



Diet sources of the endangered Attwater's prairie-chicken in Texas: evidence from $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and Bayesian mixing models

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Abstract. The Attwater's prairie-chicken (APC; *Tympanuchus cupido attwateri*) was listed as an endangered species in 1967, and since then multiple studies have addressed ecological and human factors that may have limited their population recovery. In this study, we used stable isotopes of carbon and nitrogen ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) in conjunction with Bayesian mixing models to determine current diets of APCs at the Attwater Prairie Chicken National Wildlife Refuge (APCNWR), and to compare them with past diets derived from the isotopic composition of feathers from museum specimens (1894–1965) to help elucidate potential factors limiting APC recovery. We collected feathers, blood, and feces from APCs at the APCNWR, and feathers from museum specimens. In addition, potential food items (vegetation, spiders, and insects) were collected from APCNWR and analyzed for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. A stable isotope mixing model (MixSIAR) was used to determine diet source contribution to past and present APC populations. $\delta^{13}\text{C}$ values in APC blood were significantly greater in the fall than in the summer. Blood $\delta^{15}\text{N}$ values were significantly greater in the summer than in the winter. $\delta^{13}\text{C}$ values in feathers from museum and contemporary APCs were not different, although $\delta^{13}\text{C}$ values in contemporary feathers were enriched by 0.5‰ relative to museum specimens. Results indicated that insects were the predominant food source in the summer and fall (61–65%) and C_3 vegetation was predominant in the winter (64%). Using isotope values from feathers, the model predicted that the predominant food source during the period of feather growth were insects (62%) followed by C_3 plants (17–18%), spiders (12–18%), and C_4 plants (2–3%). Feathers from contemporary birds had $\delta^{15}\text{N}$ values 0.9‰ lower than those from museum specimens, suggesting potential shifts in diets in APCs currently in the wild relative to the past. We hypothesize that the abundance and species richness of various arthropods may have changed at the APCNWR resulting in current APCs in the wild feeding on arthropods with lower $\delta^{15}\text{N}$ values than in the past. Orthopterans comprised about 23% of the APCs diet currently in the wild, while they represented only about 19% in the past.

Key words: Attwater's prairie-chicken; carbon; diets; endangered species; mixing models; nitrogen; stable isotopes.

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INTRODUCTION

The Attwater's prairie-chicken (APC; *Tympanuchus cupido attwateri*) is an endangered ground-dwelling grouse native to Texas and Louisiana. Estimates suggest that historical populations may have approached 1 million

individuals on 2.4 million ha of habitat, but by 1937 they numbered only an estimated 8700 (Lehmann 1941). This decline in APC numbers has been attributed to loss of native coastal prairie from agricultural conversion, urban and industrial expansion, overgrazing, and woody plant encroachment (Lehman 1941, Morrow et al.

1996). APC numbers decreased to less than 500 birds from 1937 to 1992 (Lehman and Mauer-mann 1963, Silvy et al. 1999, Lockwood et al. 2005). More recently, adverse impacts of invasive red imported fire ants (RIFA; *Solenopsis invicta*) on invertebrates required by APC chicks have been identified as a major limiting factor for contemporary APC population growth, and likely contributed to APC population declines after RIFA invasion of APC habitat circa 1970 (Morrow et al. 2015). The wild APC population has remained below an estimated 150 individuals since 1995 despite intensive recovery actions including habitat restoration, captive breeding and release, and a number of research projects focused on identifying factors limiting population growth (U.S. Fish and Wildlife Service, *unpublished manuscript*; Morrow et al. 2015; Attwater Prairie Chicken National Wildlife Refuge, *unpublished data*). The Attwater's Prairie-Chicken Recovery Plan identified poor survival of chicks in the wild as "...the single-most factor limiting significant progress toward recovery" (U.S. Fish and Wildlife Service, *unpublished manuscript*).

Less than 1% of coastal prairie grasslands once supporting APCs remain in relatively pristine condition (Smeins et al. 1991). Remaining grasslands have become increasingly fragmented and subjected to a number of anthropogenic perturbations since European settlement that undoubtedly have affected ecosystem function and community structure (Lehmann 1965, Smeins et al. 1991, McKinney 1996). These perturbations, which include alteration of fire regimes, grazing practices, hydrology, and introduction of exotic species, have led to ecosystem changes which may affect the ability of APCs to meet their life requisites.

Previous research concluded that adult APCs are primarily herbivorous, with seeds and insects important during some seasons (Cogar 1980). Young prairie-chickens are primarily insectivorous during the first weeks of life (Lehmann 1941, Jones 1963, Savory 1989, Morrow et al. 2015). Previous studies of APC food habits analyzed crop, ventriculus, and fecal dropping contents (Lehmann 1941, Cogar 1980). A retrospective assessment of dietary composition is rarely possible for museum specimens using these techniques since the gastrointestinal tract is

usually discarded during specimen preparation. Stable isotopes of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) are useful in ecological studies because they can help determine an animal's diet from current and/or museum specimen samples (Peterson and Fry 1987, Thompson et al. 1995). Carbon stable isotopes are beneficial in environmental studies since plants differing in photosynthetic pathway (C_3 vs. C_4) have unique $\delta^{13}\text{C}$ values (Brugnoli and Farquhar 2000). Plants with the C_3 pathway have average $\delta^{13}\text{C}$ values of -27‰ , while average values for C_4 plants are -13‰ (Boutton et al. 1999). Plant $\delta^{15}\text{N}$ values range from approximately -20 to $+20\text{‰}$ and vary with respect to geography, N-fixing status, mycorrhizal status, plant functional type, and landscape position (Bai et al. 2009, Craine et al. 2009, 2018, Zhou et al. 2018). These variations in plant $\delta^{15}\text{N}$ values can be useful in reconstructing animal food habits (Caut et al. 2009, Hartman 2011). In addition, $\delta^{15}\text{N}$ values of animals can be used as an indicator of their trophic position because each trophic level is approximately 2–4‰ more enriched in ^{15}N compared with the trophic level immediately below it (DeNiro and Epstein 1981, Post et al. 2000, Caut et al. 2009).

