often. A similar finding of differential prey size selection was found for Sharp-shinned Hawks migrating through New Mexico (DeLong et al. 2013) and juvenile Merlins (*Falco columbarius*) migrating along the California Coast (Bourbour et al. 2021). *Accipiter* hawks exhibit a high degree of reverse sexual size dimorphism among raptors, and Sharp-shinned Hawks display the highest degree of sexual size difference among all *Accipiter* hawks (Meyer 1987). Various theories have been posed about the ultimate cause of reverse sexual size dimorphism, and most are related to productivity on the breeding grounds (Wheeler and Greenwood 1983; Krüger 2005; Pérez-Camacho et al. 2015). Partitioning prey during migration

does not directly influence productivity but may be advantageous by influencing survival along a migration route, especially if each sex hunts different prey more successfully in certain habitats (Rosenfield and Evans 1980; Meyer 1987).

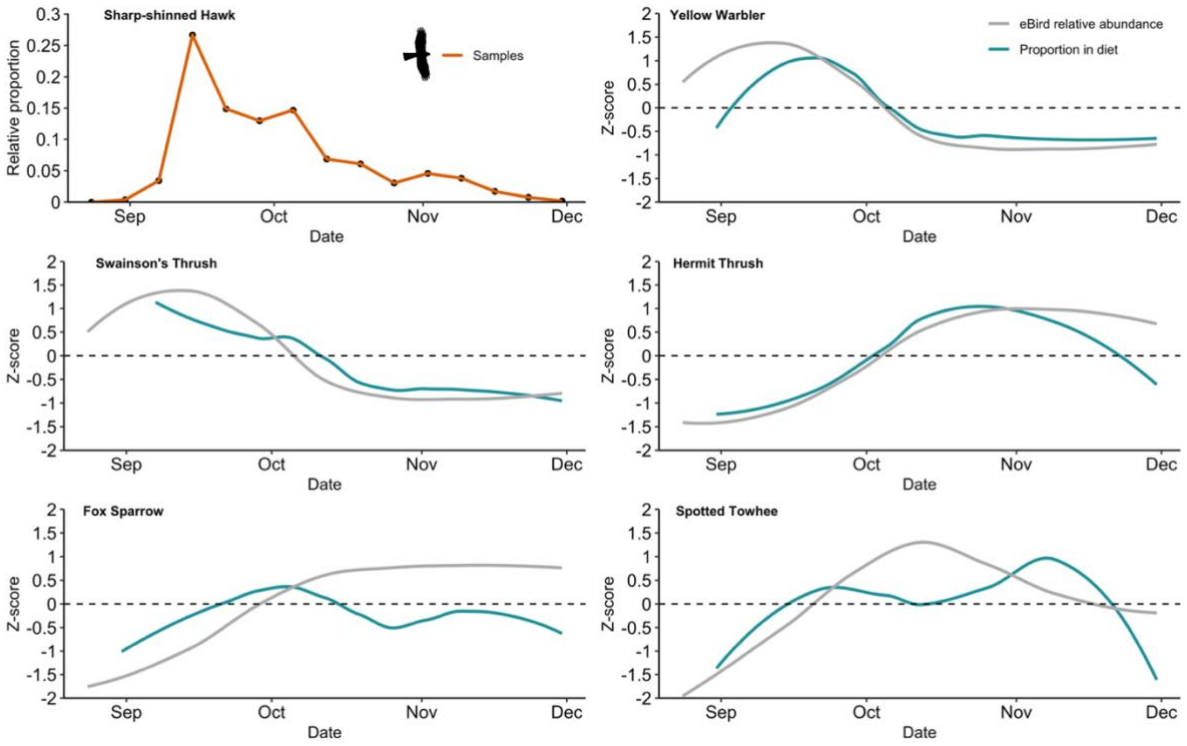

Figure 9: The top 5 prey species shared by both male and female Sharp-shinned Hawks. Each plot shows standardized proportion in diet and relative abundance on the landscape within the study area over the fall migration season. The top left panel shows distribution of hawks sampled. Peak Sharp-shinned Hawk migration movement occurs between September 14th and October 11th.

Male Sharp-shinned Hawk body size is, on average, 60% that of females, and their preference for smaller prey could influence their preference for hunting in more dense habitats. We found that male Sharp-shinned Hawks select prey associated with woodland, forested, and shrubland habitats more than females during fall migration. Differential habitat use among the sexes has been previously described for Sharp-shinned Hawks (Bildstein et al. 2020). In the non-breeding season, females have been found using open areas and human-dominated areas more

often compared to males (Clark 1985; Meyer 1987). Along the Central Coast of California within our study region, males were the majority of all mist-net captures of Sharp-shinned Hawks at a songbird banding station in shrubland and forested riparian habitat (Culliney and Gardali 2011). Understanding habitat use during migration is necessary for conservation and management planning (Bayly et al. 2018), especially when males and females of the same species exhibit differential use of habitats within the same migration corridor.

Migratory birds spend a major portion of their annual cycle on active migration, and we currently know very little about the interactions occurring within migrating avian food webs. While this study provides data to explore theories and hypotheses about predator-prey interactions within a migration corridor, it also confirms that migrating *Accipiter* hawks rely on the availability of specific prey species in specific habitats. Raptors are ecologically important, necessitating an understanding of their year-round dietary and habitat needs (Sergio et al. 2005; Sergio et al. 2006). Continued utilization of new technologies to address data gaps for migratory birds is essential for conservation in the 21st century (Greenberg and Marra 2005). Allocating resources to study complex ecological interactions has the potential to further our knowledge about the co-evolution of migration strategies and predator-prey dynamics, but most importantly it will allow us to develop baseline information about the critical resources that migratory birds require (Marra et al. 2015). This study demonstrated that the logistical challenges of documenting predator diet and prey availability within a migration corridor can be alleviated through the use of forensic techniques at established migration monitoring stations and big data generated by citizen science platforms. Taken together, this research has provided a framework for revealing the ecological and evolutionary stories that have remained elusive in migratory systems around the world.

Variables	Estimate	se	t	*p*-value
Female prey choice				
eBird relative abundance	0.225	0.031	7.347	**<0.001**
Medium sized	0.504	0.132	3.822	**<0.001**
Small sized	-0.150	0.140	-1.065	0.287
Woodland/Shrubland habitat	-0.069	0.145	-0.476	0.634
Mixed-species flocks	0.565	0.151	3.751	**0.000**
Aerial lifestyle	-2.257	0.712	-3.171	**0.002**
Invertivore	-0.250	0.094	-2.652	**0.008**
Migratory	-0.008	0.090	-0.086	0.931
Male prey choice				
eBird relative abundance	0.546	0.052	10.574	**<0.001**
Medium sized	3.157	0.553	5.711	**<0.001**
Small sized	2.738	0.544	5.033	**<0.001**
Woodland/Shrubland habitat	0.748	0.268	2.791	**0.006**
Mixed-species flocks	1.543	0.271	5.686	**<0.001**
Aerial lifestyle	-2.636	1.015	-2.597	**0.010**
Invertivore	0.124	0.118	1.052	0.293
Migratory	0.249	0.120	2.077	**0.038**

Table 1: Model summaries of multinomial logistic models. Reference levels for each variable are as follows: Medium – large, small – large, Woodland/Shrubland habitat – other habitats, Mixed-species flocks – gregarious flocks, Aerial lifestyle – other lifestyles, Invertivores – non-invertivores, Migratory – resident/sedentary. Bolded p-values are statistically significant at the $\alpha < 0.05$ level.

Photo caption: A Sharp-shinned Hawk hunting the edge of a farm field during fall in the Coast Range of California, USA.

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Chapter 1 Supplementary Materials:

Sample ID	Raptor	Recovered COI Sequence	Prey	BLAST Accession #
2015-217	SSHA	CGGCAGACTGGGCCACCTGGCGCCCTTCTAGGAGACGACCAAGTCTAT AACGTAGTCGTCACAGCTCATGCTTTCGTAATAATCTTCTTTATAGTTA TGCCAATCATAATCGGAGGATTCGGAAACTGACTAGTTCCCCTAATAA TTGGAGCCCCGGACATAGCATTCCCACGAATAAACAACATAAGCTTCT GACTTCTTCCCCCATCCTTCCTACTCCTCTTAGCATCCTCTACTGTTGAA GCAGGCGTCGGAACAGGCTGAACAGTGTACCCCCCACTAGCAGGCAA CCTGGCCCACGCCGGAGCCTCAGTCGATCTTGCAATCTTCTCCCTACAC CTAGCCGGTATCTCTTCAATCCTAGGAGCAATCAACTTCATTACAACA GCAAATTAATATGAAACCACCTGCCTTATCACTTTAACCATA	*Passerella iliaca*	HM033630.1
2015-184	SSHA	AGGCCACCCGGCGCCCTTCTGGGAGACGACCAAGTCTACAACGTAGTC GTCACGGCCCATGCCTTCGTAATAATTTTCTTTATAGTTATGCCAATTA TGATCGGAGGATTCGGAAACTGACTAGTCCCACTAATAATCGGAGCCC CAGACATAGCATTCCCACGAATAAACAACATAAGCTTCTGACTACTCC CACCATCATTCCTTCTTCTCCTAGCATCCTCCACAGTAGAAGCCGGAGC AGGAACAGGATGAACCGTGTACCCCCCACTAGCTGGCAACCTAGCCCA TGCCGGAGCTTCAGTCGACCTTGCTATTTTCTCTTTACACTTAGCCGGA ATTTCCTCAATCCTAGGGGCGATCAACTTCATTACTACAGCAATTAACA TGAAACCACCTGCCCTATCA	*Setophaga townsendi*	HM033442.1
2015-192	SSHA	CCGGCAGACTAGGCCACCATGCAGCCCTCTCTGGGAGACGACCAAGTC TATAACGTAATCGTCACGGCCCATGCTTTCGTAATAATCTTTTTTATAG TTATGCCAATTATAATCGGAGGATTCGGAAACTGACTAGTTCCCCTAA TAATTGGAGCCCCGGACATAGCATTCCCACGAATAAACAATATAAGCT TCTGACTACTACCCCCATCCTTCCTACTCCTCCTAGCATCCTCTACTG	*Passerella iliaca*	JN850753.1

