

THE ROLE OF FOOD QUANTITY AND PREY TYPE IN NESTLING DEVELOPMENT OF AMERICAN KESTRELS

ALLISON CORNELL¹

Department of Biology, Penn State Altoona, 3000 Ivyside Park, Altoona, PA 16601 USA

and

Department of Biological Sciences, Cedar Crest College, 100 College Drive, Allentown, PA 18104 USA

MELINDA A. FOWLER

Department of Biology/Chemistry, Springfield College, 263 Alden Street, Springfield, MA 01109 USA

COURTNEY ZIMMERMAN AND ZAINAB KHAKU

Department of Biological Sciences, Cedar Crest College, 100 College Drive, Allentown, PA 18104 USA

JEAN-FRANÇOIS THERRIEN

Acopian Center for Conservation Learning, Hawk Mountain Sanctuary, 410 Summer Valley Road, Orwigsburg, PA 17961 USA

ABSTRACT.—Diet is an important component of life history that can vary with, and ultimately determine, individual variation in phenotypically plastic traits. American Kestrels (*Falco sparverius*) generally have low post-fledging survival rates, which suggests a need to better understand what ecological factors, such as diet, influence nestling maturity. The generalist diet of kestrels makes it unlikely that all nestlings in a population receive the same diet. We investigated how breeding phenology and nestling sex ratio interact with diet metrics (diet diversity, percentage of prey types, rate of prey biomass delivery) and relate to nestling maturity (mass, tarsus length, wing length, hematocrit, hemoglobin concentration). We hypothesized that: (1) phenology and nestling sex ratio would correlate with inter-nest diet variation; (2) diet metrics would be predictive of nestling development; and (3) the manipulation of food quantity through food supplementation would lead to nestlings with greater developmental maturity. We found that inter-nest variation in diet was correlated with breeding phenology and nestling sex ratio, independently. However, the variation in diet was unrelated to nestling maturity. In response to food supplementation, kestrel parents decreased their food-provisioning rate, indicating that food quantity regulates parental care. Male nestlings appeared to benefit from supplementation while females did not. Our data demonstrated high inter-brood variation in nestling diet, and suggested that diet variation interacts with sex to influence growth and development of nestlings, which could potentially be linked to population decline.

KEY WORDS: *American Kestrel; Falco sparverius; diet; diet diversity; diet specialization; nestling; sexual dimorphism.*

EL PAPEL DE LA CANTIDAD DE ALIMENTO Y EL TIPO DE PRESA EN EL DESARROLLO DE LOS POLLUELOS DE *FALCO SPARVERIUS*

RESUMEN.—La dieta es un componente importante de la historia de vida que puede variar con, y en última instancia determinar, la variación individual en los rasgos fenotípicamente plásticos. *Falco sparverius* generalmente tiene bajas tasas de supervivencia después de emplumar, lo que sugiere la necesidad de comprender mejor qué factores ecológicos, como la dieta, influyen en la madurez de los polluelos. La dieta generalista de *F. sparverius* hace poco probable que todos los polluelos de una población reciban la misma dieta. Investigamos cómo la fenología reproductiva y la proporción de sexos de los polluelos interactúan con las métricas de la dieta (diversidad de la dieta, porcentaje de tipos de presas, tasa de aporte de biomasa de presas) y cómo se relacionan con la madurez de los polluelos (masa, longitud del tarso, longitud del ala,

¹ Email address: aec5780@psu.edu

hematocrito, concentración de hemoglobina). Hipotetizamos que: (1) la fenología y la proporción de sexos de los polluelos se correlacionarían con la variación de la dieta entre nidos; (2) las métricas de la dieta serían predictivas del desarrollo de los polluelos; y (3) la manipulación de la cantidad de alimento a través de alimentación suplementaria conduciría a polluelos con mayor madurez. Encontramos que la variación en la dieta entre nidos se correlacionó de modo independiente con la fenología reproductiva y con la proporción de sexos de los polluelos. Sin embargo, la variación en la dieta no estuvo relacionada con la madurez de los polluelos. En respuesta a la alimentación suplementaria, los progenitores de *F. sparverius* redujeron su tasa de suministro de alimentos, lo que indica que la cantidad de alimento regula el cuidado parental. Los polluelos machos parecieron beneficiarse de la suplementación mientras que las hembras no. Nuestros datos demostraron una alta variación entre nidadas en la dieta de los polluelos y sugirieron que la variación de la dieta interactúa con el sexo para influir en el crecimiento y desarrollo de los polluelos, lo que podría estar potencialmente relacionado con la disminución de la población.

[Traducción del equipo editorial]

INTRODUCTION

Diet can be an important indicator of habitat quality and can affect phenotypically plastic traits in wild birds. Birds consume different diets based on their life history stage (Bairlein and Gwinner 1994, Martins et al. 2013), age (Rutz et al. 2006, Nadjafzadeh et al. 2016), and sex (Forero et al. 2002, Shaw 2009, Bravo et al. 2016, Catry et al. 2016). Food availability undoubtedly affects the diet of consumers and varies naturally based on phenology (Rodríguez et al. 2010, Garcia-Heras et al. 2017b), weather (Catry et al. 2012, Garcia-Heras et al. 2017b), and habitat quality (Valkama et al. 1995, Boratynski and Kasprzyk 2005, Byholm and Kekkonen 2008). Variation in the availability of certain prey can determine the nutritional quality of the diet (Eeva et al. 2009, Arnold et al. 2010, Razeng and Watson 2015) and ultimately affect fitness through changes in long-term productivity (Annett and Pierotti 1999), clutch size (Catry et al. 2012), fecundity (Rutz et al. 2006), and offspring body size (Forero et al. 2002). Diet during nestling development may be particularly important in altricial birds because of the high mortality rate immediately following fledging (reviewed in Naef-Daenzer and Gruebler 2016).

