EVALUATING THE IMPACTS OF METALS ON AQUATIC BIRDS IN LAKE CHAPALA, AND THE USE OF STABLE ISOTOPES FOR PREDICTING THE ATTWATER'S PRAIRIE-CHICKEN DIET

A Dissertation

by

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Submitted to the Office of Graduate and Professional Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

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August 2017

Major Subject: Wildlife and Fisheries Sciences

ABSTRACT

The largest tropical lake in Mexico, Lake Chapala, is a major fishery and a recipient of many contaminants (industrial and agricultural) via the Lerma River. The objectives were to evaluate concentrations of mercury (Hg), aluminum (Al), barium (Ba), copper (Cu), manganese (Mn), strontium (Sr), vanadium (V), and zinc (Zn) in fish and wildlife of Lake Chapala. I also used stable isotopes carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) to determine trophic differences between the 3 collected fish species. I collected water, sediment, fish, and feather samples and measured these samples for Hg and other metals.

Mercury concentrations in water were higher compared to other lakes around the world, but not as high as those determined from the Jose Antonio Alzate reservoir in Mexico. Sediment Hg concentrations were similar to those reported by other studies from Lake Chapala. Also, the Hg concentrations measured in fish were similar to those from other studies. Feather samples collected had a wide range of δD values; therefore using these values were not useful for predicting significant relationships between areas of feather growth and areas of Hg acquisition. Concentrations of other metals in water, sediments, and fish were also similar to those reported in previous studies.

An Attwater's prairie-chicken (APC) study was conducted to determine the diet of wild APC populations once released from captivity with the use of stable isotope analysis of $\delta^{13}C$ and $\delta^{15}N$. I also compared historic and contemporary APC feather $\delta^{13}C$

and $\delta^{15}N$ values to one another. I collected vegetation, insect, fecal, and blood samples from APCs on the Attwater Prairie Chicken National Wildlife Refuge (APCNWR).

The stable isotope analysis revealed the mixing model produces different results dependent on the number of diet sources used. When analyzing $\delta^{13}C$ and $\delta^{15}N$ from blood, the main diet source is C_3 vegetation (forbs) when 3, 4, and 5 diet sources are used. Historic feather's $\delta^{13}C$ and $\delta^{15}N$ values showed that spiders (3 and 4 diet sources) and rice (5 diet sources) contributed the most to APC diet. Contemporary feather $\delta^{13}C$ and $\delta^{15}N$ values determined insects (3 sources), forbs (3 sources), and C_4 vegetation (grasses; 5 sources) contributed the most.

DEDICATION

This written work is dedicated to my family: Anna Guerrero, Jorge Torres, and Juanita Garcia. They have continuously encouraged me to follow my desires in pursuing an advanced degree. Without their support and understanding I would not be where I am today. I love you all very much.

ACKNOWLEDGEMENTS

I would like to acknowledge and thank my committee members for helping with my PhD projects throughout these years, Drs. Miguel Mora, Thomas Boutton, Nova Silvy, and Robert Taylor.

I would like to thank the following museums for donating Attwater's prairie-chicken feather samples for this study: Texas A&M University's Biodiversity Research and Teaching Collections, Delaware Museum of Natural History, Museum of Comparative Zoology – Harvard University, and the University of Kansas Natural History Museum. I also would like to thank the Houston Zoo for providing recent blood and feathers from captive Attwater's prairie-chickens, plus recent feed samples.

Thanks to all my past undergraduate helpers for all of their hard work both in the lab and field. In particular: Brittany Srilla, Chas Holder, Cassandra LaFleur, Christen Warkoczewski, and Cecilia Fonseca.

Of course, I would like to acknowledge my past and present lab mates: Judlyn Telesford-Checkley, Alejandra Maldonado, and Hannah Ertl. You all are a great support team and thanks for offering guidance and friendship throughout my PhD adventure.

CONTRIBUTORS AND FUNDING SOURCES

Contributors

Faculty Committee Recognition

These projects were guided by the expertise from my dissertation committee consisting of Dr. Miguel Mora (chair) and Dr. Nova Silvy of the Department of Wildlife and Fisheries Sciences, Dr. Thomas Boutton of the Department of Ecosystem Science and Management, and Dr. Robert Taylor of the Department of Veterinary Integrative Biosciences.

Collaborator Contributions

Dr. Miguel Mora of the Department of Wildlife and Fisheries Sciences contributed many comments and revisions to all the contents of the dissertation. Dr. Robert Taylor of the Department of Veterinary Integrative Biosciences contributed his knowledge and lab to have samples analyzed for metal concentrations, which contributed to the Lake Chapala study and the below chapters, “Hazard Assessment of Mercury to Waterbirds at Lake Chapala, Mexico” and “Metal Concentrations in Water, Sediment, and Fish from Lake Chapala, Mexico”. Dr. Thomas Boutton of the Department of Ecosystem Science and Management offered his knowledge and lab for stable isotope analysis of the samples collected for chapter, “Predicting diet sources of Attwater’s prairie-chicken in Texas”. Dr. Nova Silvy contributed his vast knowledge about the Attwater’s prairie chicken for the above chapter of this dissertation.

Drs. Dioselina Alvarez-Bernal and Héctor Buelna-Osben from Centro Interdisciplinario de Investigacion para Desarrollo Intergral Regional (CIIDIR) were instrumental in facilitating the collection of fish and feathers from birds from Lake Chapala for chapters, "Hazard Assessment of Mercury to Waterbirds at Lake Chapala, Mexico" and "Metal Concentrations in Water, Sediment, and Fish from Lake Chapala, Mexico". Dr. Masami Fujiwara of the Department of Wildlife and Fisheries Sciences contributed help with determining which statistical analysis to be used in chapter, "Metal Concentrations in Water, Sediment, and Fish from Lake Chapala, Mexico".

All of the below help assisted with the completion of the chapter titled, "Using Stable Isotopes to Determine Diet Sources of the Endangered Attwater's Prairie Chicken (*Tympanuchus cupido attwateri*) in Texas". Dr. Mike Morrow and Rebecca Chester of the U.S. Fish and Wildlife Service assisted with collecting vegetation and arthropod samples in the field. My undergraduate helpers, Brittany Srilla, Chas Holder, Cassandra LaFleur, Christen Warkoczewski, and Cecilia Fonseca all helped with sample collection, weighing, and/or sorting for this chapter. Drs. Fred Smeins and Stephan Hatch of the Department of Ecosystem Science and Management identified vegetation samples collected, and Edward Riley of the Department of Entomology performed arthropod identification. Lastly, Dr. Ayumi Hyodo of the Department of Ecosystem Science and Management performed stable isotope analysis for all samples collected from the three chapters mentioned previously.

All other work presented in this dissertation was completed by me, the student, independently.

Funding Sources

The following fellowships: Louis Stokes Alliance for Minority Participation-Bridge to the Doctorate, Hispanic Leaders in Agriculture and the Environment, and Tom Slick, provided financial support toward the completion of this dissertation. I also would like to thank Drs. Miguel Mora, David Reed, David Briske, and Hsiao-Hsuan (Rose) Wang for assisting me with funding in the form as a research/graduate/teaching assistant position.

The Lake Chapala project was funded by a grant from Consejo Nacional de Ciencia y Tecnologia (CONACYT) and Texas A&M University. The Attwater's prairie chicken project was funded by a grant from the U.S. Geological Survey.

NOMENCLATURE

Hg	Mercury
Al	Aluminum
Ba	Barium
Cu	Copper
Mn	Manganese
Sr	Strontium
V	Vanadium
Zn	Zinc
$\delta^{13}C$	Stable isotope carbon
$\delta^{15}N$	Stable isotope nitrogen
APC	Attwater's prairie-chicken
APCNWR	Attwater Prairie Chicken National Wildlife Refuge

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CHAPTER I

INTRODUCTION

Mercury and Other Metal Contaminations

The presence of mercury (Hg) in the atmosphere is due to both anthropogenic and natural sources (Angot et al. 2016). Major anthropogenic sources of Hg include: solid waste incineration, coal and oil combustion, pyrometallurgical processes, and gold production (Pirrone et al. 1996, Pai et al. 2000). Gold and silver mining releases Hg into the environment through the metal's amalgamation with those mined metals (Lacerda 1997). Since the beginning of the industrial period, there has been a significant increase in atmospheric Hg levels caused by industrial activities (Wang et al. 2004). Domestic sewage discharge, without being treated properly, increases Hg concentrations in aquatic systems (Hermanson 1998). Globally, Asia contributes the most anthropogenic emission of Hg by 54%; Africa contributes 18%, and then Europe with 15% (Pacyna et al. 2006). Naturally occurring Hg sources consist of degassing from Hg mineral deposits, volcanic emissions, and forest fires (Biswas et al. 2007, Pirrone et al. 2010). Pirrone et al. (2010) suggested that approximately 342 mg/yr of Hg is emitted annually from forest fires around the world. Natural and re-emitted Hg emissions have a wide range distribution, which differ from anthropogenic Hg sources. This makes estimating emission amounts and applying control methods a more complicated process (Wang et al. 2004).

The natural Hg cycle (e.g. volcanoes, forest fires, etc) is disturbed by human activity, which causes an increased amount of Hg found in the environment (Roulet et al.

2000). Aquatic systems become contaminated with Hg by both sources through surface run-off and atmospheric deposition (Ullrich et al. 2001). Once in the aquatic system, Hg can be methylated by anaerobic microorganisms, becoming methylmercury (MeHg) (Hsu-Kim et al. 2013). Methylmercury can be stored in fatty tissues (Ravichandran 2004), causing it to become biomagnified throughout the food web (Scheuhammer et al. 2007).

Mercury Toxicity to Fish

Methylmercury is a cause for concern since it is a known neurotoxin that can affect fish, wildlife, and humans (Wolfe et al. 1998, Crump and Trudeau 2009, Angot et al. 2013). Fish exposed to MeHg affects their behavior, biochemistry, growth, reproduction, development, and survival (Sorensen 1990, Wiener and Spry 1996). Exposure of dietary MeHg to fish in long-term laboratory studies, suffered loss of coordination, decreased swimming activity, starvation, and increased mortality (Wiener et al. 2003). Fish populations also may be affected by low concentrations of Hg indirectly by impairment of physiological processes (Crump and Trudeau 2009). At current Hg levels found in aquatic ecosystems, the most observed effect of Hg on wild-fish is reduced reproductive success (Wiener and Spry 1996). Sex steroid levels can be reduced by apoptosis in steroidogenic gonadal cells in fish exposed to MeHg (Crump and Trudeau 2009). Interstitial cells in fish secrete androgens, which mediate gonadotropic regulation of spermatogenesis and spermiogenesis (Yaron 1995). When male Walking catfish's (*Clarias batrachus*) were exposed to Hg, their interstitial cells became inactive and had signs of degeneration (Kirubagaran and Joy 1992). Male Nile

tilapia (*Oreochromis niloticus*) had a decrease in spermatogenesis and atrophied seminiferous tubules after being exposed to MeHg for 7 months. In female fish, Hg can inhibit steroid hormone synthesis, affect ovarian morphology, and hinder oocyte development (Crump and Trudeau 2009). Furthermore, female fish's fecundity and spawning can be altered when they are exposed to Hg. Kihlstrom et al. (1971) found that Zebrafish (*Danio rerio*) produced fewer eggs after being exposed to a mercurial fungicide. Additionally, MeHg may be transferred to eggs and embryos maternally and potentially reduce hatching success (Crump and Trudeau 2009).

Mercury Toxicity to Birds

In birds, MeHg can penetrate the blood-brain barrier causing central nervous system dysfunctions and brain lesions (Wolfe et al. 1998). Acute MeHg poisoning can lead to birds experiencing reduced food intake, advanced weakness in wings and legs, trouble flying, walking and standing, and reduced muscle coordination (Scheuhammer 1987). Inorganic Hg causes major toxic effects to bird kidneys, which happens when the proximal tubular cells undergo necrosis (Ware et al. 1975). The avian kidney may be more vulnerable to Hg toxicity because birds have a renal portal system. This means bird's venous blood travels from the digestive tract to the kidney, instead of traveling to the liver to be filtered (Wolfe et al. 1998).

Reproductive effects in birds due to Hg toxicity include reduced hatchability, thinning of eggshells, decreased clutch size, a greater chance of eggs being laid outside nests, abnormal behavior and impaired hearing of juveniles (Stoewsand et al. 1971, Heinz 1975, Scott 1977, Heinz 1979). Mallard ducks fed MeHg over 3 generations were

found to have decreased reproductive success and ducklings showed a change in behavior (Heinz 1974). Male quail also experienced delayed development of their testicles (Scheuhammer 1987).

Other Metals Toxicity to Fish and Birds

There are other metals besides Hg that can be detrimental to fish and wildlife at certain concentrations. Sparling and Lowe (1996) found toxic and sublethal effects to fish due to high concentrations of aluminum (Al), such as asphyxiation. Eisler (1998) determined behavior and growth defects due to high concentration of copper (Cu), and Eisler (1993) noticed hemorrhaging due to zinc (Zn). In birds, vanadium (V) concentrations (0.5 mg/kg) can affect the metabolism of mallards (*Anas platyrhynchos*; White and Dieter 1978). Zinc toxicity studies conducted on ducks (*Anas* spp.) determined reduced survival when their diet contained 742 Zn/kg body weight (force-fed zinc metal shot equivalent) (Grandy et al. 1968), but domestic chickens (*Gallus* sp.) had a higher tolerance (2,000 > Zn/kg ration affected chicks negatively; Stahl et al. 1990).

Carbon, Nitrogen, and Deuterium Stable Isotopes in Ecological Studies

Past ecological studies have used stable isotopes carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) to investigate trophic relationships within a food web (Kelly 2000, Mora 2008, Boecklen et al. 2011). $\delta^{13}C$ can help distinguish between a consumer diet based on autochthonous or allochthonous carbon sources (Watanabe et al. 2008). Due to an enrichment of the $\delta^{15}N$ isotope (approximately 2.2 - 3.4‰) with every trophic level increase (McCutchan et al. 2003); it has been used in animal diet studies to determine the trophic position of an animal within the food chain (Fry 2006). Previous studies also

have analyzed aquatic biota's metal concentrations and $\delta^{13}C$ and $\delta^{15}N$ to determine if bioaccumulation, biomagnification, and/or biodilution occur (Capelli et al. 2008 and Watanabe et al. 2008). Deuterium (δD) has been used in past avian studies to determine from which area the feathers were grown (Hobson 1999). This is possible since δD values in feathers reflect δD values present in precipitation from around the North American continent (Hobson 2005).

Lake Chapala Study

Few studies have been conducted on the impact of Hg as well as other metal contamination on fish and surrounding wildlife in Lake Chapala (Fig. 1). A study conducted at Lake Chapala assessed Hg contamination in fish (i.e., carp (*Cyprinus carpio*), whitefish or silverside (*Chirostoma* spp.), and tilapia (*Oreochromis* spp.)), sediments, and human hair (Trasande et al. 2010). The researchers studied routes of MeHg exposure to the human fishing population of Lake Chapala. They concluded the lake's carp had enough Hg to be a cause of concern for locals who consume fish. Recently, Stong et al. (2013) conducted a lake wide survey of carp from Lake Chapala to acquire total Hg concentration information. They found the majority of carp were safe to consume on a limited basis due to the detected Hg concentrations below 1.0 ppm total Hg. In addition, they determined Hg concentrations decreased the further away fish were collected from the Lerma River. They concluded that a large sample size, comprising the whole lake, would be needed for dependable results to be obtained. Both studies focused on fish, but neither looked at the Hg levels from the wildlife surrounding Lake Chapala.

Figure 1: American white pelican colony at Lake Chapala, Mexico.

I analyzed metal concentrations (Hg and others) in fish that could be consumed by the aquatic avian community of Lake Chapala. The main objectives for this study were to (1) determine concentrations of metals (Hg, Al, Ba, Cu, Mn, Sr, V, and Zn) in water, sediments, fish, and birds (Hg only), (2) evaluate potential problems that could be associated with metal concentrations to fish and birds (Hg only), (3) analyze $\delta^{13}C$ and $\delta^{15}N$ from fish fillet samples (tilapia and common carp) and silverside (composite whole body), and δD in feathers collected.

Fish and Wildlife of Lake Chapala

For this study, I collected 3 different fish species from Lake Chapala (Fig. 2) Silversides (*Chirostoma* spp.) are endemic to this lake and consume cladocerans (*Bosmina*, *Ceriodaphnia*, and *Daphnia*), copepods (*Cyclops*), and other small biota in the lake (Moncayo-Estrada et al. 2011). They were the smallest fish collected for this study and can measure from 28–104 mm total length (Mercado-Silva et al. 2015). Tilapias (*Oreochromis mozambique*) are known to eat smaller vertebrates including small fish and crayfish (Mercado-Silva et al. 2015). Carp (*Cyprinus carpio*), the largest fish collected in this study, are benthic feeders and consume detritus from the bottom of the lake as well as silverside eggs and fingerlings (Trasande et al. 2010, Burton 1997). Fish metal levels were monitored because they are a main source of protein for the surrounding human population.

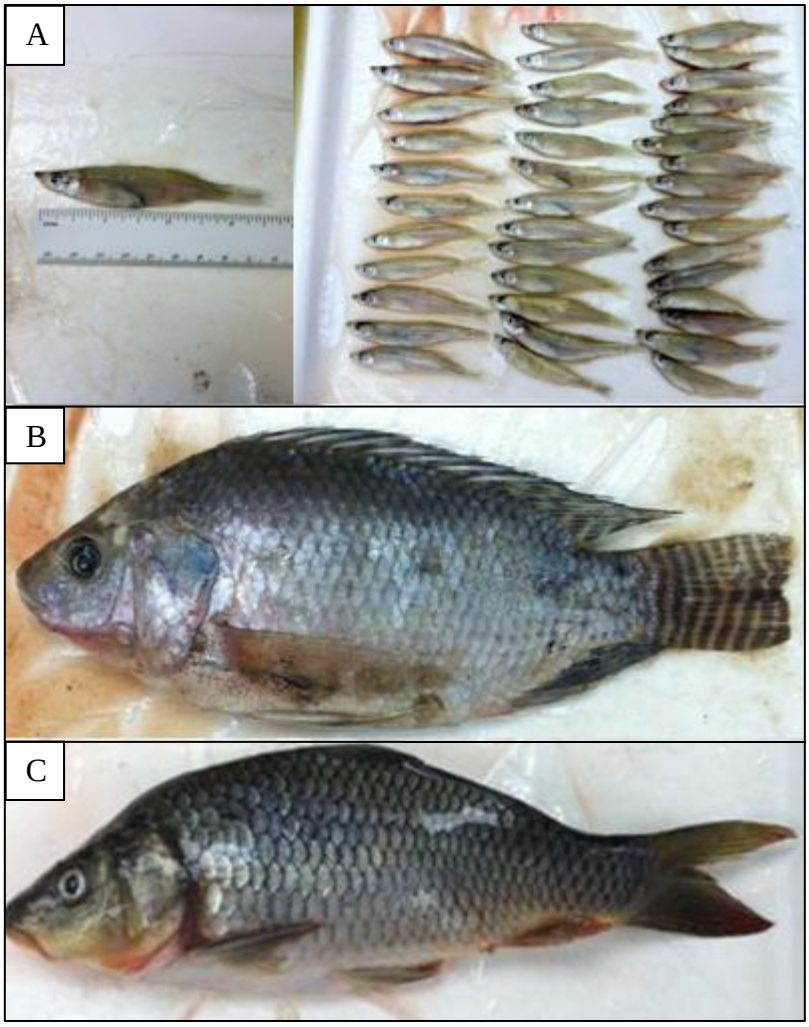

Figure 2: Silversides (A), tilapia (B), and carp (C) samples collected from Lake Chapala.

Aquatic birds residing at Lake Chapala are the resident great (GREG; *Ardea alba*) and snowy egrets (SNEG; *Egretta thula*), plus a migratory species, the American white pelican (AWPE; *Pelecanus erythrorhynchos*) (Villamagna 2009). American white pelicans breed in Canada and in the northern United States during spring, and then travel south for winter (Knopf and Evans 2004). They prefer freshwater environments such as

lakes and rivers opposed to more open waters like oceans, but are found there as well (Findholt and Anderson 1995). This is due to their foraging habits, unlike brown pelicans (*Pelecanus occidentalis*), they do not dive for food, but instead swim in a group on the surface of the water corralling fish underneath them and then placing their bills into the water and scooping up fish (Findholt and Anderson 1995). The 2 egrets utilize different foraging techniques compared to the AWPE as well as target smaller fish for their diet. The foraging technique by the GREG consists of walking slowly, standing-and-waiting, and uses peering techniques at usually fresh water and wetland habitats (Mccrimmon et al. 2011). The SNEG uses a wide range of foraging behaviors, greater than the GREG, and can be seen sometimes chasing its prey (Parsons and Master 2000).

Attwater's Prairie-chickens in Texas

The Attwater's prairie-chicken (APC; *Tympanuchus cupido attwateri*) population has been declining since the early 1900s (Lehmann and Mauermann 1963). Their decline is mainly due to habitat loss caused by conversion of prairie to agricultural fields, woody plant encroachment, urban development, and overgrazing (Lehman 1941). The current wild APC population is estimated to be less than 200 birds, compared to their historic numbers of 300,000 to 1 million (Lehman 1941, Hammerly et al. 2013). Lehmann and Mauermann (1963) reported an 85% decrease of the APCs population (8,700 in 1937 to 1,335 in 1963), and soon after they were listed as an endangered species in 1967. Since their listing as an endangered species, there have been numerous research efforts to save this species.

A captive breeding program was started in 1992 in order to prevent extinction due to low numbers of wild APCs (432 birds; Lockwood et al. 2005). This program was met with some difficulties in the form of disease (REV) and malformations of chick feet and leg growth (Griffin 1998). Nonetheless, the captive rearing program has persevered and has helped prevent the extinction of APCs. With the use of captive birds to supplement the wild population a danger of loss of genetic variability could occur (Ellsworth et al. 1994). In addition, proper records must be maintained of the APCs released back into the wild so that genetic variability is kept and no inbreeding is present (Hammerly et al. 2013).

Once released back into the wild, proper precaution must be maintained to ensure APC survival. Predator management for APCs nest predators took place during 1980-1981 and it was determined their removal resulted in an increase of nest success. However, the researchers saw that coyotes (*Canis latrans*) and birds of prey began to target rabbits and adult APCs more since there was a reduction of small mammals on the refuge (Lawrence and Silvy 1995). For future control methods to be effective they suggest targeting nest predators as well as those that prey on adult APCs. All of these studies were conducted to help understand the APCs decline, as well as to increase the wild population of APCs by supplementing them with captive reared birds.

Use of Carbon and Nitrogen Stable Isotopes in Ecological Studies

As mentioned earlier, stable isotopes are commonly used to determine animal diets. Carbon is useful for distinguishing between those animals that consume C_3 or C_4 vegetation. This is due to plants having different photosynthetic pathways. C_3 plants fix

CO_2 with the enzyme ribulose bisphosphate carboxylase (RUBISCO) while C_4 plants fix CO_2 with carboxylate phosphoenolpyruvate (PEP; O'Leary 1988). Some examples of C_3 plants include trees, shrubs, and grasses and have a $\delta^{13}C$ value of approximately -26.7‰ ± 2.3‰ (range -22‰ to -30‰), while C_4 plants consists of corn, sugar cane, and dryland grasses with an approximate value of -12.5‰ ± 1.1‰ (range -10‰ to -14‰) (Cerling et al. 1997, Fry 2006). The trophic increase of $\delta^{15}N$ is caused by the isotopically light nitrogen (^{14}N) being excreted in the urine, leaving the heavier (^{15}N) isotope in the consumer, causing a retention of ^{15}N and thus an increase between different trophic levels. An increase of $\delta^{15}N$ values also happens when an animal is water and nutritionally stressed (Kelly 2000).

$\delta^{13}C$ and $\delta^{15}N$ stable isotopes have been used in previous studies to compare historic feather samples, collected from museums, to contemporary ones in order to determine any diet changes. For example, Thompson et al. (1995) used the northern fulmar (*Fulmarus glacialis*) contemporary and historic feather samples, and compared their isotopic signatures ($\delta^{13}C$ and $\delta^{15}N$). They found that both stable isotopes declined over time, showing long-term changes to bird diet. Another study used seabird's feathers to determine if their diet was altered by environmental change over a span of 150 years (Blight et al. 2015). The authors indicated that there was a decline in diet quality of this seabird caused by either decrease of fish abundance or other human impacts.

Other studies have used $\delta^{13}C$ and $\delta^{15}N$ in stable isotope mixing models, which are used to infer the composition of the animal's assimilated diet (Phillips et al. 2014).

Recently, MixSIAR has been used to study a variety of animal including: sea turtles (Hall et al. 2015), platypus (*Ornithorhynchus anatinus*; Klamt et al. 2015), and invertebrates and fishes (Schroeter et al. 2015). One study looked at the diet of an endangered penguin, and determined that penguins targeted both squids and fish as prey sources (Connan et al. 2016). They stressed this finding since these penguins use squids to sustain themselves, while they feed fish to their chicks. With this new information, they recommend fish and squid stocks to be monitored to ensure penguin population recovery.

Stable Isotopes Study

This study aims to determine current APC populations preferred diet through the use of stable isotope analysis of $\delta^{13}C$ and $\delta^{15}N$ of potential sources (Fig. 3). Additionally, I examined $\delta^{13}C$ and $\delta^{15}N$ values in feathers of museum and current APC feather to determine potential shifts in diet of historic versus contemporary prairie chickens. The specific objectives of this study were to: (1) determine $\delta^{13}C$ and $\delta^{15}N$ stable isotope signatures in wild APCs feathers, blood, and feces collected from the Attwater Prairie Chicken National Wildlife Refuge (APCNWR) in Eagle Lake, Texas, (2) collect vegetation and arthropod samples to determine their $\delta^{13}C$ and $\delta^{15}N$ stable isotope signatures, and (3) determine and compare $\delta^{13}C$ and $\delta^{15}N$ isotopic signatures in feathers of historic and contemporary APCs.

Figure 3: A male (left) and female (right) Attwater prairie-chicken at the APCNWR.

CHAPTER II

HAZARD ASSESMENT OF MERCURY TO WATERBIRDS AT LAKE CHAPALA, MEXICO[1]

Summary

Lake Chapala is the largest lake in Mexico and serves as a fishery for the surrounding communities. This study was conducted to determine mercury (Hg) concentrations in fish and aquatic birds from Lake Chapala and evaluate for bioaccumulation. From the 3 species of fish collected, their Hg concentrations ranged from 0.021 to 0.568 µg/g wet weight. Fish Hg concentrations were positively and significantly correlated with total fish length ($R^2 = 0.4434$, $P < 0.05$). I also analyzed fish tissues for stable isotopes $\delta^{13}C$ and $\delta^{15}N$, and determined fish $\delta^{15}N$ values to be significantly correlated with Hg concentrations from Lake Chapala and San Antonio Guaracha Reservoir ($R^2 = 0.6936$, $P < 0.001$ and $R^2 = 0.4032$, $P < 0.05$). Compared to other lakes, this study's fish Hg concentrations were within the same values reported. As for the feather Hg concentrations, no significant differences were determined between years, locations, nor among species. Feather δD values showed a great range (-163‰ to -11‰) and had Hg concentrations ranging from 0.805 to 18 µg/g dw, which suggests exposure for aquatic birds (American white pelicans and egrets) are

[1] Reprinted with permission from "Accumulation and hazard assessment of mercury to waterbirds at Lake Chapala, Mexico" by Torres, Zaria, Miguel A. Mora, Robert J. Taylor, Dioselina Alvarez-Bernal, Hector R. Buelna, and Ayumi Hyodo, 2014. Environmental Science & Technology, 48, 6359-6365, Copyright 2014 by the American Chemical Society.

widespread. The next step would be to monitor contaminant exposure to breeding aquatic birds at Lake Chapala to help determine potential effects of Hg on these resident birds.

Introduction

Mercury (Hg) emissions from anthropogenic sources have contributed to the contamination of aquatic ecosystems all over the world (Wang et al. 2004). Mercury occurs in the aquatic environment in inorganic and organic forms; however, the predominant form of Hg is dependent on suspended organic matter (Meili 1997). Mercury methylation occurs naturally in an aquatic environment through acidification, and methylmercury (MeHg) is more easily accumulated through the food web (Scheuhammer and Blancher 1994). Aquatic systems become contaminated with Hg in many ways, but primarily by soil erosion, runoff, and atmospheric deposition (Roulet et al. 1999). Anthropogenic sources of Hg include solid waste incineration, coal and oil combustion, pyrometallurgical processes, and production of Hg and gold mining (Pirrone et al. 1996, and Pai et al. 2000). Domestic sewage discharge, also contributes to increased Hg concentrations in aquatic systems (Hermanson 1998). Non-anthropogenic sources of Hg include volcanic emissions and forest fires, as well as degassing from Hg mineral deposits, and degassing from Hg contaminated aquatic and terrestrial systems (Nriagu and Pacyna 1988, Lindqvist 1991, Nriagu 1994, and Camargo 2002). Brunke et al. (2001) estimate that approximately 590–930 metric tons of Hg is emitted annually from forest fires around the world.

Fish exposed to MeHg could be affected in their behavior, growth, reproduction, development, and survival (Sorensen et al. 1990, Wiener and Spry 1996). The most commonly observed effect of Hg on wild-fish is reduced reproductive success (Weiner and Spry 1996). Male Nile tilapia (*Oreochromis niloticus*) had a decrease in spermatogenesis and atrophied seminiferous tubules after being exposed to MeHg for 7 months (Crump and Trudeau 2009). In female fish, Hg can inhibit steroid hormone synthesis, affect ovarian morphology, and hinder oocyte development (Crump and Trudeau 2009). Kihlstrom et al. (1971) found that Zebrafish (*Danio rerio*) produced less eggs after being exposed to a mercurial fungicide.

