IBIS
BOU international journal of avian science
Ibis (2021) doi: 10.1111/ibi.12963

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

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During autumn migration, bird-eating raptors are thought to rely on flocks of migrant songbirds (Passeriformes) as a critical resource to fuel 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 on 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 autumn of 2015 and 2016 at a 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. We found that juvenile males selected smaller prey on average compared with juvenile females. Of the prey species detected, over 80% were migratory songbird species within the Pacific Flyway. In 2015, we detected a greater proportion of irruptive migrants in juvenile Merlin diet compared with 2016. In 2016, we found that the proportion of annual migrants consumed by Merlins corresponded to the timing of peak annual 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.

Keywords: community science, DNA metabarcoding, *Falco columbarius*, migrating food web, migration, predator–prey interactions.

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 focused on predator–prey interactions during migration has often been prey-centric, mainly revolving around predator-avoidance behaviours in relation to food and safety at refuelling sites (Alerstam & Lindström 1990, Ydenberg *et al.* 2007, Newton 2010). Consequently, the foraging ecology

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of migrating predators remains relatively unexplored in the field of ornithology (Lima 1998, Ydenberg *et al.* 2007), resulting in essential life-history 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 is spatially and temporally heterogeneous throughout the migration season, and prey abundances are continually shifting along a route and may vary year-to-year (i.e. 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 the long-distance journey.

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 being used to investigate the 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, Tobe *et al.* 2020). To date, DNA metabarcoding remains an under-utilized 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 that exceed the capabilities of direct field observations (Bourbour *et al.* 2019).

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 high energetic demands (Bildstein & Zalles 2005), making them ideal candidates to study how bird-eating raptors respond to the dynamic prey landscape during autumn migration. Our objectives were to collect dietary information from migrant juvenile Merlins to (1) describe the composition of avian prey species consumed, (2) test the long-standing hypothesis that migratory avian prey are an important 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. The results are discussed 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 autumn migration in 2015 ($n = 44$) and 2016 ($n = 28$), from 15 August to 15 December. 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), 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 facilitate 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 vulgaris* 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 clean (see Bourbour *et al.* 2019 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).

DNA extraction, amplification and sequencing

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

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.

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 Supporting Information Table S1 for primer and adapter sequences). 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, to test primers initially and 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 PCRs were performed in 25 µL with the following components: 12.5 µL of 2× 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 for 45 s, followed by a final extension of 5 min at 72 °C. A subset of PCR amplicons was visualized with 2% agarose electrophoresis to ensure amplification and all samples were then purified using Ampure beads (following the manufacturer's guidelines; Agencourt). For the Index PCR, we used 18 (8 forward, 10 reverse) barcoded primers (Illumina 2013; see Table S1). Index PCRs were performed in 50 µL with the following components: 25 µL of 2× 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 eight 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, Waltham, MA, USA) with an FLx800 Fluorescence Reader (BioTek Instruments, Winooski, VT, USA) 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. Ardeids) Merlin prey ($n = 205$) that broadly range in the Pacific Flyway according to species account range maps (Rodewald 2015; see Supporting Information Table S2). We used the R package *PrimerMiner-0.11* (Elbrecht & Leese 2017) to batch download all publicly available COI barcode sequences from the 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 cut-off 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 autumn 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 the estimated average mass of prey and migratory tendency using species accounts published in the Birds of North America online database (Rodewald 2015; see Supporting Information Table S3). 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 behaviour 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-effect 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 compared with 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 autumn migration within a migration corridor (MacMynowski & Root 2007), we tested for changes in the proportions of prey species detections in the diet of juvenile

Merlins over the autumn migration season using a generalized additive model (GAM; Hastie 2017) with the R package *mgcv* (Wood & Wood 2015). For the GAM, we analysed 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 $\gamma = 1$.

RESULTS

We obtained 13 million total raw reads with an average of 169 000 ± 175 000 sd per sample (see Supporting Information Table S4 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* or *Troglodytes pacificus* (see Table S2 for reference library summary). After matching sequences to our custom reference library, the average sample had approximately 70 00 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% cut-off 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* and Yellow Warbler. We did not detect Merlin DNA in our samples, possibly due to careful sampling or primer bias.

We detected the presence of 42 prey species with 251 prey species detections (Table S3) on 87.5% (63/72) of the migrant Merlins we sampled in 2015 ($n = 44$) and 2016 ($n = 28$); 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 to detect the majority of the avian prey species migrant Merlins were consuming along the Pacific Coast in western North America (Fig. 3).