Stable isotope mixing models have been used to estimate the relative proportions of food sources that contribute to an animal's diet (Phillips 2001, 2012, Boecklen et al. 2011, Ward et al. 2011). These mixing models have improved from simple linear mixing models (Fry and Sherr 1984) to multiple-source mixing models (Ben-David et al. 1997) and, more recently, to Bayesian mixing models like MixSIAR (Stock and Semmens 2016). Bayesian mixing models allow the inclusion of source uncertainty, concentration dependence, multiple sources, and prior information (Hopkins and Ferguson 2012, Phillips et al. 2014). Dietary studies of APCs have been conducted in the past (Lehman 1941, Cogar 1980), but no one to our knowledge has used $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ stable isotopes to characterize APC diets from feathers and blood. Over the last 20 yr or so, stable isotope techniques have been widely used in the study of diets of many vertebrate taxa, including birds. The main objectives of this study were to (1) use $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ stable isotopes from potential food sources and from blood and feathers of free-living animals in conjunction with Bayesian mixing models to

determine current diets of APCs at the Attwater Prairie Chicken National Wildlife Refuge (APCNWR); and (2) compare $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ stable isotope values with past diets derived from the isotopic composition of feathers from museum specimens (1894–1965) to evaluate whether habitat changes may have resulted in dietary modifications that could influence population viability and help elucidate potential factors limiting APC recovery. Recent studies have suggested that dietary shifts could occur in bird species based on habitat changes and different distribution and abundance of prey species (English et al. 2018).

MATERIALS AND METHODS

Study area and sample collection

The APCNWR (4265 ha) is located between Sealy and Eagle Lake, Texas ($29^{\circ}40' \text{N}$, $-96^{\circ}16' \text{W}$; Fig. 1), and consists of Gulf coastal prairie habitat with surrounding agricultural fields, primarily rice (Kessler 1978, Lockwood et al. 2005). Climate of the region is subtropical with a growing season of ≥ 229 d (Smeins et al.

1991, Brown 2006). Precipitation averages approximately 1057 mm annually; average daily temperatures range from 10°C in January to 29°C in August. The APCNWR includes one of the largest remnants of coastal prairie remaining in Texas and is managed specifically to provide habitat for the APC with prescribed fire, grazing, and application of herbicides to control invading brush (Fig. 1). In addition, Extinguish Plus has been applied to increasingly larger blocks of refuge grassland habitat since 2009 to suppress RIFA abundance: 308 ha in 2009, 527 ha in 2010–2012, and 1491 ha in 2013 (Morrow et al. 2015).

Most APCs occurring on APCNWR were released from captive rearing facilities located at several Texas zoological institutions within 515 km of the refuge or were progeny of previously released birds. Releases generally occurred in July–September of each year, although on occasion birds were released in other months. Most birds were released at 6–12+ weeks of age, although a smaller proportion of older birds were released as necessary to manage genetics and age distributions of the captive flock. APCs

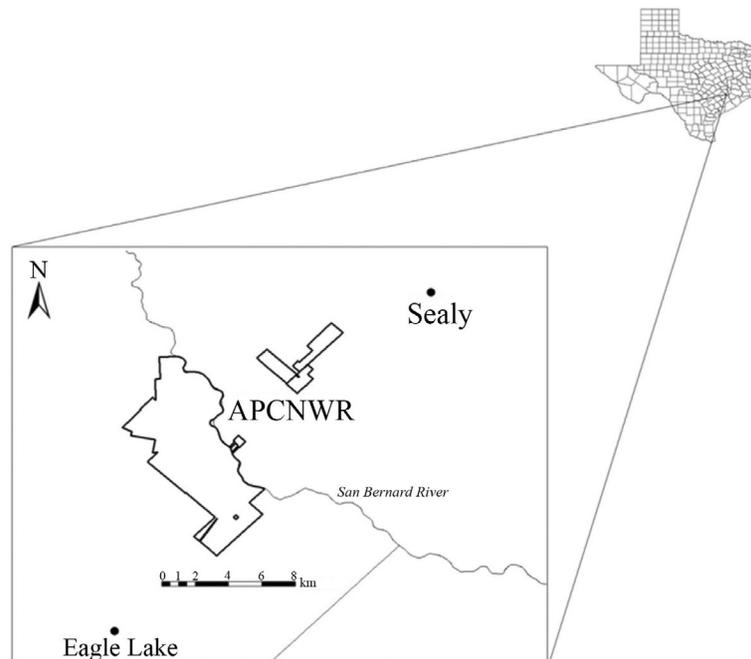


Fig. 1. Location of the Attwater Prairie Chicken National Wildlife Refuge (APCNWR) near Eagle Lake and Sealy, Texas, USA.

were placed in acclimation pens at the release site for 2 weeks, and then, doors were opened to allow the birds to exit into adjacent grasslands. Food and water were provided outside of the acclimation pens for up to 1 month to help facilitate the post-release transition. Most released birds were equipped with poncho-mounted radio-transmitters ($\leq 3\%$ of body mass) with tuned-loop antennas (Telemetry Solutions, Walnut Creek, California, USA; Advanced Telemetry Systems, Isanti, Minnesota) incorporated into the poncho (Amstrup 1980; Toepfer, *personal communication*). Movements, home ranges, and habitat use of released captive-reared APCs have been comparable to previous studies on wild Attwater's prairie-chickens (Lockwood 1998, Lockwood et al. 2005).