In birds, MeHg has been associated with brain lesions, spinal cord deterioration, and central nervous system dysfunctions (Wolfe et al. 1998). Methylmercury in birds also leads to reduced food intake, advanced weakness in wings and legs, trouble flying, walking and standing, and an inability to coordinate muscle movements (Scheuhammer 1987). Reproductive effects of Hg in birds include reduced hatchability, decreased clutch size, abnormal behavior of juveniles, and possible impaired hearing of juveniles (Heinz 1979, Heinz 1975, Stoewsand et al. 1971, and Scott 1977).

The Lerma-Chapala Basin concentrates about 10% of Mexico's human population. Industrial, agricultural, and urban settings along the basin contribute a great variety of contaminants to the Lerma River which discharges its waters into Lake Chapala, the largest tropical lake in Mexico. Lake Chapala represents a major fishery and recreation resource for various communities surrounding the lake, as well as for tourists from many parts of the country (SEMARNAT 2009). It also is the ultimate

receptor of a great variety of contaminants from the sub-basin, including pesticides, industrial residues, oils, detergents, and heavy metals such as, chromium, lead, zinc, and Hg (Hansen and Van Afferden 2001, Jay and Ford 2001). Local sources of pollution also are noticeable; Chapala County generates over 95 tons/day of trash which is deposited without treatment in an open pit (SEMARNAT 2009). Despite concerns for the effects of pollutants on human and ecosystem health, studies addressing contaminant issues in Lake Chapala are few.

Important fishes to the fishing community and are among the most harvested and consumed include: silverside (*Chirostoma* spp., commonly known as charal), common carp (*Cyprinus carpio*), and tilapia (*Oreochromis* spp.; Lind et al. 2000). One recent study suggests that pollution in Lake Chapala has led to differences in relative abundance of tolerant and non-tolerant fish species, with the most tolerant showing an increase in relative abundance (Becerra-Munoz et al. 2003). Contaminant studies in Lake Chapala have focused primarily on metal pollution. Studies show a seasonal variability in the accumulation of metals in water, with potential increases during the dry season (likely because of evaporation) and decreases during the rainy season, because of dilution (Ford et al. 2000). Elevated concentrations of copper (Cu) were reported for tilapia and carp (Ford et al. 2000). In 1993, elevated concentrations of chromium, nickel, and Cu were reported in sediments (Hansen and Van Afferden 2001). Elevated concentrations of Hg were reported previously in silverside (up to 4.9 µg/g dry weight (dw)) from Lake Chapala (Ford et al. 2000, and Jay and Ford 2001). High

concentrations of Hg also were reported recently in carp (0.87 µg/g wet weight (ww), Trasande et al. 2010).

Lake Chapala has been recognized as one of the most important wetlands of Mexico and in 2011 was designated as a Ramsar site (Ramsar 2011). More than 80 species of aquatic birds have been reported for Lake Chapala and it is one of the largest wintering areas for American white pelicans (AWPE; *Pelecanus erythrorhynchos*) in Mexico. It is estimated that 20,000–30,000 AWPEs winter and stay about 5 months from October to March in Lake Chapala (D.W. Anderson pers. comm.). Currently, to my knowledge, there are no studies which have evaluated the impacts of metals and other contaminants on fish-eating birds and other wildlife in Lake Chapala. Given the importance of Lake Chapala as a Ramsar site, understanding the effects of pollution on aquatic wildlife are important. The AWPE is a species of special concern in the United States and is protected under the Migratory Bird Treaty Act. The objectives of this study were to determine bioaccumulation of Hg in fish and to evaluate the potential impacts of Hg in the diet of aquatic birds, particularly the AWPE, in Lake Chapala. I also measured stable isotopes of $\delta^{13}C$ and $\delta^{15}N$ in fish tissue to determine trophic differences among the 3 fish species and predict potential Hg movement from water and sediments to fish. Mercury also was analyzed in feathers of AWPE wintering at Lake Chapala to determine Hg exposure in their breeding and molting grounds in the north and to compare with an AWPE colony from North Padre island, Texas, as well as with resident aquatic species such as great and snowy egrets (GREG; *Adrea alba*, and SNEG; *Egretta thula*).

Methods

Study area

Lake Chapala is located on the border of Jalisco and Michoacán, Mexico, with the majority (114,659 ha) being located in Jalisco. It is south of the major city, Guadalajara and is 1,510 m above sea level (Moncayo-Estrada 2011; Fig. 4).

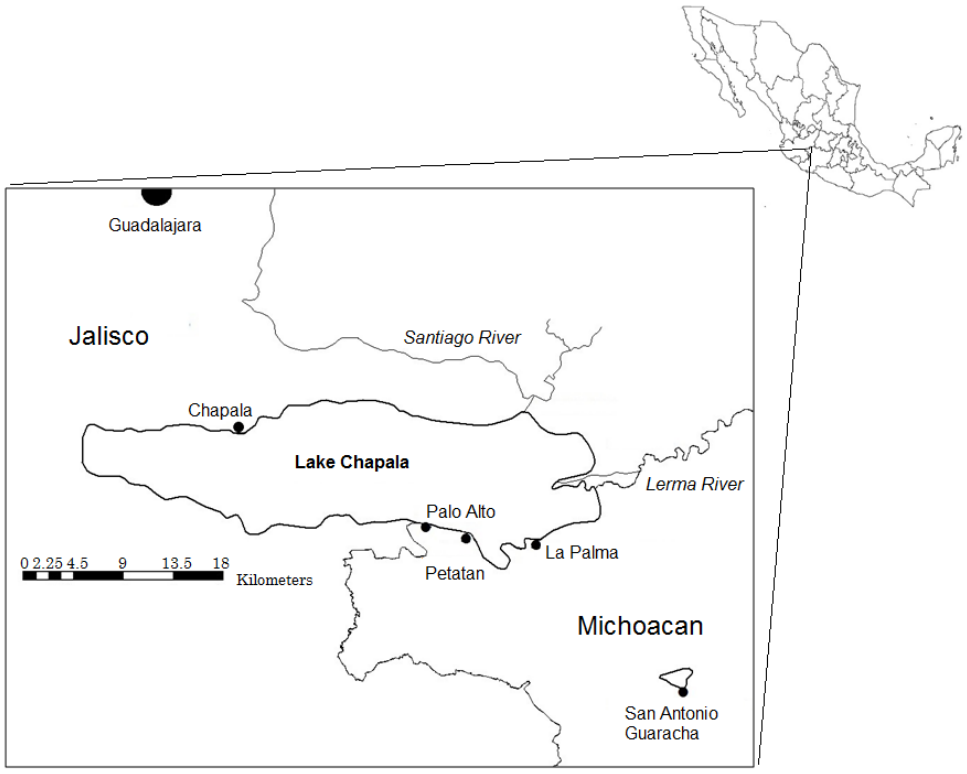

Figure 4: Lake Chapala and study sites located in Jalisco, Mexico.

Sample Collection

Six water and 6 sediment samples were collected per year in the winter of 2011 and 2012 from 3 locations southeast of Lake Chapala near the cities of Petatan, La Palma, and Palo Alto. During 2011, water was collected at each location in duplicate with pre-cleaned 125 ml LDPE bottles for clean metals and 125 ml pre-combusted flint glass bottles, preserved with BrCl, to maintain Hg(II) ions in solution and to oxidize organic Hg so that total Hg could be measured. Fish were purchased from fisherman right after they came out of the lake in the towns of La Palma and Petatan during 2011 and 2012 at the same time of the water and sediment collection. Tilapia and carp also were collected from a reference location in San Antonio Guaracha about 25 km southeast of Lake Chapala. Sediment and fish samples were placed in Ziploc bags and stored on ice until taken to an ultra-cold freezer and stored at -80°C. Primary feathers of American white pelicans and egrets (mostly great egrets), were collected from roosting areas along the shore in Lake Chapala near the towns of La Palma and Petatan southeast of the lake during 2011 and 2012. The feathers were collected haphazardly and were stored in Ziploc bags until analysis. Additionally, feathers from adult AWPEs were collected right after the breeding season from Padre Island National Seashore in 2011 only (North Padre Island, Texas) for comparison with those collected in Lake Chapala.

Chemical Analyses

All the samples were analyzed for Hg at the Trace Element Research Laboratory, College of Veterinary Medicine, Texas A&M University. Water was analyzed for mercury using EPA method 1631 revision E, with an automated sampling analysis

system (Tekran 2600). The amount of Hg in samples is calculated by comparing the detector response with that of known calibration standards that are processed and analyzed identically to the samples. The sediment samples were freeze dried and then ground up using a mortar and pestle. Approximately, 0.5 g dried, powdered sediment were dissolved in nitric acid, hydrochloric acid, and then brought to a final volume of 50ml with deionized water. Prior to analysis, all carp and tilapia were measured for total length. Carp and tilapia were filleted on each side with the scales intact and then 1 fillet portion was freeze dried and homogenized with a titanium blade grinder. Silversides (approximately 12 mm in length) were pooled (8 individuals for each sample) for analysis and also were homogenized with a titanium blade grinder. Primary feathers of AWPE from Lake Chapala and North Padre Island and egrets from Lake Chapala (10 from each species) were washed in an ionized water bath for 5 minutes each and then oven dried at 30°C over night. Once dried, they were cut into 4 sections and grounded up into a homogenized sample.

The sediments, fish, and feather samples were analyzed for total mercury by a Direct Mercury Analyzer (DMA-80) equipped with a 40 position auto sampler and a dual cell detector. Samples were weighed on pre-combusted boats and placed into an auto sampler carousel. The boats were then subjected to a sequence of heating steps while under a constant flow of oxygen. After the samples were combusted and the Hg collected for a sufficient time, the gold trap was heated in order to release the trapped Hg as a concentrated slug into the gas stream. The released Hg was swept into a 2-stage absorption cell where free Hg0 atoms absorb light from a Hg vapor lamp. Mercury

concentrations in samples were quantitatively measured by comparing peak absorption with that of known calibration standards. Accuracy was verified by analyzing a blank and a certified reference material. Precision was evaluated by analyzing replicate samples. The lowest limit of detection for Hg was 0.0000002 µg for water, 0.006 µg for sediments, and 0.004 µg for fish. The Hg QA/QC results for water, sediments, fish, and feathers from Lake Chapala can be found in Appendix A.

Stable Isotope Analysis of Fish Tissue and Avian Feathers

Approximately 10 g of previously homogenized fish muscle was further grounded in a ball mill grinder (Retsch MM400) for 30 seconds (30.0 frequency/ seconds). Afterwards, approximately 1 mg of ground fish homogenate was placed in tin capsules (4×6 mm, Costech) and then in a sample tray. The capsules were analyzed for $\delta^{13}C$ and $\delta^{15}N$ with a Delta V Advance stable isotope ratio mass spectrometer (Thermo Scientific®) coupled to an Elemental Combustion System (EA) (Costech) and Conflo IV. Deuterium isotopes (δD) were measured in avian feathers to determine potential differences in molting origin of AWPE wintering in Lake Chapala relative to patterns observed in AWPE breeding in North Padre Island. Feathers were washed in an ultrasonic bath for 5 minutes in deionized water, before any analysis. Each feather was then ground, in its entirety, with a Retsch MM400 mill grinder. Two grams of each ground sample were then washed of any debris and surface oils by a 2:1 chloroform to methanol solution for 24 hours and then allowed to air dry for 48 hours. Once dried, a portion (0.5mg) of each feather sample was placed in a silver capsule. Both standards and samples were left in room temperature for exchange with ambient vapor (Wassenaar

and Hobson 2003) for 7 days, and kept in a desiccator for at least 5 days prior to analysis. Feather samples also were analyzed in a Delta V Advance stable isotope ratio mass spectrometer (Thermo Scientific) coupled to a High Temperature Conversion Elemental Analyzer (TC/EA; Thermo Scientific) and Conflo IV (Thermo Scientific). Standards used were KHS and CBS (keratin standards from Environment Canada), and USGS42 and USGS43 (from U.S. Geological Survey). Isotopes results are reported as permil (‰) relative to Vienna Standard Mean Ocean Water (VSMOW).

Statistical Analyses

The data for water and sediment data were normally distributed as indicated by the Shapiro-Wilk test, and then were analyzed by ANOVA to determine significant differences comparing locations. The Tukey-Kramer HSD test was used to determine which means were significantly different. Fish and feather data were log transformed to meet the normality assumptions and equality of variance. ANOVA of log transformed data were used for comparisons by using the Tukey-Kramer HSD, which also was used to determine significant difference among locations. A linear regression analysis was done between log transformed Hg values and total fish length. Mercury concentrations in avian feathers from AWPEs and GREG/SNEG were compared by ANOVA and t-tests. Feather deuterium and Hg values also were compared by a linear regression analysis. The level of significance used in this study was set at $\alpha = 0.05$ (JMP®, Version 12.1. SAS Institute Inc., Cary, NC).

Results

Mean Hg concentrations in water collected in January 2011 from Lake Chapala were 0.015 ± 0.002 ng/ml (n = 6, range 0.01–0.019). Mean Hg concentrations in sediments collected from the same locations during 2011 and 2012 ranged from 0.4 to 1.0 µg/g dry weight (n = 12, $\bar{x}$ = 0.597 ± 0.190 µg/g dw). Mercury concentrations in fish were, for the most part, below 0.2 µg/g ww in the 3 species collected in Chapala and San Antonio during both years, except during 2011, when carp had mean Hg concentrations = 0.357 µg/g ww (Table 1). Mercury concentrations in carp collected during 2011 from Lake Chapala were significantly greater ($P < 0.0001$) than carp collected during 2012; they also had greater Hg concentration than silverside and tilapia from Lake Chapala during both years of collection. Lake Chapala carp collected in 2011 also were significantly greater than carp collected from San Antonio Guaracha Reservoir from both years, and tilapia (2012 only). Silverside collected in 2011 in Lake Chapala also had significantly higher concentrations of Hg than those collected in 2012. Similarly, Hg concentrations in silverside during both years were significantly greater than Hg concentrations in tilapia from Chapala, and carp and tilapia from San Antonio. Carp collected in 2012 in Chapala also had significantly higher concentrations of Hg than in tilapia collected both years in Chapala as well as in tilapia collected in San Antonio in 2012. Overall, concentrations of Hg in carp and silverside were significantly greater than those in tilapia. Mercury in fish was positively and significantly correlated with fish length (R^2 = 0.4434, $P < 0.05$, Fig. 5).

Table 1. Total Hg levels (geometric mean and range, µg/g wet weight) in fish collected from Lake Chapala and San Antonio Reservoir in 2011 and 2012. Mean values not sharing the same letter are significantly different.

Year	Location	Species	*n*	Hg (µg/g ww)
2011	Chapala	Carp	8	0.357 A
				(0.265-0.568)
		Tilapia	10	0.035 E
				(0.021-0.108)
		Silverside	8*	0.150 B
				(0.126-0.172)
	San Antonio	Carp	5	0.073 CD
				(0.042-0.134)
2012	Chapala	Carp	6	0.101 BC
				(0.056-0.215)
		Tilapia	6	0.036 DE
				(0.024-0.064)
		Silverside	8*	0.076 C
				(0.067-0.091)
	San Antonio	Carp	3	0.072 BCDE
				(0.033-0.11)
		Tilapia	3	0.031 DE
				(0.027-0.033)

* Composite samples (8 individuals each)

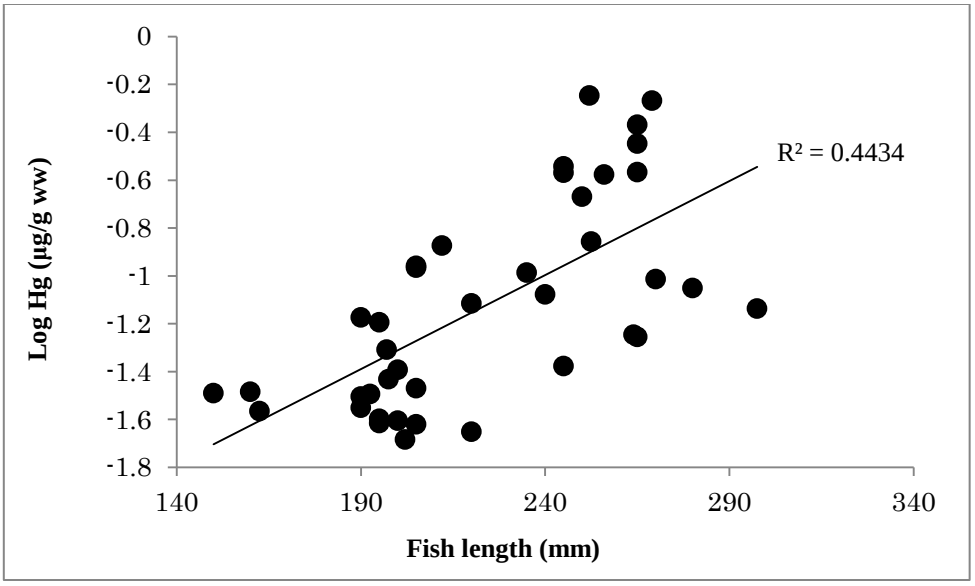

Figure 5: Relationship between fish length (mm) and Hg concentrations (silverside fish not included).

Stable isotopes $\delta^{13}C$ and $\delta^{15}N$ in fish tissue varied among species, primarily between carp and tilapia from Lake Chapala with those in the San Antonio Reservoir (Table 2). Silverside which grows to an average of 90 mm, approximately 3 times smaller (in length) than carp, had $\delta^{15}N$ values very similar to those of carp (Table 2, Fig. 6). The $\delta^{15}N$ values in these 2 species were nearly 1 trophic level above (difference in $\delta^{15}N$ of 2.69 ‰ for carp, and 2.48 ‰ for silverside) the level observed for tilapia in Chapala. Also, $\delta^{15}N$ values in carp and tilapia from Lake Chapala were greater (difference in $\delta^{15}N$ of 5.46 ‰ and 5.07 ‰, respectively) than those in carp and tilapia from the San Antonio Reservoir (Table 2, Fig. 6). The $\delta^{15}N$ was a very good predictor of Hg concentrations both in Lake Chapala and in the San Antonio Reservoir (Fig. 7); the coefficient of determination was highly significant for the fish in Chapala (R^2 =

0.6936, $P \leq 0.001$) and also for the San Antonio reservoir ($R^2 = 0.4032$, $P \leq 0.05$). The predictive equation for Hg based on $\delta^{15}N$ values in fish from Chapala was: Log Hg = -4.3 + 0.19 ($\delta^{15}N$). The $\delta^{13}C$ values were somewhat broader in carp from San Antonio (range -20.2 to -30.93 ‰) than in carp from Chapala (-25.06 to -27.26‰). The 3 fish species from Chapala had much narrower $\delta^{13}C$ values than the fish from San Antonio (Table 2, Fig. 6).

Table 2. Stable isotope ratios ($\bar{x} \pm SD$) of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) in fish from Lake Chapala, Jalisco, and San Antonio Guaracha Reservoir, Michoacán, Mexico.

Location	Species	*n*	Length (mm)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
Lake Chapala	Carp	14	263 ± 14	-26.11 ± 0.63	18.03 ± 1.37
	Tilapia	16	199 ± 8	-26.86 ± 1.68	15.35 ± 0.95
	Silverside	16*	90 ± 0	-26.65 ± 0.19	17.83 ± 1.16
San Antonio	Carp	8	244 ± 40	-25.79 ± 3.63	12.57 ± 1.03
	Tilapia	3	158 ± 7	-30.77 ± 0.64	10.27 ± 0.43

* Composite samples.

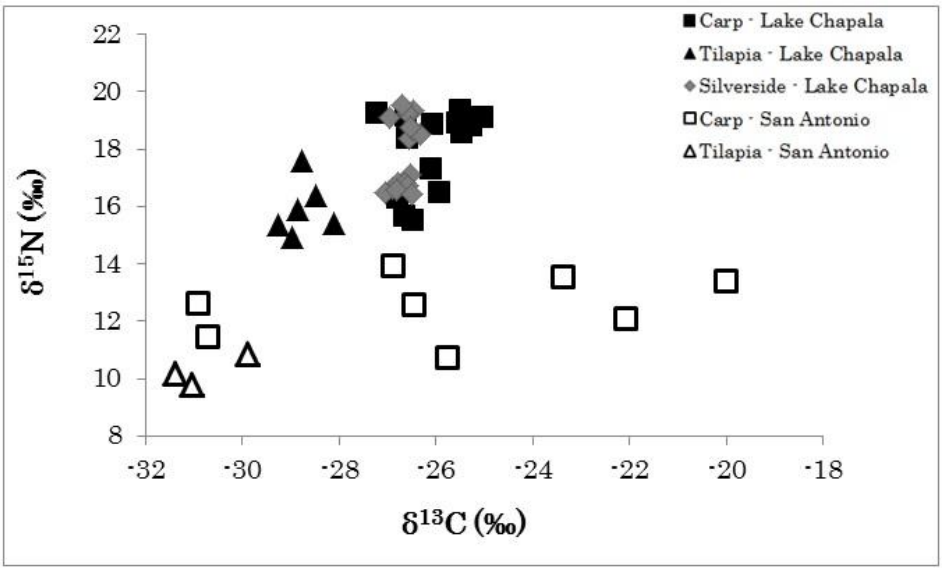

Figure 6: Relationship between $\delta^{13}C$ and $\delta^{15}N$ in fish from Lake Chapala and San Antonio Reservoir.

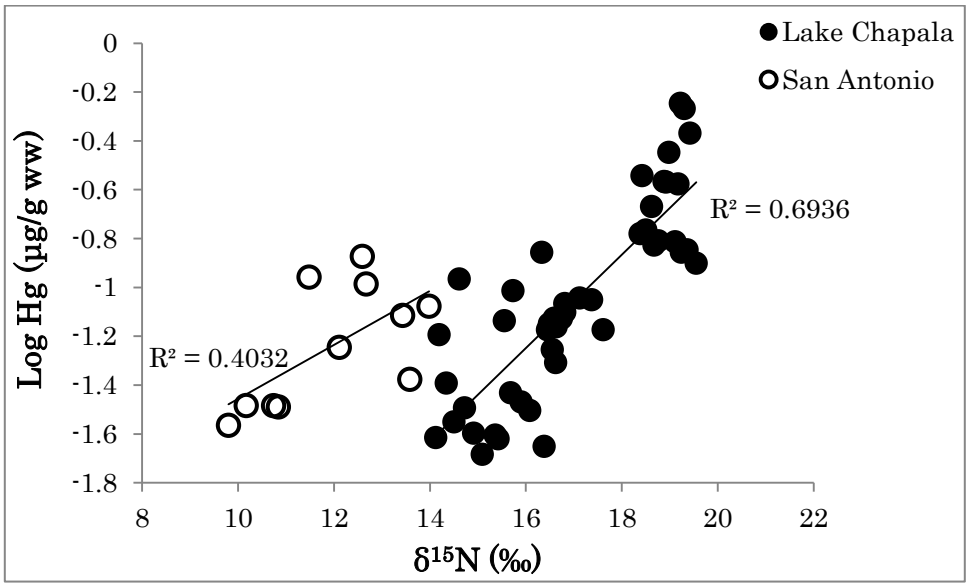

Figure 7: Relationship between Hg concentration and $\delta^{15}N$ in fish muscle from Lake Chapala and San Antonio Reservoir.

Mercury concentrations in bird feathers were not significantly different ($F_{4,45}$ = 2.1, P = 0.09) among locations or among species; however, they were slightly higher in egrets from Chapala and were much lower in AWPE from Padre Island National Seashore than in AWPE from Lake Chapala (Table 3). The δD in the same feathers analyzed for Hg from the 3 species were quite variable and ranged from -11 to -161‰ suggesting many locations of feather growth (Table 3, Fig. 8). Only a few δD values in feathers were close to the δD values in water from Lake Chapala ($\bar{x}$ = –25.9 ± 0.5‰). There was a slightly significant negative relationship ($P \leq 0.05$, R^2 = 0.14) between Hg in feathers and δD showing there was a tendency for higher Hg accumulation in feathers which grew in more northern or inland locations.

Table 3. Total mercury levels (geometric mean and range, µg/g dry weight) and δD range values (‰) in feathers of American white pelicans from Lake Chapala, Mexico and North Padre Island National Seashore, Texas, and egrets from Lake Chapala.

		2011			2012		
			Hg			Hg	
Species	Location	*n*	(µg/g dw)	δD range (‰)	*n*	(µg/g dw)	δD range (‰)
AWPE	Lake Chapala	10	3.37 (0.81-9.57)	-46 to -163	10	4.02 (1.17-18.0)	-62 to -135
	North Padre Island National Seashore	10	2.75 (1.56-4.21)	-11 to -123			
GREG/SNEG	Lake Chapala	10	4.54 (2.36-11.5)	-62 to -120	10	5.69 (1.37-16.3)	-62 to -131

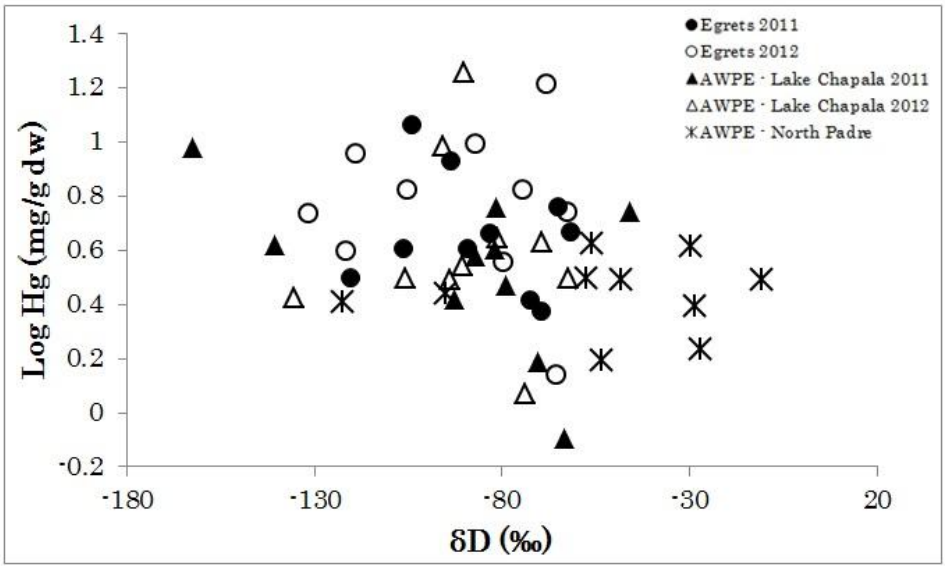

Figure 8: Relationship between Hg concentration (µg/g ww) and δD values (‰) in feathers of egrets and American white pelicans from Lake Chapala, Mexico, and North Padre Island National Seashore, Texas.

Discussion

Total Hg values in water from lake Chapala were somewhat high compared with results from other freshwater lakes; however, the highest Hg values (18.8 ppt) were lower than those measured in the Jose Antonio Alzate Reservoir, Mexico in 1995 (104 ppt), which is formed by the Lerma river upstream of Lake Chapala (Avila-Perez et al. 1999). However, that study did not report their method for Hg analysis, thus their results may not be comparable to mine. Notwithstanding, Hg levels in water from Lake Chapala were higher than those observed in Minnesota, Nova Scotia, eastern Massachusetts, Lake Michigan, and in south Brazil (Dennis et al. 2005, Wiener et al. 2006, Mirlean et al. 2008, Gabriel et al. 2009, and Jeremiason et al. 2009). Lake

Chapala is a highly alkaline lake (pH = 9.6) which likely influenced the Hg concentrations in water. Accordingly, in alkaline lakes there is less assimilation or methylation of Hg by bacteria than in more acidic lakes (Kelly et al. 2003).

Sediment Hg concentrations in Lake Chapala were similar to those reported in previous studies (Hansen and Van Afferden 2001, and Trasande et al. 2010); however, sediment samples collected from deeper sites showed higher Hg concentrations (up to 1.28 ppm; Trasande et al. 2010). Mercury concentrations in sediments from Lake Chapala were much greater than those observed in some Mississippi lakes (Huggett et al. 2001) and in Lake Ontario (Marvin et al. 2004).

Mercury concentrations in fish from Lake Chapala were within values reported in many parts of the world (Huggett et al. 2001, and Mirlean et al. 2008). Hg concentrations in silverside were higher in previous years (0.704–4.937 µg/g dw) (Ford et al. 2000, and Trasande et al. 2010) than what I observed (0.257–0.626 µg/g dw) in 2011–2012. Higher Hg values also were reported for carp in previous years (0.87 µg/g ww, Trasande et al. 2000) relatively to what I observed in 2011–2012. Previous studies indicate that most Hg in fish is actually MeHg (Evers et al. 2005), which is highly toxic to aquatic and terrestrial organisms. Common carp was the only species with mean Hg values above the fish tissue residue criterion for freshwater and estuarine fish of 0.3 mg MeHg/kg fish wet weight recommended by the U.S. EPA (USEPA 2001), but only during 2011. In all cases, total Hg geometric mean values were well below the FDA action level of 1 mg/kg wet weight of MeHg in fish (USFDA 2000). The high pH value

of Lake Chapala also could help explain the lower than expected Hg concentrations in fish (Wiener et al. 2006, and Burgess and Meyer 2008).