Of the 63 juvenile Merlin individuals analysed, males weighed (mean ± sd) an average of 150.6 ± 8.8 g ($n = 37$) and females an average of 210.7 ± 12.7 g ($n = 26$). 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, $F_{1,\ 208} = 20.7$, $P < 0.001$), with male Merlins on average (mean ± se) selecting smaller prey species (23.7 ± 3.5 g) compared with females (39.6 ± 2.7 g; Fig. 4).

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 (four species; 41 species detections) and 15.2% were residents (11 species; 32 species detections). 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. Of 22 Merlins sampled in 2016, 21 (95.5%) yielded detections for regular migrants, 13 (59.1%) for residents and seven (31.8%) for irruptive migrants. We found that irruptive migrants made up a greater proportion of juvenile Merlin diet in 2015 than in 2016 ($z = 2.51$, $df = 1$, $P = 0.0120$) and did not detect differences between years for regular migrant ($z = 0.536$, $df = 1$, $P = 0.592$) or resident ($z = 1.64$, $df = 1$, $P = 0.101$) 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 $R^2 = 0.720$, GCV = 0.0311, deviance explained = 75.8%) and 2016 (adjusted $R^2 = 0.908$, GCV = 0.0146, deviance explained = 92.9%).

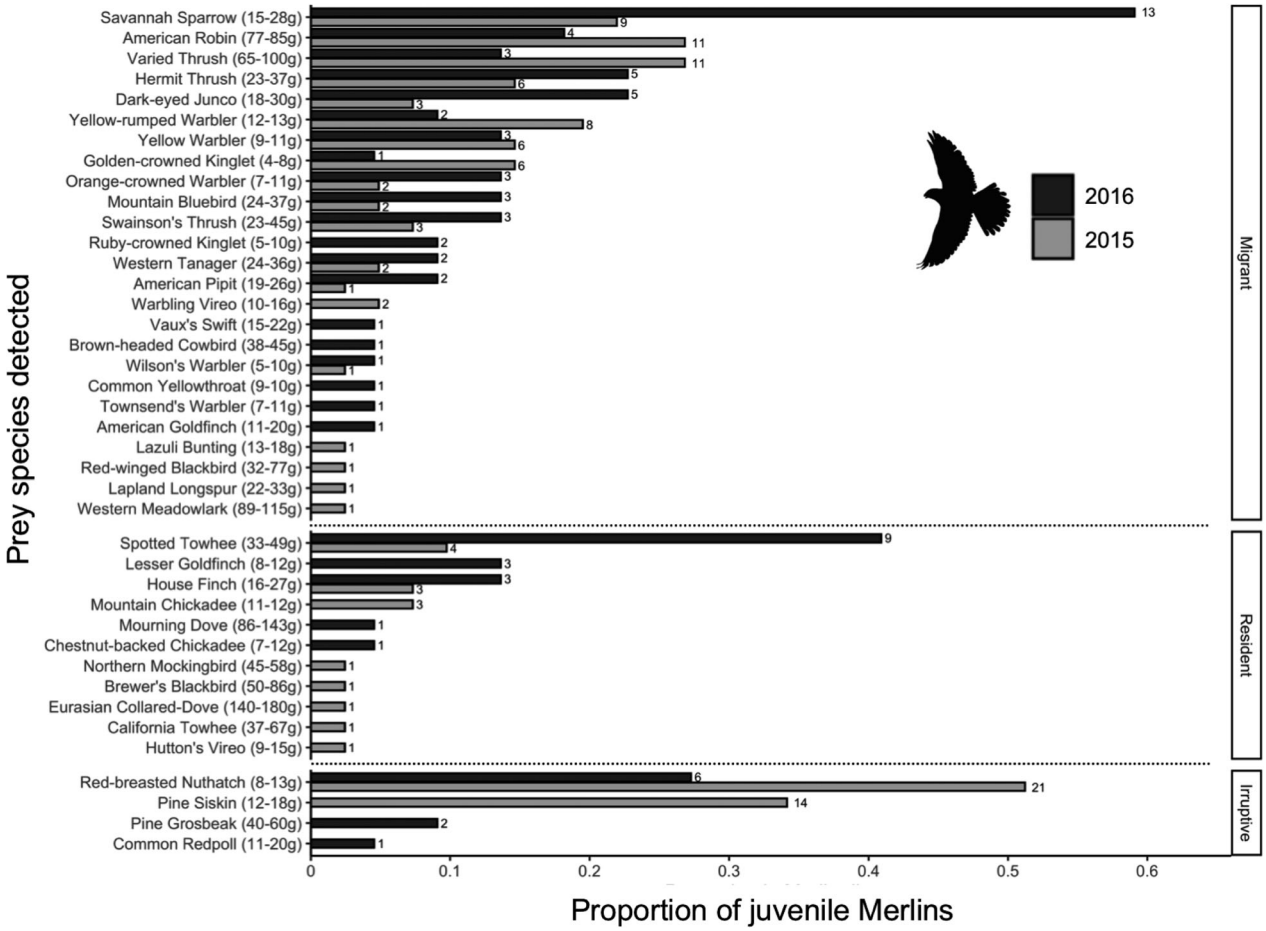

Figure 2. Proportion of migrant juvenile Merlins with prey species detections in 2015 and 2016. We detected 127 prey items on 41 individual Merlins in 2015 and 83 prey items on 22 individual Merlins in 2016. Prey species are grouped by migratory tendency in the Pacific Flyway: resident, regular migrant (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.