2015-010	SSHA	CAGCCCCTAGCCTTCTCTCCGGCAGACTAGGTCAACCCGGAGCCCTCC TAGGAGACGACCAAGTCTACAACGTAATCGTCACGGCCCATGCTTTCG TTATAATCTTCTTCATAGTTATACCCATCATAATCGGAGGATTCGGAAA CTGACTAGTTCCTCTAATGATCGGAGCCCCAGACATAGCATTCCCACG AATAAATAACATAAGCTTCTGACTACTTCCCCCATCATTCCTCCTCCTA CTAGCATCTTCCACCGTAGAAGCAGGTGTTGGTACAGGCTGAACAGTA TACCCTCCACTAGCTGGTAACCTAGCTCATGCCGGAGCTTCAGTTGACT TAGCAATTTTCTCCCTACACTTAGCCGGTATCTCTTCAATCCTAGGCGC AATCAACTTCATTACAACAGCAATCAATATAAAACCACCTGCCCTATC CTT	*Spinus tristis*	FJ236301.1
2015-107	SSHA	GGGAAACCCTGGGAGCCCCTTCCTAGGCAGACGACCAAGTATTAAAAT GTAGTCGTCACTGCCCATGCTTTCGTAATAATCTTCTTTATAGTTATGC CAATTATGATCGGAGGGTTTGGAAACTGACTAGTCCCCCTAATAATTG GAGCCCCAGACATAGCATTCCCACGAATAAATAATATGAGCTTCTGAC TGCTACCCCCATCCTTCCTGCTACTCCTGGCATCCTCCACCGTAGAATC AGGAGCAGGAACAGGCTGAACCGTGTACCCACCTCTAGCCGGCAACCT AGCCCACGCTGGAGCTTCCGTAGACCTAGCCATCTTTTCCCTGCATCTA GCTGGCATTTCTTCCATCCTAGGAGCCATTAACTTTATTACAACAGCAA TTAACATAAAACCACCTGCCCTATCCTT	*Toxostoma redivivum*	JN806017.1

2015-271	SSHA	CCATGCACCTAGCCTCCTCTCCGGCCGAACTAGGCCACCCGGAGCCCT CCTGGGAGACGACCAAGTTTACAACGTAGTCGTCACGGCCCATGCTTT CGTGATAATCTTCTTCATAGTTATACCTATTATAATCGGAGGATTTGGA AACTGACTAGTCCCCCTAATAATCGGAGCCCCAGACATAGCATTCCCA CGAATAAACAACATAAGCTTCTGACTACTTCCCCCATCCTTCCTACTCC TCCTAGCATCTTCTACCATTGAAGCAGGTGTCGGCACAGGCTGAACAG TATACCCCCCACTTGCCGGCAACCTAGCACACGCTGGAGCCTCAGTCG ATCTCGCAATTTTCTCTCTACACCTAGCCGGTATCTCTTCAATCCTAGG AGCAATCAACTTCATCACAACAGCAGTCAACATGAAACCACCTGCCCT ATCACTTAAGCATA	*Melozone crissalis*	DQ433097.1
2015-101	SSHA	CCTGCTCTAGCCTTCTCTTCCGGCAGACTAGGCCACCCGGCGCACCTAC CTAGGGAGACGACCAAATCTACAATGTAGTTGTCACCGCCCATGCCTT CGTAATGATTTTCTTTATAGTTATGCCAATCATGATTGGGGGGTTCGGA AACTGGCTAGTCCCATTAATAATCGGAGCCCCAGACATAGCATTCCCC CGAATAAACAACATAAGCTTCTGACTTCTCCCACCATCATTCCTCCTTC TCCTAGCCTCCTCCACAGTAGAAGCAGGAGCAGGAACAGGATGAACC GTGTACCCACCCCTAGCTGGCAACCTAGCACACGCAGGAGCTTCAGTC GACCTAGCTATTTTCTCCCTACACTTAGCAGGAATCTCCTCAATCCTAG GGGCCATCAACTTCATTACTACAGCAATCAACATAAAACCACCTGCCC TATCAATAA	*Catharus ustulatus*	HM033291.1
2015-171	SSHA	CAGCACCTAGTCCACTTTTCCGGCAGACTAGGCCACCAGGCGCCCTCT TGGGAGACGACCAAGTATATAACGTAATCGTCACGGCCCATGCTTTCG TAATAATCTTTTTTATAGTTATGCCAATTATGATTGGAGGATTTGGAAA CTGACTAGTTCCTCTAATAATTGGAGCACCTGACATAGCATTCCCACG AATGAACAACATAAGCTTCTGACTTCTACCCCCATCCTTTCTTCTCCTA CTAGCCTCCTCTACAGTAGAGGCCGGAGTAGGAACAGGATGAACTGTG TATCCTCCCCTGGCTGGTAATTTAGCTCACGCCGGGGCGTCAGTTGATT TAGCAATTTTCTCCCTACATCTAGCAGGAATTTCATCTATCCTAGGGGC AATCAATTTCATTACCACTGCAATTAACATAAAACCACCTGCCCTATCA ATTAGCATA	*Sitta canadensis*	DQ434090.1

2015-073	SSHA	CTAGGCCACCAGGCGCCCTCTTGGGAGACGACCAAATTACAACGTAAT CGTCACGGCCCATGCTTTCGTAATAATCTTTTTTATAGTTATGCCAATT ATGATCGGAGGATTCGGAAACTGACTAGTTCCTCTAATAATTGGAGCC CCTGACATAGCATTCCCACGAATGAACAACATAAGCTTCTGACTTCTA CCCCCATCCTTTCTTCTTCTACTAGCCTCCTCCACAGTAGAGGCAGGAG CAGGAACAGGATGAACTGTGACCCTCCCCCTGCTGGCAACCTTACCCA CGCCCGAGCCGCCGTATACCTGGCTATCTTTTTCCTTACATTAACCAGG ATTTTCTCATATCTAAGGGCCATCAACTTCATTTCCCCCTGAATTAAAA TAAAAACACCTGGCCTTTTACC	*Sitta canadensis*	JN850725.1
2015-100	SSHA	AGCCCTCCTAGGAGACGACCAAGTCTATAACGTAGTCGTCACAGCCCA CGCCTTCGTAATAATCTTCTTCATAGTTATACCAATTATAATCGGAGGA TTTGGAAACTGACTAGTTCCACTAATAATCGGAGCCCCGGACATAGCA TTCCCGCGAATAAATAACATAAGTTTTTGACTACTCCCCCCATCCTTTC TCCTCCTCCTAGCATCCTCTACCATTGAAGCAGGTGTCGGCACAGGCTG AACAGTATACCCCCCACTAGCAGGCAACCTAGCCCACGCTGGAGCCTC AGTCGACCTCGCAATCTTCTCCCTACACTTAGCCGGCATCTCCTCAATC CTAGGGGCCATCAACTTCATCACAACAGCAATCAACATAAAACCACCT G	*Junco hyemalis*	KX461113.1
2015-228	MERL	GTAGTCGTCACCGCCCACGCTTTTGTCATAATCTTCTTCATAGTAATGC CAATCATAATCGGGGGGTTCGGAAACTGACTGGTGCCCTTAATAATTG GAGCTCCAGACATAGCCTTCCCCCGAATAAACAACATGAGCTTCTGAC TACTTCCCCCATCCTTCCTACTACTATTAGCCTCCTCCACAGTAGAAGC AGGGGCGGGAACAGGATGAACCGTCTATCCCCCCCTAGCCGGAAACCT AGCACATGCAGGAGCCTCAGTGGACCTAGCTATTTTTTCCCTACACCTA GCAGGGATTTCCTCAATCCTGGGGGCTATCAACTTCATTACTACAGCA ATTAACATAAAACCACCTGCCCTATC	*Ixoreus naevius*	JN850755.1

2015-237B	MERL	GGAGATGACCAAGTTTACAACGTAGTTGTCACAGCCCATGCTTTCGTG ATAATCTTCTTTATAGTTATGCCAATTATAATTGGGGGATTCGGAAACT GACTAGTCCCACTGATAATTGGAGCACCAGACATAGCATTCCCACGAA TAAACAACATAAGCTTCTGACTGCTACCCCCATCCTTCCTCCTGCTACT AGCATCCTCCACCGTAGAAGCGGGGGCCGGCACCGGATGAACAGGAT ACCCCCCTCTAGCCGGCAACCTGGCCCACGCCGGAGCCTCAGTAGACC TAGCAATCTTCTCCCTGCACTTAGCAGGGATTTCTTCAATCTTAGGGGC AATCAACTTTATTACAACAGGAATCAACATAAAACCACCTGCCCTAT	*Passer domesticus*	KM078784.1
2015-237T	MERL	TCTACACCTAGTCTTACTTATCTGATCAATACTATGCAACCTGCGCTCT CTTGGGAGACGACCTATACCTACTACTTTCGTAATAATCTTTTTTATAG TTATGCCAATTATGATTGGAGGATTTGGAAACTGACTAGTTCCTCTAAT AATTGGAGCACCTGACATAGCATTCCCACGAATGAATAATATAAGCTT CTGACTTCTACCCCCATCCTTTCTTCTTCTACTAGCCTCCTCTACAGTAG AGGCCGGAGTGGGAACAGGATGAACTGTGTATCCCCCCCTGGCTGGTA ATTTAGCCCACGCCGGGGCGTCAGTTGATTTAGCAATTTTCTCCCTACA TCTAGCAGGAATTTCATCTATCCTAGGAGCAATCAATTTCATTACCACT GCAATTAACATAAAACCACCTGCCCTATCA	*Sitta canadensis*	HM033802.1
2015-034B	MERL	ACGGCCCATGCCTTCGTAATAATCTTCTTTATAGTTATGCCCATTATGA TCGGAGGGTTCGGAAACTGACTAGTCCCACTGATAATCGGAGCCCCAG ACATAGCATTCCCACGAATAAACAACATAAGCTTCTGACTACTTCCCC CATCCTTCCTTCTACTCCTAGCATCCTCTACCGTAGAAGCAGGGGTTGG ACAGGATGAACAGTATACCCCCCACTAGCTGGTAACTTAACCCATGCC GGAGCCTCAGTTGACTTAACAATCTTCTCCCTACACCTAGCTGGTATCT CTTCAATCCTAGGAGCAATTAACTTTATTACCACAGGAATCAATATAA AACCACCTG	*Haemorhous mexicanus*	JN850723.1