During 2012 and 2013, we collected potential APC diet sources including plant species (forbs, grasses, trees, and shrubs) from 25 different families, and arthropods (insects and spiders) from 11 orders at the APCNWR. Individual plants were collected in their entirety (excluding roots) and stored in paper bags. Arthropods were collected with sweep nets, placed in paper bags, and stored in zipper storage bags at -20°C . All plant material and arthropods collected for this study were identified with assistance from Drs. Fred Smeins and Stephan Hatch from the Department of Ecology and Conservation Biology and Dr. Edward Riley from the Department of Entomology at Texas A&M University.

Approximately 0.5–3.0 cc of blood was collected from the jugular vein of APCs, captured throughout the year during 2011–2013 by night-lighting from roosting locations as necessary (usually $\leq 1 \times / \text{year}$) to replace radio-transmitters. Blood was collected using 25-gauge needles with a non-heparinized 3-cc syringe, placed in 2-mL cryogenic vials (Nalgene, Thermo Fisher Scientific, Waltham, Massachusetts, USA), and stored at -80°C until analysis. Only blood samples from birds that were hatched in the wild or had been released from captivity >2 months prior were used in our study. Fresh (a few hours old at most) fecal samples were also collected from birds captured at roosting sites to replace the radio-transmitters during January 2012. Grouse undergo a complete postnuptial molt during their second summer of life (Johnsgard 1983). Therefore, to minimize dietary influences of

captivity for those birds not hatched in the wild, flank feathers (2–3) were obtained between 2004 and 2013 from APCs that had been in the wild for at least two years (hereafter referred to as contemporary feathers), and from APC museum specimens collected between 1894 and 1965 (names of museums are provided in acknowledgments), and were stored in paper bags at room temperature. Collection of samples from living APCs was authorized by Federal Fish and Wildlife Permit TE051839 and Texas Parks and Wildlife Scientific Research Permit SPR-0491-384 and was conducted in compliance with existing laws and regulations.

Vegetation and fecal samples were oven dried at 40°C and 60°C , respectively, for 24 h and then ground to a fine powder using a Retsch MM400 Oscillating Mixer Mill (Retsch GmbH, Haan, Germany) prior to stable isotope analysis. Arthropods were frozen for 2 weeks and then were oven dried at 40°C for 24 h. Mazuri feed ($n = 3$, Land O'Lakes, St Louis, Missouri, USA), provided to captive birds, also was oven dried at 25°C for 1 h. Blood samples were freeze-dried. All samples (plants, arthropods, blood, meal worms, and Mazuri feed) were pulverized using a mortar and pestle. Thirteen APC fecal samples were submitted fresh to Pacific Analytics (Scio, Oregon, USA) for arthropod fragment identification only since they were not prepared to identify plant material. Feathers were cleaned using a 2:1 chloroform:methanol solution to remove any surface oils (Wassenaar 2008). After drying, the right side of the flank feather's barbs (not including the vane) was cut into fine pieces.

Stable isotope analyses

Samples were analyzed for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in the Stable Isotopes for Biosphere Sciences Laboratory, Texas A&M University. Plants, arthropods, blood, fecal, and feather samples were weighed into individual 4×6 mm tin capsules (Costech Analytical Technologies, Valencia, California, USA). $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope ratios were measured on all samples using a Costech ECS 4010 elemental analyzer (Costech Analytical Technologies, Valencia, California, USA) interfaced with a Delta V Advance stable isotope ratio mass spectrometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA) via a Conflo IV interface (Thermo Fisher Scientific). Isotope

ratios are reported in delta notation as per mil (‰), according to the equation $\delta^H X = [(R_{\text{sample}}/R_{\text{standard}} - 1)] \times 1000$; where H is the mass number, X is the element of interest, and R is the ratio of the heavy to light isotope (e.g., $^{15}\text{N}/^{14}\text{N}$) in the sample and the standard. Isotope ratios are expressed relative to Vienna Pee Dee Belemnite (VPDB) for $\delta^{13}\text{C}$ (Coplen et al. 2006) and atmospheric nitrogen (AIR) for $\delta^{15}\text{N}$ (Mariotti 1983).

Determination of trophic discrimination factors with captive birds

The Houston Zoo is one of several zoological facilities in Texas that has participated in the APC recovery program and has maintained and reared APCs in captivity for many years. To determine trophic discrimination factors under controlled conditions, we selected five APCs (8 months old) that were kept in captivity at the Houston Zoo. The Mazuri gamebird maintenance diet was analyzed as part of the bird's diet. Blood (0.5 mL) was taken from the jugular vein and two flank feathers were collected at the same time in early February 2017. Blood and feather samples collected from the five individuals as well as three feed samples were analyzed for C and N isotopes. The trophic discrimination factors (TDF) were calculated using equations from Kurle et al. (2013):

$$\Delta^{13}\text{C}_{\text{tissue-diet}} = \delta^{13}\text{C}_{\text{tissue}} - \delta^{13}\text{C}_{\text{diet}}$$

$$\Delta^{15}\text{N}_{\text{tissue-diet}} = \delta^{15}\text{N}_{\text{tissue}} - \delta^{15}\text{N}_{\text{diet}}$$

Statistical analyses

We used the Shapiro-Wilk test to determine whether the data were normally distributed. The arthropod data were not normal; thus, we used an analysis of variance (ANOVA) of ranked data to test for differences in isotope composition among arthropods grouped by orders, followed by the Tukey's Studentized Range test to determine which means were significantly different. Seasonal differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in blood were also evaluated with a regular ANOVA and Tukey's test. Differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values between museum and contemporary feather samples were determined with Student's *t*-test. All statistical tests were conducted in SAS (ver 9.4) or JMP (JMP, Pro 14, SAS Institute, Cary, North Carolina,

USA). Statistical differences were set at an alpha value of 0.05.