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 autumn 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 probably 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 autumn 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 have hypothesized that these bird specialists utilize migrant songbirds as a primary

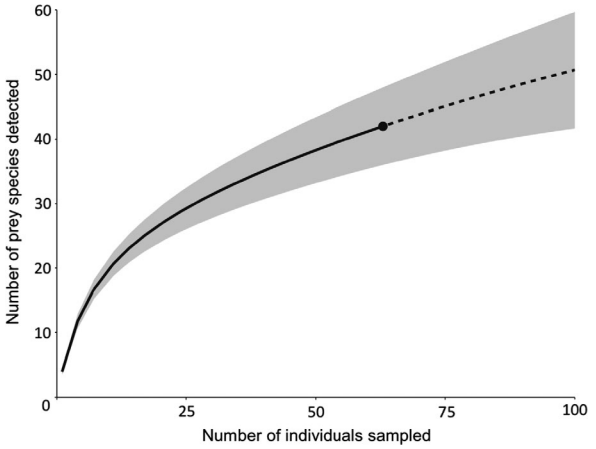

Figure 3. Rarefaction (solid line) and extrapolation (dashed line) sampling curves with a 95% confidence interval (shaded area) based on the prey DNA detected on 63 migrant Merlins in the Pacific Flyway.

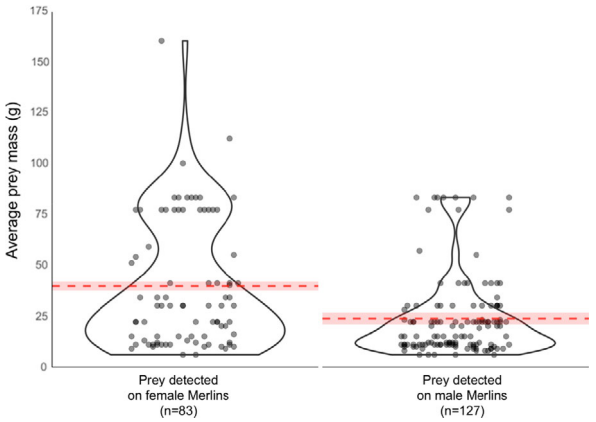

Figure 4. Violin plot showing prey size selection of female and male juvenile Merlins during the 2015 and 2016 autumn migration seasons. Over both seasons, 26 females yielded 83 prey detections and 37 males yielded 127 prey detections. The shaded red dotted line represents the modelled mean prey weight ± se. Our results show differences in prey size selection between female and male Merlins on migration, where males on average selected smaller prey species according to a linear model.

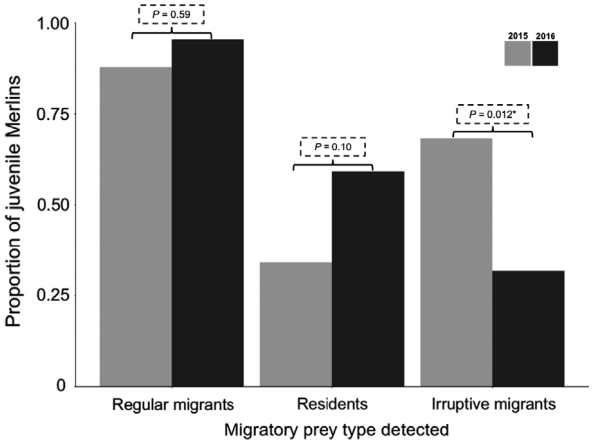

Figure 5. Proportion of Merlins with each of the migratory prey types detected. In 2015, 36 Merlins yielded detections for regular migrants (87.8% of samples), 14 for residents (34.1%) and 28 for irruptive migrants (68.3%). In 2016, 21 Merlins yielded detections for regular migrants (95.5%), 13 for residents (59.1%) and seven for irruptive migrants (31.8%). The *P*-values reported are the results of *z*-test for proportions. *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 with 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.