2015-034T	MERL	ACGACCAAGTATATAACGTAATCGTCACGGCCCATGCTTTCGTAATAA TCTTTTTTATAGTTATGCCCATTATGATTGGAGGATTTGGAAACTGACT AGTTCCTCTAATAATTGGAGCACCTGACATAGCATTCCCACGAATGAA TAACATAAGCTTCTGACTTCTACCCCCATCCTTCCTTCTTCTACTAGCCT CCTCTACAGTAGAGGCCGGAGTAGGAACAGGATGAACTGTGTATCCTC CCCTGGCTGGTAATTTAGCTCACGCCGGGGCGTCAGTTGATTTAGCAA TTTTCTCCCTACATCTAGCAGGAATTTCATCTATCCTAGGAGCAATCAA TTTCATTACCACTGCAATTAACATAAAA	*Sitta canadensis*	KJ467141.1
2015-055B	MERL	AACGTAGTCGTCACGGCCCATGCTTTCGTAATAATTTTCTTTATAGTTA TGCCCATCATAATTGGAGGATTCGGAAACTGACTAGTCCCTCTGATAA TCGGAGCCCCAGACATAGCATTCCCACGAATAAACAACATAAGCTTCT GACTACTCCCACCATCGTTCCTTCTCCTTCTAGCGCCCTCCACGGTTGA AGCAGGAGTAGGTACAGGCTGAACAGTGTACCCCCCACTAGCCGGTA ACCTGGCCCACGCCGGAGCCTCAGTCGACCTCGCAATCTTCTCTCTACA CCTAGCCGGTATTTCCTCAATCCTAGCGCAATCAACTTCATTACAACAG CAATTAACATGAAACCACCTGCCCTATCA	*Setophaga petechia*	JN850722.1
2015-055T	MERL	CGTCACGGCCCATGCTTTCGTAATAATTTTCTTTATAGTTATACCCATC ATAATTGGAGGATTCGGAAACTGACTAGTTCCCCTAATAATCGGAGCC CCAGACATAGCATTCCACGATAAACAACATAAGCTTCTGACTACTCCC ACCATCGTCCTTTCCTTTAGCGCCTCCACGGTTGAAGCGGAGTAGGTAC AGGCTGAACAGTGACCCCCCACTAGCCGGAACCTGGCCCACGCCGGA GCCTCAGTCGACCTGGCAATCTTCTCTCTACACCTAGCCGGTATTTCCT CAATCCTAGGAGCAATCAACTTCATTACAACAGCAATTAACATGAAAC CACCTGCCCTATC	*Setophaga petechia*	JN850722.1

2015-334B	MERL	GCTCTCCTAGGTGACGACCAAATCTACAACGTGGTTGTCACCGCCCAT GCTTTCGTAATAATCTTCTTCATAGTTATACCAATTATGATCGGAGGGT TCGGAAACTGACTAGTCCCCCTAATAATCGGAGCCCCAGACATAGCAT TCCCCCGAATAAACAACATAAGCTTTTGACTCCTTCCCCCATCCTTCCT TCTCCTCCTAGCCTCCTCCACAGTAGAAGCTGGGGCAGGGACAGGTTG AACCGTCTACCCACCCCTCGCCGGCAACCTAGCACACGCAGGGGCTTC AGTAGACTTGGCCATTTTCTCCCTACACTTAGCAGGGATCTCCTCAATC CTAGGGGCCATCAACTTCATCACAACAGCAATCAACATAAAACCACCT GCCCTAT	*Turdus migratorius*	KJ909198.1
2015-334T	MERL	CTCTACTAGGAGACGACCAAATCTACAACGTAGTAGTTACCGCTCACG CCTTCGTAATAATCTTCTTTATGGTTATGCCTATCATAATCGGAGGGTT CGGAAACTGACTAGTGCCCCTAATAATCGGAGCCCCAGACATAGCATT CCCTCGAATAAACAACATAAGCTTCTGACTTCTCCCCCCATCCTTCCTA CTCCTCCTAGCCTCCTCCACAGTCGAAGCAGGGGTTGGAACAGGCTGA ACCGTCTACCCCCCTCTGGCTGGCAACCTCGCCCACGCTGGGGCCTCA GTAGACCTCGCTATCTTCTCCCTCACCCTGGCAGGGATCTCCTCAATCC TAGGGGCTATTAACTTCATCACACCCGAAATAAACAAAAAACCACCTG CCCTA	*Sturnus vulgaris*	GU571639.1

Sample ID	Control	Recovered Sequence	Prey detected on swab	BLAST Accession #
Control 1	YEWA	CCATCTCCTAGCCTCCTCTTCGGCAGACTAGGCCACCCGGAGCCCTTCT GGGAGACGACCAAGTCTATAATGTAGTCGTCACGGCCCATGCCTTCGT AATAATTTTCTTTATAGTTATGCCAATTATAATTGGAGGATTCGGAAAC TGACTAGTCCCTCTAATAATCGGAGCCCCAGACATAGCATTCCCACGA ATAAACAACATAAGCTTCTGACTACTCCCACCATCGTTCCTTCTCCTTC TAGCGTCCTCCACGGTTGAAGCAGGAGTAGGTACAGGCTGAACAGTGT ACCCCCCACTAGCCGGTAACCTGGCCCACGCCGGAGCCTCAGTCGACC TCGCAATCTTCTCTCTACACCTAGCCGGTATTTCCTCAATCCTCGGAGC AATCAACTTCATTACAACAGCAATTAACATGAAACCACCTGCCCTA	*Setophaga petechia*	HM033412.1

Control 2	OCWA	CACTCCCCTAGCCTCTTTATCCGGCAGACTAGGCAACCCGGAGCCCTTC TGGGAGACGACCAAGTCTACAATGTAGTTGTCACGGCCCATGCTTTCG TAATAATTTTCTTTATAGTCATACCGATTATAATCGGAGGATTCGGAAA CTGACTAGTTCCCCTAATAATCGGAGCCCCAGACATAGCATTCCCACG AATAAACAACATAAGCTTCTGACTACTCCCACCATCATTCCTTCTCCTA CTAGCATCCTCCACAGTTGAAGCAGGTGTCGGCACAGGTTGAACAGTG TACCCTCCACTAGCTGGCAACCTAGCCCACGCCGGAGCCTCCGTCGAC CTTGCAATTTTCTCTCTACACCTGGCTGGTATTTCCTCAATCCTCGGGG CGATCAACTTCATTACAACAGCAATCAACATGAAACCACCTGCCCTAT C	*Vermivora celata*	FJ236284.1
Control 3	SWTH	CCTTGCCCTAGCCTTCTATCCGGCAGACTAGGCCACCAGGCGCACTAC TAGGTGACGACCAAATCTACAATGTAGTTGTCACCGCCCACGCCTTCG TAATGATTTTCTTTATAGTTATGCCAATCATGATTGGGGGGTTCGGAAA CTGGCTAGTCCCATTAATAATCGGAGCCCCAGACATAGCATTCCCCCG AATAAACAACATAAGCTTCTGACTTCTCCCACCATCATTCCTCCTTCTC CTAGCCTCCTCCACAGTAGAAGCAGGAGCAGGAACAGGATGGACCGT CTATCCACCCCTCGCTGGCAACCTAGCACACGCAGGAGCCTCAGTCGA CCTAGCTATTTTCTCCCTCCACTTAGCAGGAATCTCCTCAATCCTAGGG GCCATCAATTTCATTACTACAGCAATCAACATAAAACCACCTGCCCT	*Catharus ustulatus*	HM033284.1

Chapter 2 Supplementary Materials:

Supplementary Table 1. Primers used in library preparation two-step PCR for Illumina Miseq (Illumina 2013, 2018)

Step 1: Amplicon PCR

Amplicon primers	Overhang/Linker sequence	Target amplicon sequence
COI-fsdF2	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG	GCATGAGCCGGAATAGTRGG
COI-fsdR2	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG	TGTGAKAGGGCAGGTGGTTT

Step 2: Index PCR

Index 1 ID	P5 adapter (forward)	i5 indexes	Forward Overhang/Linker sequence
S502	AATGATACGGCGACCACCGAGATCTACAC	CTCTCTAT	TCGTCGGCAGCGTC
S503	AATGATACGGCGACCACCGAGATCTACAC	TATCCTCT	TCGTCGGCAGCGTC
S505	AATGATACGGCGACCACCGAGATCTACAC	GTAAGGAG	TCGTCGGCAGCGTC
S506	AATGATACGGCGACCACCGAGATCTACAC	ACTGCATA	TCGTCGGCAGCGTC
S507	AATGATACGGCGACCACCGAGATCTACAC	AAGGAGTA	TCGTCGGCAGCGTC
S508	AATGATACGGCGACCACCGAGATCTACAC	CTAAGCCT	TCGTCGGCAGCGTC
S510	AATGATACGGCGACCACCGAGATCTACAC	CGTCTAAT	TCGTCGGCAGCGTC
S511	AATGATACGGCGACCACCGAGATCTACAC	TCTCTCCG	TCGTCGGCAGCGTC
Index 2 ID	**P7 adapter (reverse)**	**i7 indexes**	**Reverse Overhang/Linker sequence**
N716	CAAGCAGAAGACGGCATACGAGAT	TAGCGAGT	GTCTCGTGGGCTCGG
N718	CAAGCAGAAGACGGCATACGAGAT	GTAGCTCC	GTCTCGTGGGCTCGG
N719	CAAGCAGAAGACGGCATACGAGAT	TACTACGC	GTCTCGTGGGCTCGG
N720	CAAGCAGAAGACGGCATACGAGAT	TACTACGC	GTCTCGTGGGCTCGG
N721	CAAGCAGAAGACGGCATACGAGAT	GCAGCGTA	GTCTCGTGGGCTCGG
N722	CAAGCAGAAGACGGCATACGAGAT	CTGCGCAT	GTCTCGTGGGCTCGG
N723	CAAGCAGAAGACGGCATACGAGAT	GAGCGCTA	GTCTCGTGGGCTCGG
N724	CAAGCAGAAGACGGCATACGAGAT	CGCTCAGT	GTCTCGTGGGCTCGG
N726	CAAGCAGAAGACGGCATACGAGAT	GTCTTAGG	GTCTCGTGGGCTCGG
N727	CAAGCAGAAGACGGCATACGAGAT	ACTGATCG	GTCTCGTGGGCTCGG

Supplementary Table 2. Custom Reference Library compiled. Reported are the publicly available COI barcode sequences harvested via the R package 'PrimerMiner'. Out of 205 avian species, we were able to compile 199 barcode sequences for our reference library.