MixSIAR model analyses

To estimate the most important APC diet sources, we used the stable isotope mixing model, MixSIAR (Stock and Semmens 2016), which is part of the statistical software R (R Core Team 2015). This model accounts for uncertainty in sources measured (Moore and Semmens 2008), categorical or continuous covariates (Semmens et al. 2009, Parnell et al. 2013), and prior information (Moore and Semmens 2008). It also accounts for variability of isotopic values in consumer, sources, and tissue-diet discrimination factors (Phillips et al. 2014). In addition, it provides summary statistics that report the probability distributions (2.5–97.5%) for individual diet sources in relation to the stable isotope values of the consumer (APCs). Prior to running the model, we calculated specific trophic discrimination factors (TDF, $\Delta_{\text{tissue-diet}}$) using $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope values in known blood, feathers, and diet samples from APCs at the Houston Zoo. Also, because there was great variation in the stable isotope carbon and nitrogen values for arthropods we used a self-organizing map (SOM) technique to combine the data for plants, insects, and spiders in clusters and reduce the number of potential food sources as recommended by the MixSIAR model. The SOM uses K-means clustering procedure and provides the optimal number of clusters when a cubic clustering criterion has been achieved. Using this procedure, we determined that four clusters provided the optimal cubic clustering criterion (Appendix S1: Fig. S1).

Prior to the MixSIAR analyses, we conducted a Monte Carlo simulation using the diet source (assembled in clusters) and the consumer's TDFs, to determine whether a mass balance was established (Smith et al. 2013). The simulation helps to determine whether the TDF values and the diet source values are valid to use in a mixing model. Validation of the diet sources is achieved when all the consumer tissue isotope values fall within the 95% mixing region. If some of the consumer tissue isotope values fall outside the mixing region, they can either be excluded from the model or we can reject the use of the mixing model with the data. We first ran the MixSIAR model in normal mode to

make sure all the Markov Chain Monte Carlo (MCMC) chains converged as determined by the Gelman-Rubin and Geweke diagnostic reports (Stock and Semmens 2016). If there was no convergence, we then used long and very long run lengths. We ran the MixSIAR model considering four cluster sources with isotope data for blood (seasonal and year-round) and feathers (contemporary and museum). For the winter season, we included only the isotope values of insects collected in November since we could not collect insects in the months of December, January, and February.

RESULTS

$\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of potential food sources at APCNWR

Details on the type and number of vegetation (forbs, grass, trees/shrubs), arthropods (insects and spiders), blood, feathers, and fecal samples are provided in Tables 1–3. Overall, we collected 58 plant species from 25 different families, and arthropods from 11 orders. Vegetation samples were classified as either C_3 ($\delta^{13}\text{C}$ values within -22‰ to -30‰ range) or C_4 ($\delta^{13}\text{C}$ values in the -10‰ to -14‰ range). Collected plant species composition was dominated by members of the Asteraceae, Fabaceae, and Poaceae families (Table 1). $\delta^{13}\text{C}$ values of individual plant species ranged from -31.9‰ to -22.4‰ for C_3 plants and -12.8‰ to -11.9‰ for C_4 plants. $\delta^{15}\text{N}$ values ranged from -6.2‰ to 9.5‰ for C_3 plants and 1.5‰ to 1.9‰ for C_4 plants. Arthropods were separated by insects and spiders because spiders had significantly higher $\delta^{15}\text{N}$ values than most of the insects collected. The most common arthropods were from the orders Orthoptera, Hemiptera, and Coleoptera (Table 2). There were significant differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values among the arthropod species. The highest $\delta^{13}\text{C}$ values were observed in spiders (Araneae) and were significantly greater than those in Lepidoptera and Orthoptera ($F_{10, 207} = 3.42$, $P < 0.001$, Table 2), but were similar to other orders. $\delta^{13}\text{C}$ values for Mantodea and Neuroptera were also high but the sample size was small ($n = 2$). $\delta^{15}\text{N}$ values were significantly greater in Araneae than in Hemiptera, Lepidoptera, and Orthoptera ($F_{10, 207} = 10.12$, $P < 0.0001$, Table 2).

$\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in blood, feathers, and fecal samples

$\delta^{13}\text{C}$ values in APC blood were significantly greater in the fall than in the summer or winter ($F_{2,97} = 7.53$, $P = 0.0009$, Table 3). $\delta^{15}\text{N}$ values were significantly greater in blood collected in the summer than in the winter but were similar during fall ($F_{2,97} = 8.55$, $P = 0.0004$, Table 3). $\delta^{13}\text{C}$ values in feathers from museum and contemporary APCs were not significantly different. However, mean values in contemporary feathers were enriched by 0.5‰ relative to museum specimens. Also, the range in $\delta^{13}\text{C}$ stable isotope values was broader in contemporary (-13.4‰ to -26.5‰) than in museum (-18.7‰ to -23.8‰) feathers. Feathers from museum specimens had significantly greater $\delta^{15}\text{N}$ values than those from recent individuals in the wild ($P = 0.0001$, Table 3). Mean $\delta^{13}\text{C}$ values in fecal material were, on average, 5.5‰ more depleted than values in blood and $8.5\text{--}9.0\text{‰}$ more depleted than values in museum and contemporary feathers. Similarly, $\delta^{15}\text{N}$ values in fecal samples were 2.3‰ more depleted than average values in blood, and $4.2\text{--}5.0\text{‰}$ more depleted than values in contemporary and museum feathers, respectively (Table 3).

$\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values obtained from controlled feeding study

Blood and feathers were collected from 5 individual APCs maintained in captivity at the Houston Zoo. The birds were fed a diet of Mazuri Game bird maintenance for several months prior to the sample collection; the individual and average stable isotope values are provided in Table 4. These values were used to calculate the trophic discrimination factors: blood $\Delta_{\text{tissue-diet}} \delta^{13}\text{C}$: 0.2 ± 0.2 , $\Delta^{15}\text{N}$: 3.4 ± 0.3 ; feather $\Delta_{\text{tissue-diet}} \delta^{13}\text{C}$: 1.1 ± 0.3 , $\Delta^{15}\text{N}$: 3.5 ± 0.5 (Table 4).