American Kestrel (*Falco sparverius*) populations have been declining since the 1980s and speculated causes include pesticide exposure, predation by larger raptors such as Cooper's Hawks (*Accipiter cooperii*), and habitat loss, though none have been supported as the sole cause of declines (Smallwood et al. 2009). Low recruitment rates reported by nest box monitoring programs (Steenhof and Heath 2013) and high variation in nestling developmental maturity (defined as mass, tarsus length, wing chord, hematocrit, hemoglobin concentration at day 21) among nests (Cornell et al. 2021) suggest that

declining nestling maturity may be a component of the observed decline. During development, limited food availability can constrain growth and reduce the maturity of nestlings, potentially constraining fitness (Gebhardt-Henrich and Richner 1998). Previous studies have shown that kestrels reared on diets restricted to 70–80% of controls' *ad libitum* diet had slower growth rates, reduced fat stores, and shorter feather lengths (Lacombe et al. 1994). But quality of food or prey type may also be a limiting factor: kestrels hand reared on high-fat, laboratory mice (*Mus musculus*) had larger fat reserves and wing-loading at fledging, but slower growth rates compared to those fed on protein-rich cockerels (*Gallus domesticus*; Lavigne et al. 1994). However, some kestrel diet studies rely on data from prey remains or pellets (see review [table 1] in Boal et al. 2021), which do not account for food quantity and make it difficult to compare the relative roles of food quantity and prey type in a natural setting. Additionally, the effects of food abundance can vary depending on ecological context, such as annual variation, weather, and breeding phenology (Dewey and Kennedy 2001, Ritz et al. 2005, Cornell and Williams 2017).

Nutritional needs of nestlings may vary with sex in dimorphic species (Anderson et al. 1993b), so variation in brood sex ratio may affect diet delivered to the nest (Rutz 2012). Captive studies of kestrel nestling diet show that the type of prey delivered to the nest determines the competitive advantage of female nestlings over males (Anderson et al. 1993a). This can lead to differential effects of supplemental feeding in nests with different sex ratios; only male-biased broods benefitted from supplemental feeding of Lesser Kestrels (*Falco naumanni*; Soravia et al. 2021). Among American Kestrels, sex ratio of nestlings can vary with breeding phenology, poten-

tially reflecting changes in prey availability (Smallwood and Smallwood 1998, Griggio et al. 2002). However, the relationship between diet and nestling sex is not well understood in American Kestrels and other raptors.

In this study we examined the nestling diet of a regionally declining, sexually dimorphic, generalist predator, the American Kestrel. We hypothesized that (1) components of the nest environment, including breeding phenology (laying date of the first egg) and nestling sex ratio would correlate with inter-nest variation in nestling diet as measured by percentage of prey types, diet diversity, and rate of biomass delivered to nestlings; (2) diet metrics would be predictive of nestling growth and development morphologically (mass, tarsus length, wing length) and physiologically (hematocrit, hemoglobin concentration); and (3) manipulation of diet with food supplementation would lead to nestlings of higher developmental maturity.

METHODS

Field Sites and Monitoring. In 2019, we studied natural variation in diet at 10 American Kestrel nests in southeastern Pennsylvania. In 2021, we conducted a supplemental feeding experiment at 13 nests in central Pennsylvania. The general habitat was similar at the two sites; both were rural areas that included agriculture (corn, soybean, or hayfields) and pastures with livestock, with nest boxes mounted on utility poles, trees, or barns. Nest box dimensions and mounting details at both sites followed Katzner et al. (2005). In both years, from May to July we monitored nest boxes for kestrel nests with eggs. Nests were checked for hatching every other day beginning 28 d after the last egg was laid, and hatch date was recorded to age nestlings. If not observed, the laying date of the first egg was estimated by back-calculating from hatch day, allowing 28 d for incubation and 2 d for the laying of each egg in the clutch. Any nest, at which at least one nestling hatched, was monitored until fledging and brood size at fledging was recorded, hereafter referred to as fledging success.

Natural Variation in Diet. In 2019, we used a GoPro Hero7 Black camera mounted to the outside of the nest box to record 10 nests for an average of 122 min/d (range: 60–253 min/d) starting 6 d after nestlings hatched: days 5 and 6, 12 and 13, and 19 and 20 (hatch day = day 0). Two nests had only 5 d of recording due to equipment malfunction; however, longer recordings on other days mitigated these

differences such that there was no effect on overall time recorded (t -test, $t = -0.14$, $P = 0.91$). A dummy GoPro was mounted to the box whenever we were not recording to minimize effects of the camera on natural behavior. We classified the weather during recording as sunny, cloudy, light rain, or steady rain, but >90% of recordings were completed with no precipitation. We viewed all video recordings and recorded the number of parental food deliveries per hour. We used one-way ANOVAs to test the effects of weather and time of day on the number of parental food deliveries per hr. We classified the prey items into the following categories: mammal, passerine, arthropod, annelid, and other (Supplemental Material Table S1). The “other” category included two prey items observed only once each: a green frog (*Rana clamitans*) and a northern fence lizard (*Sceloporus undulatus*).

We collected data on nestling development between the hours of 0800–1200 H at 21 d after hatch. American Kestrels typically fledge 28 d after hatch (Smallwood and Bird 2020). During sampling, we removed nestlings from their nest and collected blood from the brachial vein ($\leq 1\%$ of total body mass) into a heparinized microcentrifuge tube. We typically sampled nestlings within 4 min of removal from the nest box. Whole blood samples were immediately put on ice until laboratory analysis. On day 7, we marked individuals with unique color bands for individual identification (Darvic wrap-around 1FB-5.5mm, Avinet SKU: 1FBD-bl). We removed the color bands on day 21 and replaced them with USGS bands. We sexed the nestlings by examining primary feather colors (blue for males and brown for females) on day 14. Mass was measured to the nearest 0.01 g using a digital scale, tarsus length to the nearest 0.01 mm using digital calipers, and flattened wing chord to the nearest 1 mm using a wing ruler.