Stable isotopes $\delta^{13}C$ and $\delta^{15}N$ in fish tissue were useful to establish trophic differences among the 3 fish species as well as establish differences in Hg accumulation based on $\delta^{15}N$ levels. There were noticeable differences in $\delta^{15}N$ values particularly for carp and tilapia from Lake Chapala and the San Antonio Guaracha Reservoir. Within reservoirs, the fish species' diet was very similar in the 2 years of sampling (Table 2). Carp and silverside from Lake Chapala appeared to be feeding almost at the same trophic level, or close to 1 trophic level above ($\delta^{15}N$ difference of 2.72 ‰ and 2.27 ‰, respectively) the level observed in tilapia in Lake Chapala. The fish $\delta^{15}N$ values differences between the 2 reservoirs were quite great. Carp and tilapia in Lake Chapala seem to be feeding at nearly 2 trophic levels higher ($\delta^{15}N$ difference of 5.7 ‰ and 5.3 ‰, respectively) than carp and tilapia in the San Antonio Guaracha Reservoir. However, caution should be taken on labeling this as such; I address this in Chapter III. Clearly, both silverside and carp in Lake Chapala are feeding at a much higher level than carp in San Antonio. These feeding differences help explain differences in Hg accumulation between the species in Lake Chapala and those from San Antonio Guaracha Reservoir. The most surprising finding was that silverside, a small fish growing no more than 90 mm, seemed to be feeding almost at the same level than carp, which can grow up to 3 times higher or more.

The high accumulation of Hg in carp and silverside in Chapala has implications for the accumulation and impacts of Hg on fish-eating birds, such as the AWPE and

GREG/SNEG. The AWPE in Lake Chapala feeds primarily on scraps from tilapia provided by fisherman; however, they also feed on carp or other available fish. The silversides are probably too small for the AWPE to pursue them as part of their diet. Great and snowy egrets are more likely to feed on smaller fish, such as silverside, suggesting that Hg intake from eating this smaller fish species is quite high. This intake is probably reflected in the observed higher levels of Hg in egrets' feathers. The highly positive significant relationship between ($\delta^{15}N$) and Log Hg (Fig. 7) suggests this isotope could be a good predictor of Hg concentration in fish in Lake Chapala. This is particularly important for tropical lakes such as Lake Chapala to allow for more continuing monitoring of pollutants, such as Hg, with the use of less expensive analyses such as stable isotopes. Because of a smaller sample size, the relationship between Hg and $\delta^{15}N$ for fish from San Antonio was not as strong as that for Chapala.

The highest Hg concentrations in feathers were from egrets which were considered resident birds. However, the δD values in water from Lake Chapala were at least 3 times less negative (-25.9 ± 0.5) than the δD values observed in egrets' feathers, suggesting that perhaps the egrets wintering in Chapala were not resident species, but migrants and molted elsewhere in the north. Feathers of AWPE wintering in Lake Chapala had intermediate levels of Hg, whereas feathers of AWPE nesting in North Padre island had the lowest. The Hg in feathers suggests a wide pattern of exposure for AWPE with differences in locations relative to the North Padre Island colony. I analyzed δD values in feathers with the overall purpose to be able to predict potential breeding or molting areas for AWPE and egrets. Unfortunately, the δD values were too

broad and inconclusive. The δD values in egret feathers were not different from those in feathers of AWPE wintering in Chapala. Surprisingly, even the δD values in feathers of AWPE from North Padre, believed to have been grown there, did not have a consistent δD pattern and the values were much broader than those that would be expected from North Padre Island. The above suggests that with the available data it is difficult to determine where the AWPE wintering in Chapala came from, although more likely from western breeding colonies and Pacific coastal areas in the north (Anderson and Anderson 2005). Similarly, the egrets may have migrated from northern areas in the United States. Recent studies have pointed out the use of δD values to infer origin of molting and breeding bird location should be taken with caution given there are other variables which influence δD (Wolf et al. 2013). For this study, the slightly significant negative correlation between Hg and δD suggests birds that grew feathers in the north or in areas with more negative δD values were more likely to have greater concentrations of Hg than those that grew their feathers in dryer areas with less negative δD values. Hg concentrations in feathers of AWPE wintering in Lake Chapala were within the lower range of those reported previously for AWPE from various regions of Nevada, Idaho, and Oregon (3.7–20 µg/g dw; Wiemeyer et al. 2007). Similarly, Hg values in carp from Chapala were within the ranges observed in fish regurgitated from various colonies in Nevada and Oregon in 1996 (Wiemeyer et al. 2007)

Lewis and Furness (1991) estimated that primary feathers of black-headed gulls (*Larus ridibundus*) accumulated between 1.33 and 4.67 µg/g dw MeHg when chicks were dosed with 20 and 100 µg MeHg, respectively. There was a progressive reduction

in the concentration of Hg in the primary feathers as growth continued. AWPE consume an average of 1.8 kg fish/da or 20–40% of their body mass (Knopf and Evans 2004). If pelicans in Chapala were feeding exclusively on carp, the Hg intake would have been on average, 0.182 to 0.642 mg Hg/da for 2012 and 2011, respectively. This estimate indicates that Hg intake by AWPE could lead to much higher residues in feathers. However, AWPE are often seen in big groups near fisherman which throw out the remains of tilapia and carp after removing the muscle to be sold commercially in nearby towns. AWPE feeding on tilapia would be expected to ingest lesser concentrations of Hg. Exposure of AWPE to Hg in their diet while wintering in Lake Chapala could be of concern depending on the variability of Hg in fish. Lake Chapala is a very shallow lake and the total volume of water in the lake could oscillate significantly based on drought and the amount of water taken out by different municipalities and the city of Guadalajara. Thus, it is expected that Hg concentrations in water, sediments, and biota undergo considerably annual variations. Scheuhammer (1991) has suggested that diets of about 1 µg/g ww in birds can result in Hg concentrations in feathers near 20 µg/g dry weight. He also indicates that in raptorial birds normal Hg concentrations in feathers are around 1-5 µg/g dry weight. Clearly, there is some variability in Hg accumulation in bird feathers. Contaminant exposure in aquatic birds in Lake Chapala during the breeding season should be monitored to better determine the potential effects of Hg on aquatic birds.

CHAPTER III

METAL CONCENTRATIONS IN WATER, SEDIMENT, AND FISH FROM LAKE CHAPALA, MEXICO [2]

Summary

Anthropogenic sources of pollution to Lake Chapala include metals, pesticides, industrial residues, and polycyclic aromatic hydrocarbons. My main objective was to measure metal (aluminum, barium, copper, manganese, strontium, vanadium, and zinc) concentrations in water, sediment, and fish from Lake Chapala and a nearby reference location to determine potential negative effects on wildlife, particularly fish-eating birds. Fish metal concentrations ranged from 0.05 µg/g wet weight (ww) for Al and Cu to 64.70 µg/g ww for Sr. There was a positive and significant correlation between fish length and metals particularly for Ba, Cu, Mn, and Zn in Lake Chapala ($P < 0.05$). However, there were no significant correlations between metal concentrations and $\delta^{15}N$ values in fish suggesting that most metals did not biomagnify through the food chain. Overall, metal concentrations in water, sediments, and fish were similar and in some cases below those that have been reported for Lake Chapala over the last 20 years. Also, metal concentrations were below those that could be of concern for negative effects on fish and wildlife using this ecosystem.

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[2] Reprinted with permission from "Tracking Metal Pollution in Lake Chapala: Concentrations in Water, Sediments, and Fish" by Torres, Zaria, Miguel A. Mora, Robert J. Taylor, and Dioselina Alvarez-Bernal, 2016. Bulletin of Environmental Contamination and Toxicology, 97, 418-424, Copyright 2016 by Springer Science + Business Media.

Introduction

Out of Mexico's 310 hydrologic basins, the Lerma-Chapala basin is the most important and receives attention from the federal, state, and municipal government levels (Mestre 1997). This basin contains Lake Chapala, which has the Lerma River draining into it and the Santiago River flowing westward from it into the Pacific Ocean, serving as a natural drainage for the lake (Mestre 1997). Recently, however, the Santiago River's water outflow from Lake Chapala has been reduced (Hansen and van Afferden 2001). Lake Chapala, Mexico's largest freshwater lake, serves as more than a source of water to its surrounding cities (Mestre 1997, and Stong et al. 2013). It also provides a livelihood for the fishermen and serves a recreational source for retired residents (Shine et al. 1998). The fish commonly harvested from the lake are silverside (*Chirostoma spp.*), tilapia (*Oreochromis spp*), and common carp (*Cyprinus carpio*). These fish are frequently consumed by the local community (Lind et al. 2000). In the Lerma-Chapala basin, there is regional pollution from surface run-off of irrigation and discharge of untreated effluents (Mestre 1997), which can then lead to Lake Chapala. Natural sources of metals coming into rivers are rock weathering, soil erosion, and dissolution of water-soluble salts (Hansen and van Afferden 2001). Other forms of pollution that affect Lake Chapala include: metals, pesticides, industrial residues, and polycyclic aromatic hydrocarbons (Ford et al. 2000, Hansen and van Afferden 2001, Jay and Ford 2001). Fish metal levels were monitored because of concern for effects on fish-eating wildlife and the surrounding human population who use fish as a main source of protein. Aluminum (Al), barium (Ba), copper (Cu), manganese (Mn) strontium (Sr), vanadium

(V), and zinc (Zn) could be associated with a wide range of health effects on fish and wildlife (Eisler 1998, and Mora 2003). Elevated concentrations of Al have been shown to affect salmonids growth and swim speed (Price 2013). Copper is known to cause sensory and physical impairments to fish, such as damage to their olfactory system and reduction of growth (Price 2013), as well as cause hyperglycemia in carp (5 mg/L; Asztalos et al. 1990). Studies of Zn exposure in salmonids showed that fish avoided Zn polluted areas, which could prevent them from reaching their rearing habitat (Price 2013).

Stable isotopes carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) have been used in aquatic studies to determine feeding relationships between fish species (Beaudoin et al. 2001, Power et al. 2002, and Syvaranta et al. 2006). The $\delta^{13}C$ isotope can help distinguish between consumers who prefer pelagic or benthic algae since the each algae type have distinct carbon signatures from one another (Hecky and Hesslein 1995). In addition, $\delta^{15}N$ can also help elucidate the presence of biomagnifcation of contaminants, if any, within freshwater food webs (Atwell et al. 1998). Thus, both $\delta^{13}C$ and $\delta^{15}N$ can be used to determine an animal's trophic position and how it might relate to their metal concentration.

Even though there are concerns about the pollution with regards to human and ecosystem health, few studies have been conducted measuring metals in fish tissue from Lake Chapala (Ford and Ryan 1995, Shine et al. 1998, and Ford et al. 2000). The main objectives of the present study were to (1) determine concentrations of metals in water, sediments, and fish, and (2) evaluate potential adverse effects on fish and wildlife.

Methods

The sample locations and methods were the same as Chapter II; however, for this chapter I did not analyze feather samples. Water was collected in pre-cleaned 125 ml LDPE bottles. Sediment samples were taken at the surface of the lake (5–10 cm top layer) at 0.47, 0.59, and 0.71 m depths. Silverside, tilapia, and carp were collected with the assistance of local fishermen. Sediments and fish were placed in Ziploc plastic bags, stored on ice to be transported to the lab, and then stored in a -80^{o}C freezer. All samples were transported from Mexico to Texas A&M University, College Station, Texas and were kept at -80^{o}C until analysis at the Trace Elements Research Lab, Texas A&M University.

I analyzed all samples for the following metals: Al, Ba, Cu, Mn, Sr, V, and Zn. Water samples collected in 2011 and 2012 were acidified and then were analyzed with inductively coupled plasma mass spectrometry (ICP-MS) and inductively coupled plasma optical emission spectrometry (ICP-OES). For the ICP-OES method a Spectro CirOS instrument equipped with an axial torch was utilized. Ytterbium was used as an internal standard, and calibration was based on a blank and at least 3 standards. Off-peak background correction and correction for inter-element spectral overlap were used. Calibration was verified with an independent standard and it was monitored after every 10 samples as well as at the end of the run. For the ICP-MS analysis, I used a Perkin Elmer DRC 2 instrument, operated in "dynamic reaction cell" mode to correct for mass spectral overlaps. The same procedure for calibration and calibration verification and valid checks was utilized for the ICP-OES method.

Sediment samples were freeze dried, then ground with a mortar and pestle. After grinding, the samples were sifted and only the finely ground sediment was used for analysis. Ground sediment samples were weighed out to 0.5 g subsamples then dissolved in nitric and hydrochloric acid at 95^{o} C, and brought to a final volume of 50 ml with deionized water. Carp and tilapia were filleted on each side with their scales left on, and then fillets were freeze dried and homogenized using a Retsch mill grinder (Retsch ZM200). Due to their small size of ~12 mm, silversides were pooled as 8 whole individuals per sample set. They were additionally freeze dried and then homogenized. For analysis, the homogenized fish samples were weighed out to 0.2 g subsamples and dissolved in nitric acid, hydrogen peroxide, hydrochloric acid, and brought to a final volume of 20 ml with deionized water. The Quality Assurance and Quality Control (QA/QC) results for Al, Ba, Cu, Mn, Sr, V, and Zn are reported in Appendix B.

Carbon and nitrogen ($\delta^{13}C$ and $\delta^{15}N$) stable isotope values were obtained from fish fillets and composite of silverside samples (same as Chapter II methods) and were used to compare with the 7 metal concentrations.

Statistical analysis

Less than 50% of the metal values were below the limit of detection, thus, I used one-half the limit of detection value for the statistical analysis. A Shapiro-Wilks test was used to test for normality of water, sediment, and fish data. This was used because the data were not normally distributed, and a non-parametric Wilcoxon rank sums test was performed for both water and sediments to determine the differences in the metal concentrations between the 2 years of sample collection. Fish samples were analyzed

using Kruskal-Wallis test on the 3 fish species from Lake Chapala, followed by a Steel-Dwass post-hoc test for each pair, with alpha = 0.05. A linear regression analysis was conducted between each metal (Al, Ba, Cu, Mn, Sr, V, and Zn) and $\delta^{13}C$ and $\delta^{15}N$ values to determine significant relationships between them (JMP®, Pro 11, SAS Institute Inc., Cary, NC).

Results

Median and range metal concentrations in water (µg/L), sediments (mg/kg dry weight (dw)), and fish (µg/g wet weight (ww)) are provided in Table 4. For the most part, concentrations of metals in water and sediment were not significantly different between 2011 and 2012 for both Lake Chapala and San Antonio reservoir. However, concentrations of Al, Mn, V, and Zn in water from Chapala were higher ($P < 0.05$) in 2011 than in 2012; whereas concentrations of Cu and V in sediments were higher ($P < 0.05$) in 2012. Most concentrations of metals in water, sediments, and fish were below levels of concern for effects on biota, except for a few cases (water Al concentrations). There was a positive and significant correlation between fish length and metal concentrations (Fig. 9; $P < 0.05$) for Ba, Cu, Mn, and Zn (Lake Chapala) and V (San Antonio Reservoir).

Table 4. Median (range) metal concentrations in water (µg/L), sediment (mg/kg dw), and fish muscle (µg/g wet weight) collected from Lake Chapala and San Antonio Guaracha reservoir, Mexico, in 2011 and 2012.

Year	Location	Sample Type		Al	Ba	Cu	Mn	Sr	V	Zn
2011	Lake Chapala	Water		3,815	205.5	2.8	39	544	33.2	4.65
		(*n* = 6)		(3,630-3,860)	(202-207)	(2.70-2.80)	(36.80-49.10)	(541-552)	(32.50-34.90)	(4.50-4.80)
		Sediment		66,400	218.5	18.95	698.5	86.05	54.65	77.9
		(*n* = 6)		(23,100-70,500)	(138-270)	(10.80-22.60)	(335-936)	(74.80-99.90)	(35.20-56.50)	(35.80-92.90)
		Fish	Silverside[a]	0.05	2.63	0.14	0.94	17.89	0.26	18.16
			(*n* = 8)	(0.05-0.06)	(2.30-2.88)	(0.12-0.23)	(0.79-1.74)	(15.84-18.92)	(0.24-0.30)	(16.30-19.52)
			Tilapia	0.41	0.9	0.16	0.5	29.75	0.16	8.04
			(*n* = 10)	(0.05-1.59)	(0.72-1.64)	(0.05-0.20)	(0.27-0.76)	(23.54-64.70)	(0.14-0.27)	(6.75-11.07)
			Carp	0.42	2.61	0.3	0.95	22.44	0.15	24.56
			(*n* = 8)	(0.14-0.73)	(2.05-3.44)	(0.23-0.42)	(0.74-1.20)	(11.90-26.18)	(0.11-0.17)	(15.20-29.27)
	San Antonio		Carp	0.05	4.18	0.22	1.86	22.44	0.18	16
			(*n* = 5)	(0.04-3.39)	(3.70-8.64)	(0.16-0.45)	(1.21-3.00)	(15.92-31.68)	(0.15-0.25)	(11.45-22.22)
2012	Lake Chapala	Water		124	213	2.8	8.63	596	32	2.6
		(*n* = 9)		(2.50-474)	(132-222)	(2.70-2.80)	(3.05-28.50)	(351-608)	(24-34)	(1.20-5.90)
		Sediment		61,800	222	22.1	768	80	57.9	81.4
		(*n* = 9)		(47,800-68,200)	(190-262)	(21-24.20)	(569-906)	(76.70-86.40)	(53.20-63.20)	(59.80-88.40)

Table 4. Continued

	Fish	Silverside[a]	9.26	3.44	0.47	2.15	18.84	0.2	21.83
		(n = 8)	(6.50-30.60)	(3.03-3.88)	(0.38-0.49)	(1.67-2.52)	(17.61-21.54)	(0.08-0.34)	(21.00-27.62)
		Tilapia	6.24	1.26	0.38	0.7	24.16	0.37	9.46
		(n = 6)	(3.92-26.80)	(0.95-2.97)	(0.26-0.46)	(0.61-1.83)	(18.60-37.25)	(0.24-0.41)	(7.53-12.18)
		Carp	13.59	3.28	0.55	1.29	23.47	0.2	30.43
		(n = 6)	(4.83-18.27)	(2.45-4.56)	(0.39-0.65)	(0.86-1.90)	(20.31-31.01)	(0.14-0.28)	(22.41-35.03)
San Antonio		Carp	2.45	3.77	0.57	2.01	20.52	0.18	22.71
		(n = 3)	(1.39-2.55)	(3.67-4.33)	(0.55-0.87)	(1.63-2.02)	(15.84-23.79)	(0.18-0.27)	(17.42-25.27)
		Tilapia	2.72	2.42	0.25	3.38	16.89	0.16	11.04
		(n = 3)	(2.64-3.05)	(1.51-3.07)	(0.25-0.28)	(3.26-3.52)	(15.38-22.45)	(0.13-0.19)	(10.11-11.50)

[a]Composite sample of 8 individuals each.

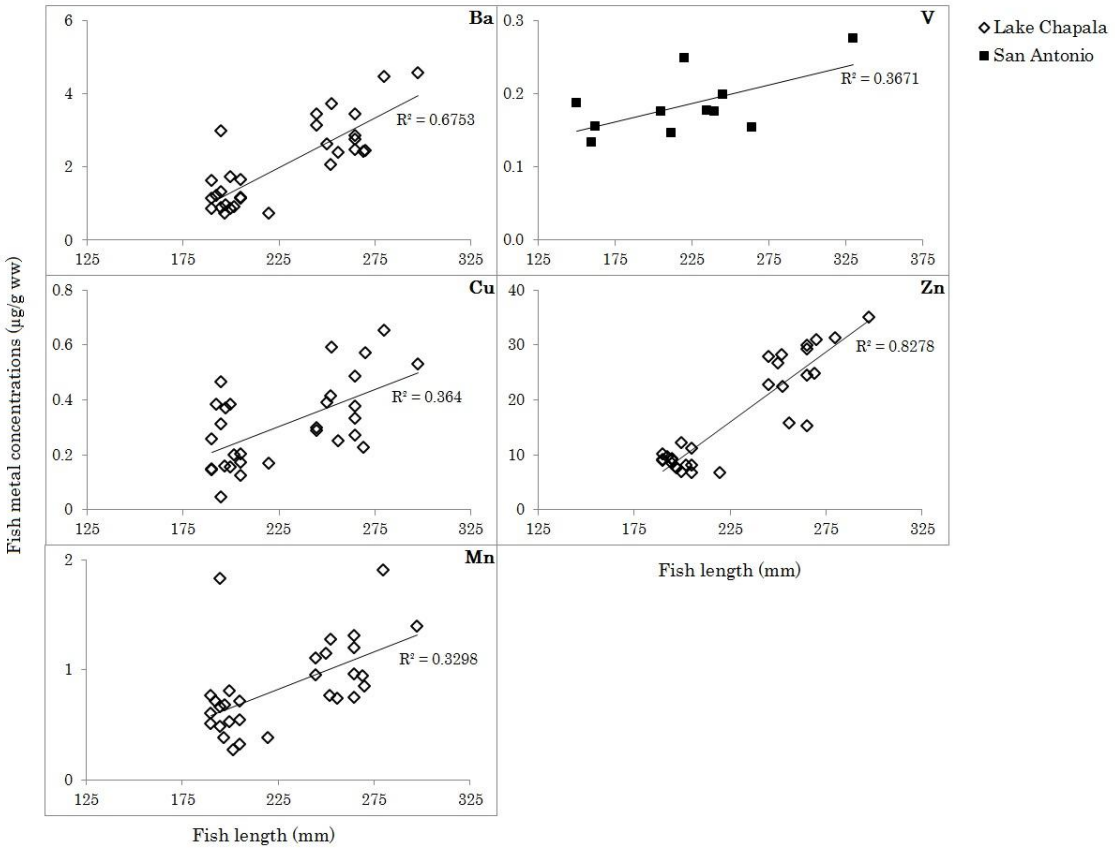


Figure 9: Relationship between fish length (mm) and Ba, Cu, Mn, V, and Zn concentrations (silverside fish not included).

Discussion

In 2011, Al concentrations in water ($\bar{x}$ = 3,796.67 µg/L) from Lake Chapala exceeded the United States Environmental Protection Agency's (USEPA) freshwater Al criteria maximum concentration (CMC; 750 µg/L) and criterion continuous concentration (CCC: 87 µg/L; USEPA 1988). High concentrations of Al in water could be associated with the lake's high alkalinity (Gundersen et al. 1994); since the pH varied between 9.5–9.6 during the 2 years of study. High concentrations of Al could become

toxic to aquatic biota; however, the concentrations measured in fish are well below those at which some sublethal effects have been reported (Sparling and Lowe 1996).

Copper concentrations in water were similar to those reported previously (Ford and Ryan 1995, Shine et al. 1998) suggesting that inputs and outputs of Cu to the lake have not changed much over the last 20 years. However, Cu concentrations from the Alzate reservoir upstream of the Lerma River were much higher (Avila-Perez et al. 1999). Copper concentrations in sediments were nearly 2 times lower in my study than those reported earlier for the lake (Rosales-Hoz et al. 2000, Trujillo-Cardenas et al. 2010), and for the Alzate reservoir (Avila-Perez et al. 1999). Also, Cu concentrations in the 3 fish species analyzed in the present study were similar or lower to those reported in the same species in the late 1990s (Ford and Ryan 1995, Shine et al. 1998, Ford et al. 2000). Copper concentrations in carp (1.65 µg/g dw) were about 2–4 times lower than those reported by Ford and Ryan (1995), and Shine et al. (1998). The results in fish suggest that Cu inputs to the lake may have decreased over the last 2 decades, in contrast with the results observed in water which suggested no change over time. Copper concentrations in fish also were below levels at which sublethal or chronic effects could be observed in fish or wildlife (Eisler 1998).

Zinc concentrations in water decreased nearly 20% over a 20 year period (Ford and Ryan 1995, Trujillo-Cardenas et al. 2010). Zinc concentrations in water also were lower than those observed in water from the Alzate reservoir (Avila-Perez et al. 1999). Similarly, Zn concentrations in sediments were 2-3 times lower than those reported in the 1990s and early 2000s (Rosalez-Hoz et al. 2000, Trujillo-Cardenas et al. 2010). It

appears the Alzate reservoir, upstream of the Lerma River is or was much more contaminated with metals than Lake Chapala. Zinc concentrations in fish from the present study also were somewhat lower than those reported in previous studies (Ford and Ryan 1995, Shine et al. 1998, Ford et al. 2000) and at levels that are not of concern for effects on fish or wildlife (Eisler 1993).

The significant correlation between fish length and metal concentrations suggests these metals (Ba, Cu, Mn, V, and Zn) are subject to bioaccumulation with age (Quinn et al. 2003, Pereira et al. 2010). However, there were not significant correlations between metal concentrations and $\delta^{15}N$ values. The lack of correlations suggests most metals do not biomagnify through the food chain. The $\delta^{15}N$ values are often used as indicators of trophic structure; thus, greater values generally indicate a higher position in the food web (Kelly 2000).

Barium, Mn, and Zn concentrations were greater in carp and tilapia from San Antonio (reference site) than in those from Lake Chapala (Table 4). San Antonio reservoir is a much smaller lake than Chapala and carp and tilapia from the reservoir appear to be feeding at a lower trophic level ($\delta^{15}N$ = 10.27‰ and 12.57‰, for tilapia and carp respectively) than in Lake Chapala ($\delta^{15}N$ = 15.35‰ and 18.03‰; Chapter II). However, various studies also have shown $\delta^{15}N$ values can increase in aquatic systems that have anthropogenic input of sewage (Cabana and Rasmussen 1996, Wayland and Hobson 2001). Thus, even though the tilapia and carp from Lake Chapala have higher $\delta^{15}N$ values than those from San Antonio, it may not necessarily indicate that they are at a higher trophic level, but may be exposed to enriched $\delta^{15}N$ sources in their

environment. It has been pointed out that $\delta^{15}N$ baseline values could vary between different systems and $\delta^{15}N$ values should only be used to compare to biota within the same food web (Cabana and Rasmussen 1996, Atwell et al. 1998).

Overall, metal concentrations in water, sediments, and fish from Lake Chapala were similar or lower than those reported in previous studies suggesting metal pollution in Lake Chapala has stayed consistent and in some cases (Cu and Zn) decreased over the last 20 years. Most of the metals analyzed do not exceed the USEPA's recommended levels for aquatic life, except for Al. However, even though Al is highly concentrated in the water column it does not seem to be bioaccumulating or biomagnifying up the food chain. Fish-eating wildlife in Lake Chapala may not be at risk from most metal exposure; however, mercury could be of concern for some fish-eating birds, such as the American white pelican (*Pelecanus erythrorynchos*) wintering in Lake Chapala (Chapter II).

CHAPTER IV

PREDICTING DIET SOURCES OF ATTWATER'S PRAIRIE-CHICKEN IN TEXAS

Summary

The Attwater's prairie-chicken (APC; *Tympanuchus cupido attwateri*) once ranged throughout the gulf coastal prairies of Texas and Louisiana with numbers approaching 1,000,000 individuals. It has been listed as an endangered species since 1967 and ranged from the gulf coastal prairies of Texas (Nueces River) to Louisiana (Abbeyville). Since its listing as an endangered species, multiple research studies have been conducted to recover the wild population. In this study I used stable isotope techniques to determine current diets of APCs at the Attwater's Prairie Chicken National Wildlife Refuge (APCNWR) and to correlate with diets from individuals formerly occurring at these ranges, and based on feathers from museum specimens. I collected APC feathers, blood and feces at the APCNWR, and APC feathers from selected museums (1894–1965) and analyzed them for stable carbon and nitrogen isotopes ($\delta^{13}C$ and $\delta^{15}N$). Vegetation and insect samples also were analyzed for $\delta^{13}C$ and $\delta^{15}N$. The stable isotope results were used in a mixing model (MixSIAR), to help determine the preferred diet of the APCs. MixSIAR produced different results depending on how many diet sources were used (e.g., 4 diet sources indicated that C3 vegetation was contributed the most to APC diet). The $\delta^{15}N$ values in feathers of historical samples were greater than those in feathers taken from individuals currently in the wild, for both males and females. However, $\delta^{13}C$ values in feathers were not significantly different

between historic and current specimens; although, $\delta^{13}C$ values from current specimens had a much broader range. The observation of lower $\delta^{15}N$ values and a broader range of $\delta^{13}C$ values in feathers of current APCs compared to historic values, suggest the APCs are currently utilizing different sources of food or that grasses and forbs have changed from what was available in the past.

Introduction

The Attwater Prairie Chicken National Wildlife Refuge (APCNWR) was established in 1972 to protect the endangered Attwater's prairie-chicken (APC; *Tympanuchus cupido attwateri*) population in Texas. This species was listed as endangered in 1967 when their population was approximately 1,070 birds (USFWS 2010). This was a dramatic decrease from when their peak numbers ranged from 300,000 to 1 million birds (Lehmann 1941, Lehmann 1965). The decline in APC numbers dates to the early 1900s and corresponds mainly with the loss of their habitat, native coastal prairie. This decline of coastal prairie habitat was brought about by agricultural conversion, urban and industrial expansion, overgrazing, and woody plant encroachment (Lehmann 1941). As their habitat declined, APC numbers followed suit and, from 1937 to 1992, APCs were reduced from 8,700 to 432 birds (Lehmann and Mauermann 1963, Silvy et al. 1999, Lockwood et al. 2005). Due to such low numbers in 1992, and in efforts to prevent extinction, the Attwater's Prairie-Chicken Recovery Team began a captive-breeding program to supplement the wild APC population with pen-reared birds (Lockwood et al. 2005). Seal (1994) reported that wild APC

populations would go extinct by 2000 without the addition of supplementation of captive-reared birds. Currently, the wild APC population ranges from 50–110 and is supplemented by captive-reared APCs (Hammerly et al. 2013).