Genus species	BOLD # of seq	GB # of seq	Mito # of seq	sum
Acanthis flammea	105	142	2	249
Accipiter cooperii	7	6	0	13
Accipiter striatus	27	16	0	43
Actitis macularius	12	11	0	23
Aegolius acadicus	15	15	0	30
Aeronautes saxatalis	4	3	0	7
Agelaius phoeniceus	31	12	4	47
Agelaius tricolor	2	2	0	4
Aimophila ruficeps	5	3	0	8
Ammodramus savannarum	1	1	0	2
*Ammospiza nelson**	0	0	0	0
Anthus rubescens	10	8	0	18
Aphelocoma californica	22	19	0	41
Arenaria interpres	36	16	2	54
Arenaria melanocephala	3	2	0	5
Artemisiospiza belli	5	5	0	10
Athene cunicularia	10	9	0	19
Baeolophus inornatus	7	4	0	11
Bombycilla cedrorum	8	7	1	16
Bombycilla garrulus	18	15	0	33
Bonasa umbellus	11	7	0	18
Calcarius lapponicus	14	13	0	27
Calidris alba	20	12	0	32
Calidris alpina	29	11	0	40
Calidris bairdii	12	6	0	18
Calidris canutus	29	15	0	44
Calidris mauri	23	17	0	40
Calidris melanotos	27	23	0	50
Calidris minutilla	25	17	0	42
Calidris subruficollis	5	3	0	8
Calidris virgata	3	0	0	3
Callipepla californica	8	8	0	16
Calypte anna	8	3	1	12
Cardellina pusilla	38	32	0	70
Catharus guttatus	36	36	0	72
Catharus ustulatus	46	30	0	76

Catherpes mexicanus	2	2	0	4
Certhia americana	16	10	0	26
Chamaea fasciata	3	3	0	6
Charadrius montanus	6	5	0	11
Charadrius nivosus	0	3	0	3
Charadrius semipalmatus	16	8	0	24
Charadrius vociferus	13	8	0	21
Chondestes grammacus	2	2	0	4
Cinclus mexicanus	9	9	0	18
Cistothorus palustris	11	10	0	21
Coccothraustes vespertinus	0	3	2	5
Coccyzus americanus	5	4	0	9
Colaptes auratus	30	20	0	50
Columba livia	75	56	10	141
Contopus cooperi	6	6	0	12
Contopus sordidulus	14	13	0	27
Corvus brachyrhynchos	24	16	2	42
Cyanocitta stelleri	12	11	0	23
*Cypseloides niger**	0	0	0	0
Dendragapus fuliginosus	2	2	0	4
*Dryobates albolarvatus**	0	0	0	0
Dryobates nuttallii	13	13	0	26
Dryobates pubescens	30	18	2	50
Dryobates villosus	0	22	0	22
Empidonax difficilis	12	9	0	21
Empidonax hammondii	10	9	0	19
Empidonax oberholseri	7	7	0	14
Empidonax traillii	3	7	0	10
Empidonax wrightii	5	5	0	10
Eremophila alpestris	28	26	0	54
Euphagus cyanocephalus	20	8	2	30
Falco columbarius	24	12	2	38
Falco sparverius	35	14	2	51
Fulica americana	6	5	0	11
Gallinago delicata	11	10	0	21
Gallinula galeata	0	1	0	1
Geococcyx californianus	9	2	3	14
Geothlypis tolmiei	14	11	1	26
Geothlypis trichas	26	25	0	51
Glaucidium gnoma	2	2	0	4
Haemorhous cassinii	17	5	2	24

Haemorhous mexicanus	29	17	3	49
Haemorhous purpureus	16	15	0	31
Himantopus mexicanus	10	6	0	16
Hirundo rustica	58	49	2	109
Icteria virens	10	6	0	16
Icterus bullockii	7	5	1	13
Icterus cucullatus	6	5	0	11
Junco hyemalis	142	139	1	282
*Lanius borealis**	0	0	0	0
Lanius ludovicianus	9	6	0	15
Laterallus jamaicensis	1	1	0	2
Leucosticte tephrocotis	4	4	0	8
Limnodromus griseus	13	7	0	20
Limnodromus scolopaceus	8	6	0	14
Loxia curvirostra	34	23	2	59
Megaceryle alcyon	5	4	0	9
Megascops kennicottii	13	10	0	23
Melanerpes formicivorus	14	10	0	24
Melanerpes lewis	5	5	0	10
Meleagris gallopavo	27	11	3	41
Melospiza georgiana	13	10	0	23
Melospiza lincolnii	29	27	0	56
Melospiza melodia	36	35	1	72
Melozone crissalis	2	2	0	4
Mimus polyglottos	12	10	0	22
Mniotilta varia	21	16	0	37
Molothrus ater	10	10	0	20
Myadestes townsendi	10	9	0	19
Myiarchus cinerascens	5	5	0	10
Nucifraga columbiana	20	8	2	30
Numenius americanus	4	3	0	7
Numenius phaeopus	26	16	2	44
Oreothlypis celata	0	23	1	24
Oreothlypis ruficapilla	0	10	0	10
*Oreotyx pictus**	0	0	0	0
Passer domesticus	81	63	3	147
Passerculus sandwichensis	25	22	0	47
Passerella iliaca	27	22	0	49
Passerina amoena	10	9	1	20
Passerina caerulea	4	4	0	8
Passerina cyanea	9	7	0	16

Patagioenas fasciata	5	3	2	10
Perisoreus canadensis	5	5	0	10
Petrochelidon pyrrhonota	4	4	0	8
Phainopepla nitens	3	2	0	5
Phalaenoptilus nuttallii	7	6	0	13
Phalaropus fulicarius	10	8	0	18
Phalaropus lobatus	37	33	1	71
Phalaropus tricolor	7	3	0	10
Phasianus colchicus	40	18	5	63
Pheucticus melanocephalus	14	12	1	27
Pica nuttalli	5	5	0	10
Picoides arcticus	4	6	0	10
Pinicola enucleator	37	22	2	61
Pipilo chlorurus	3	3	0	6
Pipilo maculatus	18	15	1	34
Piranga ludoviciana	9	8	4	21
Plectrophenax nivalis	12	8	0	20
Pluvialis dominica	14	6	0	20
Pluvialis fulva	20	14	2	36
Pluvialis squatarola	27	15	0	42
Podiceps nigricollis	5	5	0	10
Poecile gambeli	10	11	0	21
Poecile rufescens	11	11	0	22
Polioptila caerulea	11	7	0	18
Pooecetes gramineus	3	3	0	6
Porzana carolina	5	4	0	9
Progne subis	24	24	0	48
Psaltriparus minimus	11	6	0	17
Psiloscops flammeolus	5	3	0	8
Pyrocephalus rubinus	14	12	0	26
Quiscalus mexicanus	18	12	0	30
Rallus limicola	7	7	0	14
Recurvirostra americana	7	7	0	14
Regulus calendula	50	37	2	89
Regulus satrapa	22	19	0	41
Riparia riparia	27	23	0	50
Salpinctes obsoletus	3	3	0	6
Sayornis nigricans	6	6	0	12
Sayornis saya	3	3	0	6
Selasphorus calliope	4	4	0	8
Selasphorus rufus	5	5	0	10

Selasphorus sasin	3	3	0	6
Setophaga coronata	44	75	1	120
Setophaga nigrescens	20	18	0	38
Setophaga occidentalis	13	12	0	25
Setophaga palmarum	17	15	0	32
Setophaga petechia	43	33	0	76
Setophaga ruticilla	25	23	0	48
Setophaga townsendi	19	18	0	37
Sialia currucoides	5	5	0	10
Sialia mexicana	9	8	0	17
Sitta canadensis	11	10	0	21
Sitta carolinensis	24	9	2	35
Sitta pygmaea	6	6	0	12
Sphyrapicus ruber	9	9	0	18
Spinus lawrencei	3	3	0	6
Spinus pinus	20	0	0	20
Spinus psaltria	16	2	2	20
Spinus tristis	19	17	1	37
Spizella atrogularis	4	2	1	7
Spizella passerina	22	21	0	43
Stelgidopteryx serripennis	7	5	0	12
Streptopelia decaocto	74	75	3	152
Sturnella neglecta	8	7	1	16
Sturnus vulgaris	48	30	3	81
Tachycineta bicolor	21	8	2	31
Tachycineta thalassina	15	3	2	20
Thryomanes bewickii	14	11	0	25
Toxostoma redivivum	7	7	0	14
Tringa flavipes	13	12	0	25
Tringa incana	3	3	0	6
Tringa melanoleuca	10	9	0	19
Tringa semipalmata	11	9	2	22
Tringa solitaria	14	13	0	27
Troglodytes aedon	97	71	0	168
*Troglodytes pacificus**	0	0	0	0
Turdus migratorius	49	31	2	82
Tyrannus verticalis	1	1	0	2
Vireo cassinii	10	9	0	19
Vireo gilvus	38	36	0	74
Vireo huttoni	12	6	0	18
Xanthocephalus xanthocephalus	8	7	0	15

Ixoreus naevius	6	4	0	10
Zenaida macroura	19	16	2	37
Zonotrichia albicollis	29	28	0	57
Zonotrichia atricapilla	7	7	0	14
Zonotrichia leucophrys	33	30	1	64

Supplementary Table 3. Prey detection summary

Prey detected on migrant Merlin beaks & talons	Merlins with detections (2015, 2016)	Average mass	Migratory
European Starling (*Sturnus vulgaris*) *	26 (15, 11)	77-93g	partial migrant
Red-breasted Nuthatch (*Sitta canadensis*)	27 (21, 6)	8-13g	irruptive migrant
Savannah Sparrow (*Passerculus sandwichensis*)	22 (9, 13)	15-28g	partial migrant
House Sparrow (*Passer domesticus*) *	15 (8, 7)	27-30g	resident
Spotted Towhee (*Pipilo maculatus*)	13 (4, 9)	33-49g	resident
Pine Siskin (*Spinus pinus*)	14 (14, 0)	12-18g	irruptive migrant
American Robin (*Turdus migratorius*)	15 (11, 4)	77-85g	partial migrant
Hermit Thrush (*Catharus guttatus*)	11 (6, 5)	23-37g	migrant
Varied Thrush (*Ixoreus naevius*)	14 (11, 3)	65-100g	partial migrant
Yellow Warbler (*Setophaga petechia*)	9 (6, 3)	9-11g	migrant
Yellow-rumped Warbler (*Setophaga coronata*)	10 (8, 2)	12-13g	partial migrant
Golden-crowned Kinglet (*Regulus satrapa*)	7 (6, 1)	4-8g	partial migrant
Swainson's Thrush (*Catharus ustulatus*)	6 (3, 3)	23-45g	migrant
Dark-eyed Junco (*Junco hyemalis*)	8 (3, 5)	18-30g	partial migrant
House Finch (*Haemorhous mexicanus*)	6 (3, 3)	16-27g	resident
Mountain Bluebird (*Sialia currucoides*)	5 (2, 3)	24-37g	partial migrant
Western Tanager (*Piranga ludoviciana*)	4 (2, 2)	24-36g	migrant
Orange-crowned Warbler (*Oreothylpis celata*)	5 (2, 3)	7-11g	partial migrant
Lesser Goldfinch (*Spinus psaltria*)	3 (0, 3)	8-12g	resident
Pine Grosbeak (*Pinacola nucleator*)	2 (0, 2)	40-60g	irruptive migrant
American Pipit (*Anthus rubescens*)	3 (1, 2)	19-26g	migrant
Ruby-crowned Kinglet (*Regulus calendula*)	2 (0, 2)	5-10g	migrant
Mountain Chickadee (*Poecile gambeli*)	3 (3, 0)	11-12g	resident
Chestnut-backed Chickadee (*Poecile rufescens*)	1 (0, 1)	7-12g	resident
Warbling Vireo (*Vireo gilvus*)	2 (2, 0)	10-16g	migrant
Wilson's Warbler (*Cardellina pusilla*)	2 (1, 1)	5-10g	migrant
Red-winged Blackbird (*Agelaius phoeniceus*)	1 (1, 0)	32-77g	resident
Common Redpoll (*Acanthis flammea*)	1 (0, 1)	11-20g	irruptive migrant
Lapland Longspur (*Calcarius lapponicus*)	1 (1, 0)	22-33g	migrant