Experimental Manipulation of Food Quantity. In 2021, we randomly assigned nests to either treatment ($n = 7$ nests [29 nestlings]) or control ($n = 6$ nests [31 nestlings]) on hatch day. We used data from Anderson et al. (1993b) to approximate the mean of 20% of the food mass consumed daily per nestling. We supplemented treatment nests by delivering this amount of food (proportionate to brood size) every other day. Thus, nestlings in treatment broods received 10% extra food overall from hatch day until day 21. Supplements were dead mice (*Mus musculus*) purchased from RodentPro.com, stored in a freezer and thawed the day before

each feeding. To minimize disturbance, we used a telescoping pole to deliver food through the nest box hole. Nestlings regularly made begging calls when food was dropped. We collected data on nestling development using the same methods as in 2019. If nestling sampling took place on a day scheduled for supplemental feeding, we supplemented the nest after sampling. On both days 19 and 20, we used binoculars to make 1-hr long observations from a distance of at least 20 m, with a vehicle as a blind. We recorded the number of times the male and female parent brought food to the nest, as well as the type of prey delivered. Observations for treatment nests were completed before supplemental food was delivered.

Laboratory Methods. We measured hematocrit by centrifuging the blood in a capillary tube at 13,000 revolutions per min for 5 min. We measured packed cell volume and total volume using digital calipers to the nearest 0.01 mm. We determined hemoglobin concentration (g/dL whole blood) using the cyanmethemoglobin method (Drabkin and Austin 1932) with modifications for use with a microplate spectrophotometer. We added a 5 μ L aliquot of whole blood to 1.25 mL Drabkin's reagent (Sigma Aldrich D5941) and measured absorbance values in triplicate as a measure of intra-assay variation at 540 nm in Fisher Multiskan FC Model 357 plate reader in 2019 and a BioTek Epoch plate reader in 2021. Inter-assay variation of a pooled sample across plates was 5% in 2019 and <1% in 2021, coefficient of variation within repeated samples was 5% in 2019 and 3% in 2021.

Statistical Analyses. We report all values as mean \pm SD. We grouped related terms as follows: diet metrics included diet diversity, percentage of prey types (by biomass), rate of prey biomass delivery per nestling; nestling maturity referred to the day 21 values of mass, tarsus length, wing chord, hematocrit, and hemoglobin concentration. Nestling sex ratio was based on day 7 values. We calculated percent mass of each prey type in the diet using values of average mass for each prey type found in the literature (Table S1) and dividing by the total mass of all prey items observed at the nest and multiplying by 100. We arcsine-transformed percent of each prey type. We calculated rate of total prey biomass delivered per nestling by summing the total estimated mass of prey items delivered at a nest, divided by the total hours of observation, divided by the number of nestlings in the nest. We used the Shannon-Wiener diversity index to estimate diet

diversity using the R package *vegan* (Oksanen et al. 2020), and Pearson's product-moment correlation tests to test the relationship between nestling sex ratio and laying date. We used principal component analysis to synthesize nestling maturity into a morphology component (mass, tarsus, wing length) and physiology component (hematocrit, hemoglobin concentration) in R (R Core Team 2020). We used stepwise regression in linear and linear mixed effects models, starting with the full model and all interactions, but removing all nonsignificant predictor variables until we arrived at the final models reported in the tables (Table S4). Subsequently we tested reduced versus full models using ANOVAs (linear models) or *F*-tests (linear mixed effects models) to ensure there were no significant differences in the reduced models' explanation of variance. We used linear models to test the relationship between diet metrics (as response variables) and breeding phenology and nestling sex ratio (as predictor variables) in R (R Core Team 2020). This analysis only included the natural variation year (2019). We tested the effects of diet metrics on fledging success in 2019 and 2021 using linear models. We tested the relationship between diet metrics (percentages of each prey type, diet diversity, and rate of prey biomass delivered per nestling) and experimental treatment group as predictor variables, and nestling morphology and physiology as response variables using linear mixed effects models with the *nlme* package (Pinheiro et al. 2020). We included nest ID as a random factor and sex as a covariate for the model of morphology principal component due to female nestlings being 10% larger than males. We also included sex as a covariate in the morphology model. We used an unpaired two-tailed *t*-test to compare per-nestling provisioning rate in 2021 between treatment and control nests. We reported correlations of nestling maturity with breeding phenology and nestling sex ratio in another study (Cornell et al. 2021). We ran a multiple regression power analysis in R on a sample size of 10 nests at a power of 80% and significance of 0.05 (R Core Team 2020).

RESULTS

Natural Variation in Diet (2019). We analyzed 117 hr of video and recorded a total of 229 prey items provisioned to the 10 study nests. We found a mean of 2.5 ± 2.2 deliveries per nest per hr (range: 0–12). Prey types varied by nest and included mammals (seven nests), passerines (seven nests), arthropods

Table 1. Results of linear models testing the relationship between nest environment and natural variation in American Kestrel nestling diet (2019).