Diet predictions using a stable isotope mixing model

We ran the MixSIAR model considering four isotope clusters as major food sources using tissue-diet discrimination factors (TDF) for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for blood and APC feathers. Four clusters provided the optimal cubic clustering criterion using the K-means procedure and a self-organizing map function as described earlier

Table 1. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ stable isotope values (‰, mean \pm SD) of vegetation collected at the Attwater Prairie Chicken National Wildlife Refuge, Colorado County, Texas during 2012–2013.

Family	Species	<i>n</i>	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	
Asteraceae	<i>Ambrosia psilostachya</i>	7	-29.7 ± 1.0	0.9 ± 3.4	
	<i>Cirsium</i> spp.	1	-30.9	-2.2	
	<i>Coreopsis tinctoria</i>	1	-30.8	0.5	
	<i>Dracopis amplexicaulis</i>	3	-28.5 ± 0.2	-0.2 ± 1.1	
	<i>Euthamia</i> sp.	1	-30.8	0.1	
	<i>Helenium amarum</i>	1	-28.5	1.6	
	<i>Iva annua</i>	2	-29.4 ± 0.6	5.0 ± 4.8	
	<i>Krigia</i> sp.	2	-22.4 ± 0.1	0.4 ± 0.1	
	<i>Nothocalais</i> sp.	2	-30.8 ± 0.8	4.3 ± 4.8	
	<i>Rudbeckia</i> spp.	1	-30.1	1.7	
	<i>Symphotrichum</i> spp.	3	-29.4 ± 1.7	2.3 ± 2.3	
	<i>Liatris mucronata</i>	1	-29.1	0.7	
	Acanthaceae	<i>Ruellia humilis</i>	1	-29.7	0.3
		<i>Ruellia</i> spp.	3	-28.9 ± 0.7	2.1 ± 4.9
Apiaceae	<i>Eryngium yuccifolium</i>	1	-26.8	5.7	
Brassicaceae	<i>Lepidium</i> sp.	1	-27.6	5.7	
Campanulaceae	<i>Triodanis perfoliata</i>	3	-29.3 ± 0.5	2.8 ± 2.7	
Commelinaceae	<i>Tradescantia</i> sp.	2	-29.4 ± 0.1	8.0 ± 0.1	
Euphorbiaceae	<i>Croton capitatus</i>	7	-29.1 ± 1.1	1.7 ± 3.1	
	<i>Euphorbia</i> spp.	2	-29.9 ± 0.1	4.5 ± 0.4	
Fabaceae	<i>Medicago lupulina</i>	1	-28.2	-0.8	
	<i>Medicago polymorpha</i>	3	-30.1 ± 0.3	-0.1 ± 0.2	
	<i>Mimosa nuttallii</i>	4	-30.0 ± 0.8	-1.3 ± 1.2	
	<i>Mimosa</i> spp.	3	-30.4 ± 0.5	-1.4 ± 0.7	
	<i>Neptunia lutea</i>	2	-29.6 ± 0.6	-0.5 ± 1.6	
	<i>Neptunia</i> spp.	1	-25.7	-2.2	
	<i>Baptisia bracteata</i>	1	-27.9	0.8	
	<i>Chamaecrista fasciculata</i>	10	-30.4 ± 0.8	-0.6 ± 0.6	
	<i>Vicia ludoviciana</i>	1	-31.1	2.0	
	<i>Vicia</i> spp.	2	-30.4 ± 0.6	0.2 ± 0.1	
	<i>Tephrosia onobrychooides</i>	1	-28.4	-0.6	
	Gentianaceae	<i>Sabatia campestris</i>	2	-29.6 ± 0.0	2.7 ± 0.2
	Geraniaceae	<i>Geranium carolinianum</i>	1	-31.1	3.1
Iridaceae	<i>Sisyrinchium</i> spp.	2	-29.7 ± 0.3	3.5 ± 1.2	
Juncaceae	<i>Juncus</i> sp.	1	-29.7	2.2	
Liliaceae	<i>Nothoscordum bivalve</i>	4	-29.0 ± 0.9	1.8 ± 1.2	
	<i>Hypoxis</i> sp.	1	-29.8	1.5	
Lythraceae	<i>Lythrum</i> sp.	2	-29.4 ± 3.5	5.0 ± 2.5	
Malvaceae	<i>Callirhoe involucrata</i>	2	-29.4 ± 0.4	1.6 ± 0.2	
Onagraceae	<i>Oenothera laciniata</i>	1	-31.6	2.2	
	<i>Oenothera</i> spp.	4	-27.4 ± 0.9	2.6 ± 2.2	
Oxalidaceae	<i>Oxalis</i> spp.	4	-30.2 ± 0.1	4.6 ± 2.7	
	<i>Oxalis stricta/corniculata</i>	2	-30.2 ± 0.1	3.8 ± 0.0	
Plantaginaceae	<i>Plantago</i> sp.	1	-23.0	2.4	
Poaceae	<i>Chloris</i> sp.	1	-12.2	1.9	
	<i>Briza</i> sp.	1	-28.0	1.6	
	<i>Dichanthelium oligosanthes</i>	2	-29.8 ± 0.0	-0.7 ± 0.9	
	<i>Dichanthelium</i> spp.	8	-29.5 ± 1.0	0.9 ± 2.5	
	<i>Hordeum pusillum</i>	1	-28.3	4.5	
	<i>Phalaris</i> sp.	1	-29.3	0.6	
	<i>Tridens strictus</i>	1	-12.8	1.5	
Polemoniaceae	<i>Phlox</i> sp.	1	-30.8	0	
Polygonaceae	<i>Rumex</i> spp.	1	-29.8	1.1	

(Table 1. Continued.)