The prolonged small APC population size contributed to a loss of genetic variability and, thus, has been an important factor in determining which APCs get released into the wild (Burns-Cusato and Morrow 2003). The APCs selected for release into the wild are those that will increase genetic diversity even though these birds may not have the innate fear responses that will aid in predator avoidance (Burns-Cusato and Morrow 2003). Early issues APCs faced in captivity were the contraction and spread of reticuloendotheliosis (RE) and reticuloendotheliosis virus (REV; Drew et al. 1998). Environmental stochasticity, such as excessive rainfall, also has affected survival of post-released captive APCs into the wild (Morrow et al. 1996, Silvy et al. 1999, Morrow et al. 2004). The most recent obstacle the APC is facing in the wild is the introduction and expansion of the red imported fire ant (RIFA; *Solenopsis invicta*) onto the APCNWR (Allen et al. 1994, Morrow et al. 2013). These ants reduce the abundance of invertebrates that would otherwise be available to adult and chick APCs (Morrow et al. 2015). Undoubtedly, APC population decline is not due to 1 factor alone, but rather consists of several factors affecting recovery (Morrow et al. 1996).

Stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) are useful tools in ecological studies since 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 that use different

photosynthetic pathways (i.e., C_3 and C_4 plants) have distinguishable $\delta^{13}C$ values (Brugnoli and Farquhar 2000). C_3 and C_4 plants have $\delta^{13}C$ means of -27‰ and -13‰, respectively (Boutton 1991). Nitrogen stable isotopes also have distinguishable values between different plant types (nitrogen-fixing versus non-nitrogen-fixing; Kelly 2000), but in most cases they are used to determine the trophic level of an organism (DeNiro and Epstein 1981, Post et al. 2000). This is due to an organism being more enriched in $\delta^{15}N$ compared to their diet by 2 to 4‰ between each trophic level (Post 2002).

Mixing models have been used to measure the proportion of sources that contribute to an animal's diet (Phillips 2001, Ward et al. 2011, Phillips 2012). These mixing models have grown from the simple linear mixing models utilized by Fry and Sherr (1984) to a multiple-source mixing model (Ben-David et al. 1997) and, more recently, to Bayesian mixing models like MixSIAR (Stock and Semmens 2013). Bayesian mixing models have improved upon the original simple linear mixing models by including source uncertainty, concentration dependence, multiple sources, and prior information (Hopkins and Ferguson 2012, Phillips et al. 2014).

Diet studies for APCs have been conducted in the past, but not since the start of the captive breeding program and the release of APCs from captivity into the wild (Lehmann 1941, Cogar 1980). In the present study, my objectives were to (1) determine the potential diets of APCs once they are released into the APCNWR using $\delta^{13}C$ and $\delta^{15}N$ stable isotopes, and (2) compare potential differences and similarities in diets between historic APC specimens and contemporary individuals using stable isotopes.

Methods

Study Area

This study was conducted on the APCNWR, located between Sealy and Eagle Lake, Texas (29^{o} 40'N, -96^{o} 16'W), an area consisting of gulf coastal prairie habitat (3,248 ha) with surrounding agriculture fields, primarily rice (Kessler 1978, Lockwood et al. 2005; Fig. 10). The refuge contains the largest remnants of coastal prairie left in Texas.

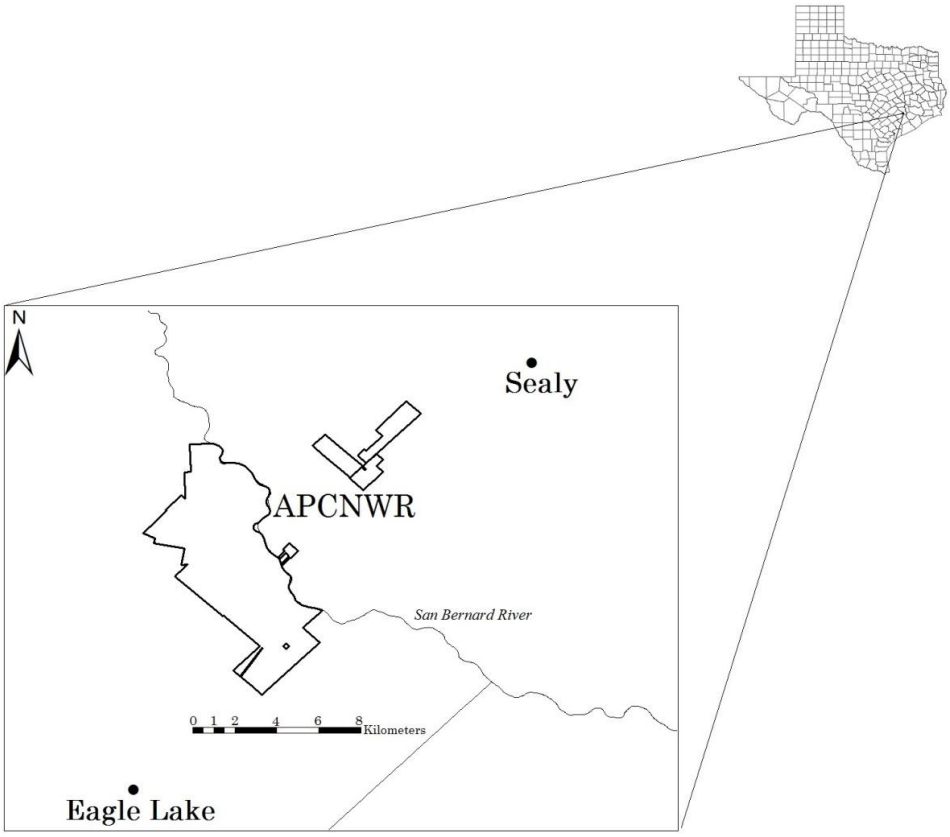


Figure 10: Location of the APCNWR near Eagle Lake and Sealy, Texas.

During 2012 and 2013 I collected possible diet sources of the APCs including vegetation (forbs: n = 112, grass: n = 13, rush: n = 1), and arthropod (insect: n = 219, and spiders (Araneae): n = 24) samples (Appendix C and D). Attwater's blood (n = 86) and fecal (n = 35) samples were collected during 2012 and 2013 as well (Appendix E). Flank feather samples were obtained from wild APCs (2004–2013; hereafter referred to as contemporary feathers), and from museum specimens (APCs), obtained from the museums listed in the acknowledgement section (1894–1965; hereafter referred to as historic feathers). The blood, feather, and fecal samples from APCs were collected by biologists from the APCNWR. The feather samples were kept in paper bags while blood samples (0.5–3.0 cc) were collected using 25 gauge needles with a non-heparinized 3 cc syringe and placed in 2 ml tubes (Nalgene® cryogenic vials) and stored in a -80^{o} C freezer, and the fecal samples were collected fresh (M.E. Morrow, personal communication).

Vegetation samples were collected in their entirety, excluding roots, and were stored in labeled paper bags. These samples were oven dried at 40^{o} C for 24 hours and then ground using a Retsch Oscillating Mixer Mill (MM400) prior to stable isotope analysis. Arthropod samples were collected using sweep nets and placed into labeled paper bags which were then placed in Ziploc bags. Once taken to the lab, arthropods were frozen for 2 weeks and then were oven dried at 40^{o} C for 24 hours to remove any extra moisture and to prepare the insects to be ground for stable isotope analysis. Captive diet sources, meal worms (n = 4), and Mazuri feed were oven dried at 25^{o} C for 1 hour and ground using a mortar and pestle. Blood samples were freeze dried and

ground using a mortar and pestle. Fifteen APC fecal samples were prepared for stable isotope analysis. These fecal samples were oven dried at 60^{o} C (24 hours), then ground using the Retsch Oscillating Mixer Mill prior to stable isotope analysis. Thirteen additional APC fecal samples were submitted to Pacific Analytics in Scio, Oregon, for arthropod fragment identification.

Stable Isotope Analyses

Samples were weighed and analyzed at the Stable Isotopes for Biosphere Sciences Laboratory, Texas A&M University. Arthropod, blood, and fecal samples were weighed to 1 mg, vegetation to ~2.2 mg, and then placed into individual tin capsules (4 x 6 mm, Costech). Both historic and contemporary APC feathers were cleaned using a 2:1 chloroform to methanol solution to remove any surface oils. Then the right side of the flank feather's barbs (not the vane) was cut finely to be prepared for stable isotope analysis. A portion (0.5 mg) of each feather sample was placed in tin capsules. $\delta^{13}C$ and $\delta^{15}N$ isotope ratios were measured for all samples using an Elemental Analyzer (EA Costech) coupled to a Delta V Advance stable isotope ratio mass spectrometer (Thermo Scientific) via a Conflo IV interface (Thermo Scientific). These isotope ratios were reported as per mil (‰), relative to Vienna Pee Dee Belemnite (VPDB) for $\delta^{13}C$, and atmospheric nitrogen (AIR) for $\delta^{15}N$, respectively.

Statistical Analyses

I used the Shapiro-Wilk test to determine if the data were normally distributed. If the data were normal I used an analysis of variance (ANOVA) to test for differences of $\delta^{13}C$ and $\delta^{15}N$ between vegetation and arthropod samples, respectively, then a Tukey-

Kramer HSD test (post-hoc) to determine significant differences. When the data was determined to be non-normal, I used the Kruskal-Wallis and Steel-Dwass tests for comparisons. For both $\delta^{13}C$ and $\delta^{15}N$ values an ANOVA and Student's *t*-test was conducted to determine if there were any differences between historic and contemporary APC feather samples. I also looked at potential seasonal changes in vegetation and arthropod $\delta^{13}C$ and $\delta^{15}N$ values on the refuge using the above tests. All statistical tests were conducted in JMP (JMP®, Pro 11, SAS Institute Inc., Cary, NC). Statistical differences were set at an alpha value of 0.05.

MixSIAR Model Analyses

To estimate which sources contribute to the APC diet, I used the stable isotope mixing model, MixSIAR (Stock and Semmens 2013), which is part of the statistical software R (R core team 2015). This model has improved upon the original linear mixing model by accounting for uncertainty in sources measured (Moore and Semmens 2008), categorical or continuous covariates (Semmens et al. 2009, Parnell et al. 2013), as well as prior information (Moore and Semmens 2008). MixSIAR accounts for variability of isotopic values in consumer, sources, and tissue-diet discrimination factors (Phillips et al. 2014). It also has summary statistics that report the probability distributions (2.5 to 97.5%) for individual diet sources in relation to the stable isotope values of the consumer (APCs).

For the different model scenarios, I chose the run length to be "normal" for all models to make sure all the Markov Chain Monte Carlo (MCMC) chains converged. If any did not properly converged I then used "long" and "very long". Convergence was

checked by running Gelman-Rubin and Geweke diagnostic reports (Gelman et al. 2014, Geweke 1991). Stable isotope transfer from a diet source to the consumer cannot be accounted for directly, thus the need to use diet-tissue discrimination factors (TDF) for both $\delta^{13}C$ and $\delta^{15}N$ stable isotopes in this model (Hobson and Clark 1992, Tieszen et al. 1983). If TDF values are not used, then the assessment of the consumer's diet will be misinterpreted (Therrien et al. 2011). To calculate specific TDF values ($\Delta_{diet\text{-}tissue}$) for APCs, I used blood, feathers, and diet samples from APCs in captivity (Houston Zoo) and analyzed them for $\delta^{13}C$ and $\delta^{15}N$. The resulting discrimination factors were: Blood $\Delta_{diet\text{-}tissue}$ $\delta^{13}C$: $+0.2 \pm 0.2$, $\delta^{15}N$: $+3.9 \pm 0.27$; Feather $\Delta_{diet\text{-}tissue}$ $\delta^{13}C$: $+1.14 \pm 0.28$, $\delta^{15}N$: $+3.46 \pm 0.53$.

I also used other TDF values from the literature to compare specifically with those calculated in this study (Healy et al. 2017; Caut et al. 2009; Hobson and Clark 1992). All the results using the 3 different TDF values gathered from these articles are presented in Appendix F. As mentioned by Ben-David and Schell (2001), the TDF value is the most important parameter in a mixing model, since these values can sometimes significantly alter the model output. In this study, if I had used a proxy TDF value instead of the determining APC's specific TDF values, I would have received very different outputs for contributing diet sources. The proxy TDF values derived from Caut et al. (2009), produced similar results as to when the model was run with the APC actual TDF values.

For MixSIAR analysis, APC blood samples were analyzed separately by season of collection (summer, fall, and winter) and then grouped together (hereafter referred to

as combined blood). Feathers were analyzed separately as historic and contemporary. Potential vegetation sources were classified as either C_3 ($\delta^{13}C$ values within the -22‰ to -30‰ range) or C_4 ($\delta^{13}C$ values in the -10‰ to -14‰ range). Arthropods were separated by insects and spiders. This was done since spiders had significantly higher $\delta^{15}N$ values compared to other insects when grouped together.

The MixSIAR model was run using 3 potential diet source combinations. The first model used $\delta^{13}C$ and $\delta^{15}N$ values from 3 potential sources: C_3 vegetation, spiders, and insects. After I conducted this model, there were some blood and feather samples that fell outside the mixing polygon (see model verification); this suggested there were potential missing diet sources. During certain periods (July to mid-October; Dr. Morrow personal communication) corn (C_4 plant), along with sweet and black-eyed peas are provided as extra food to newly released APCs. Accordingly, I performed another model that had the same diet sources from the first model, plus the addition of C_4 vegetation. The third model used the same sources as the second model, plus rice, as an extra source, using $\delta^{13}C$ and $\delta^{15}N$ values reported by others (Alisauskas and Hobson 1993). I included rice as a diet source in the last model since rice fields are near the refuge, and APCs have been known to forage there (M. E. Morrow personal communication). For all MixSIAR model results, the median percentage values will be reported in the results section.

Verifying Model Fit Using Mixing Polygons

Prior to performing any MixSIAR analyses, I performed a model evaluation using the Monte Carlo simulation developed by (Smith et al. 2013). By using the means (± standard deviation) of each diet source (C_3 and C_4 vegetation, spiders, insects, and rice), along with the consumer's (APC) TDF values, this simulation provides information if mass balance is established. Since the TDF values used in this simulation are from APCs and are not proxy values (e.g. quail TDF values from Hobson and Clark 1992), I checked if the diet sources selected are validated to be used in a mixing model. Validation of the diet sources is when all of the consumer samples fall within the 95% mixing region, meaning I can then use this data in a mixing model. If some of the consumers fall outside this mixing region, I can either exclude those consumers, or reject to use a mixing model with the data.

The model using only 3 potential sources was run using blood values separated by seasons (summer and fall) and with all seasons combined. The mixing polygon simulation indicated that most data were adequate to use for the MixSIAR analysis, except for 3 samples from the combined blood analysis (Fig. 11). Both summer and fall blood samples fell inside the 95% mixing polygon and all were included in the analysis (Fig. 12). Blood samples collected in the winter did not fall within the 95% mixing polygon, thus, they were not used in the analysis. For feather data, the mixing polygon simulation suggested that values from 3 historic and 14 contemporary feathers were not adequate to include in the model and they were excluded (Fig. 13).

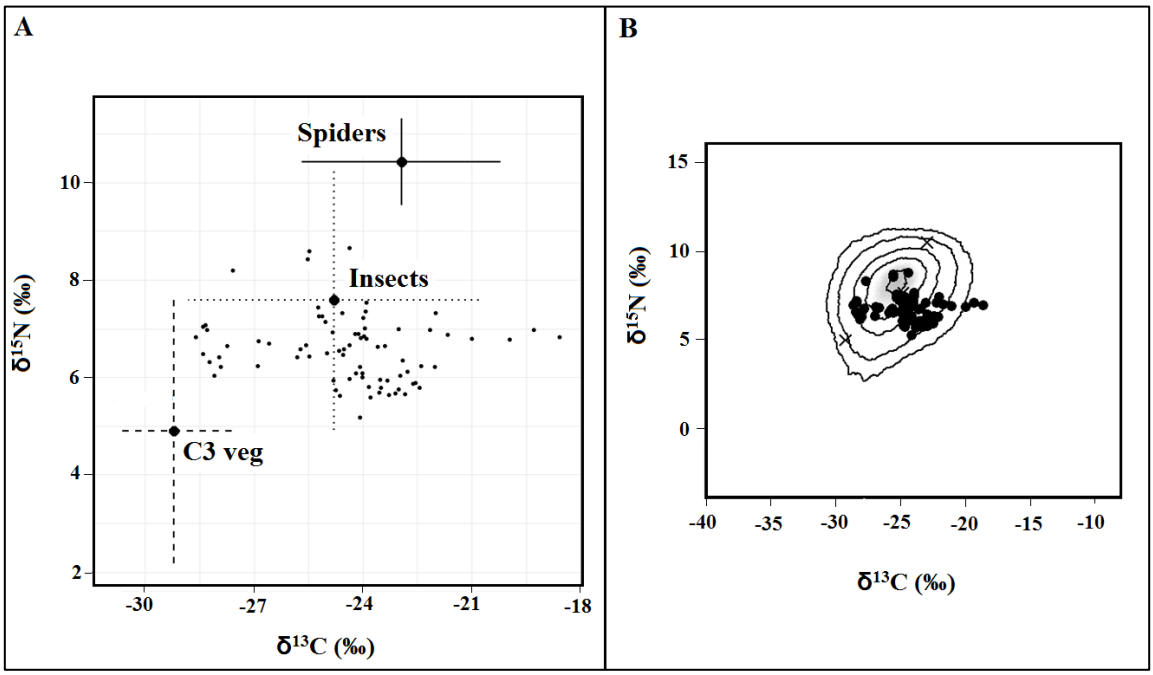


Figure 11: (A) Isospace plot of the $\delta^{13}C$ and $\delta^{15}N$ values for combined blood and 3 sources, and (B) the simulated mixing region for the isospace plot in (A).

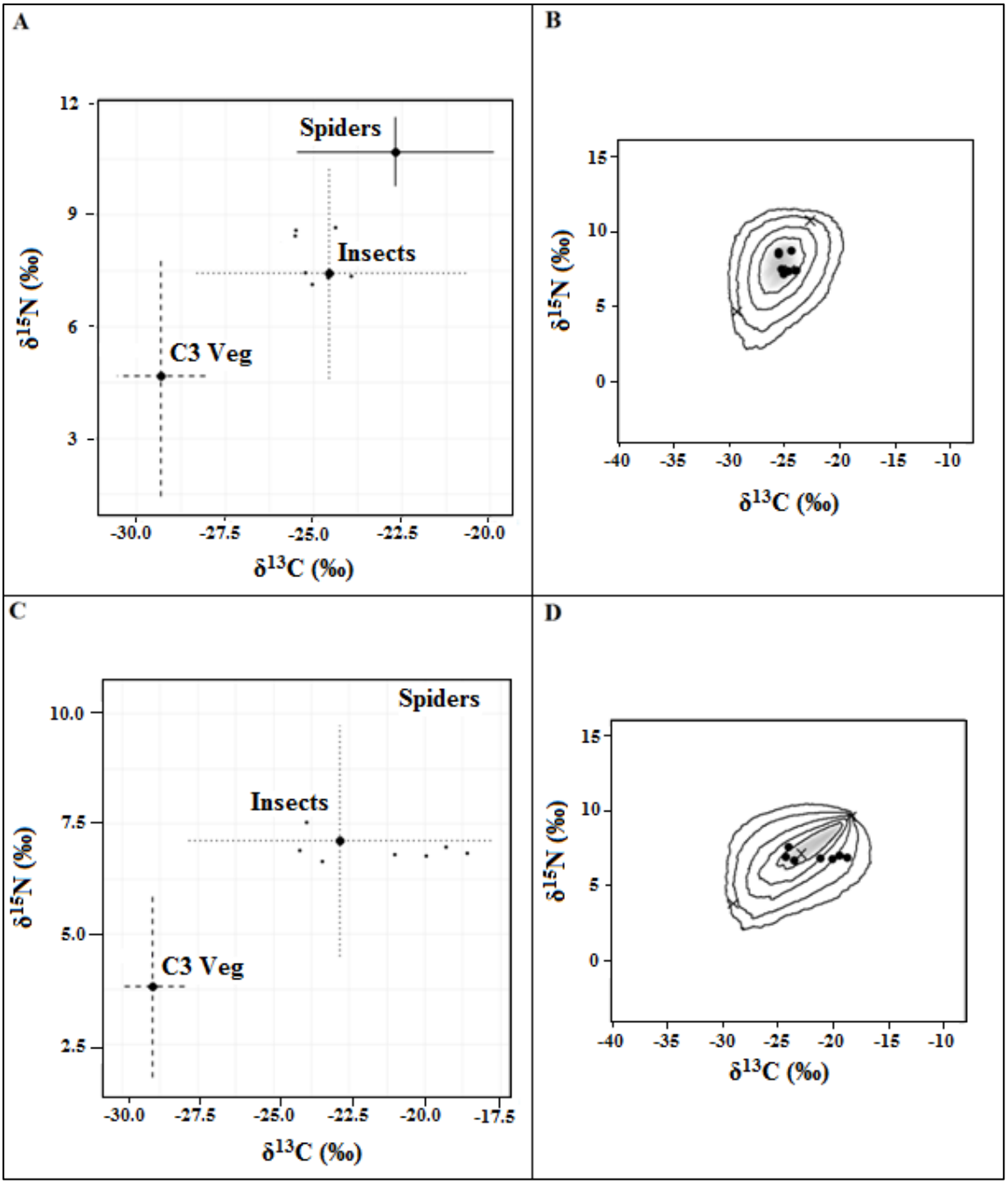


Figure 12: Isospace plot of the $\delta^{13}C$ and $\delta^{15}N$ values for summer (A) and fall (C) blood and 3 sources, and the simulated mixing region for their respective isospace plots (summer [B], and fall [D]).

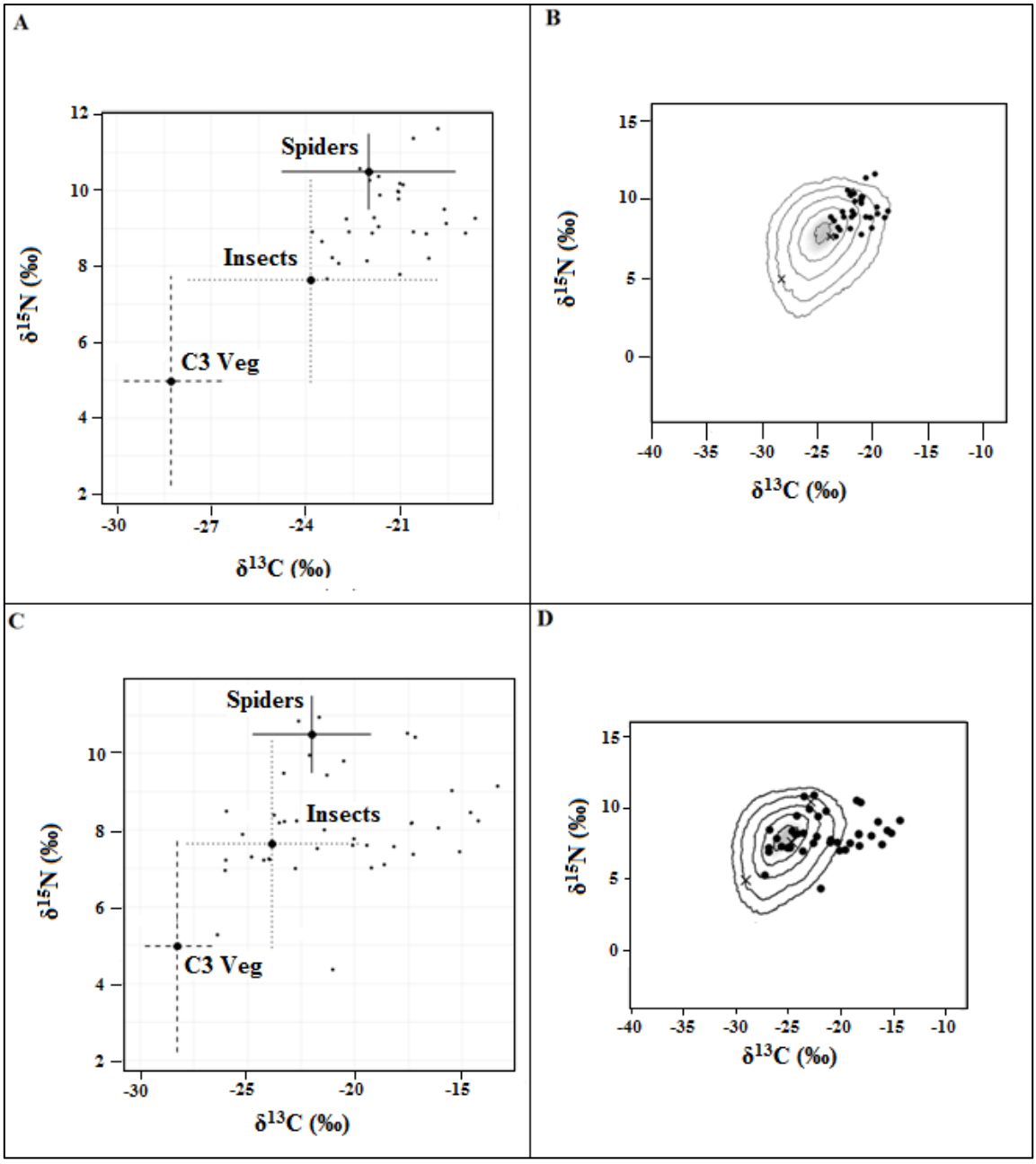


Figure 13: Isospace plot of the $\delta^{13}C$ and $\delta^{15}N$ values for historic (A) and contemporary (C) feathers and 3 sources, and the simulated mixing region for their respective isospace plots (historic [B], and contemporary [D]).

When 1 extra source was added, the data from combined blood samples, as well as those collected in the summer and fall fell within the 95% mixing polygon and none were excluded from the model (Fig. 14). Blood and diet sources collected during winter did not fall within the 95% mixing polygon, and were not analyzed with the model. In the case of feathers, only 1 historic and 2 contemporary feathers were excluded from the model (Fig. 15). For the model with 5 sources the same sets of blood samples also were within the 95% mixing polygon (Fig. 16), and only 2 contemporary feathers had to be excluded (Fig. 17).

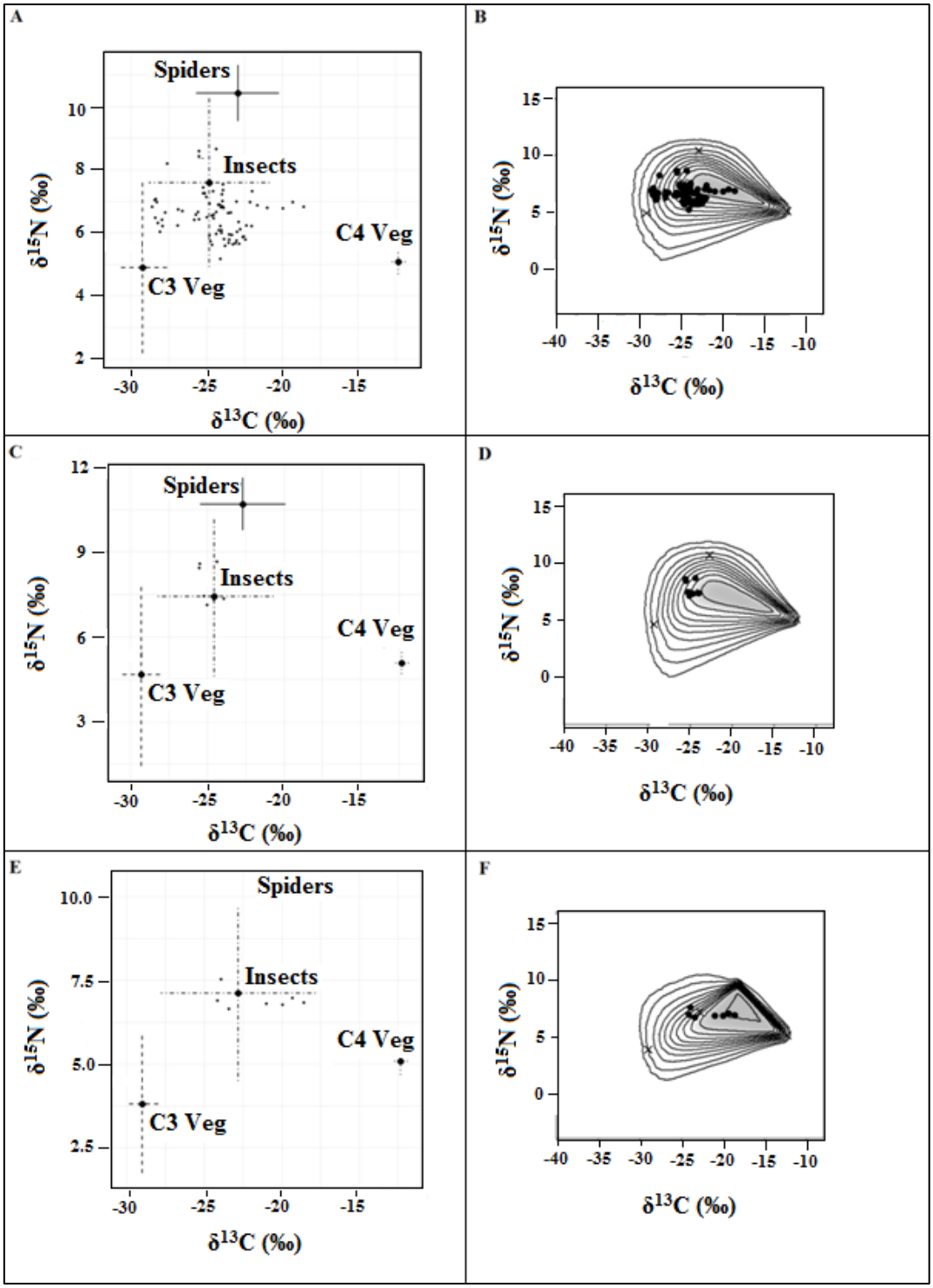


Figure 14: Isospace plot of the $\delta^{13}C$ and $\delta^{15}N$ values for combined (A), summer (C), and fall (E) blood and 4 sources, and the simulated mixing region for their respective isospace plots (combined [B], summer [D], and fall [F]).