DIET METRIC	LAYING DATE	NESTLING SEX RATIO
Percent mammal	No relationship	+ Related to males
Percent passerine	No relationship	+ Related to females
Percent arthropod	+ correlation	No relationship
Percent annelid	No relationship	No relationship
Diversity of prey (Shannon index)	No relationship	No relationship
Biomass of food per nestling per hour	No relationship	No relationship

(nine nests), annelids (seven nests), as well as one frog and one lizard each at different nests (Fig. S1). Diet breadth varied among broods: at one nest we recorded only mammalian prey items delivered, whereas at others the dominant prey item by mass made up <50% of total biomass provisioned (Fig. S1). Brood sizes ranged from 1–5 nestlings and averaged 4.4 ± 1.1 nestlings, and fledging success was 0–5 nestlings, with 3.5 ± 1.9 nestlings on average. The only mortalities were a single dead nestling (of a brood of five) in one box between days

14 and 21 and a single dead nestling in another box on day 28 when we checked the box for fledging. Sex ratios ranged from 0–100% female nestlings and averaged $54 \pm 18\%$ female. Sex ratio of the nestlings and laying date were not significantly correlated ($P > 0.10$). Weather (categorical) had no effect on the number of parental food deliveries to the nest per hr (one-way ANOVA, $F_{3, 421} = 1.5, P = 0.20$). Time of day also had no effect on parental deliveries to the nest per hr (linear mixed effects model, nest as a random effect, $F_{1, 47} < 0.1, P = 0.88$). The results of the power analysis showed that for a sample size of 10 nests, the effect size would have to be 0.80 to reach a power of 80% at a significance of 0.05.

Kestrels with later laying dates delivered a higher percentage of arthropod prey (Table 1; $df = 1, 8$, estimate = 0.02, $R^2 = 0.5, P = 0.02$). However no aspect of diet was related to fledging success (Table S2). Nests with a higher proportion of female nestlings were fed a greater percentage of passerine prey ($df = 1, 7$, estimate = 1.40, $R^2 = 0.5, P = 0.03$), and nests with a higher proportion of male nestlings were fed a greater percentage of mammalian prey ($df = 1, 7$, estimate = -1.75, $R^2 = 0.6, P < 0.01$; Fig. 1). When the two single-sex broods were removed from the models, the relationship between females and passerine prey remained significant ($df = 1, 5$, estimate = 3.11, $R^2 = 0.6, P = 0.04$), though the relationship between males and mammalian prey became nonsignificant ($df = 1, 5$, estimate = -2.43, $R^2 = 0.4, P = 0.12$). There were no other significant relationships between diet and nestling sex ratio or phenology (Table 1, Table S3).

The first principal component of morphology metrics (mass, tarsus, and wing length) explained 56% of the variation. The first principal component of the physiology metrics (hematocrit, hemoglobin concentration) explained 64% of the variation. Neither of these principal components was significantly related to any of the diet metrics (Table S4).

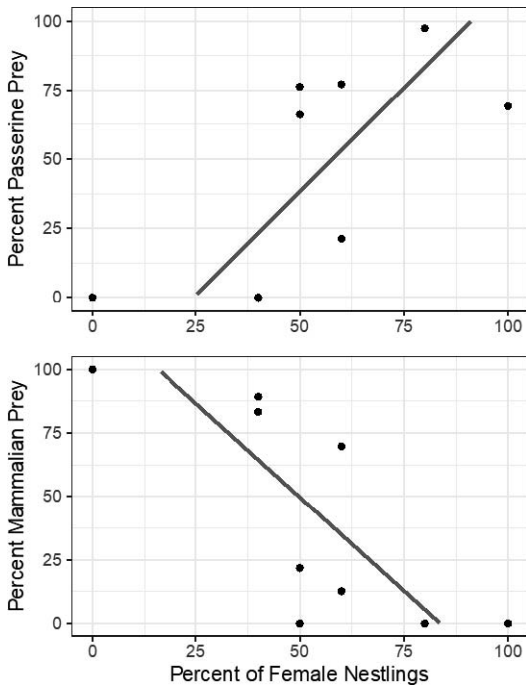


Figure 1. Nests with a higher percentage of female American Kestrel nestlings had a higher percentage of passerine prey in diet provisioned to the nest. Nests with a higher percentage of male nestlings had a higher percentage of mammal prey provisioned to the nest.

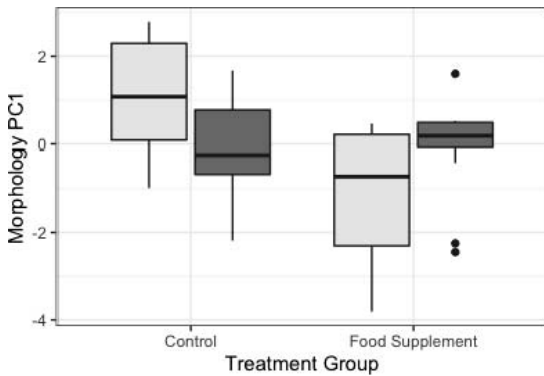


Figure 2. Morphology principal component 1 of male (dark gray bars) and female (light gray bars) American Kestrel nestlings in control and treatment (food-supplemented) nests.

The time of day we collected the blood sample was not significantly related to nestling maturity (linear mixed effects models controlling for nest ID as a random factor and age and sex as covariates where significant, $P > 0.27$).

Response to Supplemental Feeding (2021). We observed 26 hr of parental care behavior and recorded a total of 87 deliveries to the 13 study nests. We observed a mean of 2.6 ± 3.0 deliveries per nest per hr (range: 0–11). Parents responded to supplemental feeding by reducing provisioning rate ($t_{17.8} = -2.3$, $P = 0.03$). This effect was driven by a reduction in the number of food deliveries per nestling per hr by the female parent ($t_{14.9} = -2.8$, $P = 0.01$), with the per-nestling provisioning rate for controls (0.83 ± 0.69 deliveries per nestling per hr) over three times greater than that of supplemented nests (0.23 ± 0.30 deliveries per nestling per hr), while the male parent's provisioning rate did not differ between groups ($t_{18.9} = 1.0$, $P = 0.84$). There was no effect of time of day on per-nestling provisioning rate (linear mixed effects model, nest as a random effect, $F_{1, 17} = 2.3$, $P = 0.14$). There was a significant effect of treatment, nestling sex, and an interaction of treatment and sex on the morphology principal component (Table S5; Fig. 2). Supplemental feeding appeared to benefit males but not females: body mass at day 21 was sexually dimorphic in control nests, with males (119.3 ± 10.0 g) smaller than females (131.4 ± 11.1 g), but no sexual dimorphism in treatment nests (males: 127.1 ± 10.7 g; females: 124.4 ± 16.0). There was no effect of treatment on the physiology principal component.