Family	Species	<i>n</i>	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$
Primulaceae	<i>Anagallis arvensis</i>	4	-31.4 ± 0.4	2.6 ± 1.3
Ranunculaceae	<i>Anemone caroliniana</i>	3	-29.0 ± 0.1	3.1 ± 1.2
Rosaceae	<i>Rosa bracteata</i>	1	-28.0	-6.2
	<i>Rubus</i> spp.	1	-29.0	1.9
Scrophulariaceae	<i>Linaria</i> sp.	1	-29.9	0.2

Table 2. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ stable isotope values (‰, mean ± SD) for arthropods collected at the Attwater Prairie Chicken National Wildlife Refuge, Colorado County, Texas, during 2012–2013.†

Order	<i>N</i> ‡	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$
Araneae	24	-23.4 ± 2.7 A	6.9 ± 0.9 A
Coleoptera	29	-25.1 ± 3.1 AB	5.3 ± 2.4 AB
Diptera	4	-24.5 ± 3.0 AB	6.5 ± 1.8 AB
Hemiptera	42	-24.7 ± 4.5 AB	2.8 ± 2.3 B
Hymenoptera	11	-24.4 ± 4.3 AB	6.8 ± 3.8 AB
Lepidoptera	18	-26.1 ± 4.8 B	5.0 ± 2.8 B
Mantodea	2	-18.4 ± 1.4	5.5 ± 1.1
Neuroptera	2	-23.4 ± 3.0	4.0 ± 0.1
Orthoptera	63	-25.0 ± 3.8 B	3.7 ± 2.1 B
Phasmida	2	-27.2 ± 0.9	5.5 ± 2.7
Sternorrhyncha	1	-28.9	-0.5

† Within columns, rows not sharing the same letter are significantly different ($P < 0.05$).

‡ Orders with $n \leq 2$ were not included in the statistical analysis.

(Table 5). For blood (seasonal and year-round), insects were the predominant food source in the summer and fall (61–65%) and C_3 vegetation was predominant in the winter (Table 6). Spiders comprised 8.6–9.9‰ of the diet in the summer and fall. C_4 plants peaked at 5.7‰ in the winter (Table 6). The isospace plots in all cases showed that values for blood were inside the 95% mixing polygon and that it was acceptable to include all the values in the run (Fig. 2). When we ran the model with the same isotope clusters representing food sources and those from contemporary and museum feathers, we determined that the predominant food source during the period of feather growth were insects (62–65%) followed by C_3 plants (17–18%), spiders (13–17%), and C_4 plants (2–3%; Table 6). The MixSIAR model results indicated that the top two insect taxa consumed by APCs were Orthopterans (9–26%) and Hemipterans (13–16%), particularly during the summer and fall (Table 7). During these two

seasons, approximately 40% of the total APC diet consisted of two taxa, but decreased to 20% during the winter (Table 7).

Arthropod remains in APC feces

Arthropod remains from seven orders and 10 families were identified in feces collected during January 2012 (Fig. 3). The most abundant insects were grasshoppers (Orthoptera:Acrididae), butterflies/moths (Lepidoptera), and weevils (Coleoptera:Curculionidae; Fig. 3).

DISCUSSION

Our results using the MixSIAR model with blood isotope values suggested that insects were the primary food source for APCs during the summer and fall, and C_3 plants during the winter (Table 6). Lehmann (1941) and Cogar (1980) reported that adult APCs consumed more insects during the summer, but vegetation was the primary food source throughout the year based on their analysis of stomach contents and fecal material. Plant species they reported (e.g., Acanthaceae, Fabaceae, and Liliaceae) as used by APCs throughout the year, are mostly C_3 species based on their carbon isotope values (Boutton et al. 1999). However, it should be noted that food habit studies based on samples collected during or after passage from the gastrointestinal tract could be subject to biases associated with differential digestibility of food items (Codron et al. 2011). Our analysis was based on samples presumed to be eaten by APCs, but not taken from the stomach or gastrointestinal tract, which could contribute to the differences we observed between or among studies.

Analysis of feathers from museum and contemporary specimens predicted similar diets except that the proportion of spiders in the diet of museum specimens was slightly greater by 4%.

Table 3. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ stable isotope values (‰, mean \pm SD) in blood, feathers, and feces of Attwater's prairie-chickens primarily from the Attwater Prairie Chicken National Wildlife Refuge.†

Tissue	Category	<i>n</i>	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$
Blood‡	Summer	15	-25.3 ± 1.6 B	6.8 ± 1.9 A
	Fall	15	-22.1 ± 2.6 A	6.5 ± 0.5 AB
	Winter	70	-24.7 ± 1.9 B	6.4 ± 0.5 B
	Year-round	100	-24.4 ± 2.2	6.5 ± 0.9
Feathers	Contemporary§	33	-20.8 ± 3.8 A	8.1 ± 1.4 B
	Museum	30	-21.4 ± 1.3 A	9.3 ± 1.0 A
Feces¶		22	-29.8 ± 1.7	4.2 ± 1.1

† Letters represent separate comparisons for blood and feathers; rows not sharing the same letter are significantly different ($P < 0.05$).

‡ Collected 2011–2013.

§ Collected 2004–2013.

¶ Collected only in January 2012 from night roosts.

Table 4. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ stable isotope values (‰) in feed (Mazuri), blood, and feathers of APCs raised in captivity at the Houston Zoo.