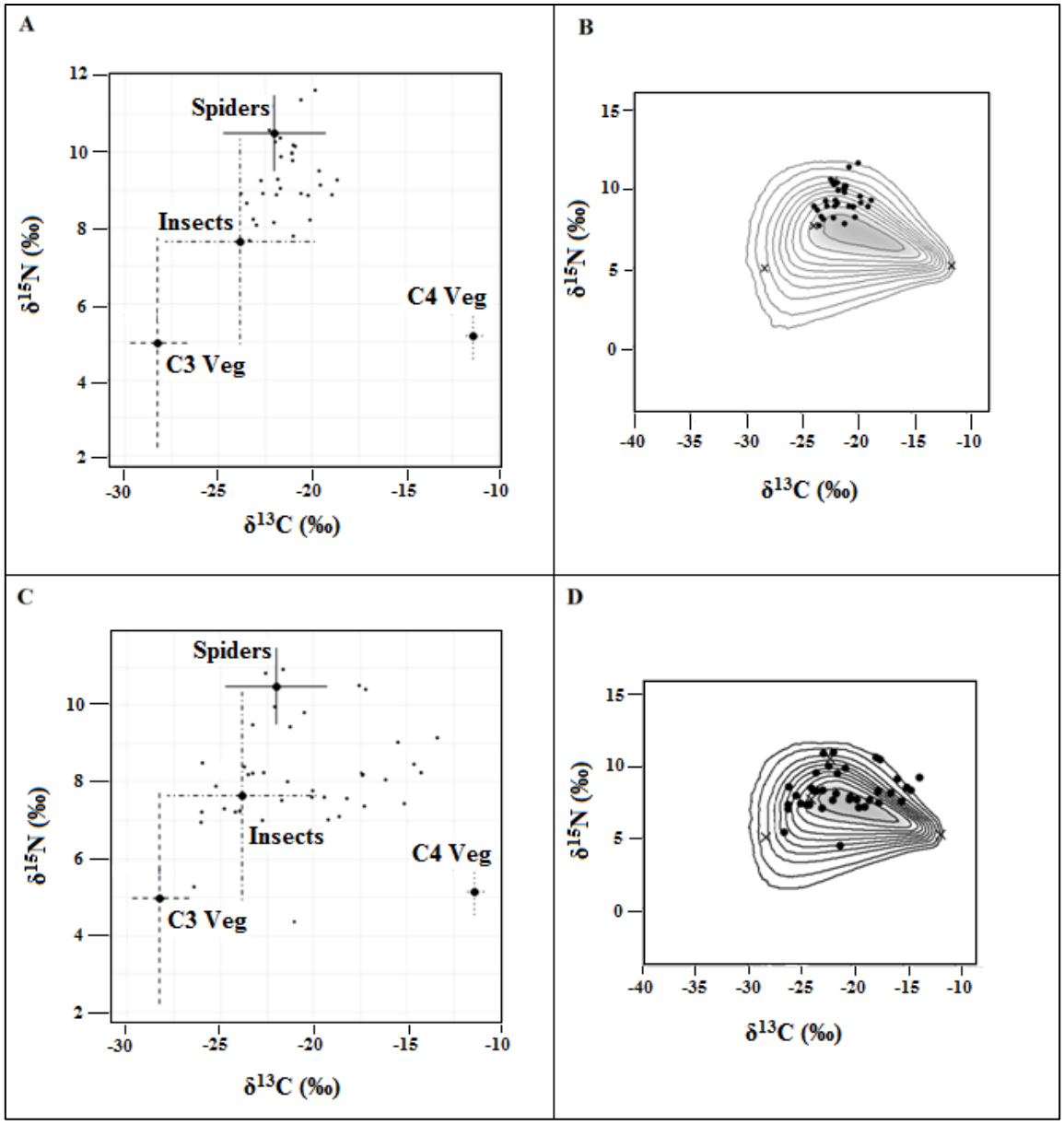


Figure 15: Isospace plot of the $\delta^{13}C$ and $\delta^{15}N$ values for historic (A) and contemporary (C) feathers and 4 sources, and the simulated mixing region for their respective isospace plots (historic [B], and contemporary [D]).

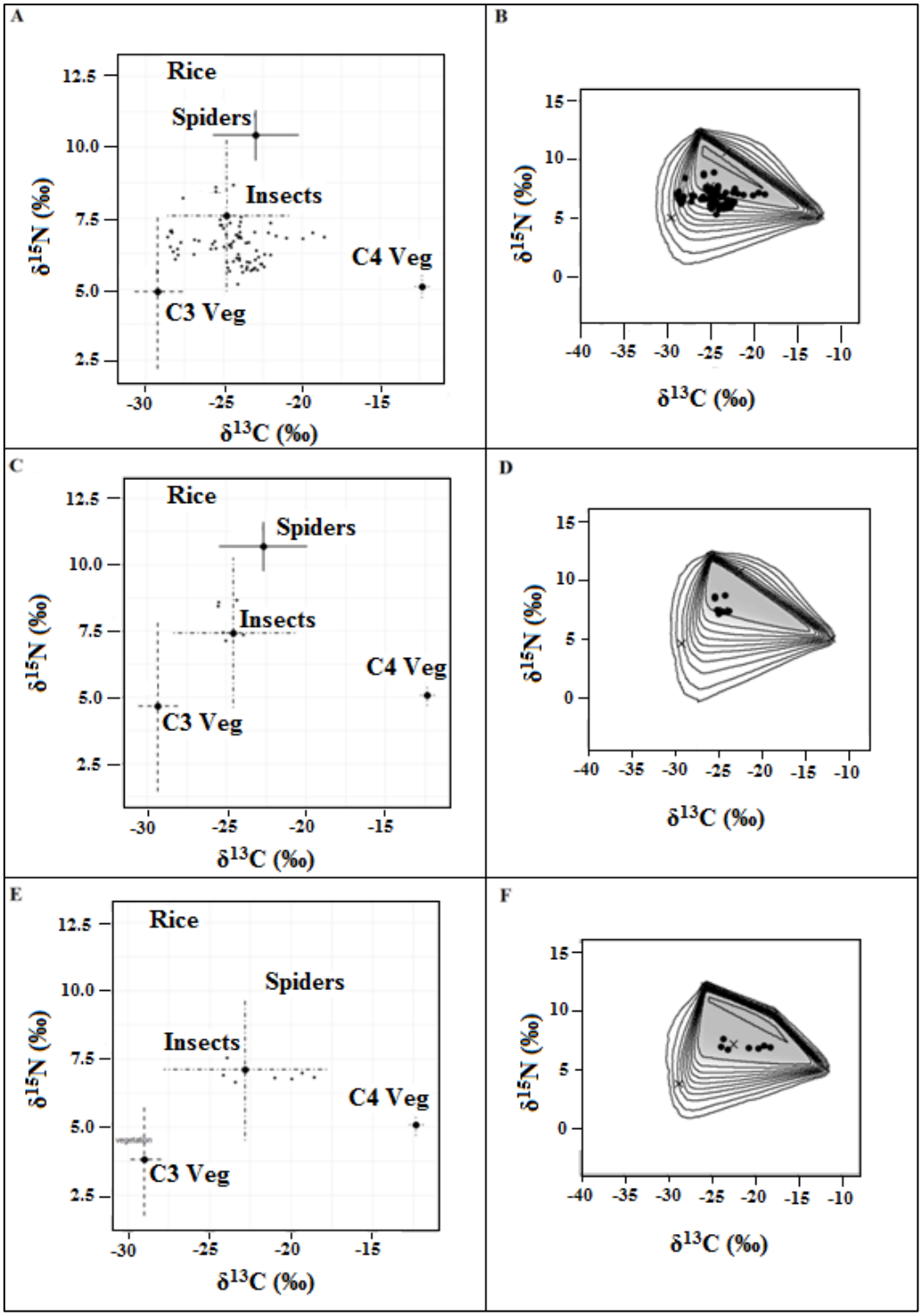


Figure 16: Isospace plot of the $\delta^{13}C$ and $\delta^{15}N$ values for combined (A), summer (C), and fall (E) blood and 5 sources, and the simulated mixing region for their respective isospace plots (combined [B], summer [D], and fall [F]).

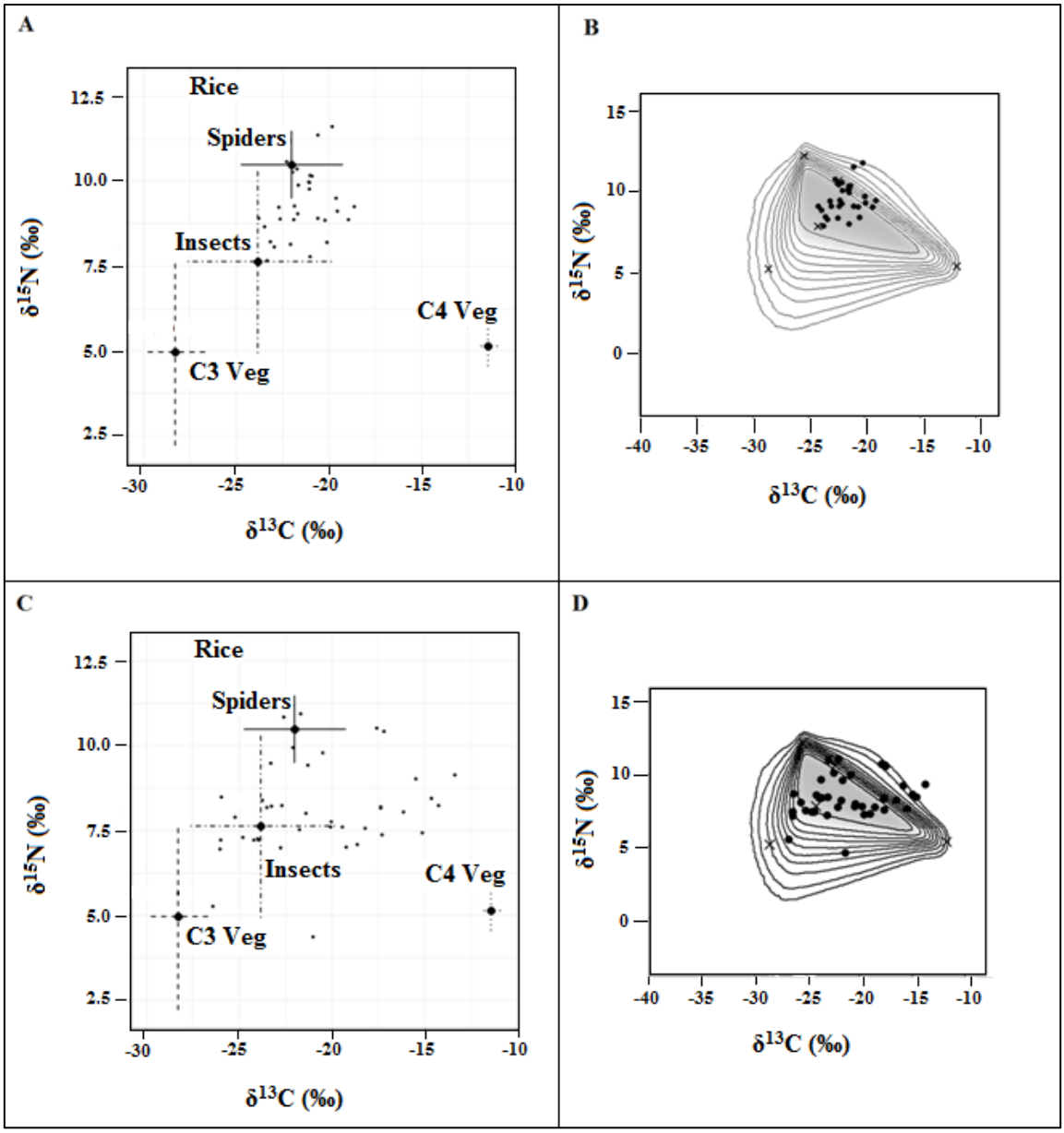


Figure 17: Isospace plot of the $\delta^{13}C$ and $\delta^{15}N$ values for historic (A) and contemporary (C) feathers and 5 sources, and the simulated mixing region for their respective isospace plots (historic [B], and contemporary [D]).

Results

Vegetation and Arthropods

A detailed list of vegetation and arthropod samples collected from the APCNWR and their $\delta^{13}C$, $\delta^{15}N$, and carbon and nitrogen percent concentration values is provided in Appendix C&D. I collected arthropods from 11 orders, and vegetation from 26 different families. Vegetation samples more commonly obtained were from the families Asteraceae, Fabaceae, and Poaceae (Table 5). The arthropods most often collected were from the order Orthoptera, Hemiptera, and Coleoptera (Table 5).

Table 5. The $\delta^{13}C$ and $\delta^{15}N$ mean (± standard deviation) values of vegetation and arthropods collected from the Attwater Prairie Chicken National Wildlife Refuge during 2012-2013.

Source	n	Family	$\delta^{13}C$	$\delta^{15}N$	%C	%N
Vegetation						
Ambrosia psilostachya	7	Asteraceae	-29.74	0.95	42.61	1.47
			(-1.04)	(-3.41)	(-1.52)	(-0.42)
Anagallis arvensis	4	Primulaceae	-31.44	2.56	42.2	2.08
			(-0.39)	(-1.27)	(-2.95)	(-0.51)
Anemone caroliniana	3	Ranunculaceae	-28.98	3.01	42.11	2.59
			(-0.06)	(-1.21)	(-2.03)	(-0.22)
Baptisia bracteata	1	Fabaceae	-27.88	0.75	46.02	4.27
Briza sp.	1	Poaceae	-27.96	1.56	42.1	1.13
Callirhoe involucrata	2	Malvaceae	-29.38	1.62	40.19	1.52
			(-0.37)	(-0.16)	(-1.13)	(-0.01)
Chamaecrista fasciculata	10	Fabaceae	-30.42	-0.64	49.34	2.16
			(-0.8)	(-0.62)	(-1.56)	(-0.22)
Chloris sp.	1	Poaceae	-12.23	1.89	43.42	0.3
Cirsium spp.	1	Asteraceae	-30.91	-2.16	32.98	2.2
Coreopsis tinctoria	1	Asteraceae	-30.77	0.5	44.37	1.55
Croton capitatus	7	Euphorbiaceae	-29.06	1.7	44.73	1.93
			(-1.08)	(-3.12)	(-0.7)	(-0.59)
Dichanthelium oligosanthes	2	Poaceae	-29.78	-0.72	43.73	0.97
			(-0.04)	(-0.94)	(-0.4)	(-0.15)

Table 5. Continued

Source	n	Family	$\delta^{13}C$	$\delta^{15}N$	%C	%N
Dichanthelium spp.	8	Poaceae	-29.54	0.87	41.8	1.96
			(-0.98)	(-2.51)	(-3.42)	(-1.26)
Dracopis amplexicaulis	3	Asteraceae	-28.49	-0.15	46.46	1.77
			(-0.21)	(-1.11)	(-1.25)	(-0.07)
Eryngium yuccifolium	1	Apiaceae	-26.85	5.71	41.58	1.12
Euphorbia spp.	2	Euphorbiaceae	-29.97	4.53	44.97	3.18
			(-0.08)	(-0.35)	(-1)	(-0.35)
Euthamia sp.	1	Asteraceae	-30.83	0.07	45.66	2.59
Geranium carolinianum	1	Geraniaceae	-31.12	3.09	41.75	3.6
Helenium amarum	1	Asteraceae	-28.5	1.63	45.21	1.27
Hordeum pusillum	1	Poaceae	-28.28	4.45	42.75	0.92
Hypoxis sp.	1	Liliaceae	-29.84	1.53	42.76	1.63
Iva annua	2	Asteraceae	-29.39	4.98	38.88	1.61
			(-0.58)	(-4.79)	(-1.64)	(-0.83)
Juncus sp.	1	Juncaceae	-29.72	2.23	44.58	0.74
Krigia sp.	2	Asteraceae	-22.42	0.43	44.04	1.06
			(-0.03)	(-0.07)	(-0.08)	(-0.01)
Lepidium sp.	1	Brassicaceae	-27.65	5.68	38.31	2.38
Liatris mucronata	1	Asteraceae	-29.12	0.7	44.42	4.16
Linaria sp.	1	Scrophulariaceae	-29.9	0.16	39.96	0.68
Lythrum sp.	2	Lythraceae	-29.41	4.96	40.96	2.58
			(-3.54)	(-2.5)	(-5.22)	(-2.04)
Medicago lupulina	1	Fabaceae	-28.18	-0.81	44.48	2.79
Medicago polymorpha	3	Fabaceae	-30.13	-0.08	43.47	4.16
			(-0.31)	(-0.18)	(-1.24)	(-0.48)
Mimosa nuttallii	4	Fabaceae	-30.04	-1.28	45.1	2.39
			(-0.76)	(-1.19)	(-0.68)	(-1.13)
Mimosa spp.	3	Fabaceae	-30.39	-1.35	46.18	1.87
			(-0.54)	(-0.74)	(-0.71)	(-0.41)
Neptunia lutea	2	Fabaceae	-29.58	-0.54	46.1	1.92
			(-0.58)	(-1.64)	(-0.33)	(-0.61)
Neptunia spp.	1	Fabaceae	-25.74	-2.25	44.05	7.48
Nothocalais sp.	2	Asteraceae	-30.83	4.27	42.48	3.21
			(-0.78)	(-4.83)	(-0.5)	(-2.79)
Nothoscordum bivalve	4	Liliaceae	-29.02	1.77	37.26	3.09
			(-0.91)	(-1.22)	(-4.55)	(-1.19)
Oenothera laciniata	1	Onagraceae	-31.56	2.16	41.48	1.34
Oenothera spp.	4	Onagraceae	-27.35	2.65	44.49	1.24
			(-0.91)	(-2.25)	(-0.73)	(-0.4)
Oxalis spp.	4	Oxalidaceae	-30.23	4.62	41.76	2.92
			(-0.12)	(-2.72)	(-3.89)	(-0.97)
Oxalis stricta/corniculata	2	Oxalidaceae	-30.16	3.81	45.21	2.9
			(-0.04)	(-0.02)	(-0.07)	(-0.01)
Phalaris sp.	1	Poaceae	-29.28	0.59	39.67	1.08

Table 5. Continued

Source	n	Family	$\delta^{13}C$	$\delta^{15}N$	%C	%N
Phlox sp.	1	Polemoniaceae	-30.85	0	43	1.1
Plantago sp.	1	Plantaginaceae	-23	2.36	42.61	1.14
Rosa bracteata	1	Rosaceae	-27.99	-6.2	43.34	2.33
Rubus spp.	1	Rosaceae	-28.96	1.88	45.24	1.19
Rudbeckia spp.	1	Asteraceae	-30.06	1.69	45.91	1.19
Ruellia humilis	1	Acanthaceae	-29.68	0.26	39.62	2.24
Ruellia spp.	3	Acanthaceae	-28.94	2.11	41.33	1.58
			(-0.72)	(-4.93)	(-0.16)	(-0.46)
Rumex spp.	1	Polygonaceae	-29.84	1.08	43.08	0.95
Sabatia campestris	2	Gentianaceae	-29.58	2.68	44.77	1.43
			(-0.01)	(-0.23)	(-0.08)	(-0.01)
Sisyrinchium spp.	2	Iridaceae	-29.75	3.53	43.6	1.38
			(-0.32)	(-1.2)	(-2.05)	(-0.22)
Symphyotrichum spp.	3	Asteraceae	-29.45	2.34	35.13	3.41
			(-1.74)	(-2.32)	(-5.89)	(-0.71)
Tephrosia onobrychoides	1	Fabaceae	-28.38	-0.58	45.41	2.68
Tradescantia sp.	2	Commelinaceae	-29.41	7.98	41.24	6.52
			(-0.09)	(-0.11)	(-3.11)	(-0.6)
Tridens strictus	1	Poaceae	-12.84	1.48	40.85	1.18
Triodanis perfoliata	3	Campanulaceae	-29.33	2.77	40.33	1.36
			(-0.5)	(-2.67)	(-5.46)	(-0.27)
Vicia ludoviciana	1	Fabaceae	-31.1	1.98	40.79	3.92
Vicia spp.	2	Fabaceae	-30.39	0.2	43.53	4.07
			(-0.59)	(-0.08)	(-1.63)	(-1.94)
Arthropods						
Araneae	22		-22.26	6.58	48.23	10.63
			(-3.98)	(-1.71)	(-2.51)	(-3.11)
Coleoptera	29		-25.13	5.32	50.93	10.23
			(-3.12)	(-2.37)	(-2.48)	(-0.97)
Diptera	4		-24.48	6.46	48.25	10.49
			(-3.03)	(-1.81)	(-0.46)	(-0.9)
Hemiptera	42		-24.72	2.77	50.81	10.82
			(-4.48)	(-2.31)	(-2.7)	(-1.09)
Hymenoptera	11		-24.4	6.84	48.9	12.46
			(-4.27)	(-3.75)	(-0.82)	(-1.3)
Lepidoptera	18		-26.14	4.97	48.97	10.78
			(-4.81)	(-2.76)	(-3.24)	(-2.35)
Mantodea	2		-18.38	5.48	49.76	11.59
			(-1.41)	(-1.11)	(-2.76)	(-1.13)
Neuroptera	2		-23.39	4.01	49.19	9
			(-2.97)	(-0.06)	(-1.19)	(-2.06)
Orthoptera	63		-25.01	3.74	49.7	10.94
			(-3.8)	(-2.14)	(-6.67)	(-2.03)

Table 5. Continued

Source	n	Family	$\delta^{13}C$	$\delta^{15}N$	%C	%N
Phasmida	2		-27.22	5.5	47.7	11.05
			(-0.89)	(-2.75)	(-2.08)	(-1.2)
Sternorrhyncha	1		-28.93	-0.49	51.72	6.82

Overall, vegetation $\delta^{13}C$ values ranged from -31.91‰ to -12.23‰, and $\delta^{15}N$ values ranged from -6.20‰ to 8.36‰. No significant differences in isotope values were observed for vegetation samples among seasons or years of collection; however, when vegetation was grouped into legumes and non-legumes, $\delta^{15}N$ values in legumes were significantly greater in winter than in spring ($P = 0.0171$; Appendix C), but $\delta^{13}C$ values were not different. Seasonal differences were determined in spiders and insects $\delta^{13}C$ and $\delta^{15}N$ values. During spring, $\delta^{13}C$ values in Orthoptera were significantly greater than in Lepidoptera ($P = 0.0179$). Also in spring, spiders had significantly greater $\delta^{13}C$ values than Lepidoptera and Orthoptera ($P < 0.05$) and greater $\delta^{15}N$ values than Hemiptera and Orthoptera ($P < 0.05$; Appendix D).

Captive diet (Mazuri APC feed and meal worms) was ~1.17 times more enriched in $\delta^{13}C$ ($\bar{x}$ = -22.71‰) and ~1.20 times less enriched in $\delta^{15}N$ ($\bar{x}$ = 2.79‰) compared to vegetation and arthropods stable isotope values ($\bar{x}$: $\delta^{13}C$ = -26.47‰, $\delta^{15}N$ = 3.35‰) collected from the refuge.

Blood and Feathers

A list of all $\delta^{13}C$ and $\delta^{15}N$ blood, feathers, and feces is provided in Appendix E. The $\delta^{13}C$ or $\delta^{15}N$ values in APC blood were not significantly different between sexes throughout 2012–2013. However, 1 female had very low $\delta^{15}N$ values (3.78‰), compared with the rest. The $\delta^{13}C$ values in APC blood ranged from -28.6‰ to -18.35‰, and the $\delta^{15}N$ values ranged from 3.78‰ to 8.66‰. When separated by season, $\delta^{15}N$ values in blood were more enriched in the summer than in the fall ($P = 0.0105$) and winter ($P = 0.0010$). The $\delta^{13}C$ values also were more enriched in the fall compared to summer and winter blood samples ($P = 0.0015$; Fig. 18).

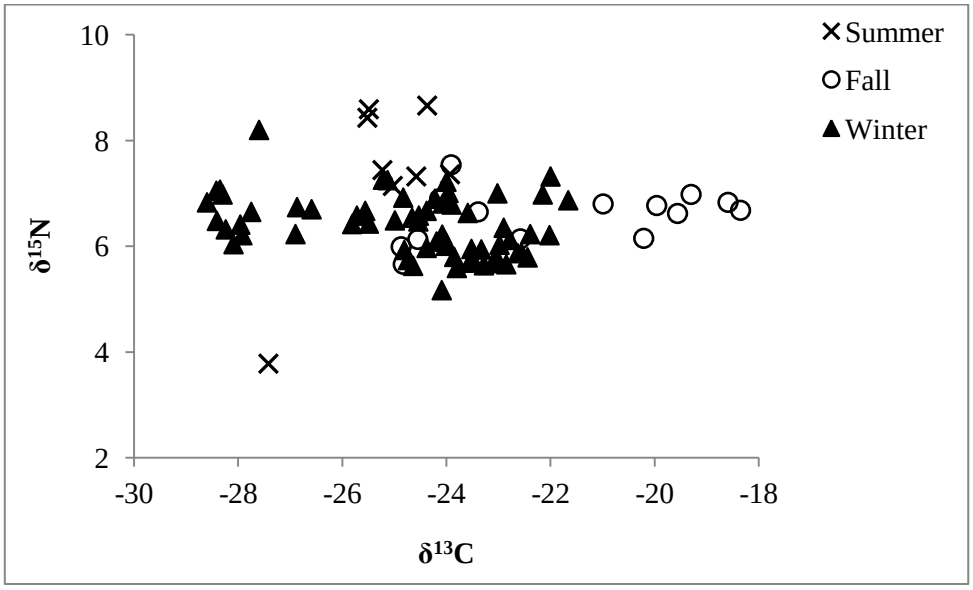


Figure 18: The $\delta^{13}C$ and $\delta^{15}N$ values for Attwater's prairie-chicken blood samples collected from the APCNWR by season.

Feather $\delta^{13}C$ and $\delta^{15}N$ values collected from historic and contemporary APCs in the wild, were rather similar; however the range for $\delta^{13}C$ values was more widespread for recent individuals than for historic specimens, whereas feathers from historic specimens had greater $\delta^{15}N$ values compared to recent individuals (Table 6). Also, no differences were observed for feather $\delta^{13}C$ and $\delta^{15}N$ values between APC males and females. However, ranges in $\delta^{13}C$ values in feathers of contemporary individuals from the refuge were much broader than in those from historic specimens. In contrast, feather $\delta^{15}N$ values were significantly more enriched in those from historic specimens compared to contemporary individuals ($P = 0.0001$). The $\delta^{13}C$ values in fecal samples did not vary much and ranged from (-30.58‰ to -29.19‰); however, $\delta^{15}N$ values had a wider range (2.38‰ to 6.4‰; Table 6).

Table 6. Attwater's prairie-chicken mean (± standard deviation) $\delta^{13}C$ and $\delta^{15}N$ values from blood, feathers (historic and contemporary), and feces.

Sex	Sample collected	$\delta^{13}C$	$\delta^{15}N$
Male	Blood	-24.73 (1.76)	6.86 (0.80)
	Contemporary Feathers	-21.14 (4.04)	8.00 (1.10)
	Historic Feathers	-21.37 (1.50)	9.08 (0.90)
	Feces	-30.12 (0.49)	3.71 (1.22)
Female	Blood	-24.27 (2.25)	6.42 (0.59)
	Contemporary Feathers	-20.26 (3.15)	8.28 (1.63)
	Historic Feathers	-21.42 (1.07)	9.81 (1.00)
	Feces	-30.12 (0.42)	4.11 (1.12)

Arthropod Fragments in Fecal Samples

A large array of arthropods was found in the 13 APC fecal samples analyzed by Pacific Analytics. A total of 8 families within 7 Orders was identified in the fecal material: Araneae: Lycosidae; Coleoptera: Curculiondiae, and Elateridae; Hemiptera: Coreidae, and Lygaeidae; Homoptera: Aphididae; Hymenoptera: Formicidiae; Orthoptera: Acrididae; and Lepidoptera. The most abundant insects found were grasshoppers (25%; Orthoptera:Acrididae), butterflies/moths (21.6%; Lepidoptera), and weevils (20.5%; Coleoptera:Curculionidae; Fig. 19).

Figure 19: Total arthropod remains found in Attwater's prairie chicken feces. Numbers above bars represent total taxa found in feces.

Diet Predictions Using a Stable Isotope Mixing Model

The MixSIAR model using $\delta^{13}C$ and $\delta^{15}N$ values for combined blood and 3 sources (vegetation, insects, and spiders) suggested insects were the main contributor to diet (87.2%; Fig. 20A). Insects also were the main contributor to diet for blood collected during the summer (57.3%) and fall (42.7%; Figs. 20B, 20C). For combined blood and fall blood samples, the second most prevalent diet source was C_3 vegetation (11.4% and 25.5%, respectively), and third was spiders (0.9% and 22.7%, respectively). For blood and diet samples collected during summer, the model results indicated spiders were the second most abundant diet source (21.9%), followed by C_3 vegetation (20.6%).