One nest failed due to predation (no nestlings survived) and two nests had partial mortality prior to fledging, but there was no effect of treatment on fledging success ($F_{1,10} = 0.7$, $P = 0.42$).

DISCUSSION

We investigated the relationships between nestling diet (food quantity and prey type), nestling development (morphological and physiological), and components of nest environment (breeding phenology and nestling sex ratio) in American Kestrels. Although some other raptors have a specialist feeding strategy (e.g., Garcia-Heras et al. 2017a), including some species of kestrels (Korpimäki 1985), the American Kestrels we studied had high inter-brood variation in diet (Fig. S1). However, no diet metrics were correlated with nestling maturity. Our experimental results indicated that parental care was adjusted when supplemental food was provided, suggesting that food quantity was important. The experimental effects on nestlings may have benefitted the smaller sex (males), perhaps by releasing male nestlings from a food limitation. However, the small sample size of this study limited our ability to find significant effects. Nonetheless, the data presented here suggest some trends that may be pertinent to the American Kestrel decline and should be investigated further.

The percentage of arthropods in the nestling diet was significantly higher in nests with later laying dates. Many insects have a strongly seasonal component to their development, during which avian insectivores may coordinate their reproduction to optimize insect predation opportunities (e.g., Naef-Daenzer et al. 2000, Tremblay et al. 2003). The possibility of phenological mismatch between predators and prey has been a major concern in avian conservation (Visser et al. 2012, Visser and Gienapp 2019). However, the fine-tuning of breeding phenology may be less important for a generalist predator, and despite the fact that some American Kestrel populations rely on arthropods for up to 71% of prey items (by frequency) during the breeding period (Liébaná et al. 2009), the biomass contributed by arthropods is generally quite small. Kestrels have also shown significant shifts toward earlier laying dates in the last few decades (Smith et al. 2017). Whether these phenological shifts are linked to changes in diet is unknown. Regardless of possible phenological mismatch, the decline of insects (Wagner 2020) throughout the range of the American Kestrel may be linked to kestrel decline if

arthropods are or historically were a major component of diet. For the kestrels we studied, arthropods only made up $8 \pm 9\%$ of prey biomass on average and this percentage was unrelated to both fledging success and nestling development. A higher percentage of arthropods in the diet also had no relationship to nestling sex ratio, despite the higher proportion of female kestrel nestlings in late-season nests reported elsewhere in some (Smallwood and Smallwood 1998, Griggio et al. 2002) but not all American Kestrel populations (Wiebe and Bortolotti 1992). The seasonal increase in arthropods was unrelated to both diet diversity and rate of prey biomass delivered per nestling.

In our study population, the preferred prey type appears to vary in relation to nestling sex ratio: nests with more males received higher proportions of mammalian prey and nests with more females received higher proportions of passerine prey (Fig. 1). Previous studies of kestrel prey nutritional composition have shown that avian prey has the highest gross energy and fat content, compared to arthropod and mammalian prey items (Bird et al. 1982). Given the faster growth rate of female kestrels, it follows that nests with more females may require more energy-rich, avian prey (Anderson et al. 1997, Cornell et al. 2021). It is interesting that our rodent food supplementation seemed to benefit male nestlings only. One possible explanation is that passerine prey may be more supportive of female development and mammalian prey has greater benefits to male development, given the nutritional differences required for growth of each sex (Anderson et al. 1993b) and the natural variation in diet relative to nestling sex ratios (Rutz 2012). Therefore, the rodent supplement may have replaced parent feeding visits that might otherwise have brought passerine prey, benefitting the males but decreasing the supply of passerine prey preferential for females. The experiment would need to be repeated with a passerine prey supplement for conclusive assessment. An unresolved factor in interpreting our results is the unknown physiological mechanism for facultative adjustment of sex ratios at the time of egg laying (Pike and Petrie 2003), as sex-specific nestling mortality is unlikely to be a mechanism in our system given the low partial brood mortality.

Results from food supplementation showed that parents, in particular female parents, regulate provisioning rate based on quantity of food. However, male raptors often hand off prey to the female parent to deliver to the nest (Sonerud et al. 2013)

and our data could not account for such handoffs; therefore, we cannot assume there was no effect on male parental care. Reduced provisioning at supplemented nests could be facilitated by changes in begging intensity of nestlings or the presence of the food supplement itself. Although reductions in parent provisioning have been found in other kestrel supplemental feeding studies (Wiehn and Korpimäki 1997, Dawson and Bortolotti 2002), most similar manipulations in raptors do not document effects on parent provisioning rate (e.g., Kennedy and Ward 2003, González et al. 2006, Wellicome et al. 2013, Perrig et al. 2014), so it is unclear whether this behavioral response is unique to kestrels. It is possible that the reduced provisioning is responsible for the minimal effects we found on nestlings: treatment nestlings may have received the same quantity of food as controls, as in Dawson and Bortolotti (2002). At the least, we likely manipulated diet composition, because our supplements were exclusively rodents.