Table 1. GAM model output with approximate statistical significance of smooth terms for 2015 and 2016 migration seasons analysed 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

	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			
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

energetic resource. Our study demonstrates that these correlations in migration timing are related to the composition of juvenile Merlin diet during autumn migration and provide support for a migrating food web hypothesis (i.e. raptors migrating with migrant 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

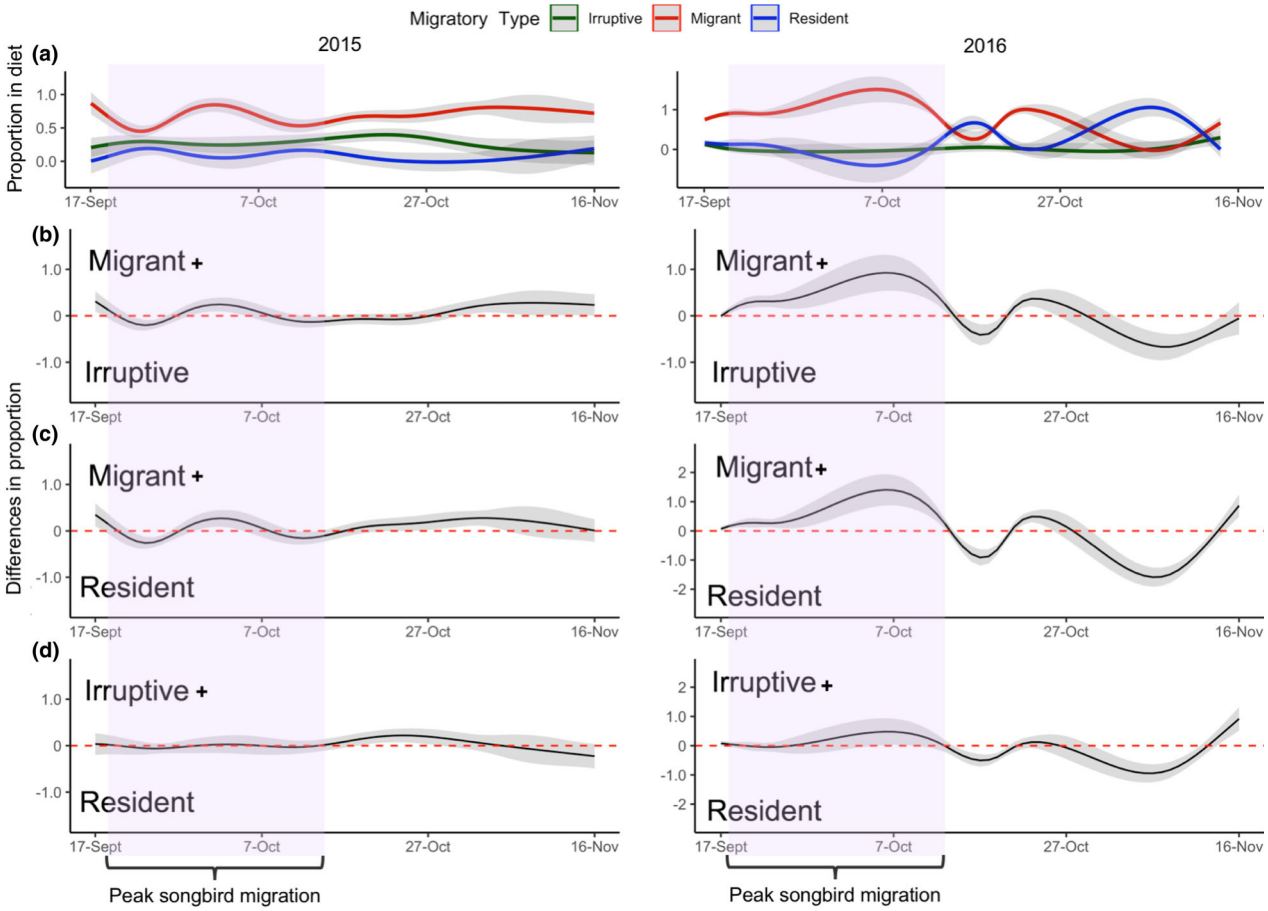

Figure 6. GAM model visualizations for the 2015 and 2016 migration seasons. (a) Variation in the proportion of prey by migratory type consumed by all Merlins sampled in each year. (b–d) Differences in proportions between migratory prey types. +, the black line is above the red dotted line, the proportion of that prey type is greater than the prey type compared below. Where the 95% confidence does not overlap with the red dotted line, the proportion differences between the two migratory prey types are 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.