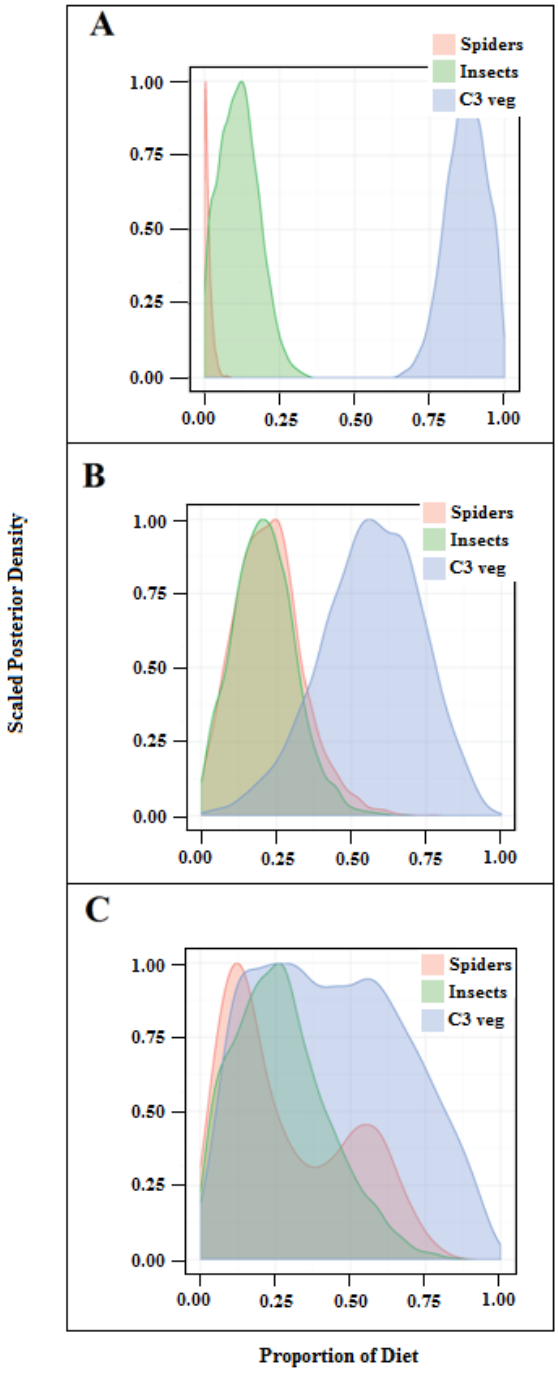


Figure 20: Posterior density plot for combined (A), summer (B), and fall (C) blood using 3 sources (C_3 vegetation, Spiders, and Insects).

Using historic feathers $\delta^{13}C$ and $\delta^{15}N$ values and 3 sources, the model results indicated spiders had the greatest contribution to diet (63.2%), then insects (32.3%), and C_3 vegetation contributed the least (3.5%; Fig. 21A). Using values from feathers collected from contemporary specimens, the mixing model indicated insects had the greatest contribution to the APC diet (63.4%), then spiders (26.4%), and C_3 vegetation (9.1%; Fig. 21B).

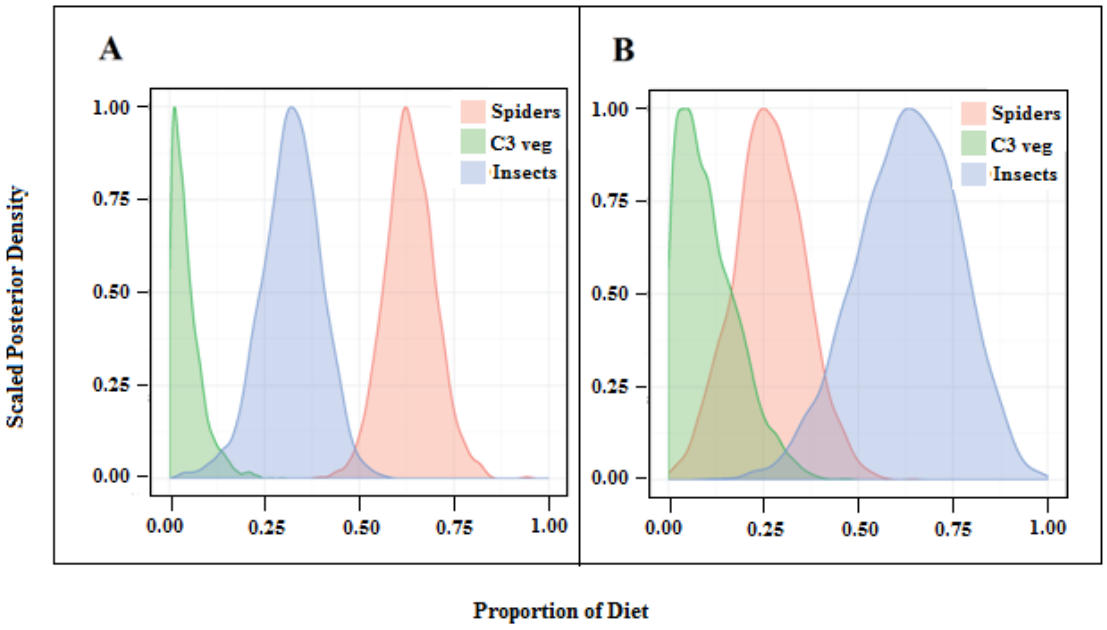


Figure 21: Posterior density plot for historic (A) and contemporary (B) feathers using 3 sources (C_3 vegetation, Spiders, and Insects).

Results for combined blood $\delta^{13}C$ and $\delta^{15}N$ values and 4 sources (C_3 vegetation, insects, spiders, and C_4 vegetation) indicated the following 3 sources contribute the most to APC diet: C_3 vegetation, C_4 vegetation, and insects (60.8%, 24.6%, and 11.2%, respectively; Fig. 22A). For summer blood samples, the main 3 contributors were: C_3 vegetation, insects, and spiders (43.4%, 27.7%, and 14.7%, respectively; Fig. 22B). For fall blood samples, C_3 vegetation, C4 vegetation, and insects contributed 36.6%, 33.7%, and 19.4%, respectively to APC diet (Fig. 22C).

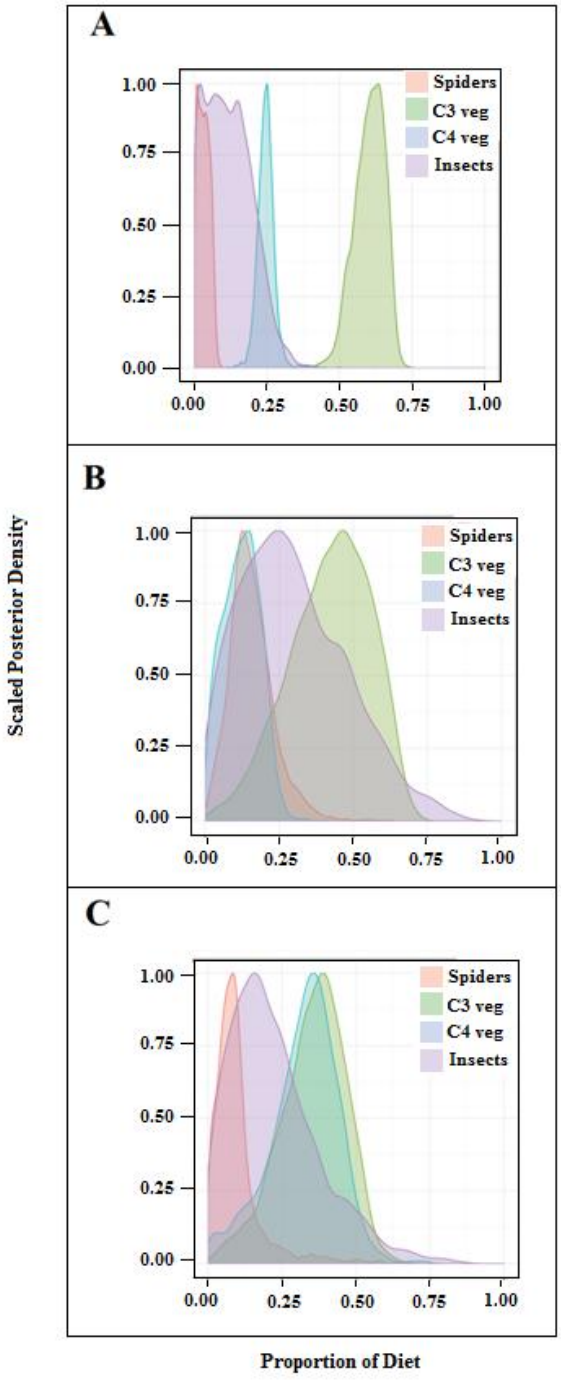


Figure 22: Posterior density plot for combined (A), summer (B), and fall (C) blood using 4 sources (C_3 and C_4 vegetation, Spiders, and Insects).

By using the $\delta^{13}C$ and $\delta^{15}N$ values from historic feathers with 4 sources, the model results indicated spiders (37.1%) contributed the most to the diet, then C_4 vegetation (23.3%), C_3 vegetation (22.8%), and insects (15.7%; Fig. 23A). Using $\delta^{13}C$ and $\delta^{15}N$ values with contemporary feathers and 4 sources, the model suggests C_4 vegetation contributed the most (32.7%; Fig. 23B) to the diet, followed by C_3 vegetation (32.1%), insects (19.6%), and spiders (14.8%).

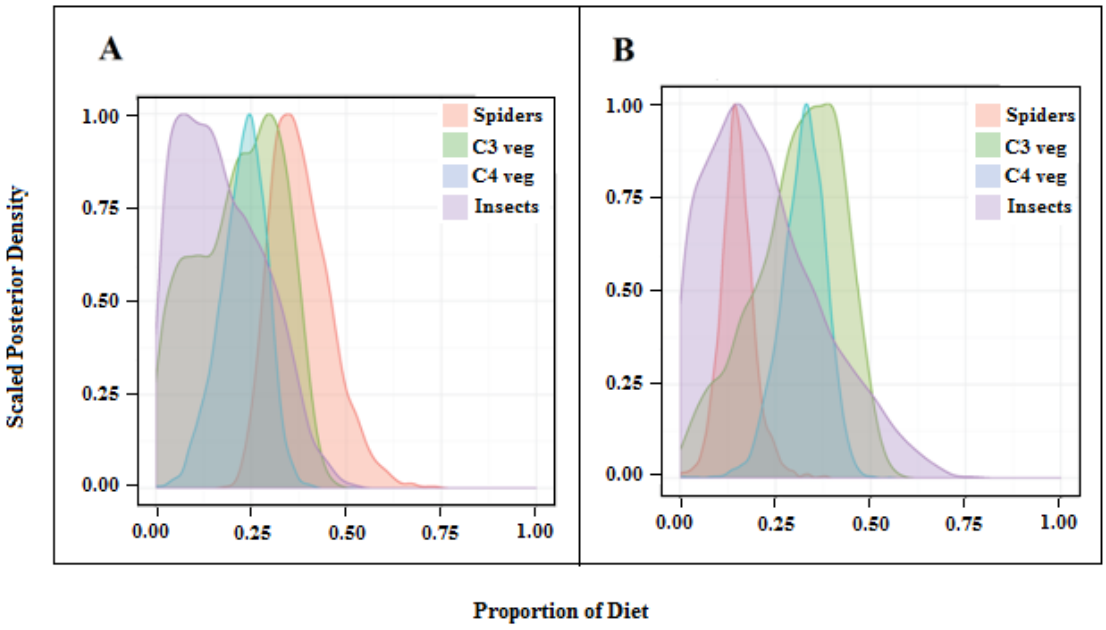


Figure 23: Posterior density plot for historic (A) and contemporary (B) feathers using 4 sources (C_3 and C_4 vegetation, Spiders, and Insects).

When 5 sources (C_3 vegetation, insects, spiders, C_4 vegetation, and rice) were considered and the $\delta^{13}C$ and $\delta^{15}N$ values for combined blood, the model indicated C_3

vegetation (53.6%) contributed the most to APC diet, followed by C_4 vegetation (23.1%), rice (10.9%), insects (7.8%), and spiders (1.9%; Fig. 24A). When only the isotope values from blood samples collected in the summer were used, the model showed different results with rice (34.2%) contributing the most to APC diet, followed by C_3 vegetation (25.6%), insects (18.8%), C_4 vegetation (11.2%), and spiders (6.6%; Fig. 24B).

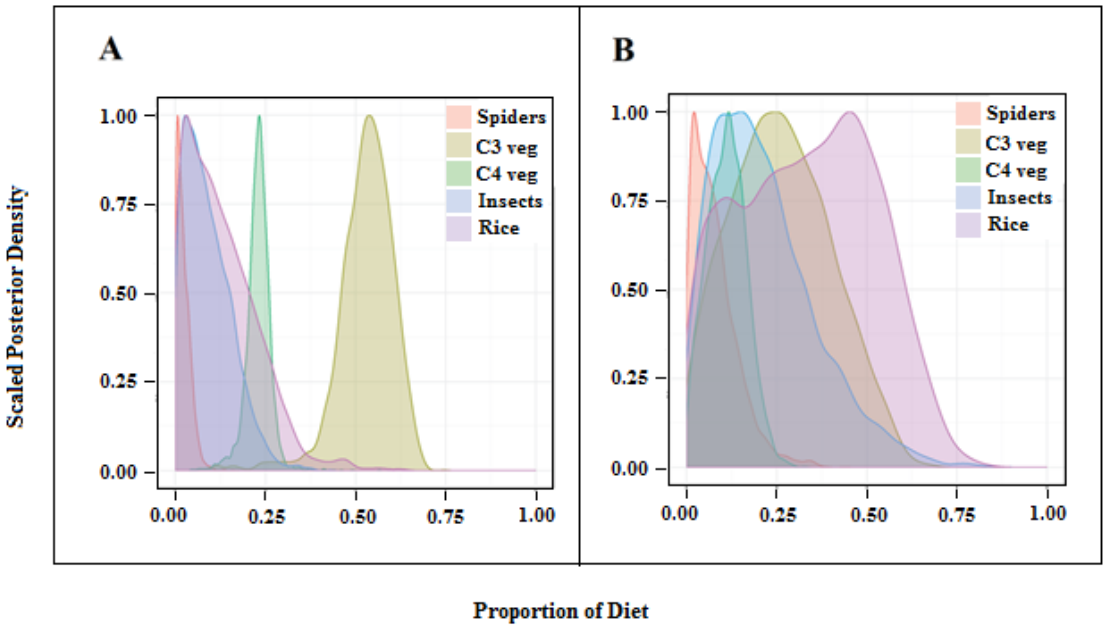


Figure 24: Posterior density plot for combined (A) and summer (B) blood using 5 sources (C_3 and C_4 vegetation, Spiders, Insects, and Rice).

Using historic feather stable isotope values with 5 sources, the model results indicated almost half of their diet was comprised of rice (45.3%), followed by C_4

vegetation (25.6%), spiders (12.3), C_3 vegetation (9.4%), and insects (6.4%; Fig. 25A). Contemporary feather $\delta^{13}C$ and $\delta^{15}N$ values used in the model indicated C_4 vegetation (30.2%) contributed the most to APC diet, followed by insects (19.9%), C_3 vegetation (18.9%), rice (18.7%), and then spiders (8.7%; Fig. 25B).

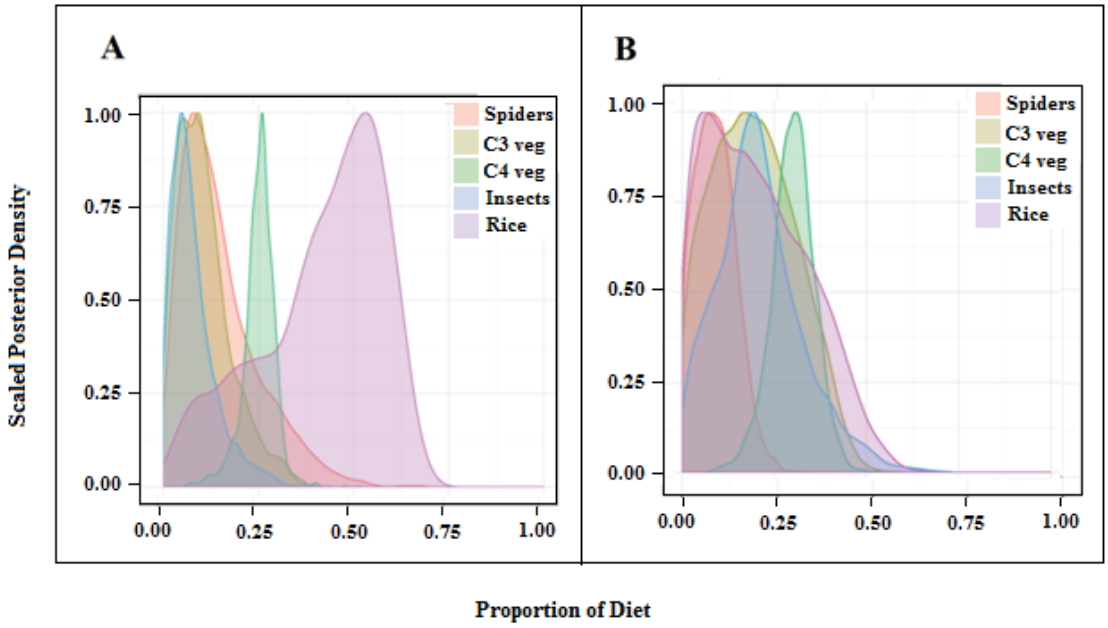


Figure 25: Posterior density plot for historic (A) and contemporary (B) feathers using 5 sources (C_3 and C_4 vegetation, Spiders, Insects, and Rice).

Discussion

One of the main objectives of this study was to determine if stable isotopes carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) could be used to predict the diet of wild APCs on the

APCNWR. Assessing diet using stable isotopes could be useful to determine seasonal abundance and distribution of the most important diet sources for APCs once they are released at the refuge. The results from the MixSIAR model suggest diverse contribution of sources which are quite variable and not easy to interpret. Using only 3 sources (vegetation, insects, and spiders) and isotope values for contemporary APC feathers and blood, the model suggests APCs have a diet high in insects (87.2%), followed by C_3 vegetation (11.4%) then spiders (0.9%); however, these results should be taken with caution because when the isotope values for sources and compartments were plotted, some of those values fell off the triangle recommended for proper analysis (Fig. 13), which suggested that some additional sources were needed. This is when I considered adding C_4 plants, although I collected only a few C_4 plants at the refuge. The majority of plants and forbs at the refuge are C_3. Additionally, finding insects as the predominant food source runs opposite to what Lehman (1941) and Cogar (1980) reported in previous studies (1936-1980) , which indicate that plant matter (foliage and seeds) is the dominant food choice, with insects comprising less than 20% of their diet. However, both studies reported APCs consumed more insects during summer. This can explain why analysis of summer blood indicates a high percentage of insects contributing to the diet during that time. However, it does not explain the results for feathers and fall blood samples. When an additional source (C_4 plants) was added to the model vegetation (C_3 plants) was observed to be the main component to APCs' overall diet. These results are similar to Lehman (1941) and Cogar (1980) and the consumed vegetation stable isotope values had similar signatures to C_3 plants. Even though APCs

are historically known to eat primarily forbs (C_3 vegetation), no surrounding crops (e.g. corn) were noticed near the refuge with C_4 plants during the collection period. However, corn and a mixture of peas (sweet or black-eyed peas) are given to captive-reared APCs during (inside enclosure) and after (outside enclosure) acclimation onto the refuge (USFWS 2010). Millet, a C_4 crop, was planted on the refuge during 2011 and 2012, but was stunted, so it could have been eaten by APCs there at that time. However, whether the birds sampled in this study were exposed to C_4 plants or not, remains uncertain. Using 5 sources to run the model I observed C_3 (53.6%), C_4 plants (23.1%), and rice (10.9%) contributed the most to APC diet. Rice was considered a separate diet source since it had high $\delta^{15}N$ values compared to the other sources. Rice crops surround the refuge, an area where APCs are known to forage (USFWS 2010). The availability of corn, peas, and rice to current APCs likely explains why the stable isotope analysis indicates a much greater contribution of these additional sources in comparison to naturally available dietary sources from the refuge. The major diet source for APCs changes between each model, which suggests that the source variability is important. The results from the models using 4 and 5 sources provide information about APC diet that may be more reliable based on experience and previous studies.

There may be unintended consequences of having extra food provided to APCs once on the refuge, particularly corn. A study done by Feret et al. (2003) showed that wild geese eating a corn based diet had a better body condition compared to geese that did not. Thus, a shift in diet from a high energy food source like corn (Gauthier et al. 1992) to a diet of forbs and insects that are less enriched in $\delta^{13}C$ values may cause a

problem for the overall health of newly released APCs (Krapu et al. 1995, Feret et al. 2003) unless it is continuously made available throughout the year.

Burns-Cusato and Morrow (2003) pointed out that introducing captive APCs to the wild has limited success, most likely because released birds are not prepared for their new, wild environment. Hess et al. (2005) suggest rearing APC chicks with forbs that APCs are known to eat and also use as natural cover. Enriching the APCs' captive environment with natural food (forbs and insects from the refuge) could help APCs learn important foraging strategies early on, skills they can then utilize once released to the wild (Carlstead and Shepherdson 1994). Accordingly, chicks reared in a semi-natural environment may have an increased chance for survival, though more experiments should be done to further explore this possibility for APCs.

Even though there were no statistically significant differences for $\delta^{13}C$ values of APC historic feathers compared to contemporary feathers, I observed $\delta^{13}C$ values in feathers of APCs currently in the wild to have a wider range of values (-13.37‰ to -26.41‰) than those in historic specimens. Other studies that have observed this shift in birds suggest the wide variation in $\delta^{13}C$ values could be due to changes in the birds' foraging strategies (Bearhop et al. 2006). However, I do not have data from this study to fully support this observation. Food plots (peanuts, corn, and rice) used to be prevalent around the APCNWR and were utilized by APCs as food sources in the past (Lehman 1941). Both peanuts and rice are C_3 crops (Teramura 1983, Rajwade et al. 2015), and APCs primarily ate peanuts and rice during the fall (Kessler 1978). Currently, peanut crops are no longer present around the APCNWR due to the risk of aflatoxins forming

and potentially harming the APCs (USFWS 1992, 2010). Rice fields have decreased due to drought (Baddour 2014) and some have even been acquired by the APCNWR for eventual conversion into APC habitat (Morrow et al. 2004). However, they do surround the refuge today (Werner et al. 2016).

The decrease in $\delta^{15}N$ values of contemporary APC feathers when compared to historic APC feathers is not enough to be considered a decrease in trophic level (Kelly 2000), but it is still statistically significant. There can be a few reasons for the observed decrease in APC feather $\delta^{15}N$ values when comparing historic to contemporary. One reason could be potential diet shifts based on the current available food choices on the refuge, such as the supplemental corn and peas provided. Another reason could be due to the introduction of red imported fire ants (RIFA; *Solenopsis invicta*) to the United States in the 1930s (Allen et al. 1994). Since their introduction, RIFA have spread across the southern U.S., including Texas and the APCs' natural habitat (Morrow et al. 2013). Invertebrate numbers can decrease in the presence of RIFA since they prey upon invertebrates as they scavenge (Holway et al. 2002). By making invertebrates relatively scarce, the presence of RIFA in the refuge could account in part for why $\delta^{15}N$ values in contemporary feathers are less enriched than in the past. Another reason for the decrease in APC feather $\delta^{15}N$ values when comparing historic to contemporary could be that some areas of the APCNWR were previous cropland that had fertilizer applied. Sometimes there is a decrease in $\delta^{15}N$ values of plant samples collected when there is an application of fertilizer since it is depleted in $\delta^{15}N$ itself (Hogberg et al. 1995).

This study is the first to present $\delta^{13}C$ and $\delta^{15}N$ values for the endangered APCs and their diet sources. However, some caveats should be addressed, including the use of captive APC TDF values for the mixing models used. Since wild APCs may be under nutritional stress compared to their captive counterparts, their TDF values could differ (Phillips and Koch 2002). Also, I did not collect and analyze any current Texas rice samples for $\delta^{13}C$ and $\delta^{15}N$ analyses in this study, instead using values obtained by Alisauskas and Hobson (1993). Consequently, the $\delta^{13}C$ and $\delta^{15}N$ values for my rice source may be different from current rice crops surrounding the refuge. Also, due to limited arthropod collection during winter, the mixing model was unusable for determining APC diet during that time.

Overall, when using 3 diet sources (C_3 vegetation, insects and spiders) and contemporary feather $\delta^{13}C$ and $\delta^{15}N$ values, the model suggests that insects contribute mainly to APC diet. This result is possibly misleading since so many APC feather samples fell outside the mixing polygon. When the model was run with the same contemporary feather $\delta^{13}C$ and $\delta^{15}N$ values and 4 (C_3, C_4 vegetation, insects, and spiders) and 5 diet sources (C_3, C_4 vegetation, insects, spiders and rice), the results shifted to vegetation (C_3 and C_4) plants contributing the most to APC diet. The results of C_3 plants (forbs) comprising the most to APC diet are similar to what past APC diet studies have noted; however, C_4 is a new main contributor to APC diet. In the past corn (C_4 plant) was foraged by APCs opportunistically and in this study did not show to comprise much of historic APCs diet. The opposite was determined for contemporary APCs, and C_4 plants contributed more so compared to past APC diet.

CHAPTER V

DISCUSSION AND CONCLUSIONS

The results from Chapter II, "Hazard Assessment of Mercury to Waterbirds at Lake Chapala, Mexico" indicated that there was a positive and significant correlation between fish Hg concentrations and fish length. Fish $\delta^{15}N$ values also were significantly correlated with fish Hg concentrations from both Lake Chapala and the San Antonio Guaracha Reservoir (reference site). When I compared fish Hg concentrations from this study with the results of other studies, I found fish Hg concentrations were similar to what others reported. No significant differences for feather Hg concentrations were determined between years, locations, or species. The feather δD values had a wide range (-163‰ to -11‰). The majority of them had more depleted δD values, which suggests these feathers were grown in more northerly regions.

For Chapter III, "Metal Concentrations in Water, Sediment, and Fish from Lake Chapala, Mexico", I found a significant correlation between fish metal concentrations (Ba, Cu, Mn, and Zn) and fish length. As opposed to Chapter II, no fish metal concentrations were significantly correlated with $\delta^{15}N$ values, suggesting no biomagnification occurring with these particular metals. Also, I found Lake Chapala metal concentrations in water, sediments, and fish were similar to, or below past metal concentrations recorded in previous studies. All metal concentrations I analyzed were below levels of concern for both fish and wildlife. This study is unique in that it reports

recent metal data not only in the water, sediments, and fish from Lake Chapala, but from aquatic birds as well.

In Chapter IV, "Using Stable Isotopes to Determine Diet Sources of the Endangered Attwater's Prairie Chicken (*Tympanuchus cupido attwateri*) in Texas", I found C_3 vegetation (mostly forbs) contributed over 50% to APC diet when blood was grouped from all seasons (summer, fall, and winter). When the model was run using summer APC blood samples it indicated APCs mainly consume rice, then forbs (legumes more than non-legume sources), insects, C_4 vegetation (primarily grasses and possibly corn), and spiders during this time.

I also found there was an apparently wider range in contemporary feather $\delta^{13}C$ values (-26.41‰ to -13.37‰) compared to historic individuals (-23.80‰ to -18.65‰). Feather $\delta^{15}N$ values were significantly lower in contemporary compared to historic APCs. When I used contemporary APC feather $\delta^{13}C$ and $\delta^{15}N$ values in the MixSIAR model, I determined C_4 vegetation was predominantly consumed compared to the other diet sources available (forbs, rice, spiders, and insects). This differed from historic APC feather stable isotope values, which determined rice was the main contributor to APC diet, then grasses/corn, spiders, forbs, and insects.

Conclusions

For the Lake Chapala study, metal concentrations in water, sediment, and fish were similar, or to some extent lower, compared to those reported in previous studies. Lake Chapala is an alkaline lake, which could contribute to the low metal concentrations

in water and possibly fish since there is less assimilation of Hg and other metals by bacteria compared to more acidic lakes. This lake is also very shallow and concentrations of contaminants in it can fluctuate greatly depending on the weather. Hence, metal concentrations in water and biota in Lake Chapala could experience annual variations.

The Attwater's prairie chicken study determined different sources were being assimilated by the bird depending on which diet sources were used in the mixing model. I used the three initial diet sources (forbs, insects, and spiders) since I was directed by the refuge biologists in my selection of those samples. After running the mixing polygon simulation, I determined that I should add the grasses to my model since there seemed to be a missing diet source with similar $\delta^{13}C$ values. This then lead me to add rice for my last mixing model, since there showed to be a missing diet source with high $\delta^{15}N$ values. All of the described mixing models results are possible predictions for current APC diet. However, the models with grasses as an added diet source are better predictors for APC diet since those stable isotope values fill in a possible missing APC diet source. Forbs were shown to contribute mainly to APC diet (when using blood $\delta^{13}C$ and $\delta^{15}N$ values). These results are similar to past APC diet studies conducted by Lehman (1941) and Cogar (1980). When looking at the fecal arthropod fragments, Orthoptera, Lepidotera, and Coleoptera were the most consumed by APCs during the winter months. Even though I was not able to conduct a mixing model analysis during this period, I know APCs were consuming these particular insects; however, I cannot

determine if these insect sources were assimilated by APCs. The results of this study reaffirm that APCs are omnivorous and opportunistic foragers.

LITERATURE CITED

Alisauskas, R.T., and K.A. Hobson 1993. Determination of lesser snow goose diets and winter distribution using stable isotope analysis. The Journal of Wildlife Management 57:49–54.