We found high inter-brood variation in prey types delivered to nestlings, significantly related to phenology and nestling sex ratio. Although diet did not correlate with nestling development, supplemental feeding down-regulated parental care and may have had some benefits to male nestlings. Given the declines reported in American Kestrels (Smallwood et al. 2009) and the possibility of pesticides among the potential causes (McClure et al. 2017), differences in diet between sexes could lead to differential exposure to pesticides. For example, more passerine prey provisioned to nests with more females could lead to females with higher neonicotinoid concentrations. More data are needed to identify whether sexual differences in diet lead to differences in pesticide load. Future studies should explore how kestrel population declines relate to food, habitat quality, pesticide exposure, and land use, with potential sexual differences in mind.

SUPPLEMENTAL MATERIAL (available online). Table S1: Representative mass values used in the calculations of prey mass provisioned to the nest. Table S2: Results from linear model showing how natural variation in diet metrics relate to fledging success during 2019. Table S3: Statistical values of linear models testing the relationship between nest environment and natural variation in nestling diet (2019). Table S4: Results from linear mixed effects models showing how diet metrics relate (as fixed effects) to natural variation in nestling maturity (in 2019). Table S5: Results from linear mixed effects

models showing how supplemental feeding affected nestling maturity (day 21 values) and developmental trajectory in 2021. Table S6: Results of *F*-tests on full compared to reduced linear mixed effects models reported in Table S4. Figure S1: Diet composition by prey item across nests.

ACKNOWLEDGMENTS

Studies during 2019 were conducted under the Cedar Crest College IACUC permit #2018-01 and USGS permit #22749. Studies during 2021 were conducted under Pennsylvania State University IACUC protocol #202001707 and USGS permit #24268. This work was funded by a Blake-Nuttall Award to A. Cornell, and other awards to Hawk Mountain Sanctuary from the Giorgi Family Foundation, the Robert F. Schumann Foundation, the Pennsylvania Game Commission, and the Wild Resource Conservation Program of the Pennsylvania Department of Conservation and Natural Resources. Thank you to Jon Kauffman, Shaver's Creek Environmental Center, lab assistants Kerice Kelly and Gizelle Abanador, field technician Matt Bowers, field assistants Mercy Melo and Jaime Matson, as well as the many Hawk Mountain Sanctuary staff, trainees, and volunteers who assisted on this project. We would also like to thank the numerous private landowners of Berks, Lehigh, Schuylkill, Centre, Huntingdon, and Mifflin Counties for allowing us to conduct this study on their properties. This is Hawk Mountain Sanctuary contribution to conservation science number 378.

LITERATURE CITED

- Anderson, D. J., C. Budde, V. Apanius, J. E. Martinez Gomez, D. M. Bird, and W. W. Weathers (1993a). Prey size influences female competitive dominance in nestling American Kestrels (*Falco sparverius*). *Ecology* 74:367–376.
- Anderson, D. J., J. Reeve, and D. M. Bird (1997). Sexually dimorphic eggs, nestling growth and sibling competition in American Kestrels *Falco sparverius*. *Functional Ecology* 11:331–335.
- Anderson, D. J., J. Reeve, J. E. Martinez Gomez, W. W. Weathers, S. Hutson, H. V. Cunningham, and D. M. Bird (1993b). Sexual size dimorphism and food requirements of nestling birds. *Canadian Journal of Zoology* 71:2541–2545.
- Annett, C. A., and R. Pierotti (1999). Long-term reproductive output in Western Gulls: Consequences of alternate tactics in diet choice. *Ecology* 80:288–297.
- Arnold, K. E., S. L. Ramsay, L. Henderson, and S. D. Larcombe (2010). Seasonal variation in diet quality: Antioxidants, invertebrates and Blue Tits *Cyanistes caeruleus*. *Biological Journal of the Linnean Society* 99:708–717.
- Bairlein, F., and E. Gwinner (1994). Nutritional mechanisms and temporal control of migratory energy accumulation in birds. *Annual Review of Nutrition* 14:187–215.
- Bird, D. M., S. K. Ho, and D. Paré (1982). Nutritive values of three common prey items of the American Kestrel. *Comparative Biochemistry and Physiology Part A: Physiology* 73:513–515.
- Boal, C. W., M. A. Thornley, and S. D. Mullican (2021). Food habits of American Kestrels in the southern High Plains of Texas. *Journal of Raptor Research* 55:574–583.
- Boratyński, Z., and K. Kasprzyk (2005). Does urban structure explain shifts in the food niche of the Eurasian Kestrel (*Falco tinnunculus*)? *Buteo* 14:11–17.
- Bravo, C., C. Ponce, L. M. Bautista, and J. C. Alonso (2016). Dietary divergence in the most sexually size-dimorphic bird. *The Auk* 133:178–197.
- Byholm, P., and M. Kekkonen (2008). Food regulates reproduction differently in different habitats: Experimental evidence in the goshawk. *Ecology* 89:1696–1702.
- Catry, I., T. Catry, M. Alho, A. M. A. Franco, and F. Moreira (2016). Sexual and parent-offspring dietary segregation in a colonial raptor as revealed by stable isotopes. *Journal of Zoology* 299:58–67.
- Catry, I., A. M. A. Franco, and W. J. Sutherland (2012). Landscape and weather determinants of prey availability: Implications for the Lesser Kestrel *Falco naumanni*. *Ibis* 154:111–123.
- Cornell, A., M. Melo, C. Zimmerman, and J.-F. Therrien (2021). Nestling physiology is independent of somatic development in a common raptor, the American Kestrel (*Falco sparverius*). *Physiological and Biochemical Zoology* 94:99–109.
- Cornell, A., and T. D. Williams (2017). Variation in developmental trajectories of physiological and somatic traits in a common songbird approaching fledging. *Journal of Experimental Biology* 220:4060–4067.
- Cox, W. A., F. R. Thompson, A. S. Cox, and J. Faaborg (2014). Post-fledging survival in passerine birds and the value of post-fledging studies to conservation. *Journal of Wildlife Management* 78:183–193.
- Dawson, R. D., and G. R. Bortolotti (2002). Experimental evidence for food limitation and sex-specific strategies of American Kestrels (*Falco sparverius*) provisioning offspring. *Behavioral Ecology and Sociobiology* 52:43–52.
- Dewey, S. R., and P. L. Kennedy (2001). Effects of supplemental food on parental-care strategies and juvenile survival of Northern Goshawks. *The Auk* 118:352–365.
- Drabkin, D., and H. Austin (1932). Spectrophotometry of hemoglobin. *Journal of Biological Chemistry* 98:719–733.
- Eeva, T., S. Sillanpää, and J. P. Salminen (2009). The effects of diet quality and quantity on plumage colour and growth of Great Tit *Parus major* nestlings: A food manipulation experiment along a pollution gradient. *Journal of Avian Biology* 40:491–499.
- Forero, M. G., K. A. Hobson, G. R. Bortolotti, J. A. Donazar, M. Bertelotti, and G. Blanco (2002). Food resource utilisation by the Magellanic Penguin evaluated