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 targetted 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 than 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 (National Audubon Society 2020), and we found that the proportion of juvenile Merlins with irruptive songbird migrant prey DNA detected was greater in 2015 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 inter-annual 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.

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 more frequently on females, such as American Robins. 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 are focused on nest defence 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 autumn 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 behaviour, 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.* 2019).

Secondly, it is impossible to know how many individuals of a single species were consumed by an individual raptor – we can only determine the frequency at which a species was detected among samples. This is because amplicon read counts are not correlated with the 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 an unknown degree of DNA degradation.

Thirdly, 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 geographical areas, and when foraging cannot be observed (Bourbour *et al.* 2019). 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 (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.

The materials for this study were funded by the Henry A. Jastro Graduate Research Award, American Ornithological Society's Hesse Award, and Wilson Ornithological Society's Fuertes Grant. DNA sequencing was done by the UC Davis Genome Center. We thank Andrea Schreier, Amanda Coen, Grace Auringer, Ann Holmes, Sean O'Rourke, Shannon Kieran and the Genomic Variation Laboratory. We thank Andy & Irene Engilis and the UC Davis Museum of Wildlife & Fish Biology and Diana Humple & Reneé Cormier with Point Blue Conservation Science. We thank Ryan Phillips, Sara Kross, Megan Crane, Shannon Skalos, Emily Abernathy, Emily Phillips, Jessica Schlarbaum, Amy Miles, Molly Heal, Brian Hoover and many others for feedback and support. We thank the Golden Gate Raptor Observatory (GGRO) and all the community science volunteers who made sample collection possible. Every year, GGRO manages hundreds of community science volunteers in an effort to build long-term movement, morphological and genetic datasets that give researchers the ability to monitor population health and trends of raptors in the Pacific Flyway. We would also like to express gratitude to those who provided peer-reviews for the manuscript during the year 2020. All national and institutional guidelines for the care and use of animals were followed. All animal handling and sample collections were in compliance with the Golden Gate Raptor Observatory standards as described in their Institutional Animal Care and Use Committee protocols, California State Permit, and United States Geological Survey (IACUC permit # CA_GOGA_Ely_Raptors_2020.A3; California State permit # SCP 13739; Federal banding permit # 21827).

AUTHOR CONTRIBUTIONS

Ryan Bourbour: Conceptualization (equal); Data curation (lead); Formal analysis (lead); Funding acquisition (lead); Investigation (lead); Methodology (lead); Project administration (lead); Writing-original draft (lead); Writing-review & editing (lead). **Cody Aylward:** Formal analysis (equal); Methodology (equal); Writing-review & editing (equal). **Chris Tyson:** Data curation (equal); Formal analysis (equal); Methodology (equal); Visualization (equal); Writing-review & editing (equal). **Breanna Martinico:** Formal analysis (equal); Methodology (equal); Visualization (equal); Writing-original draft (equal); Writing-review & editing (equal). **Alisha Goodbla:** Methodology (equal); Resources (equal); Supervision (equal); Writing-review & editing (equal). **Teresa Ely:** Project administration (equal); Resources (equal); Writing-review & editing (equal). **Allen M Fish:** Project administration (equal); Resources (equal); Supervision (equal); Writing-review & editing (equal). **Angus C. Hull:** Project administration (equal); Supervision (equal); Writing-review & editing (equal). **Joshua Hull:** Conceptualization (equal); Project administration (equal); Resources (equal); Supervision (equal); Writing-review & editing (equal).

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request or will be uploaded to Dryad pending approval of data release.

Custom reference library harvested via PrimerMiner is available at https://doi.org/10.25338/B8J91X.

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Received 19 August 2020;
revision accepted 20 April 2021.
Associate Editor: Beatriz Arroyo.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Tables S1. Primers used in library preparation two-step PCR for Illumina Miseq

Tables S2. Custom Reference Library of publicly available COI barcode sequences harvested via the R package 'PrimerMiner'.

Tables S3. Summary of prey detections with average mass and migratory behavior of each prey species.

Tables S4. Summary of reads generated by R package 'dada2' for samples reported in this study.