Vaux's Swift (*Chaetura vauxi*)	**1 (0, 1)**	**15-22g**	**migrant**
Lazuli Bunting (*Passerina amoena*)	**1 (1, 0)**	**13-18g**	**migrant**
Common Yellowthroat (*Geothylpis trichas*)	**1 (0, 1)**	**9-10g**	**partial migrant**
Townsend's Warbler (*Setophaga townsendi*)	**1 (0, 1)**	**7-11g**	**migrant**
Mourning Dove (*Zenaida macroura*)	**1 (0, 1)**	**86-143g**	**partial migrant**
Western Meadowlark (*Sturnella neglecta*)	**1 (1, 0)**	**89-115g**	**partial migrant**
Brewer's Blackbird (*Euphagus cyanocephalus*)	**1 (1, 0)**	**50-86g**	**resident**
Brown-headed Cowbird (*Molothrus ater*)	**1 (0, 1)**	**38-45g**	**partial migrant**
Eurasian Collared-Dove (*Streptopelia decaocto*)	**1 (1, 0)**	**140-180g**	**resident**
Northern Mockingbird (*Mimus polyglottos*)	**1 (1, 0)**	**45-58g**	**resident**
California Towhee (*Melozone crissalis*)	**1 (1, 0)**	**37-67g**	**resident**
Hutton's Vireo (*Vireo huttoni*)	**1 (1, 0)**	**9-15g**	**resident**
American Goldfinch (*Spinus tristis*)	**1 (0, 1)**	**11-20g**	**migrant**

A list of prey species and the frequencies we detected on the beaks and talons of 63 migrant juvenile Merlins in the Pacific Flyway during 2015 (n = 41) and 2016 (n = 22) fall migration. Data on prey biomass, foraging guild, and migratory tendencies were compiled using the Birds of North America species account database (Rodewald 2015). Including lure bird species, we detected the DNA of 42 unique prey species on the beaks and talons of 63 migrant Merlins, with more than half of all detected prey species are also migratory within the Pacific Flyway. *Indicates species that are both probable prey and lure birds, therefore should be interpreted with caution. European Starling and House Sparrow were not included in statistical analyses.

Supplementary Table 4. A summary of reads for samples reported in this study. This output was generated via R package 'dada2'.

dada2_input	filtered	merged	nonchim	final_perc_reads_retained	%retained
2015-015	96564	62375	43171	27669	28.7
2015-034	396	183	71	41	10.4
2015-048	15708	9757	6422	5769	36.7
2015-055	139	57	35	35	25.2
2015-061	55458	26109	15817	14981	27
2015-062	18535	8082	4031	3919	21.1
2015-066	32388	16724	7705	7492	23.1
2015-074	26419	20182	12601	7979	30.2
2015-103	9883	7260	4979	4338	43.9
2015-112	132297	105375	69650	59657	45.1
2015-139	39927	25544	14750	13204	33.1
2015-145	33905	22121	14673	12777	37.7

2015-147	448	152	48	48	10.7
2015-162	47595	34714	20775	17834	37.5
2015-169	20550	15154	9815	7642	37.2
2015-183	20437	15127	10019	9283	45.4
2015-185	47467	31419	18231	15233	32.1
2015-190	445	177	134	134	30.1
2015-191	65411	45116	23840	20597	31.5
2015-195	10522	5367	2901	2799	26.6
2015-206	57049	43856	27076	21120	37
2015-210	125678	104243	68283	59403	47.3
2015-224	49856	34840	20334	16863	33.8
2015-225	126137	101980	62753	58364	46.3
2015-226	22982	16776	12151	9648	42
2015-228	55828	38419	22884	20420	36.6
2015-237	12393	6979	3733	3409	27.5
2015-245	58955	42685	26848	16464	27.9
2015-268	57786	43712	26104	24394	42.2
2015-270	29736	20244	12553	11847	39.8
2015-277	53290	37710	25358	21388	40.1
2015-284	69582	48727	31172	25694	36.9
2015-287	58117	38711	20867	19401	33.4
2015-290	190206	152325	94854	79740	41.9
2015-292	195393	155032	89375	72229	37
2015-300	60735	42492	28294	21446	35.3
2015-310	18022	11941	7659	6151	34.1
2015-313	72225	48862	29899	24735	34.2
2015-315	1640	874	350	350	21.3
2015-321	62284	48370	30552	20399	32.8
2015-329	55787	38600	23960	21333	38.2
2015-332	15704	11358	8154	6715	42.8
2015-333	69297	53880	34581	25218	36.4
2015-334	20436	11995	6408	6109	29.9
2015-338	39471	24140	12390	11688	29.6
2016-003	13738	9331	5863	5299	38.6
2016-045	32865	21629	17231	17223	52.4
2016-050	18178	11079	6426	5888	32.4
2016-060	25211	17883	11909	9869	39.1
2016-067	3042	1810	735	735	24.2
2016-078	68	13	0	0	0

2016-085	3009	1678	987	987	32.8
2016-086	373	212	110	110	29.5
2016-088	18290	10976	5618	4859	26.6
2016-091	122	21	0	0	0
2016-092	30104	16802	9568	8171	27.1
2016-101	23397	17124	11214	7882	33.7
2016-118	63828	41035	25295	21580	33.8
2016-120	25734	14563	7229	6773	26.3
2016-122	43085	28550	18805	15563	36.1
2016-128	32892	24222	14276	12183	37
2016-138	9062	5311	2500	2315	25.5
2016-141	20544	10791	5877	5225	25.4
2016-146	11428	6975	3637	2525	22.1
2016-169	1752	1062	729	729	41.6
2016-176	726	389	183	183	25.2
2016-196	8745	4522	1933	1775	20.3
2016-200	123725	92267	54969	43417	35.1
2016-226	160	71	33	33	20.6
2016-270	51607	34074	20698	17073	33.1
2016-275	262	118	103	103	39.3
2016-291	19343	10590	4234	4144	21.4
2016-294	319	155	118	118	37
Negative1	222	76	58	58	26.1
Negative2	231	156	152	152	65.8
OCWA	117066	88993	48392	32365	27.6
Negative3	188	92	66	66	35.1
SWTH	63145	46988	29985	24582	38.9
Negative4	304	150	108	67	22
Undetermined	3351388	261118	173208	147492	4.4

Chapter 3 Supplementary Materials:

Supplementary Table 1: Table summary of the primers and indices used for the two-step PCR Illumina (2013) protocol.

Step 1: Amplicon PCR

Amplicon primers	Overhang/Linker sequence	Target amplicon sequence
COI-fsdF2	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG	GCATGAGCCGGAATAGTRGG
COI-fsdR2	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG	TGTGAKAGGGCAGGTGGTTT

Step 2: Index PCR

Index 1 ID	P5 adapter (forward)	i5 indexes	Forward Overhang/Linker sequence
S502	AATGATACGGCGACCACCGAGATCTACAC	CTCTCTAT	TCGTCGGCAGCGTC
S503	AATGATACGGCGACCACCGAGATCTACAC	TATCCTCT	TCGTCGGCAGCGTC
S505	AATGATACGGCGACCACCGAGATCTACAC	GTAAGGAG	TCGTCGGCAGCGTC
S506	AATGATACGGCGACCACCGAGATCTACAC	ACTGCATA	TCGTCGGCAGCGTC
S507	AATGATACGGCGACCACCGAGATCTACAC	AAGGAGTA	TCGTCGGCAGCGTC
S508	AATGATACGGCGACCACCGAGATCTACAC	CTAAGCCT	TCGTCGGCAGCGTC
S510	AATGATACGGCGACCACCGAGATCTACAC	CGTCTAAT	TCGTCGGCAGCGTC
S511	AATGATACGGCGACCACCGAGATCTACAC	TCTCTCCG	TCGTCGGCAGCGTC
S513	AATGATACGGCGACCACCGAGATCTACAC	TCGACTAG	TCGTCGGCAGCGTC
S515	AATGATACGGCGACCACCGAGATCTACAC	TTCTAGCT	TCGTCGGCAGCGTC
S516	AATGATACGGCGACCACCGAGATCTACAC	CCTAGAGT	TCGTCGGCAGCGTC
S517	AATGATACGGCGACCACCGAGATCTACAC	GCGTAAGA	TCGTCGGCAGCGTC
S518	AATGATACGGCGACCACCGAGATCTACAC	CTATTAAG	TCGTCGGCAGCGTC
S520	AATGATACGGCGACCACCGAGATCTACAC	AAGGCTAT	TCGTCGGCAGCGTC
S521	AATGATACGGCGACCACCGAGATCTACAC	GAGCCTTA	TCGTCGGCAGCGTC
S522	AATGATACGGCGACCACCGAGATCTACAC	TTATGCGA	TCGTCGGCAGCGTC

Index 2 ID	P7 adapter (reverse)	i7 indexes	Reverse Overhang/Linker sequence
N701	CAAGCAGAAGACGGCATACGAGAT	TCGCCTTA	GTCTCGTGGGCTCGG
N702	CAAGCAGAAGACGGCATACGAGAT	CTAGTACG	GTCTCGTGGGCTCGG
N703	CAAGCAGAAGACGGCATACGAGAT	TTCTGCCT	GTCTCGTGGGCTCGG
N704	CAAGCAGAAGACGGCATACGAGAT	GCTCAGGA	GTCTCGTGGGCTCGG
N705	CAAGCAGAAGACGGCATACGAGAT	AGGAGTCC	GTCTCGTGGGCTCGG
N706	CAAGCAGAAGACGGCATACGAGAT	CATGCCTA	GTCTCGTGGGCTCGG
N707	CAAGCAGAAGACGGCATACGAGAT	GTAGAGAG	GTCTCGTGGGCTCGG