- through stable-isotope analysis: Segregation by sex and age and influence on offspring quality. *Marine Ecology Progress Series* 234:289–299.
- García-Heras, M. S., F. Mougeot, B. Arroyo, G. Avery, M. Avery, and R. E. Simmons (2017a). Is the Black Harrier *Circus maurus* a specialist predator? Assessing the diet of a threatened raptor species endemic to southern Africa. *Ostrich* 88:1–8.
- García-Heras, M. S., F. Mougeot, R. E. Simmons, and B. Arroyo (2017b). Regional and temporal variation in diet and provisioning rates suggest weather limits prey availability for an endangered raptor. *Ibis* 159:567–579.
- Gebhardt-Henrich, S., and H. Richner (1998). Causes of growth variation and its consequences for fitness. In *Avian Growth and Development* (J. M. Starck and R. E. Ricklefs, Editors). Oxford Univ. Press, New York, NY, USA. pp. 324–339.
- González, L. M., A. Margalida, R. Sánchez, and J. Oria (2006). Supplementary feeding as an effective tool for improving breeding success in the Spanish Imperial Eagle (*Aquila adalberti*). *Biological Conservation* 129:477–486.
- Griggio, M., F. Hamerstrom, R. N. Rosenfield, and G. Tavecchia (2002). Seasonal variation in sex ratio of fledgling American Kestrels: A long term study. *Wilson Bulletin* 114:474–478.
- Katzner, T., S. Robertson, B. Robertson, J. Klucsarits, K. McCarty, and K. L. Bildstein (2005). Results from a long-term nest-box program for American Kestrels: Implications for improved population monitoring and conservation. *Journal of Field Ornithology* 76:217–226.
- Kennedy, P. L., and J. M. Ward (2003). Effects of experimental food supplementation on movements of juvenile Northern Goshawks (*Accipiter gentilis atricapillus*). *Oecologia* 134:284–291.
- Korpimäki, E. (1985). Prey choice strategies of the kestrel *Falco tinnunculus* in relation to available small mammals and other Finnish birds of prey. *Annales Zoologici Fennici* 22:91–104.
- Lacombe, D., D. M. Bird, and K. A. Hibbard (1994). Influence of reduced food availability on growth of captive American Kestrels. *Canadian Journal of Zoology* 72:2084–2089.
- Lavigne, A. J., D. M. Bird, D. Lacombe, and J. J. Negro (1994). Growth of hand-reared American Kestrels II. Body composition and wingloading of fledglings hand-fed two different diets. *Growth, Development, and Aging* 58:203–209.
- Lepczyk, C. A., K. G. Murray, K. Winnett-Murray, P. Bartell, E. Geyer, and T. Work (2000). Seasonal fruit preferences for lipids and sugars by American Robins. *The Auk* 117:709–717.
- Liébana, M. S., J. H. Sarasola, and M. S. Bó (2009). Parental care and behavior of breeding American Kestrels (*Falco sparverius*) in central Argentina. *Journal of Raptor Research* 43:338–344.
- Martins, R. C., T. Catry, C. D. Santos, J. M. Palmeirim, and J. P. Granadeiro (2013). Seasonal variations in the diet and foraging behaviour of Dunlins *Calidris alpina* in a South European estuary: Improved feeding conditions for northward migrants. *PLoS ONE* 8:e81174. <https://doi.org/10.1371/journal.pone.0081174>.
- McClure, C. J. W., S. E. Schulwitz, R. Van Buskirk, B. P. Pauli, and J. A. Heath (2017). Research recommendations for understanding the decline of American Kestrels (*Falco sparverius*) across much of North America. *Journal of Raptor Research* 51:455–464.
- Nadjafzadeh, M., C. C. Voigt, and O. Krone (2016). Spatial, seasonal and individual variation in the diet of White-tailed Eagles *Haliaeetus albicilla* assessed using stable isotope ratios. *Ibis* 158:1–15.
- Naef-Daenzer, B., and M. U. Gruebler (2016). Post-fledging survival of altricial birds: Ecological determinants and adaptation. *Journal of Field Ornithology* 87:227–250.
- Naef-Daenzer, L., B. Naef-Daenzer, and R. G. Nager (2000). Prey selection and foraging performance of breeding Great Tits *Parus major* in relation to food availability. *Journal of Avian Biology* 31:206–214.
- Oksanen, J., F. G. Blanchet, M. Friendly, R. Kindt, P. Legendre, D. McGinn, P. R. Minchin, R. B. O'Hara, G. L. Simpson, P. Polymos, M. H. H. Stevens, et al. (2019). *vegan*: Community Ecology Package. <https://cran.r-project.org/package=vegan>.
- Parrish, J. D. (2000). Plasticity and conservation during migration. *Studies in Avian Biology* 20:53–70.
- Perrig, M., M. U. Gruebler, H. Keil, and B. Naef-Daenzer (2014). Experimental food supplementation affects the physical development, behaviour and survival of Little Owl *Athene noctua* nestlings. *Ibis* 156:755–767.
- Pike, T. W., and M. Petrie (2003). Potential mechanisms of avian sex manipulation. *Biological Reviews of the Cambridge Philosophical Society* 78:553–574.
- Pinheiro, J., D. Bates, S. DebRoy, D. Sarkar, and R Core Team (2020). *nlme*: Linear and nonlinear mixed effects models. <https://cran.r-project.org/package=nlme>.
- R Core Team (2020). *R*: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.r-project.org/>.
- Razeng, E., and D. M. Watson (2015). Nutritional composition of the preferred prey of insectivorous birds: Popularity reflects quality. *Journal of Avian Biology* 46:89–96.
- Ritz, M. S., S. Hahn, and H. U. Peter (2005). Factors affecting chick growth in the South Polar Skua (*Catharacta macrorhynchos*): Food supply, weather and hatching date. *Polar Biology* 29:53–60.
- Rodríguez, C., L. Tapia, F. Kieny, and J. Bustamante (2010). Temporal changes in Lesser Kestrel (*Falco naumanni*) diet during the breeding season in Southern Spain. *Journal of Raptor Research* 44:120–128.
- Rutz, C. (2012). Brood sex ratio varies with diet composition in a generalist raptor. *Biological Journal of the Linnean Society* 105:937–951.