Sample	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\Delta^{13}\text{C}$	$\Delta^{15}\text{N}$
Feed 1	-19.4	4.1		
Feed 2	-19.6	4.3		
Feed 3	-19.8	3.9		
Mean \pm SD	-19.6 ± 0.2	4.1 ± 0.2		
Blood 1	-19.6	7.8		
Blood 2	-19.3	7.5		
Blood 3	-19.5	7.1		
Blood 4	-19.5	7.4		
Blood 5	-19.1	7.5		
Mean \pm SD	-19.4 ± 0.2	7.5 ± 0.3	0.20	3.4
Feather 1	-18.7	7.2		
Feather 2	-18.3	8.2		
Feather 3	-18.1	7.7		
Feather 4	-18.7	7.0		
Mean \pm SD	-18.4 ± 0.3	7.5 ± 0.5	1.1	3.5

Note: Values used to calculate discrimination factors Δ .

Also, feathers from contemporary birds had $\delta^{15}\text{N}$ values that were 0.8‰ lower than those from museum specimens. The decrease suggests that APCs are currently feeding at a slightly lower level in the food web than in the past, consistent with the different proportion of spiders in the diet from museum (17%) and contemporary specimens (13%). Spiders had the highest $\delta^{15}\text{N}$ values among all the arthropods sampled, which indicates that they occupy the highest trophic position of the arthropods we sampled (Table 2). The MixSIAR model also suggested an increase of orthopterans in contemporary diets compared with museum specimens (Table 7). Orthopterans

comprised the largest arthropod proportion of the diet predicted by MixSIAR models for both feathers (contemporary and museum) and blood (except for winter; Table 7) and were the most abundant and frequently found invertebrate in fecal samples collected in January 2012 (Fig. 3).

English et al. (2018) recently suggested that museum specimens could determine diet and habitat changes in species over time. They reported a 1.4–2.8‰ decline in $\delta^{15}\text{N}$ values in feathers of whip-poor-wills (*Antrostomus vociferous*) over the last 130 yr and attributed this change to a decrease in abundance of higher trophic level prey. Our study suggests that spider densities may have decreased in APCNWR grasslands and become less available to APCs as food, while orthopteran species increased in availability to APCs. This hypothesis is consistent with reported impacts on arthropod communities following invasion by RIFA. Studies show that fire ant invasion affected the abundance and species richness of various arthropods (Porter and Savignano 1990, Holway et al. 2002, Morrow et al. 2015). Species richness of arthropods was 40% less at sites infested with fire ants than at those not infested (Porter and Savignano 1990). Porter and Savignano (1990) also found that spiders were 30% fewer while orthopterans were seven times more abundant in RIFA infested sites. Additionally, a recent study on the diet of lesser prairie-chickens (*Tympanuchus pallidicinctus*) indicated that birds consumed more spiders in native grasslands than in croplands or Conservation Reserve Prairie grasslands (Sullins et al. 2018). These findings support the results in

Table 5. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ stable isotope values (‰, mean \pm SD) from APC food sources (plants and arthropods) separated by clusters, and percent contribution by source, as determined by an optimal cubic clustering procedure using K-means and self-organizing map function in JMP.

Cluster	<i>n</i>	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	Insects (%)	Spiders (%)	C ₃ Plants (%)	C ₄ Plants (%)
1	82	-26.7 \pm 2.7	7.8 \pm 1.5	48.7	23.2	28	
2	38	-16.9 \pm 3.3	3.8 \pm 2.2	81.6	13.2		5.3
3	137	-26.9 \pm 2.0	3.5 \pm 1.2	75.8		24.4	
4	101	-29.3 \pm 1.5	0.01 \pm 1.4	19.9		80.3	

Table 6. Predicted diet sources (calculated %) for the Attwater’s Prairie Chicken, primarily from the Attwater Prairie Chicken National Wildlife Refuge, based on results from the MixSIAR model using $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ stable isotope values in blood and feathers.

Tissue	Group	Insects (%)	Spiders (%)	C ₃ Plants (%)	C ₄ Plants (%)
Blood	Year-round	57.8	7.0	33.6	1.5
	Summer	60.7	9.9	28.5	1.1
	Fall	65.4	8.6	22.2	2.4
	Winter	29.1	0	64.3	5.7
Feathers	Contemporary	65.2	13.2	17.8	2.5
	Museum	62.3	17.1	17.2	2.2

Note: Data were grouped in 4 optimal clusters using the self-organizing map function in JMP.