N710	CAAGCAGAAGACGGCATACGAGAT	CAGCCTCG	GTCTCGTGGGCTCGG
N711	CAAGCAGAAGACGGCATACGAGAT	TGCCTCTT	GTCTCGTGGGCTCGG
N712	CAAGCAGAAGACGGCATACGAGAT	TCCTCTAC	GTCTCGTGGGCTCGG
N714	CAAGCAGAAGACGGCATACGAGAT	TCATGAGC	GTCTCGTGGGCTCGG
N715	CAAGCAGAAGACGGCATACGAGAT	CCTGAGAT	GTCTCGTGGGCTCGG
N716	CAAGCAGAAGACGGCATACGAGAT	TAGCGAGT	GTCTCGTGGGCTCGG
N718	CAAGCAGAAGACGGCATACGAGAT	GTAGCTCC	GTCTCGTGGGCTCGG
N719	CAAGCAGAAGACGGCATACGAGAT	TACTACGC	GTCTCGTGGGCTCGG
N720	CAAGCAGAAGACGGCATACGAGAT	TACTACGC	GTCTCGTGGGCTCGG
N721	CAAGCAGAAGACGGCATACGAGAT	GCAGCGTA	GTCTCGTGGGCTCGG
N722	CAAGCAGAAGACGGCATACGAGAT	CTGCGCAT	GTCTCGTGGGCTCGG
N723	CAAGCAGAAGACGGCATACGAGAT	GAGCGCTA	GTCTCGTGGGCTCGG
N724	CAAGCAGAAGACGGCATACGAGAT	CGCTCAGT	GTCTCGTGGGCTCGG
N726	CAAGCAGAAGACGGCATACGAGAT	GTCTTAGG	GTCTCGTGGGCTCGG
N727	CAAGCAGAAGACGGCATACGAGAT	ACTGATCG	GTCTCGTGGGCTCGG

Supplementary Table 2: A summary of reads for Sharp-shinned Hawk samples reported on Miseq DNA sequencing Lane 1 in this study. This output was generated via R package ‘dada2’.

sampleID	dada2_input	filtered	merged	nonchim	Final % reads retained
2015-001	82106	46548	26434	24150	29.4
2015-002	37053	21670	14555	13242	35.7
2015-003	12361	7230	3959	3730	30.2
2015-004	13372	9943	6411	4867	36.4
2015-005	9934	7371	4384	3897	39.2
2015-006	8558	4316	2137	2014	23.5
2015-007	19937	5224	2588	2339	11.7
2015-008	20900	10375	7388	7236	34.6
2015-009	7965	5776	4001	2671	33.5

2015-010	80497	54218	29445	25358	31.5
2015-011	12173	6889	3375	3045	25
2015-013	19903	14348	10081	6951	34.9
2015-014	5984	2971	1437	1350	22.6
2015-016	8422	5247	2977	2636	31.3
2015-017	3539	1985	1121	1064	30.1
2015-018	12948	7010	4131	3937	30.4
2015-019	9222	4853	2928	2256	24.5
2015-020	5919	2755	903	881	14.9
2015-021	10398	5708	2839	2638	25.4
2015-022	8250	5612	3364	3187	38.6
2015-023	11213	4704	2267	2209	19.7
2015-024	11179	6710	3172	2634	23.6
2015-025	9345	5108	3042	2536	27.1
2015-026	12665	6895	4673	4453	35.2
2015-027	6218	3030	1165	1079	17.4
2015-028	11196	6187	2285	2192	19.6
2015-029	11720	2220	750	750	6.4
2015-030	51042	28773	15663	13959	27.3
2015-031	76274	46485	30217	22203	29.1
2015-032	10648	7343	4559	4110	38.6
2015-033	37360	22124	14498	13994	37.5
2015-036	8984	5399	3126	2867	31.9

2015-037	46173	27593	18978	15259	33
2015-038	45512	20230	12157	11262	24.7
2015-039	354	63	0	0	0
2015-040	7654	4467	1994	1949	25.5
2015-041	61784	24659	12178	10320	16.7
2015-042	9346	5280	2844	2483	26.6
2015-043	8428	4293	916	916	10.9
2015-044	18395	11596	7322	6474	35.2
2015-045	19700	15633	10764	9635	48.9
2015-046	4579	2235	648	599	13.1
2015-047	15462	9059	5191	4808	31.1
2015-049	74366	57031	33185	28214	37.9
2015-050	9970	6807	4587	3936	39.5
2015-051	11804	6948	4132	3726	31.6
2015-052	10550	5719	3843	3525	33.4
2015-053	12432	7771	4683	3915	31.5
2015-056	10659	7963	5541	3681	34.5
2015-057	69898	51562	33402	28790	41.2
2015-058	44961	34651	20254	17470	38.9
2015-059	10205	6610	4029	3849	37.7
2015-060	52997	28385	16940	14732	27.8
2015-063	74327	56382	37308	32451	43.7
2015-064	76369	48693	31539	28600	37.4

2015-065	14042	8189	5158	4677	33.3
2015-067	31574	15876	9066	7899	25
2015-068	12731	6504	2178	2178	17.1
2015-069	16651	9410	4961	4533	27.2
2015-070	82757	46926	28788	25268	30.5
2015-071	27919	9039	5913	4889	17.5
2015-072	38933	26292	17095	15282	39.3
2015-073	75203	43784	23831	21516	28.6
2015-075	7410	4225	1797	1797	24.3
2015-076	13481	9673	6724	5893	43.7
2015-077	67860	43390	26465	21187	31.2
2015-078	33001	17704	11849	11148	33.8
2015-079	9161	6272	4386	3849	42
2015-080	74143	53466	33384	27138	36.6
2015-081	54082	32786	20709	17986	33.3
2015-082	12907	7511	4058	3817	29.6
2015-083	14457	5729	3396	3320	23
2015-084	79108	54560	33407	26979	34.1
2015-085	17795	8398	5073	4721	26.5
2015-086	12310	7069	3836	3233	26.3
2015-087	3143	1868	1074	999	31.8
2015-088	5669	3205	2401	2327	41
2015-089	17725	11770	6717	5967	33.7

2015-090	109611	65471	39337	35734	32.6
2015-091	10356	6956	3857	3446	33.3
2015-092	7875	5332	3342	2882	36.6
2015-093	15424	7452	3063	3004	19.5
2015-094	7840	4313	2628	2043	26.1
2015-095	10142	6900	4445	3352	33.1
2015-096	10093	5521	2799	2462	24.4
2015-097	115072	71367	45200	33118	28.8
2015-098	9340	5189	2879	2463	26.4
2015-099	21962	11543	6239	5842	26.6
2015-100	18347	7892	4499	4070	22.2
2015-101	69361	45955	29986	21331	30.8
2015-102	18053	7828	5026	4593	25.4
2015-104	13815	10468	7173	5735	41.5
2015-105	29968	18212	11299	9310	31.1
2015-106	13913	7881	4756	4373	31.4
2015-107	69705	40873	24780	18510	26.6
2015-108	10716	7874	5118	4412	41.2
2015-109	12730	6877	3429	3429	26.9
2015-110	9209	3443	1938	1938	21
2015-111	46942	26774	15945	14759	31.4
2015-113	18564	9604	5643	5214	28.1
2015-114	10794	6574	4076	3462	32.1

2015-115	7800	4636	2573	2438	31.3
2015-116	20509	9779	6947	6151	30
2015-117	32786	23240	13688	11682	35.6
2015-118	14706	9810	6471	5891	40.1
2015-119	11905	6961	4144	3728	31.3
2015-120	45195	20580	7940	7908	17.5
2015-121	17237	12694	8671	8123	47.1
2015-122	57663	25587	14265	11015	19.1
2015-123	83406	63478	36090	31984	38.3
2015-124	60373	34049	20251	17531	29
2015-125	221	28	0	0	0
2015-126	14651	8814	5354	4201	28.7
2015-127	13652	7054	4068	3386	24.8
2015-128	33079	24472	15974	9844	29.8
2015-129	8757	5081	3216	2923	33.4
2015-130	15470	8536	5499	5333	34.5
2015-131	9213	6641	4443	3824	41.5
2015-132	17435	11847	7963	7509	43.1
2015-133	56763	32430	10422	9058	16
2015-134	74359	42283	23769	22369	30.1
2015-135	51672	31134	20143	17316	33.5
2015-136	81408	52024	32005	26765	32.9
2015-137	12062	6903	4182	3981	33

2015-138	80043	57711	32055	23981	30
2015-141	7987	4690	2568	2205	27.6
2015-142	70558	45662	24505	21707	30.8
2015-143	36708	22705	15167	12924	35.2
2015-144	9059	5473	2916	2662	29.4
2015-146	15696	11325	7249	5186	33
2015-149	72579	45854	29750	25054	34.5
2015-151	55828	36976	21583	20214	36.2
2015-152	74131	51802	31582	27978	37.7
2015-153	16581	11351	7003	5175	31.2
2015-154	16639	13576	9398	8792	52.8
2015-155	8469	3671	1652	1471	17.4
2015-156	89699	49682	28281	22131	24.7
2015-157	6542	4385	3283	2761	42.2
2015-158	9242	6344	3699	2798	30.3
2015-159	70047	42015	25960	22364	31.9
2015-160	14050	6517	2766	2384	17
2015-161	9950	5894	3656	2887	29
2015-163	60121	30424	14711	13937	23.2
2015-164	10779	6076	3116	2886	26.8
2015-165	39056	26451	14177	13286	34
2015-167	7221	4785	3163	2900	40.2
2015-168	8701	4549	2454	2233	25.7

2015-170	6780	2964	1276	1147	16.9
2015-171	56211	33422	18941	17625	31.4
2015-172	14128	6688	4200	3907	27.7
2015-173	32524	22197	15122	12291	37.8
2015-175	75573	47308	31231	27370	36.2
2015-176	9772	5933	3876	3289	33.7
2015-177	12345	7668	3988	3724	30.2
2015-178	15918	7936	4315	4155	26.1
2015-179	10666	5839	3389	3179	29.8
2015-180	17197	8973	5466	4966	28.9
2015-181	53890	37432	21111	18444	34.2
2015-182	10095	6723	4100	3549	35.2
2015-184	47581	30713	18998	14607	30.7
2015-186	10586	5359	2923	2643	25
2015-187	14042	8863	5499	4752	33.8
2015-188	6298	2691	1049	1049	16.7
2015-189	10706	6117	3412	3017	28.2
2015-192	69474	34384	20927	16147	23.2
2015-193	10509	6284	3490	3164	30.1
2015-194	11996	7935	5590	5045	42.1
2015-196	8172	4541	2229	2059	25.2
2015-197	88346	55063	33162	23151	26.2
2015-198	7340	3559	1988	1925	26.2