- Rutz, C., M. J. Whittingham, and I. Newton (2006). Age-dependent diet choice in an avian top predator. *Proceedings of the Royal Society B: Biological Sciences* 273:579–586.
- Shaw, T. R. (2009). Sexual differences in the diet of Little Penguins *Eudyptula minor*. Ph.D. dissertation, Univ. Pretoria, Pretoria, South Africa.
- Smallwood, J. A., and D. M. Bird (2020). American Kestrel (*Falco sparverius*), version 1.0. In *Birds of the World* (A. F. Poole and F. B. Gill, Editors). Cornell Lab of Ornithology, Ithaca, NY, USA. <https://doi.org/10.2173/bow.amekes.01>.
- Smallwood, J. A., M. F. Causey, D. H. Mossop, J. R. Klucsarits, B. Robertson, S. Robertson, J. Mason, M. J. Maurer, R. J. Melvin, R. D. Dawson, G. R. Bortolotti, et al. (2009). Why are American Kestrel (*Falco sparverius*) populations declining in North America? Evidence from nest-box programs. *Journal of Raptor Research* 43:274–282.
- Smallwood, P. D., and J. A. Smallwood (1998). Seasonal shifts in sex ratios of fledgling American Kestrels (*Falco sparverius paulus*): The early bird hypothesis. *Evolutionary Ecology* 12:839–853.
- Smith, S. H., K. Steenhof, C. J. W. McClure, and J. A. Heath (2017). Earlier nesting by generalist predatory bird is associated with human responses to climate change. *Journal of Animal Ecology* 86:98–107.
- Sonerud, G. A., R. Steen, L. M. Løw, L. T. Røed, K. Skar, V. Selås, and T. Slagsvold (2013). Size-biased allocation of prey from male to offspring via female: Family conflicts, prey selection, and evolution of sexual size dimorphism in raptors. *Oecologia* 172:93–107.
- Soravia, C., J. G. Cecere, and D. Rubolini (2021). Brood sex ratio modulates the effects of extra food on parental effort and sibling competition in a sexually dimorphic raptor. *Behavioral Ecology and Sociobiology* 75. doi:10.1007/s00265-021-02970-0.
- Steenhof, K., and J. A. Heath (2013). Local recruitment and natal dispersal distances of American Kestrels. *The Condor* 115:584–592.
- Tremblay, I., D. W. Thomas, M. M. Lambrechts, J. Blondel, and P. Perret (2003). Variation in Blue Tit breeding performance across gradients in habitat richness. *Ecology* 84:3033–3043.
- Valkama, J., E. Korpimäki, and P. Tolonen (1995). Habitat utilization, diet and reproductive success in the kestrel in a temporally and spatially heterogeneous environment. *Ornis Fennica* 72:49–61.
- Visser, M. E., and P. Gienapp (2019). Evolutionary and demographic consequences of phenological mismatches. *Nature Ecology and Evolution* 3:879–885.
- Visser, M. E., L. te Marvelde, and M. E. Lof (2012). Adaptive phenological mismatches of birds and their food in a warming world. *Journal of Ornithology* 153:75–84.
- Wagner, D. L. (2020). Insect declines in the anthropocene. *Annual Review of Entomology* 65:457–480.
- Wellicome, T. I., L. D. Todd, R. G. Poulin, G. L. Holroyd, and R. J. Fisher (2013). Comparing food limitation among three stages of nesting: Supplementation experiments with the Burrowing Owl. *Ecology and Evolution* 3:2684–2695.
- Wiebe, K. L., and G. R. Bortolotti (1992). Facultative sex ratio manipulation in American Kestrels. *Behavioral Ecology and Sociobiology* 30:379–386.
- Wiehn, J., and E. Korpimäki (1997). Food limitation on brood size: Experimental evidence in the Eurasian Kestrel. *Ecology* 78:2043–2050.

Received 24 January 2022; accepted 6 December 2022
Associate Editor: James C. Bednarz