Allen, C. R., S. Demarais, and R. S. Lutz. 1994. Red imported fire ant impact on wildlife: an overview. The Texas Journal of Science. 46:51–59.

Anderson, J. G., and K. B. Anderson. 2005. An analysis of band returns of the American white pelican, 1922 to 1981. Waterbirds 28:55–60.

Angot, H., A. Dastoor, F. De Simone, K. Gardfeldt, C. N. Gencarelli, I. M. Hedgecock, S. Langer, O. Magand, M. N. Mastromonaco, C. Nordstrom, K. A. Pfaffhuber, N. Pirrone, A. Ryjkov, N. E. Selin, H. Skov, S. Song, F. Sprovieri, A. Steffen, K. Toyota, O. Travnikov, X. Yang, and A. Dommergue. 2016. Chemical cycling and deposition of atmospheric mercury in polar regions: review of recent measurements and comparison with models. Atmospheric Chemistry and Physics 16:10735–10763.

Asztalos, B., J. Nemcsók, I. Benedeczky, R. Gabriel, A. Szabo, and O. Refaie. 1990. The effects of pesticides on some biochemical parameters of carp (*Cyprinus carpio* L.). Archives of Environmental Contamination and Toxicology 19:275–282.

Atwell, L., K. A. Hobson, and H. E. Welch. 1998. Biomagnification and bioaccumulation of mercury in an arctic marine food web: insights from stable nitrogen isotope analysis. Canadian Journal of Fisheries and Aquatic Sciences 55:1114–1121.

Avila-Pérez, P., M. Balcázar, G. Zarazúa-Ortega, I. Barceló-Quintal, and C. Dıaz-Delgado. 1999. Heavy metal concentrations in water and bottom sediments of a Mexican reservoir. Science of the Total Environment 234:185–196.

Bearhop, S., R.A. Phillips, R. McGill, Y. Cherel, D.A. Dawson, and J.P. Croxall. 2006. Stable isotopes indicate sex-specific and long-term individual foraging specialisation in diving seabirds. Marine Ecology Progress Series 311:157–164.

Beaudoin, C. P., E. E. Prepas, W. M. Tonn, L. I. Wassenaar, and B. G. Kotak. 2001. A stable carbon and nitrogen isotope study of lake food webs in Canada's Boreal Plain. Freshwater Biology 46:465–477.

Becerra-Muñoz, S., H. R. Buelna-Osben, and J. M. Catalán-Romero. 2003. Spatial patterns of ARIMA modeled rates of change of atherinids (*Chirostoma* spp.) and

goodeid *Chapalichthys encaustus* from Lake Chapala, México. Ecological Modelling 165:237–250.

Ben-David, M., R. Flynn, and D. Schell. 1997. Annual and seasonal changes in diets of martens: evidence from stable isotope analysis. Oecologia 111:280–291.

Ben-David, M., and D. M. Schell. 2001. Mixing models in analyses of diet using multiple stable isotopes: a response. Oecologia 127:180–184.

Biswas, A., J. D. Blum, B. Klaue, and G. J. Keeler. 2007. Release of mercury from Rocky Mountain forest fires. Global Biogeochemical Cycles 21:1–13.

Blight, L.K., K. A. Hobson, T. K. Kyser, and P. Arcese. 2015. Changing gull diet in a changing world: A 150-year stable isotope ($\delta^{13}C$, $\delta^{15}N$) record from feathers collected in the Pacific Northwest of North America. Global Change Biology 21:1497–1507.

Boecklen, W. J., C. T. Yarnes, B. A. Cook, and A. C. James. 2011. On the use of stable isotopes in trophic ecology. Annual Review of Ecology, Evolution, and Systematics 42:411–440.

Boutton, T. W. 1991. Stable carbon isotope ratios of natural materials: II. Atmospheric, terrestrial, marine, and freshwater environments. Pages 173–184 in D. C. Coleman and B. Fry, editors. Carbon Isotope Techniques. Academic Press, Inc., San Diego, CA, USA.

Brugnoli, E., and G. D. Farquhar. 2000. Photosynthetic fractionation of carbon isotopes. Pages 399-434 *in* R. C. Leegood, T. D. Sharkey, and S. von Caemmerer, editors. Photosynthesis. Kluwer Academic Publishers, Dordrecht.

Brunke, E. G., C. Labuschagne, and F. Slemr. 2001. Gaseous mercury emissions from a fire in the Cape Peninsula, South Africa, during January 2000. Geophysical Research Letters 28:1483–1486.

Burgess, N. M., M. W. Meyer. 2008. Methylmercury exposure associated with reduced productivity in common loons. Ecotoxicology 17:83–91.

Burns-Cusato, M. and M.E. Morrow. 2003. Fear in the captive-bred Attwater's prairie chicken as an indicator of postrelease survival. International Journal of Comparative Psychology 16:95–110.

Burton, T. 1997. Can Mexico's largest lake be saved? Ecodecision-Montreal 23:68-71.

Cabana, G., and J. B. Rasmussen. 1996. Comparison of aquatic food chains using nitrogen isotopes. Proceedings of the National Academy of Sciences of the United States of America 93:10844–10847.

Camargo, J. A. 2002. Contribution of Spanish–American silver mines (1570–1820) to the present high mercury concentrations in the global environment: a review. Chemosphere 48:51–57.

Capelli, R., K. Das, R. De Pellegrini, G. Drava, G. Lepoint, C. Miglio, V. Minganti, and R. Poggi. 2008. Distribution of trace elements in organs of six species of cetaceans from the Ligurian Sea (Mediterranean), and the relationship with stable carbon and nitrogen ratios. Science of the Total Environment 390:569–578.

Carlstead, K., and D. Shepherdson. 1994. Effects of environmental enrichment on reproduction. Zoo Biology13:447–458.

Caut, S., E. Angulo, and F. Courchamp. 2009. Variation in discrimination factors (Δ^{15}N and Δ^{13}C): the effect of diet isotopic values and applications for diet reconstruction. Journal of Applied Ecology 46:443–453.

Cerling, T. E., J. M. Harris, B. J. MacFadden, M. G. Leakey, J. Quade, V. Eisenmann, and J. R. Ehleringer. 1997. Global vegetation change through the Miocene/Pliocene boundary. Nature 389:153–158.

Cogar, V. F. 1980. Food habits of Attwater's prairie-chicken in Refugio County, Texas. Dissertation, Texas A&M University, College Station, USA.

Connan, M., G. G. Hofmeyr, and P. A. Pistorius. 2016. Reappraisal of the Trophic Ecology of One of the World's Most Threatened Spheniscids, the African Penguin. PloS one 11:e0159402.

Crump, K. L., and V. L. Trudeau. 2009. Mercury-induced reproductive impairment in fish. Environmental Toxicology and Chemistry 28:895–907.

DeNiro, M. J., and S. Epstein. 1981. Influence of diet on the distribution of nitrogen isotopes in animals. Geochimica Et Cosmochimica Acta 45:341–351.

Dennis, I. F., T. A. Clair, C. T. Driscoll, N. Kamman, A. Chalmers, J. Shanley, S. A. Norton, and S. Kahl. 2005. Distribution patterns of mercury in lakes and rivers of northeastern North America. Ecotoxicology 14:113–123.

Drew, M. L., W. L. Wigle, D. L. Graham, C. P. Griffin, N. J. Silvy, A. M. Fadly, and R. L. Witter. 1998. Reticuloendotheliosis in captive greater and Attwater's prairie-chickens. Journal of Wildlife Diseases 34:78–791.

Eisler, R. 1993. Zinc hazards to fish, wildlife, and invertebrates: a synoptic review. Biological Report Fish and Wildlife Service 10.

Eisler, R. 1998. Copper hazards to fish, wildlife, and invertebrates: A synoptic review. U.S. Geological Survey, Biological Resources Division, Biological Science Report USGS/BRD/BSR--1998-0002.

Ellsworth, D. L., R. L. Honeycutt, N. J. Silvy, K. D. Rittenhouse, and M. H. Smith. 1994. Mitochondrial-DNA and nuclear-gene differentiation in North American prairie grouse (genus *Tympanuchus*). The Auk 111:661–671.

Evers, D. C., N. M. Burgess, L. Champoux, B. Hoskins, A. Major, W. M. Goodale, R. J. Taylor, R. Poppenga, and T. Daigle. 2005. Patterns and interpretation of mercury exposure in freshwater avian communities in northeastern North America. Ecotoxicology 14:193–221.

Féret, M., G. Gauthier, A. Béchet, J. Giroux, and K. A. Hobson. 2003. Effect of a spring hunt on nutrient storage by greater snow geese in southern Quebec. The Journal of Wildlife Management 67:796–807.

Findholt, S. L., and S. H. Anderson. 1995. Foraging areas and feeding habitat selection of American White Pelicans (*Pelecanus erythrorhynchos*) nesting at Pathfinder Reservoir, Wyoming. Colonial Waterbirds 18:47–57.

Ford, T. E., R. Ika, J. Shine, L. D. Lind, and O. Lind. 2000. Trace metal concentrations in *Chirostoma* sp. from Lake Chapala, Mexico: Elevated concentrations of mercury and public health implications. Journal of Environmental Science & Health, Part A 35:313–325.

Ford, T., and D. Ryan. 1995. Toxic metals in aquatic ecosystems: a microbiological perspective. Environmental Health Perspectives 103:25–28.

Fry, B., editor. 2006. Stable isotope ecology. Springer Science & Business Medial LLC, Berlin.

Fry, B., and E. B. Sherr. 1984. $\delta^{13}C$ measurements as indicators of carbon flow in marine and freshwater ecosystems. Pages 13–47 *in* P. W. Rundel, J. R. Ehleringer, and K. A. Nagy, editors. Stable isotopes in ecological research. Springer-Verlag New York Inc.

Gabriel, M. C., R. Kolka, T. Wickman, E. Nater, and L. Woodruff. 2009. Evaluating the spatial variation of total mercury in young-of-year yellow perch (*Perca flavescens*),

surface water and upland soil for watershed–lake systems within the southern Boreal Shield. Science of the Total Environment 407:4117–4126.

Gauthier, G., J. Giroux, and J. Bédard. 1992. Dynamics of fat and protein reserves during winter and spring migration in greater snow geese. Canadian Journal of Zoology 70:2077–2087.

Gelman, A., J. B. Carlin, H. S. Stern, and D. B. Rubin. 2014. Bayesian data analysis. Chapman & Hall/CRC Boca Raton, USA.

Geweke, J. 1991. Evaluating the accuracy of sampling-based approaches to the calculation of posterior moments. Federal Reserve Bank of Minneapolis and University of Minnesota. Minneapolis, USA.

Grandy, J. W., IV, L. N. Locke, and G. E. Bagley. 1968. Relative toxicity of lead and five proposed substitute shot types to pen-reared mallards. Journal of Wildlife Management 32:483-488.

Griffin, C. P. 1998. Factors affecting captive prairie chicken production. Dissertation, Texas A&M University, College Station, USA.

Gundersen, D. T., S. Bustaman, W. K. Seim, and L. R. Curtis. 1994. pH, hardness, and humic acid influence aluminum toxicity to rainbow trout (*Oncorhynchus mykiss*) in weakly alkaline waters. Canadian Journal of Fisheries and Aquatic Sciences 51:1345–1355.

Hall, A. G., L. Avens, J. B. McNeill, B. Wallace, and L. R. Goshe. 2015. Inferring long-term foraging trends of individual juvenile loggerhead sea turtles using stable isotopes. Marine Ecology Progress Series 537:265–276.

Hammerly, S.C., M.E. Morrow, and J.A. Johnson. 2013. A comparison of pedigree-and DNA-based measures for identifying inbreeding depression in the critically endangered Attwater's prairie-chicken. Molecular Ecology 22:5313–5328.

Hansen, A. M., M. van Afferden. 2001. The Lerma-Chapala Watershed: Evaluation and Management. Springer Science & Business Media New York.

Healy, K., S.B. Kelly, T. Guillerme, R. Inger, S. Bearhop, and A.L. Jackson. 2017. Predicting trophic discrimination factor using Bayesian inference and phylogenetic, ecological and physiological data. DEsIR: Discrimination Estimation in R. Peer J Preprints 5:e1950v3.

Hecky, R., and R. Hesslein. 1995. Contributions of benthic algae to lake food webs as revealed by stable isotope analysis. Journal of the North American Benthological Society 14:631–653.

Heinz, G. H. 1974. Effects of low dietary levels of methyl mercury on mallard reproduction. Bulletin of Environmental Contamination and Toxicology 11:386–392.

Heinz, G. H. 1975. Effects of methylmercury on approach and avoidance behavior of mallard ducklings. Bulletin of Environmental Contamination and Toxicology 13:554–564.

Heinz, G. H. 1979. Methylmercury: reproductive and behavioral effects on three generations of mallard ducks. The Journal of Wildlife Management 43:394–401.

Hermanson, M. H. 1998. Anthropogenic mercury deposition to Arctic lake sediments. Water, Air, & Soil Pollution 101:309–321.

Hess, M.F., N. J. Silvy, C. P. Griffin, R. R. Lopez, and D. S. Davis. 2005. Differences in flight characteristics of pen-reared and wild prairie-chickens. Journal of Wildlife Management 69:650–654.

Hobson, K. A. 1999. Tracing origins and migration of wildlife using stable isotopes: a review. Oecologia 120:314–326.

Hobson, K. A. 2005. Stable isotopes and the determination of avian migratory connectivity and seasonal interactions. The Auk 122:1037–1048.

Hobson, K.A., and R.G. Clark. 1992. Assessing avian diets using stable isotopes II: factors influencing diet-tissue fractionation. The Condor 94:189–197.

Högberg, P., C. Johnnisson, M. Högberg, L. Högbom, T. Näsholm, and J. E. Hällgren. 1995. Measurements of abundances of ^{15}N and ^{13}C as tools in retrospective studies of N balances and water stress in forests: A discussion of preliminary results. Plant and Soil 168:125-133.

Holway, D. A., L. Lach, A. V. Suarez, N. D. Tsutsui, and T. J. Case. 2002. The causes and consequences of ant invasions. Annual Review of Ecology and Systematics 33:181–233.

Hopkins III, J. B., and J. M. Ferguson. 2012. Estimating the diets of animals using stable isotopes and a comprehensive Bayesian mixing model. PLoS One 7:e28478.

Hsu-Kim, H., K. H. Kucharzyk, T. Zhang, and M. A. Deshusses. 2013. Mechanisms regulating mercury bioavailability for methylating microorganisms in the aquatic environment: a critical review. Environmental science & technology 47:2441–2456.

Huggett, D., J. Steevens, J. Allgood, C. Lutken, C. Grace, and W. Benson. 2001. Mercury in sediment and fish from North Mississippi Lakes. Chemosphere 42:923–929.

Irwin, R. J., M. VanMouwerik, L. Stevens, M. D. Seese, and W. Basham. 1997. Environmental Contaminants Encyclopedia. National Park Service, Water Resources Division, Fort Collins, Colorado.

Jay, J. A., and T. E. Ford. 2001. Water concentrations, bioaccumulation, and human health implications of heavy metals in Lake Chapala. Pages 123–136 *in* A. M. Hansen and M. van Afferden. The Lerma-Chapala Watershed: Evaluation and Management. Springer Science & Business Media New York.

Jeremiason, J. D., L. A. Kanne, T. A. Lacoe, M. Hulting, and M. F. Simcik. 2009. A comparison of mercury cycling in Lakes Michigan and Superior. Journal of Great Lakes Research 35:329–336.

Kelly, C., J. W. Rudd, and M. Holoka. 2003. Effect of pH on mercury uptake by an aquatic bacterium: implications for Hg cycling. Environmental Science and Technology 37:2941–2946.

Kelly, J. F. 2000. Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. Canadian Journal of Zoology 78:1–27.

Kessler, W.B. 1978. Attwater prairie chicken ecology in relation to agricultural and range management practices. Dissertation, Texas A&M University, College Station, USA.

Kihlström, J., C. Lundberg, and L. Hulth. 1971. Number of eggs and young produced by zebrafishes (*Brachydanio rerio*, Ham.-Buch.) spawning in water containing small amounts of phenylmercuric acetate. Environmental Research 4:355–359.

Kirubagaran, R., and K. Joy. 1992. Toxic effects of mercury on testicular activity in the freshwater teleost, *Clarias batrachus* (L.). Journal of Fish Biology 41:305–315.

Klamt, M., J. A. Davis, R. M. Thompson, R. Marchant, and T. R. Grant. 2015. Trophic relationships of the platypus: insights from stable isotope and cheek pouch dietary analyses. Marine and Freshwater Research 67:1196–1204.

Knopf, F. L., and R. M. Evans. 2004. American White Pelican (*Pelecanus erythrorhynchos*), The Birds of North America Online (A. Poole, Ed.). Ithaca: Cornell Lab of Ornithology; Retrieved from the Birds of North America Online: http://bna.birds.cornell.edu/bna/species/057 doi:10.2173/bna.57

Krapu, G. L., K. J. Reinecke, D. G. Jorde, and S. G. Simpson. 1995. Spring-staging ecology of midcontinent greater white-fronted geese. The Journal of Wildlife Management 59:736–746.

Lacerda, L. 1997. Global mercury emissions from gold and silver mining. Water, Air, & Soil Pollution 97:209–221.

Lawrence, J. S., and N. J. Silvy. 1995. Effect of predator control on reproductive success and hen survival of Attwater's prairie-chicken. Proceedings of the Southeastern Association of Fish and Wildlife Agencies 49:275–282.

Lehmann, V. W. 1941. Attwater's prairie-chicken: its life history and management. North American Fauna 57:1–65.

Lehmann, V. W., and R. Mauermann. 1963. Status of Attwater's prairie-chicken. The Journal of Wildlife Management 27:713–725.

Lewis, S., and R. Furness. 1991. Mercury accumulation and excretion in laboratory reared black-headed gull *Larus ridibundus* chicks. Archives of Environmental Contamination and Toxicology 21:316–320.

Lind, O. T., L. Dávalos-Lind, and T. E. Ford. 2000. Clay and the movement of metals into food fishes. Journal of Environmental Science & Health Part A 35:1171–1182.

Lindqvist, O. 1991. Mercury in the Swedish environment: recent research on causes, consequences and corrective methods. Water, Air and Soil Pollution 55:1–261.

Lockwood, M. A., C. P. Griffin, M. E. Morrow, C. J. Randel, and N. J. Silvy. 2005. Survival, movements, and reproduction of released captive-reared Attwater's prairie-chicken. Journal of Wildlife Management 69:1251–1258.

Marvin, C., S. Painter, and R. Rossmann. 2004. Spatial and temporal patterns in mercury contamination in sediments of the Laurentian Great Lakes. Environmental Research 95:351–362.

Mccrimmon, Jr., D. A., J. C. Ogden, and G. T. Bancroft. 2011. Great Egret (*Ardeaalba*), The Birds of North America Online (A. Poole, Ed.). Ithaca: Cornell Lab of Ornithology; Retrieved from the Birds of North America Online: http://bna.birds.cornell.edu/bna/species/570 doi:10.2173/bna.570

McCutchan, J. H., W. M. Lewis, C. Kendall, and C. C. McGrath. 2003. Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. Oikos 102:378–390.

Meili, M. 1997. Mercury in lakes and rivers. Metal ions in Biological Systems 34:21–52.

Mercado-Silva, N., J. Lyons, R. Moncayo-Estrada, P. Gesundheit, T. J. Krabbenhoft, D. L. Powell, and K. R. Piller. 2015. Stable isotope evidence for trophic overlap of sympatric Mexican Lake Chapala silversides (Teleostei: Atherinopsidae: *Chirostoma* spp.). Neotropical Ichthyology 13:389–400.

Mestre, J. E. 1997. Case study VIII – Lerma-Chapala Basin, Mexico. Pages 371-391 *in* R. Helmer and I Hespanhol, editors. Water Pollution Control - A Guide to the Use of Water Quality Management Principles. First edition. St Edmundsbury Press, Bury St Edmunds, Suffolk.

Mirlean, N., P. Baisch, I. Machado, and E. Shumilin. 2008. Mercury contamination of soil as the result of long-term phosphate fertilizer production. Bulletin of Environmental Contamination and Toxicology 81:305–308.

Moncayo-Estrada, R., O. T. Lind, and C. Escalera-Gallardo. 2011. Trophic interactions among sympatric zooplanktivorous fish species in volume change conditions in a large, shallow, tropical lake. Neotropical Ichthyology 9:169–176.

Moore, J. W., and B. X. Semmens. 2008. Incorporating uncertainty and prior information into stable isotope mixing models. Ecology Letters 11:470–480.

Mora, M. A. 2003. Heavy metals and metalloids in egg contents and eggshells of passerine birds from Arizona. Environmental Pollution 125:393–400.

Morrow, M. E., R. E. Chester, B. M. Drees, and J. E. Toepfer. 2013. Attwater's prairie-chicken brood survival–the invertebrate and red imported fire ant connection. Grouse News 45:25–27.

Morrow, M. E., T. A. Rossignol, and N. J. Silvy. 2004. Federal listing of prairie grouse: lessons from the Attwater's prairie-chicken. Wildlife Society Bulletin 32:112–118.

Morrow, M. E., R. E. Chester, S. E. Lehnen, B. M. Drees, and J. E. Toepfer. 2015. Indirect effects of red imported fire ants on Attwater's prairie-chicken brood survival. The Journal of Wildlife Management 79:898–906.

Morrow, M. E., R. S. Adamcik, J. D. Friday, and L. B. McKinney. 1996. Factors Affecting Attwater's Prairie-Chicken Decline on the Attwater Prairie Chicken National Wildlife Refuge. Wildlife Society Bulletin 24:593–601.

Nriagu, J. O. 1994. Mechanistic steps in the photoreduction of mercury in natural waters. Science of the Total Environment 154:1–8.

Nriagu, J. O., and J. M. Pacyna. 1988. Quantitative assessment of worldwide contamination of air, water and soils by trace metals. Nature 333:134–139.

O'Leary, M. H. 1988. Carbon isotopes in photosynthesis. Bioscience 38:328–336.

Pacyna, E.G., J.M. Pacyna, F. Steenhuisen, and S. Wilson. 2006. Global anthropogenic mercury emission inventory for 2000. Atmospheric Environment 40:4048–4063.

Pai, P., D. Niemi, and B. Powers. 2000. A North American inventory of anthropogenic mercury emissions. Fuel Processing Technology 65:101–115.

Parnell, A. C., D. L. Phillips, S. Bearhop, B. X. Semmens, E. J. Ward, J. W. Moore, A. L. Jackson, J. Grey, D. J. Kelly, and R. Inger. 2013. Bayesian stable isotope mixing models. Environmetrics 24:387–399.

Parsons, K. C., and T. L. Master. 2000. Snowy Egret (*Egretta thula*). In The Birds of North America Online. A. Poole, Edition. Cornell Lab of Ornithology, Ithaca, New York.

Pereira, A., B. van Hattum, J. de Boer, P. van Bodegom, C. Rezende, and W. Salomons. 2010. Trace elements and carbon and nitrogen stable isotopes in organisms from a tropical coastal lagoon. Archives of Environmental Contamination and Toxicology 59:464–477.

Peterson, B. J., and B. Fry. 1987. Stable isotopes in ecosystem studies. Annual Review of Ecology and Systematics 18:293–320.

Phillips, D. L., R. Inger, S. Bearhop, A. L. Jackson, J. W. Moore, A. C. Parnell, B. X. Semmens, and E. J. Ward. 2014. Best practices for use of stable isotope mixing models in food-web studies. Canadian Journal of Zoology 92:823–835.

Phillips, D. L. 2001. Mixing models in analyses of diet using multiple stable isotopes: a critique. Oecologia 127:166–170.

Phillips, D. L. 2012. Converting isotope values to diet composition: the use of mixing models. Journal of Mammalogy 93:342–352.

Phillips, D. L., and P. L. Koch. 2002. Incorporating concentration dependence in stable isotope mixing models. Oecologia 130:114–125.

Pirrone, N., G. J. Keeler, and J. O. Nriagu. 1996. Regional differences in worldwide emissions of mercury to the atmosphere. Atmospheric Environment 30:2981–2987.

Pirrone, N., S. Cinnirella, X. Feng, R. B. Finkelman, H. R. Friedli, J. Leaner, R. Mason, A. B. Mukherjee, G. B. Stracher, D. G. Streets, and K. Telmer. 2010. Global mercury emissions to the atmosphere from anthropogenic and natural sources. Atmospheric Chemistry and Physics 10:5951–5964.

Post, D. M. 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology 83:703–718.

Post, D. M., M. L. Pace, and N. G. Hairston. 2000. Ecosystem size determines food-chain length in lakes. Nature 405:1047–1049.

Power, M., G. Klein, K. Guiguer, and M. Kwan. 2002. Mercury accumulation in the fish community of a sub-Arctic lake in relation to trophic position and carbon sources. Journal of Applied Ecology 39:819–830.

Price, M. H. H., editor. 2013. Sub-lethal metal toxicity effects on salmonids: a review. Smithers, British Columbia.

Quinn, M. R., X. Feng, C. L. Folt, and C. P. Chamberlain. 2003. Analyzing trophic transfer of metals in stream food webs using nitrogen isotopes. Science of the Total Environment 317:73–89.

R Core Team, 2015. R: A Language and Environment for Statistical Computing.R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Rajwade, Y., D. K. Swain, K. N. Tiwari, U. C. Mohanty, and P. Goswami. 2015. Evaluation of field level adaptation measures under the climate change scenarios in rice based cropping system in India. Environmental Processes 2:669–687.

Ramsar. 2011. Ramsar Sites Information Service, Lago de Chapala. https://rsis.ramsar.org/ris/1973. Accessed 15 May 2016.

Ravichandran, M. 2004. Interactions between mercury and dissolved organic matter–a review. Chemosphere 55:319-331.

Rosales-Hoz, L., A. Carranza-Edwards, and M. Lopez-Hernandez. 2000. Heavy metals in sediments of a large, turbid tropical lake affected by anthropogenic discharges. Environmental Geology 39:378–383.

Roulet, M., M. Lucotte, N. Farella, G. Serique, H. Coelho, C. Sousa Passos, De Jesus da Silva, E., P. Scavone de Andrade, D. Mergler, and J. R. D. Guimarães. 1999. Effects of recent human colonization on the presence of mercury in Amazonian ecosystems. Water, Air, & Soil Pollution 112:297–313.

Roulet, M., M. Lucotte, R. Canuel, N. Farella, M. Courcelles, J. R. D. Guimaraes, D. Mergler, and M. Amorim. 2000. Increase in mercury contamination recorded in lacustrine sediments following deforestation in the central Amazon. Chemical Geology 165:243–266.

Scheuhammer, A. 1987. The chronic toxicity of aluminium, cadmium, mercury, and lead in birds: a review. Environmental Pollution 46:263–295.

Scheuhammer, A. 1991. Effects of acidification on the availability of toxic metals and calcium to wild birds and mammals. Environmental Pollution 71:329–375.

Scheuhammer, A., and P. Blancher. 1994. Potential risk to common loons (*Gavia immer*) from methylmercury exposure in acidified lakes. Hydrobiologia 279:445–455.

Scheuhammer, A.M., M.W. Meyer, M.B. Sandheinrich, and M. W. Murray. 2007. Effects of environmental methylmercury on the health of wild birds, mammals, and fish. AMBIO: a Journal of the Human Environment 36:12–19.

Schroeter, R. E., T. A. O'Rear, M. J. Young, and P. B. Moyle. 2015. The Aquatic Trophic Ecology of Suisun Marsh, San Francisco Estuary, California, During Autumn in a Wet Year. San Francisco Estuary and Watershed Science 13:1–18.

Scott, M. L. 1977. Effects of PCBs, DDT, and mercury compounds in chickens and Japanese quail. Federation Proceedings 36:1888–1893.

Seal, U. 1994. Attwater's prairie-chicken population and habitat viability assessment. Apple Valley, MN: Captive Breeding Specialist Group. International Union for the Conservation of Nature 80.

SEMARNAT. 2009. Estrategia General para el Rescate Ambiental y Sustentabilidad de la Cuenca Lerma-Chapala. Informe Final; Instituto Mexicano de Tecnología del Agua: Mexico, D.F.

Semmens, B. X., E. J. Ward, J. W. Moore, and C. T. Darimont. 2009. Quantifying inter- and intra-population niche variability using hierarchical Bayesian stable isotope mixing models. PloS One 4:e6187.

Shine, J. P., D. K. Ryan, and T. E. Ford. 1998. Annual cycle of heavy metals in a tropical lake-lake Chapala, Mexico. Journal of Environmental Science & Health Part A 33:23–43.

Silvy, N. J., C. P. Griffin, M. A. Lockwood, M. E. Morrow, and M. J. Peterson. 1999. Attwater's prairie-chicken: a lesson in conservation biology research. Pages 153–162 *in* W. D. Svedarsky, R. H. Hier, and N. J. Silvy, editors. The greater prairie chicken: a national look. Minnesota Agricultural Experiment Station, Miscellaneous Publication 99–1999, St. Paul, USA.

Smith, J. A., D. Mazumder, I. M. Suthers, and M. D. Taylor. 2013. To fit or not to fit: evaluating stable isotope mixing models using simulated mixing polygons. Methods in Ecology and Evolution 4:612–618.

Sorensen, J. A., G. E. Glass, K. W. Schmidt, J. K. Huber, and G. R. Rapp Jr. 1990. Airborne mercury deposition and watershed characteristics in relation to mercury concentrations in water, sediments, plankton, and fish of eighty northern Minnesota lakes. Environmental Science & Technology 24:1716–1727.