2015-199	13152	8708	6106	5440	41.4
2015-200	14627	8610	5360	4707	32.2
2015-201	16435	11069	6800	5744	34.9
2015-202	120816	87350	54793	33742	27.9
2015-203	12176	6217	4328	3757	30.9
2015-204	9608	5042	2903	2424	25.2
2015-205	14640	6902	3427	3298	22.5
2015-207	233	19	0	0	0
2015-208	40159	22186	13251	12440	31
2015-209	81234	55449	33640	28187	34.7
2015-211	13712	9241	6462	5908	43.1
2015-212	18068	12830	8749	7941	44
2015-213	33055	19207	13384	11971	36.2
2015-214	75883	47941	29708	26846	35.4
2015-215	42303	20551	11878	10434	24.7
2015-216	23605	10993	7248	6305	26.7
2015-217	97407	57215	26388	21099	21.7
2015-218	57017	39010	24427	21543	37.8
2015-219	97231	77919	51466	43265	44.5
2015-220	59597	38547	23674	20704	34.7
2015-221	11740	8088	5351	4633	39.5
2015-222	55981	27799	14144	13716	24.5
2015-223	3890	2336	1727	1601	41.2

2015-227	14524	7305	4850	4266	29.4
2015-229	62183	42876	27325	21199	34.1
2015-230	16631	11906	8198	6879	41.4
2015-231	64450	49217	30964	27273	42.3
2015-232	10652	5731	3436	3248	30.5
2015-233	414	68	37	37	8.9
2015-238	11733	7794	4544	4264	36.3
2015-239	17252	10092	6516	5790	33.6
2015-240	13836	9483	6373	4418	31.9
2015-241	22169	10992	7836	6663	30.1
2015-242	12640	7545	4590	4368	34.6
2015-243	18424	9397	6613	6248	33.9
2015-244	9436	6290	4194	3341	35.4
2015-246	19888	14101	9477	5344	26.9
2015-247	14282	7798	5060	4379	30.7
2015-248	15813	6686	3420	3100	19.6
2015-249	262	22	0	0	0
2015-250	12553	5907	4748	4637	36.9
2015-254	11464	6248	4273	3919	34.2
2015-255	13465	5876	2856	2758	20.5
2015-256	92535	68392	45575	34372	37.1
2015-257	7265	4978	3658	3143	43.3
2015-258	92641	59009	36633	32494	35.1

2015-260	14138	10068	7336	4400	31.1
2015-261	13790	8142	4723	3767	27.3
2015-262	4943	3447	2187	2089	42.3
2015-263	13659	9428	5814	4051	29.7
2015-264	42920	28061	14567	13526	31.5
2015-266	31726	22936	15159	12354	38.9
2015-267	92269	55423	33695	28549	30.9
2015-269	29073	18953	10278	8880	30.5
2015-271	51592	21323	13211	11884	23
2015-272	68562	31301	16196	13871	20.2
2015-273	9029	5975	4302	3206	35.5
2015-274	9421	5432	2998	2643	28.1
2015-275	11445	7841	4717	3791	33.1
2015-278	30452	14552	9722	9017	29.6
2015-279	56336	35926	19521	17659	31.3
2015-280	50952	34741	22418	20311	39.9
2015-282	11412	6013	3483	2923	25.6
2015-283	18003	9873	6236	6007	33.4
2015-285	16837	11853	7695	6808	40.4
2015-286	16182	6583	3777	3522	21.8
2015-288	69589	49295	27951	25704	36.9
2015-289	13337	9278	5932	4584	34.4
2015-291	51391	30797	18081	15794	30.7

2015-293	15387	9262	5842	5326	34.6
2015-294	7187	4659	3166	3012	41.9
2015-295	66036	46938	31618	26495	40.1
2015-296	13697	7905	5905	5575	40.7
2015-297	14988	6730	3349	2855	19
2015-298	59523	47670	30774	18075	30.4
2015-299	18867	9607	5843	5677	30.1
2015-301	18442	6766	4210	3773	20.5
2015-302	87654	57881	33685	30303	34.6
2015-303	31859	10617	6764	6182	19.4
2015-304	15513	10305	7628	5138	33.1
2015-305	71959	47015	30049	23795	33.1
2015-306	10206	6838	4156	3235	31.7
2015-307	26576	17225	9802	9298	35
2015-308	86219	52621	30862	26562	30.8
2015-309	87448	58581	38213	31436	35.9
2015-311	11330	6925	4192	3680	32.5
2015-314	10389	6753	3958	3507	33.8
2015-317	17457	12368	8104	7081	40.6
2015-318	14540	10084	7249	6421	44.2
2015-319	9899	4095	1670	1609	16.3
2015-322	70423	44383	26596	23546	33.4
2015-323	6440	3563	2440	1452	22.5

2015-324	11829	8206	5620	4691	39.7
2015-325	15057	8850	4782	4575	30.4
2015-326	19180	11519	7539	6282	32.8
2015-327	13858	6764	4840	4464	32.2
2015-328	27533	16915	9134	8016	29.1
2015-335	8254	4843	2260	2218	26.9
2015-336	13857	6927	3873	3553	25.6
2015-339	10008	6029	3585	3304	33
2015-341	16378	10895	6656	5798	35.4
2015-344	206	50	0	0	0
2015-347	39803	18726	10673	9805	24.6
2015-349	89465	45522	24916	20064	22.4
2015-351	50652	27845	15557	13285	26.2
2015-352	9386	6115	3793	3143	33.5
2015-354	102624	61686	38147	35148	34.2
2015-356	86855	49424	27230	24334	28
2015-357	8894	5921	3916	3709	41.7
2015-360	44848	29801	19708	17649	39.4
2015-400	86656	54719	34840	29333	33.8
2016-345	13029	7639	4395	3956	30.4
Negative1	222	76	58	58	26.1
Negative2	231	156	152	152	65.8
OCWA-1	117066	88993	48392	32365	27.6

OCWA-2	188	92	66	66	35.1
SWTH-1	63145	46988	29985	24582	38.9
SWTH-2	304	150	108	67	22
Undetermined	3351388	261118	173208	147492	4.4
Total	8489811	5202543	3141015	2671740	8582

Supplementary Table 3: A summary of reads for Sharp-shinned Hawk samples reported on Miseq DNA sequencing Lane 2 in this study. This output was generated via R package 'dada2'.

SampleID	dada2_input	filtered	merged	nonchim	Final % reads retained
2015-234	28984	20795	14261	14240	49.1
2015-265	30140	18993	12978	12978	43.1
2015-346	57977	44194	32134	32134	55.4
2015-353	48851	35367	25163	25099	51.4
2015-355	1348	833	689	689	51.1
2016-001	30354	24079	14322	14322	47.2
2016-002	27968	18560	13230	13173	47.1
2016-004	57624	46154	35062	35036	60.8
2016-005	94032	67638	48494	47724	50.8
2016-006	108077	80797	54924	54741	50.6
2016-007	107128	80346	57341	56211	52.5
2016-008	125614	95131	71216	66466	52.9
2016-009	1850	1070	472	472	25.5
2016-010	34805	25726	20905	20675	59.4
2016-012	15195	10169	8004	8004	52.7

2016-013	55190	35850	27764	27348	49.6
2016-014	10052	5358	3837	3836	38.2
2016-015	8676	4969	2487	2487	28.7
2016-016	34114	23446	18073	18054	52.9
2016-018	75189	58098	40543	39839	53
2016-020	59390	45754	34300	34300	57.8
2016-021	69106	54488	39995	39831	57.6
2016-022	84158	56292	39689	39682	47.2
2016-023	6652	4840	3996	3957	59.5
2016-024	43494	32854	25772	25772	59.3
2016-025	70744	35789	25014	23652	33.4
2016-026	55206	39026	30538	30498	55.2
2016-027	58342	35814	27263	27263	46.7
2016-028	35643	25051	15724	15724	44.1
2016-029	45011	33034	24532	24518	54.5
2016-030	88511	63921	48602	48006	54.2
2016-031	37146	21472	16603	16603	44.7
2016-032	85034	68119	52547	52470	61.7
2016-033	26588	16909	13552	13552	51
2016-034	85738	46632	33278	33258	38.8
2016-035	40623	30058	26797	26797	66
2016-036	28690	17810	13061	13061	45.5
2016-037	14371	10683	8193	8193	57

2016-038	4367	3087	1567	1567	35.9
2016-039	98872	73756	53756	52083	52.7
2016-040	41833	30710	25988	25960	62.1
2016-041	44462	31025	23492	23248	52.3
2016-042	13690	7308	5598	5598	40.9
2016-043	15061	8659	3835	3835	25.5
2016-044	100336	72673	58394	54518	54.3
2016-045	32865	21629	17231	17223	52.4
2016-046	1705	526	297	297	17.4
2016-049	32212	22249	14608	14608	45.3
2016-051	16822	11849	9126	9126	54.3
2016-052	23513	15690	12125	12125	51.6
2016-053	360	71	18	18	5
2016-054	37504	26434	16383	16333	43.6
2016-055	31136	17856	13500	13500	43.4
2016-056	37876	26473	21623	21623	57.1
2016-057	64331	49762	41859	41846	65
2016-058	39887	30093	22497	22497	56.4
2016-059	23040	14261	11168	11168	48.5
2016-061	23635	17077	12505	12505	52.9
2016-062	27225	19088	13732	13726	50.4
2016-063	39019	26519	18717	18490	47.4
2016-064	40069	29666	21278	20860	52.1

2016-065	30069	17723	11551	11499	38.2
2016-066	45676	33309	22553	22434	49.1
2016-068	69128	50081	41127	41123	59.5
2016-069	60147	44998	34253	34052	56.6
2016-070	63495	39461	26651	26515	41.8
2016-072	52514	33638	23725	23696	45.1
2016-073	31153	18691	13205	13205	42.4
2016-074	42683	30245	25409	25409	59.5
2016-075	47660	27990	20562	20562	43.1
2016-076	31015	22341	14590	14590	47
2016-077	47975	26751	20734	20734	43.2
2016-079	32695	22661	13199	12961	39.6
2016-080	36518	25210	16677	16545	45.3
2016-081	27991	17020	12356	12356	44.1
2016-082	90674	62703	42405	39472	43.5
2016-083	26761	20277	16885	16885	63.1
2016-084	52008	37089	28056	28056	53.9
2016-087	141	42	0	0	0
2016-089	147	32	0	0	0
2016-090	45555	34977	27307	27272	59.9
2016-093	55013	39196	27618	27611	50.2
2016-094	31000	19522	14650	14650	47.3
2016-095	130	24	0	0	0