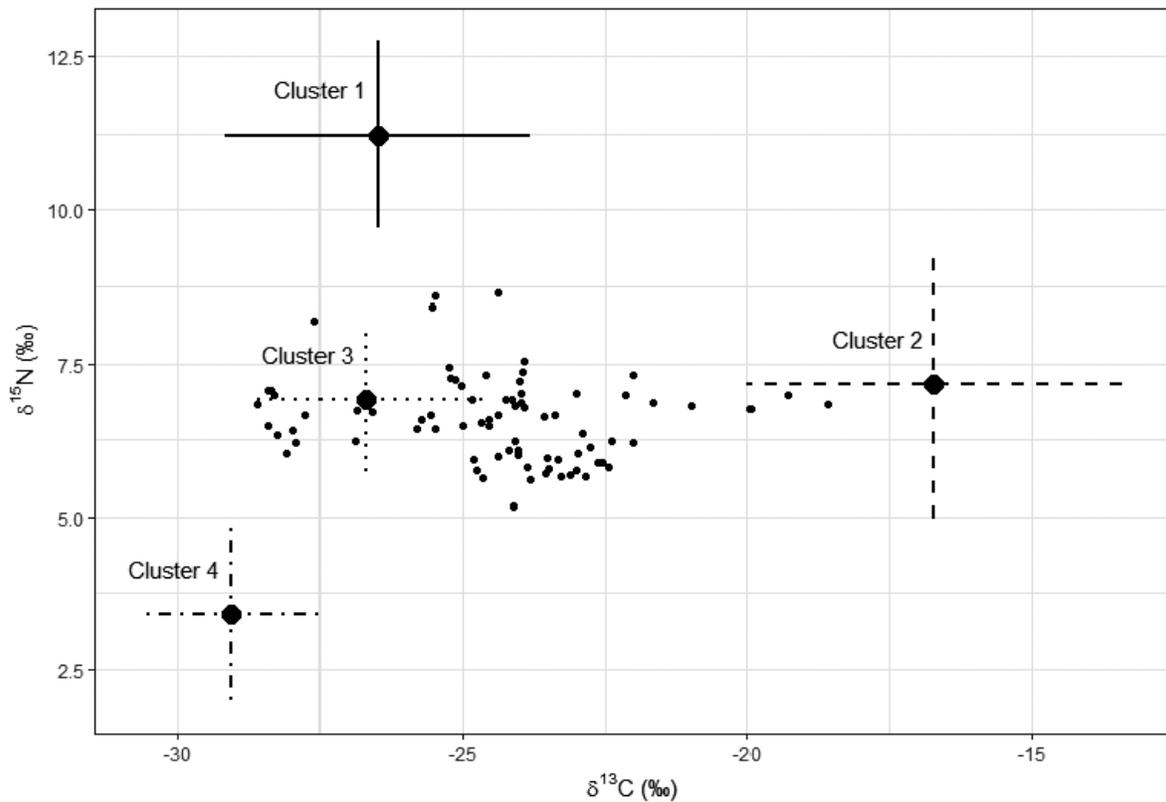


Fig. 2. Isospace plot of the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for year-round blood values with four food sources (insects, spiders, C₃ plants, and C₄ plants) as determined by clusters.

Table 7. Percent contribution of arthropods (most commonly found) in the Attwater's prairie chicken diet as determined by MixSIAR model using stable isotope clusters of spiders, insects, and plants collected during 2012–2013 at the Attwater's Prairie Chicken National Wildlife Refuge.

Tissue	Group	Aranea (%)	Coleoptera (%)	Hemiptera (%)	Hymenoptera (%)	Lepidoptera (%)	Orthoptera (%)
Blood	Year-round	7.0	7.5	14.9	2.6	5.7	22.3
	Summer	9.9	9.8	13.6	3.8	6.5	22.4
	Fall	8.6	8.1	16.2	2.8	6.4	25.7
	Winter	0	2.2	13.1	0	4.1	9.2
Feathers	Contemporary	13.2	9.8	15.0	3.8	7.3	22.7
	Museum	18.0	11.1	13.4	4.9	7.9	18.6

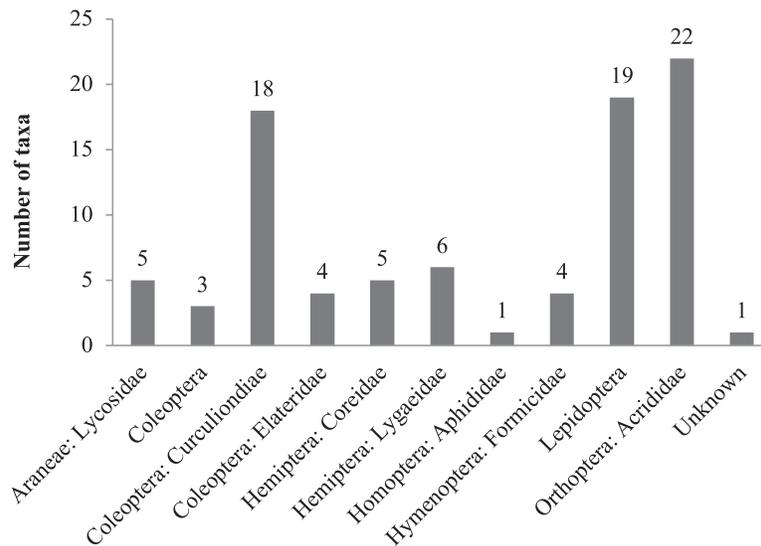


Fig. 3. Total arthropod remains found in Attwater's prairie chicken feces ($n = 22$) collected at night roosts in January 2012 at the Attwater Prairie Chicken National Wildlife Refuge. Numbers above bars represent total number of species from each family.

our study suggesting that the current grassland habitat of the APC may no longer be what it used to be in the past. Although 7–35% of refuge grasslands were treated to suppress RIFA during 2009–2013, this treatment occurred near the end of sample collection for our study. It will likely take some time for grassland invertebrate communities to fully recover from the approximately 40-year impact of RIFA.

The minor differences observed in $\delta^{13}\text{C}$ values between museum and contemporary feathers could also be associated with changes in habitat and/or foraging strategies (Bearhop et al. 2006, English et al. 2018). $\delta^{13}\text{C}$ values in feathers from current wild APCs were enriched by 0.6‰

relative to feathers from museum specimens suggesting that contemporary individuals are more associated with agriculture, consistent with observed changes in land use over the last 70 yr (McKinney 1996). Lower $\delta^{15}\text{N}$ values and a broader range of $\delta^{13}\text{C}$ values in feathers of contemporary APCs compared with museum values, suggest the APCs may be currently utilizing different sources of food or that grasses, forbs, and insects have changed to some extent from what was available in the past, at least before 1965. Attwater's prairie chickens are occasionally seen in croplands, particularly rice fields; however, they generally remain within the refuge boundaries.

Overall, the use of the MixSIAR model to determine potential food sources for the APCs has some limitations, particularly when using too many sources (Parnell et al. 2010). Notwithstanding, the results using food sources associated in clusters to optimize the distribution of isotope values suggest that APCs feed primarily on insects in the summer and fall and on C_3 plants during the winter. $\delta^{13}C$ and $\delta^{15}N$ values of additional potential food sources will further facilitate a most accurate determination of the most likely diet items of wild APCs.

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