2016-096	77363	53430	36966	36756	47.5
2016-097	79539	47434	33420	33379	42
2016-098	43386	33700	26103	25779	59.4
2016-099	30364	22966	17455	17429	57.4
2016-100	10171	6940	5891	5891	57.9
2016-102	36259	27538	21100	21100	58.2
2016-103	46148	28954	22133	22133	48
2016-104	29173	21088	15048	14995	51.4
2016-105	60794	42874	28930	28609	47.1
2016-106	25205	14934	8314	8314	33
2016-107	80152	50337	32457	31893	39.8
2016-108	49048	37658	29401	29342	59.8
2016-109	59150	45290	35981	35972	60.8
2016-110	36841	26460	19849	19832	53.8
2016-111	21854	15567	13301	13297	60.8
2016-112	38971	29249	23564	23564	60.5
2016-113	19288	12641	10443	10443	54.1
2016-114	35061	29014	18907	18907	53.9
2016-115	26974	15982	12926	12926	47.9
2016-116	43266	26716	17751	17634	40.8
2016-117	16583	9925	7320	7315	44.1
2016-121	49735	38887	28769	28769	57.8
2016-123	37616	27608	21340	21331	56.7

2016-124	22761	16261	11958	11219	49.3
2016-125	126432	92737	61948	61941	49
2016-126	81220	61255	43732	41747	51.4
2016-127	40497	31626	23617	23281	57.5
2016-129	21830	15790	11891	11785	54
2016-130	43986	32610	23712	18525	42.1
2016-131	23692	16760	11632	11632	49.1
2016-132	12380	6803	3414	3414	27.6
2016-133	9195	4704	3194	3194	34.7
2016-136	9089	4926	4133	4131	45.5
2016-137	37306	20253	4526	4486	12
2016-139	47046	33725	25853	25853	55
2016-140	68460	41998	32523	32495	47.5
2016-142	45477	24093	16341	15838	34.8
2016-143	48455	34231	20936	20765	42.9
2016-144	29868	24078	18541	18541	62.1
2016-145	78573	39857	29185	29097	37
2016-147	42935	28869	22989	22684	52.8
2016-148	23498	16294	9058	9058	38.5
2016-149	42419	31299	21766	21766	51.3
2016-150	2775	2042	1624	1624	58.5
2016-151	23226	13852	9528	9527	41
2016-152	54135	40548	29407	28413	52.5

2016-153	46034	22914	18143	18143	39.4
2016-154	673	273	167	167	24.8
2016-155	163	23	0	0	0
2016-156	52358	34237	23718	23657	45.2
2016-157	30016	18683	13511	13511	45
2016-158	78271	54633	37559	37519	47.9
2016-160	58723	39756	28306	28306	48.2
2016-161	97943	80141	53274	53054	54.2
2016-162	72947	29053	21297	21297	29.2
2016-163	42179	25626	20201	20005	47.4
2016-164	58526	44009	29885	29484	50.4
2016-165	84646	34474	28100	28100	33.2
2016-166	103129	50162	37012	37011	35.9
2016-167	40658	24638	19381	19381	47.7
2016-168	73296	35319	26154	26115	35.6
2016-170	183	32	15	15	8.2
2016-171	41644	17737	12470	12469	29.9
2016-172	34491	25853	20122	19810	57.4
2016-173	44377	30998	22427	22419	50.5
2016-174	19465	15375	11858	11858	60.9
2016-175	117755	79890	54590	52201	44.3
2016-177	70876	32669	21590	21590	30.5
2016-179	48720	34579	24073	24073	49.4

2016-181	9853	5880	4536	4536	46
2016-182	61579	39747	31297	31297	50.8
2016-183	59238	35947	26033	25886	43.7
2016-184	131899	79704	41376	40281	30.5
2016-186	48883	20633	16219	16213	33.2
2016-187	32879	25663	18033	18000	54.7
2016-188	57102	42582	30760	30716	53.8
2016-189	51818	23161	16400	16400	31.6
2016-190	214	27	0	0	0
2016-191	50529	34631	28521	28521	56.4
2016-192	36761	20425	14210	14059	38.2
2016-193	18682	13489	9507	9507	50.9
2016-194	72040	42251	28226	28226	39.2
2016-195	93857	62430	47013	46902	50
2016-197	1930	730	502	502	26
2016-198	64985	50406	41344	41174	63.4
2016-199	90467	50481	36248	36111	39.9
2016-201	42016	31676	22443	21544	51.3
2016-203	85198	56080	41626	41173	48.3
2016-204	95054	40041	32507	32507	34.2
2016-205	34518	23497	16528	16528	47.9
2016-206	328	73	0	0	0
2016-207	40068	29527	20881	20881	52.1

2016-208	44885	34220	24545	24545	54.7
2016-209	36481	26305	18368	18368	50.3
2016-210	45428	34467	24559	24511	54
2016-211	38317	27603	20563	20545	53.6
2016-212	26181	20579	14364	14359	54.8
2016-213	45659	34678	27646	27455	60.1
2016-214	20873	14499	11208	11208	53.7
2016-215	55993	40454	29097	29021	51.8
2016-216	61447	41237	33313	33271	54.1
2016-217	47260	34500	25610	25551	54.1
2016-218	282	65	0	0	0
2016-220	48471	31245	21702	21523	44.4
2016-221	60814	39473	28910	28575	47
2016-222	7011	3869	2460	2460	35.1
2016-223	24091	17428	14166	14166	58.8
2016-225	26190	17510	11989	11941	45.6
2016-227	16956	10761	9281	9281	54.7
2016-228	61279	41489	34045	34045	55.6
2016-229	10376	5990	2074	2074	20
2016-230	75584	48387	35517	35369	46.8
2016-231	24403	19294	13866	13866	56.8
2016-232	51125	24374	20042	20042	39.2
2016-237	21296	12459	10057	10057	47.2

2016-238	40176	27658	17428	15718	39.1
2016-239	42113	19714	14577	14577	34.6
2016-240	35640	19217	13505	13505	37.9
2016-241	37775	28280	21775	21766	57.6
2016-242	10148	5260	3987	3987	39.3
2016-243	65684	43949	30017	30012	45.7
2016-244	371	177	42	42	11.3
2016-245	29753	20827	16407	16407	55.1
2016-246	202	34	0	0	0
2016-248	46088	31132	24710	24691	53.6
2016-249	39007	20740	16748	16748	42.9
2016-250	27807	18242	14959	14959	53.8
2016-251-1	42045	32011	27039	26204	62.3
2016-251-2	61441	36837	18604	16695	27.2
2016-252	46758	31420	22286	21893	46.8
2016-253-1	27153	15192	10508	10508	38.7
2016-253-2	31208	20962	14431	14431	46.2
2016-259-1	25897	16429	11160	11160	43.1
2016-259-2	80587	63335	45306	45304	56.2
2016-263	7395	3765	2852	2852	38.6
2016-265	67630	49277	35844	34605	51.2
2016-266	45749	34642	25148	25148	55
2016-267	77197	59684	47117	43684	56.6

2016-268	38960	28549	22012	22012	56.5
2016-269	49501	28661	21516	21516	43.5
2016-271	149219	110736	78088	75925	50.9
2016-272	4304	2498	1647	1647	38.3
2016-273	32382	24790	17431	17431	53.8
2016-276-1	1311	558	192	192	14.6
2016-276-2	51210	32868	24458	24134	47.1
2016-277	84104	64804	51402	50714	60.3
2016-278	16541	11457	8591	8591	51.9
2016-279	76209	46865	33866	32678	42.9
2016-280	8905	5111	3581	3581	40.2
2016-281	47607	31549	27274	27213	57.2
2016-284	57432	45578	28911	28889	50.3
2016-285	38572	26109	17295	17295	44.8
2016-287	78283	63174	40268	40229	51.4
2016-288	39483	29952	22337	22337	56.6
2016-289	88817	66772	49841	45479	51.2
2016-290	67019	45333	36503	36248	54.1
2016-292	46995	32121	22207	22207	47.3
2016-295	58663	30194	19824	19815	33.8
2016-296	187	34	0	0	0
2016-297	57565	41864	32933	32831	57
2016-298	63347	42113	32803	32268	50.9

2016-299	15334	8412	5829	5829	38
2016-300	43669	33567	25778	25686	58.8
2016-304	44206	35546	24494	24413	55.2
2016-305	91806	61920	51936	50575	55.1
2016-306	73268	55738	41359	41359	56.4
2016-307	32698	24163	16684	16684	51
2016-308	91037	70700	52565	51572	56.6
2016-309	77906	61115	41209	41014	52.6
2016-310	47650	37492	24846	24846	52.1
2016-311	95303	73826	51514	51476	54
2016-312-1	26411	18869	14075	14075	53.3
2016-312-2	178301	134385	93678	89970	50.5
2016-313	1014	364	101	101	10
2016-314	635	244	83	83	13.1
2016-315	76705	58752	41027	39521	51.5
2016-316	35465	25224	17409	17215	48.5
2016-317	49150	36400	27879	27879	56.7
2016-318	38333	28538	21525	21525	56.2
2016-319	88985	42266	3887	3658	4.1
2016-323	52093	37472	20964	20962	40.2
2016-325	47610	35386	24314	24314	51.1
2016-326	47330	29002	22198	22197	46.9
2016-331	25055	17139	12652	12652	50.5

2016-337	137128	93510	68775	68697	50.1
2016-340	22942	17245	13276	13276	57.9
2016-343	39787	16804	12341	12341	31
2016-348	45179	28239	20036	19999	44.3
2016-350	17312	10761	8539	8538	49.3
2016-358	42909	28856	20751	20647	48.1
2018-019	29442	18800	13438	13438	45.6
3016-261	37349	28112	19897	19897	53.3
neg1	758	284	156	156	20.6
neg2	1311	522	308	308	23.5
neg3	627	170	51	51	8.1
neg4	969	271	69	69	7.1
neg5	1033	383	208	208	20.1
neg6	950	387	180	180	18.9
neg7	909	337	149	149	16.4
neg8	670	196	39	39	5.8
neg9	636	203	43	43	6.8
OCWA1	79637	62835	45655	45655	57.3
OCWA2	139002	109363	74109	74109	53.3
SWTH3	58800	44019	31465	31375	53.4
Undetermined	3830891	1161823	898956	850976	22.2