Sparling, D. W., and T. P. Lowe. 1996. Environmental hazards of aluminum to plants, invertebrates, fish, and wildlife. Pages 1-127 *in* G. W. Ware, editor. Reviews of Environmental Contamination and Toxicology. Springer-Verlag New York, Inc.

Stahl, J. L., J. L. Greger, and M. E. Cook. 1990. Breeding-hen and progeny performance when hens are fed excessive dietary zinc. Poultry Science 69:259–263.

Stock, B. C., and B. X. Semmens. 2013. MixSIAR GUI User Manual. Version 3.1. https://github.com/brianstock/MixSIAR/. doi:10.5281/zenodo.47719

Stoewsand, G. S., J. L. Anderson, W. H. Gutenmann, C. A. Bache, and D. J. Lisk. 1971. Eggshell thinning in Japanese quail fed mercuric chloride. Science 173:1030–1031.

Stong, T., C. A. Osuna, H. Shear, J. de Anda Sanchez, G. Ramírez, and J. de Jesús Díaz Torres. 2013. Mercury concentrations in common carp (*Cyprinus carpio*) in Lake Chapala, Mexico: A lakewide survey. Journal of Environmental Science and Health, Part A 48:1835–1841.

Syvaranta, J., H. Haemaelaeinen, and R. I. Jones. 2006. Within-lake variability in carbon and nitrogen stable isotope signatures. Freshwater Biology 51:1090–1102.

Teramura, A.H. 1983. Effects of ultraviolet-B radiation on the growth and yield of crop plants. Physiologia Plantarum 58:415–427.

Therrien, J., G. Fitzgerald, G. Gauthier, and J. Bêty. 2011. Diet–tissue discrimination factors of carbon and nitrogen stable isotopes in blood of Snowy Owl (*Bubo scandiacus*). Canadian Journal of Zoology 89:343–347.

Thompson, D. R., R. W. Furness, and S. A. Lewis. 1995. Diets and long-term changes in $\delta^{15}N$ and $\delta^{13}C$ values in northern fulmars *Fulmarus glacialis* from two northeast Atlantic Colonies. Marine Ecology Progress Series 125:3–11.

Tieszen, L.L., Boutton, T.W., Tesdahl, K.G. and N.A. Slade. 1983. Fractionation and turnover of stable carbon isotopes in animal tissues: implications for $\delta^{13}C$ analysis of diet. Oecologia 57:32–37.

Trasande, L., J. E. Cortes, P. J. Landrigan, M. I. Abercrombie, R. F. Bopp, and E. Cifuentes. 2010. Methylmercury exposure in a subsistence fishing community in Lake Chapala, Mexico: an ecological approach. Environmental Health 9:1–10.

Trujillo-Cárdenas, J. L., N. P. Saucedo-Torres, P. F. Zárate del Valle, N. Ríos-Donato, E. Mendizábal, and S. Gómez-Salazar. 2010. Speciation and sources of toxic metals in sediments of Lake Chapala, Mexico. Journal of the Mexican Chemical Society 54:79–87.

Ullrich, S.M., T. W. Tanton, and S. A. Abdrashitova. 2001. Mercury in the aquatic environment: a review of factors affecting methylation. Critical reviews in environmental science and technology 31:241–293.

USEPA. 1988. Ambient water quality criteria for aluminum. http://water.epa.gov/scitech/swguidance/standards /criteria/current/index.cfm. Accessed 16 July 2015.

USEPA. 2001. Water Quality Criterion for the Protection of Human Health. EPA-823-R01-001. USEPA Washington, DC.

USFDA. 2000. Guidance for Industry: Action Levels for Poisonous or Deleterious Substances in Human Food and Animal Feed. USFDA Washington, DC.

USFWS. 2010. Attwater's Prairie-Chicken Recovery Plan, Second Revision. Albuquerque, New Mexico.

USFWS. 1992. Attwater's Prairie Chicken Recovery Plan. Albuquerque, New Mexico.

Villamagna, A. M. 2009. Ecological effects of water hyacinth (*Eichhornia crassipes*) on Lake Chapala, Mexico. Dissertation, Virginia Polytechnic Institute and State University, Blacksburg, USA.

Wang, Q., D. Kim, D. D. Dionysiou, G. A. Sorial, and D. Timberlake. 2004. Sources and remediation for mercury contamination in aquatic systems—a literature review. Environmental Pollution 131:323–336.

Ward, E. J., B. X. Semmens, D. L. Phillips, J. W. Moore, and N. Bouwes. 2011. A quantitative approach to combine sources in stable isotope mixing models. Ecosphere 2:1–11.

Ware, R. A., P. M. Burkholder, and L. W. Chang. 1975. Ultrastructural changes in renal proximal tubules after chronic organic and inorganic mercury intoxication. Environmental Research 10:121–140.

Wassenaar, L., and K. A. Hobson. 2003. Comparative equilibration and online technique for determination of non-exchangeable hydrogen of keratins for use in animal migration studies. Isotopes in Environmental and Health Studies 39:211–217.

Watanabe, K., M. T. Monaghan, Y. Takemon, and T. Omura. 2008. Biodilution of heavy metals in a stream macroinvertebrate food web: evidence from stable isotope analysis. Science of the Total Environment 394:57–67.

Wayland, M., and K. A. Hobson. 2001. Stable carbon, nitrogen, and sulfur isotope ratios in riparian food webs on rivers receiving sewage and pulp-mill effluents. Canadian Journal of Zoology 79:5–15.

Werner, S. J., K. A. Hobson, S. L. Van Wilgenburg, and J. W. Fischer. 2016. Multi-Isotopic ($\delta^{2}H$, $\delta^{13}C$, $\delta^{15}N$) Tracing of Molt Origin for Red-Winged Blackbirds Associated with Agro-Ecosystems. PloS One 11:e0165996.

Wiemeyer, S. N., J. F. Miesner, P. L. Tuttle, E. C. Murphy, L. Sileo, and D. Withers. 2007. Mercury and selenium in American white pelicans breeding at Pyramid Lake, Nevada. Waterbirds 30:284–295.

Wiener, J. G., D. P. Krabbenhoft, G. H. Heinz, and A. M. Scheuhammer. 2003. Ecotoxicology of mercury. Pages 409-463 *in* D. J. Hoffman, B. A. Rattner, G. A. Burton, Jr., and J. Cairns, Jr., editors. Handbook of ecotoxicology. CRC Press LLC.

Wiener J., B. Knights, M. Sandheinrich, J. Jeremiason, M. Brigham, D. Engstrom, L. Woodruff, W. Cannon, and S. Balogh. 2006. Mercury in soils, lakes, and fish in Voyageurs National Park (Minnesota): importance of atmospheric deposition and ecosystem factors. Environmental Science & Technology 40:6261–6268.

Wiener, J., and D. Spry. 1996. Toxicological significance of mercury in freshwater fish. Pages 197-339 *in* W. N. Beyer, G. H. Heinz, and A. W. Redmon-Norwood.

Environmental contaminants in wildlife: Interpreting tissue concentrations. CRC Press LLC.

Wolf, N., S. D. Newsome, M. L. Fogel, and C. M. Del Rio. 2013. The relationship between drinking water and the hydrogen and oxygen stable isotope values of tissues in Japanese Quail (*Cortunix japonica*). The Auk 130:323–330.

Wolfe, M. F., S. Schwarzbach, and R. A. Sulaiman. 1998. Effects of mercury on wildlife: a comprehensive review. Environmental Toxicology and Chemistry 17:146–160.

Yaron, Z. 1995. Endocrine control of gametogenesis and spawning induction in the carp. Aquaculture 129:49–73.

APPENDIX

Appendix A

The Hg QA/QC results for water, sediments, fish, and feathers from Lake Chapala.

Sample Type	MDL (ng/g)[a]	Precision[b]	Accuracy[c]	
			Spike	CRM[d]
Water	0.0002	7.49 (*n =3*)	105 (*n = 2*)	94 (*n = 1*)
Sediment	0.68	14.5 (*n = 2*)	100 (*n = 2*)	95.5 (*n = 2*)
Fish	4.6	2.4 (*n = 5*)	99.2 (*n = 5*)	98.4 (*n = 18*)
Feathers	11.7	2.62 (*n = 4*)	100.7 (*n = 3*)	96.8 (*n = 6*)

[a] Method detection limit

[b] Relative percent difference (RPD)

[c] Average percent recovery

[d] Certified reference material (Water: NIST 1631d; Sediment: NRCC MESS-3; Fish and feathers: NIST 2976, NRCC DOLT-4)

Appendix B

The QA/QC results of water, sediment, and fish analysis from Lake Chapala.

	Sample	Al	Ba	Cu	Mn	Sr	V	Zn
MDL (µg/mL, µg/g)	Water (*n = 2*)	0.00255	0.000525	0.000125	0.000035	0.000275	0.00275	0.0002
	Sediment (*n = 1*)	0.93	0.093	0.187	0.093	0.019	0.467	0.187
	Fish (*n = 3*)	0.4487	0.0897	0.4487	0.1793	0.0449	0.4487	0.1793
Precision	Water (*n = 2*)	0	0.5	4.5	0.5	1.5	2.5	2
	Sediment (*n = 1*)	7	2	3	2	0	7	5
	Fish (*n = 3*)	15.67	1.67	20	2.33	4.33	6	2
Accuracy (Avg: Lab control sample, Spike, and SRM)	Water Al, Ba, Sr, V (*n = 6*)	92.33	98.67	96.25	100.25	98.83	100.5	100
	Cu, Mn, Zn (*n = 4*)							
	Sediment (*n = 3*)	87.67	106	96.33	99.67	76.67	82.33	96.33
	Fish Al, Cu, Mn, Zn (*n = 12*)	83.75	92.63	84.5	99.92	86.63	96.25	92.67
	Ba, Sr, V (*n = 8*)							

Appendix C

All information for vegetation collected from APCNWR during 2012–2013.

Material	*n*	Date collected	Month	Year	Season	Collection Site	Common Name	Scientific Name	Family	C3 or C4	Forb/grass/rush	Legume/non-legume	$\delta^{13}C$	$\delta^{15}N$	%C	%N
Vegetation	1	6/26/2012	June	2012	Summer	CAG	Cuman ragweed	*Ambrosia psilostachya*	Asteraceae	C3	Forb	Non-legume	-30.5	8.07	40.97	1.1
Vegetation	1	7/18/2013	July	2013	Spring	RWR	Cuman/western ragweed	*Ambrosia psilostachya*	Asteraceae	C3	Forb	Non-legume	-30.57	-0.89	41.4	1.27
Vegetation	1	7/18/2013	July	2013	Spring	CN1	Cuman/western ragweed	*Ambrosia psilostachya*	Asteraceae	C3	Forb	Non-legume	-29.97	-0.88	42.46	1.02
Vegetation	1	7/18/2013	July	2013	Spring	RN7	Cuman/western ragweed	*Ambrosia psilostachya*	Asteraceae	C3	Forb	Non-legume	-30.47	1.29	42.72	1.65
Vegetation	1	9/25/2013	September	2013	Fall	CN1	Cuman/western ragweed	*Ambrosia psilostachya*	Asteraceae	C3	Forb	Non-legume	-30.14	-2.07	41.44	1.73
Vegetation	1	9/25/2013	September	2013	Fall	RN7	Cuman/western ragweed	*Ambrosia psilostachya*	Asteraceae	C3	Forb	Non-legume	-28.56	-0.59	44.88	1.32
Vegetation	1	9/25/2013	September	2013	Fall	RWR	Cuman/western ragweed	*Ambrosia psilostachya*	Asteraceae	C3	Forb	Non-legume	-27.97	1.69	44.37	2.23
Vegetation	1	2/3/2012	February	2012	Winter	CAG	Scarlet pimpernel	*Anagallis arvensis*	Primulaceae	C3	Forb	Non-legume	-31.48	2.45	39.19	2.38
Vegetation	1	2/3/2012	February	2012	Winter	CAG	Scarlet pimpernel	*Anagallis arvensis*	Primulaceae	C3	Forb	Non-legume	-30.88	4.38	40.16	2.65
Vegetation	1	4/23/2012	April	2012	Spring	CAG	Scarlet pimpernel	*Anagallis arvensis*	Primulaceae	C3	Forb	Non-legume	-31.7	1.65	44.7	1.65
Vegetation	1	4/23/2012	April	2012	Spring	CAG	Scarlet pimpernel	*Anagallis arvensis*	Primulaceae	C3	Forb	Non-legume	-31.69	1.75	44.76	1.64
Vegetation	1	2/3/2012	February	2012	Winter	CAG	Carolina anemone	*Anemone caroliniana*	Ranunculaceae	C3	Forb	Non-legume	-29.02	1.61	42.6	2.42
Vegetation	1	2/3/2012	February	2012	Winter	CAG	Carolina anemone	*Anemone caroliniana*	Ranunculaceae	C3	Forb	Non-legume	-29.01	3.6	39.88	2.51
Vegetation	1	2/3/2012	February	2012	Winter	CAG	Carolina anemone	*Anemone caroliniana*	Ranunculaceae	C3	Forb	Non-legume	-28.92	3.81	43.86	2.83
Vegetation	1	2/8/2013	February	2013	Winter	DN4	Aster	*Symphyotrichum spp.*	Asteraceae	C3	Forb	Non-legume	-31.43	-0.19	38.12	2.74
Vegetation	1	2/8/2013	February	2013	Winter		Aster	*Symphyotrichum spp.*	Asteraceae	C3	Forb	Non-legume	-28.14	2.84	38.92	4.16
Vegetation	1	2/8/2013	February	2013	Winter		Aster Family	*Symphyotrichum spp.*	Asteraceae	C3	Forb	Non-legume	-28.78	4.36	28.35	3.32
Vegetation	1	4/5/2013	April	2013	Spring	RER	Longbract wild indigo	*Baptisia bracteata*	Fabaceae	C3	Forb	Legume	-27.88	0.75	46.02	4.27
Vegetation	1	4/23/2012	April	2012	Spring	CAG	Quaking grass	*Briza*	Poaceae	C3	Grass	Non-legume	-27.96	1.56	42.1	1.13
Vegetation	1	4/23/2012	April	2012	Spring	CAG	Wine cup	*Callirhoe involucrata*	Malvaceae	C3	Forb	Non-legume	-29.64	1.5	40.99	1.53
Vegetation	1	4/23/2012	April	2012	Spring	CAG	Wine cup	*Callirhoe involucrata*	Malvaceae	C3	Forb	Non-legume	-29.12	1.73	39.39	1.51
Vegetation	1	7/18/2013	July	2013	Spring	CN1	Partridge pea	*Chamaecrista fasciculata*	Fabaceae	C3	Forb	Legume	-31.33	-0.66	50.51	2.23
Vegetation	1	7/18/2013	July	2013	Spring	RWR	Partridge pea	*Chamaecrista fasciculata*	Fabaceae	C3	Forb	Legume	-30.44	-0.61	50.74	2.27
Vegetation	1	7/18/2013	July	2013	Spring	RWR	Partridge pea	*Chamaecrista fasciculata*	Fabaceae	C3	Forb	Legume	-30.44	-0.61	50.57	2.26
Vegetation	1	7/18/2013	July	2013	Spring	RN7	Partridge pea	*Chamaecrista fasciculata*	Fabaceae	C3	Forb	Legume	-30.23	-0.31	50.45	2.16
Vegetation	1	7/18/2013	July	2013	Spring	CN2	Partridge pea	*Chamaecrista fasciculata*	Fabaceae	C3	Forb	Legume	-31.29	0.61	48.1	2.32
Vegetation	1	9/25/2013	September	2013	Fall	CN2	Partridge pea	*Chamaecrista fasciculata*	Fabaceae	C3	Forb	Legume	-29.51	-1.67	49.4	2.32
Vegetation	1	9/25/2013	September	2013	Fall	RWR	Partridge pea	*Chamaecrista fasciculata*	Fabaceae	C3	Forb	Legume	-29.2	-0.89	49.49	1.92
Vegetation	1	9/25/2013	September	2013	Fall	CN2	Partridge pea	*Chamaecrista fasciculata*	Fabaceae	C3	Forb	Legume	-29.87	-0.62	47.49	1.67
Vegetation	1	9/25/2013	September	2013	Fall	RN7	Partridge pea	*Chamaecrista fasciculata*	Fabaceae	C3	Forb	Legume	-30.2	-0.28	50.39	2.08
Vegetation	1	4/23/2012	April	2012	Spring	CAG	Partridge pea	*Chamaecrista fasciculata*	Fabaceae	C3	Forb	Legume	-31.64	-1.34	46.24	2.41
Vegetation	1	2/8/2013	February	2013	Winter	DN4	Thistle	*Cirsium spp.*	Asteraceae	C3	Forb	Non-legume	-30.91	-2.16	32.98	2.2
Vegetation	1	4/23/2012	April	2012	Spring	CAG	Golden tickseed	*Coreopsis tinctoria*	Asteraceae	C3	Forb	Non-legume	-30.77	0.5	44.37	1.55
Vegetation	1	8/16/2012	August	2012	Summer	DN4	Hogwort	*Croton capitatus*	Euphorbiaceae	C3	Forb	Non-legume	-30.62	-1.8	43.83	0.98
Vegetation	1	7/18/2013	July	2013	Spring	CN2	Hogwort/croton	*Croton capitatus*	Euphorbiaceae	C3	Forb	Non-legume	-29.68	3.6	44.02	2.1
Vegetation	1	7/18/2013	July	2013	Spring	CN2	Hogwort/croton	*Croton capitatus*	Euphorbiaceae	C3	Forb	Non-legume	-28.79	7.07	44.39	2.99
Vegetation	1	9/25/2013	September	2013	Fall	DN4	Hogwort/croton	*Croton capitatus*	Euphorbiaceae	C3	Forb	Non-legume	-29.28	-1.58	44.8	1.88
Vegetation	1	9/25/2013	September	2013	Fall	RN7	Hogwort/croton	*Croton capitatus*	Euphorbiaceae	C3	Forb	Non-legume	-28.65	0.21	45.72	1.83
Vegetation	1	9/25/2013	September	2013	Fall	CN2	Hogwort/croton	*Croton capitatus*	Euphorbiaceae	C3	Forb	Non-legume	-27.09	1.79	45.42	1.88
Vegetation	1	9/25/2013	September	2013	Fall	CN2	Hogwort/croton	*Croton capitatus*	Euphorbiaceae	C3	Forb	Non-legume	-29.31	2.58	44.93	1.87
Vegetation	1	2/3/2012	February	2012	Winter	CAG	Rosette grass	*Dichanthelium spp.*	Poaceae	C3	Grass	Non-legume	-28.69	2.36	39.39	3.73
Vegetation	1	2/3/2012	February	2012	Winter	CAG	Rosette grass	*Dichanthelium spp.*	Poaceae	C3	Grass	Non-legume	-30.57	2.8	34.65	2.69
Vegetation	1	2/8/2013	February	2013	Winter	CN1	Rosette grass	*Dichanthelium spp.*	Poaceae	C3	Grass	Non-legume	-31.2	4.81	41.07	3.75

Appendix C Continued

Material	*n*	Date collected	Month	Year	Season	Collection Site	Common Name	Scientific Name	Family	C3 or C4	Forb/grass/rush	Legume/non-legume	$\delta^{13}C$	$\delta^{15}N$	%C	%N
Vegetation	1	7/18/2013	July	2013	Spring	RWR	Dichanthelium	*Dichanthelium oligosanthes*	Poaceae	C3	Grass	Non-legume	-29.75	-0.05	44.01	1.07
Vegetation	1	9/25/2013	September	2013	Fall	CN1	Dichanthelium	*Dichanthelium oligosanthes*	Poaceae	C3	Grass	Non-legume	-29.8	-1.38	43.45	0.86
Vegetation	1	7/18/2013	July	2013	Spring	CN1	Dichanthelium	*Dichanthelium spp.*	Poaceae	C3	Grass	Non-legume	-29.1	-2.26	43.22	1.12
Vegetation	1	7/18/2013	July	2013	Spring	RN7	Dichanthelium	*Dichanthelium spp.*	Poaceae	C3	Grass	Non-legume	-28.97	1.08	43.12	1.13
Vegetation	1	9/25/2013	September	2013	Fall	DN4	Dichanthelium	*Dichanthelium spp.*	Poaceae	C3	Grass	Non-legume	-28.32	-2.59	45.13	0.78
Vegetation	1	9/25/2013	September	2013	Fall	CN1	Dichanthelium	*Dichanthelium spp.*	Poaceae	C3	Grass	Non-legume	-29.86	-0.03	43.3	0.81
Vegetation	1	9/25/2013	September	2013	Fall	RN7	Dichanthelium	*Dichanthelium spp.*	Poaceae	C3	Grass	Non-legume	-29.62	0.75	44.51	1.64
Vegetation	1	7/18/2013	July	2013	Spring	DN4	Clasping coneflower	*Dracopis amplexicaulis*	Asteraceae	C3	Forb	Non-legume	-28.24	-1.43	47.9	1.85
Vegetation	1	7/18/2013	July	2013	Spring	CN1	Clasping coneflower	*Dracopis amplexicaulis*	Asteraceae	C3	Forb	Non-legume	-28.61	0.45	45.6	1.72
Vegetation	1	7/18/2013	July	2013	Spring	CN1	Clasping coneflower	*Dracopis amplexicaulis*	Asteraceae	C3	Forb	Non-legume	-28.61	0.53	45.88	1.73
Vegetation	1	6/26/2012	June	2012	Summer	CAG	Button eryngo	*Eryngium yuccifolium*	Apiaceae	C3	Forb	Non-legume	-26.85	5.71	41.58	1.12
Vegetation	1	7/18/2013	July	2013	Spring	CN2	Spurge	*Euphorbia spp.*	Euphorbiaceae	C3	Forb	Non-legume	-30.03	4.28	44.26	2.93
Vegetation	1	7/18/2013	July	2013	Spring	CN2	Spurge	*Euphorbia spp.*	Euphorbiaceae	C3	Forb	Non-legume	-29.91	4.78	45.67	3.43
Vegetation	1	4/5/2013	April	2013	Spring	DN4	Goldentop	*Euthamia spp.*	Asteraceae	C3	Forb	Non-legume	-30.83	0.07	45.66	2.59
Vegetation	1	8/16/2012	August	2012	Summer	CN2	Beeblossom	*Oenothera spp.*	Onagraceae	C3	Forb	Non-legume	-26.25	3.67	43.77	1.22
Vegetation	1	9/25/2013	September	2013	Fall	RWR	Beeblossom	*Oenothera spp.*	Onagraceae	C3	Forb	Non-legume	-27.13	-0.42	44.57	0.7
Vegetation	1	9/25/2013	September	2013	Fall	CN2	Beeblossom	*Oenothera spp.*	Onagraceae	C3	Forb	Non-legume	-27.57	2.5	45.46	1.44
Vegetation	1	9/25/2013	September	2013	Fall	CN2	Beeblossom	*Oenothera spp.*	Onagraceae	C3	Forb	Non-legume	-28.43	4.83	44.14	1.61
Vegetation	1	2/3/2012	February	2012	Winter	CAG	Carolina geranium	*Geranium carolinianum*	Geraniaceae	C3	Forb	Non-legume	-31.12	3.09	41.75	3.6
Vegetation	1	8/16/2012	August	2012	Summer	CN2	Sneezeweed	*Helenium amarum*	Asteraceae	C3	Forb	Non-legume	-28.5	1.63	45.21	1.27
Vegetation	1	4/23/2012	April	2012	Spring	CAG	Little barley	*Hordeum pusillum*	Poaceae	C3	Grass	Non-legume	-28.28	4.45	42.75	0.92
Vegetation	1	4/23/2012	April	2012	Spring	CAG	Star-grass	*Hypoxis*	Liliaceae	C3	Forb	Non-legume	-29.84	1.53	42.76	1.63
Vegetation	1	6/26/2012	June	2012	Summer	CAG	Annual marsh elder	*Iva annua*	Asteraceae	C3	Forb	Non-legume	-29.8	8.36	40.04	1.02
Vegetation	1	8/16/2012	August	2012	Summer	CN1	Annual marsh elder	*Iva annua*	Asteraceae	C3	Forb	Non-legume	-28.98	1.59	37.72	2.2
Vegetation	1	4/23/2012	April	2012	Spring	CAG	Rush	*Juncus*	Juncaceae	C3	Rush	Non-legume	-29.72	2.23	44.58	0.74
Vegetation	1	4/23/2012	April	2012	Spring	CAG	Dwarfdandelion	*Krigia*	Asteraceae	C3	Forb	Non-legume	-22.44	0.38	44.09	1.06
Vegetation	1	4/23/2012	April	2012	Spring	CAG	Dwarfdandelion	*Krigia*	Asteraceae	C3	Forb	Non-legume	-22.4	0.48	43.98	1.05
Vegetation	1	4/23/2012	April	2012	Spring	CAG	Pepperweed	*Lepidium*	Brassicaceae	C3	Forb	Non-legume	-27.65	5.68	38.31	2.38
Vegetation	1	2/8/2013	February	2013	Winter	RWR	Gayfeather	*Liatris mucronata*	Asteraceae	C3	Forb	Non-legume	-29.12	0.7	44.42	4.16
Vegetation	1	4/23/2012	April	2012	Spring	CAG	Toadflax	*Linaria*	Scrophulariaceae	C3	Forb	Non-legume	-29.9	0.16	39.96	0.68
Vegetation	1	6/26/2012	June	2012	Summer	CAG	Loosestrife	*Lythrum*	Lythraceae	C3	Forb	Non-legume	-26.91	3.19	44.65	1.14
Vegetation	1	2/8/2013	February	2013	Winter	CN1	Loosestrife	*Lythrum*	Lythraceae	C3	Forb	Non-legume	-31.91	6.72	37.27	4.02
Vegetation	1	4/23/2012	April	2012	Spring	CAG	Black medic	*Medicago lupulina*	Fabaceae	C3	Forb	Legume	-28.18	-0.81	44.48	2.79
Vegetation	1	2/3/2012	February	2012	Winter	CAG	Burclover	*Medicago polymorpha*	Fabaceae	C3	Forb	Legume	-30.29	-0.29	42.54	4.26
Vegetation	1	2/3/2012	February	2012	Winter	CAG	Burclover	*Medicago polymorpha*	Fabaceae	C3	Forb	Legume	-30.33	-0.02	44.88	4.59
Vegetation	1	2/3/2012	February	2012	Winter	CAG	Burclover	*Medicago polymorpha*	Fabaceae	C3	Forb	Legume	-29.78	0.06	42.99	3.64
Vegetation	1	4/23/2012	April	2012	Spring	CAG	Schrankia (sensitive briar)	*Mimosa nuttallii*	Fabaceae	C3	Forb	Legume	-29.5	0.42	44.09	4.05
Vegetation	1	7/18/2013	July	2013	Spring	CN1	Sensitive briar	*Mimosa nuttallii*	Fabaceae	C3	Forb	Legume	-30.54	-2.22	45.5	1.63
Vegetation	1	7/18/2013	July	2013	Spring	RWR	Sensitive briar	*Mimosa nuttallii*	Fabaceae	C3	Forb	Legume	-30.83	-1.98	45.48	1.77
Vegetation	1	7/18/2013	July	2013	Spring	AM150	Sensitive briar	*Mimosa nuttallii*	Fabaceae	C3	Forb	Legume	-29.28	-1.34	45.33	2.1
Vegetation	1	7/18/2013	July	2013	Spring	AM150	Sensitive briar	*Mimosa spp.*	Fabaceae	C3	Forb	Legume	-30.52	-2.17	46.12	2.34

Appendix C Continued

Material	*n*	Date collected	Month	Year	Season	Collection Site	Common Name	Scientific Name	Family	C3 or C4	Forb/grass/rush	Legume/non-legume	$\delta^{13}C$	$\delta^{15}N$	%C	%N
Vegetation	1	7/18/2013	July	2013	Spring	DN4	Sensitive briar	*Mimosa spp.*	Fabaceae	C3	Forb	Legume	-29.8	-0.72	45.5	1.6
Vegetation	1	9/25/2013	September	2013	Fall	RN7	Sensitive briar	*Mimosa spp.*	Fabaceae	C3	Forb	Legume	-30.86	-1.17	46.92	1.66
Vegetation	1	2/3/2012	February	2012	Winter	CAG	Neptunia	*Neptunia*	Fabaceae	C3	Forb	Legume	-25.74	-2.25	44.05	7.48
Vegetation	1	4/23/2012	April	2012	Spring	CAG	Neptunia (sensitive briar)	*Neptunia lutea*	Fabaceae	C3	Forb	Legume	-29.17	0.62	45.86	2.35
Vegetation	1	7/18/2013	July	2013	Spring	DN4	Yellow sensitive briar	*Neptunia lutea*	Fabaceae	C3	Forb	Legume	-29.99	-1.7	46.33	1.49
Vegetation	1	2/3/2012	February	2012	Winter	CAG	False dandelion	*Nothocalais*	Asteraceae	C3	Forb	Non-legume	-30.28	7.68	42.83	5.18
Vegetation	1	4/23/2012	April	2012	Spring	CAG	False Dandelion	*Nothocalais*	Asteraceae	C3	Forb	Non-legume	-31.38	0.85	42.12	1.24
Vegetation	1	2/3/2012	February	2012	Winter	CAG	Yellow false garlic/ crowpoison	*Nothoscordum bivalve*	Liliaceae	C3	Forb	Non-legume	-30.22	1.86	36.35	2.49
Vegetation	1	2/3/2012	February	2012	Winter	CAG	Yellow false garlic/ crowpoison	*Nothoscordum bivalve*	Liliaceae	C3	Forb	Non-legume	-29.2	1.86	40.81	3.17
Vegetation	1	2/3/2012	February	2012	Winter	CAG	Yellow false garlic/ crowpoison	*Nothoscordum bivalve*	Liliaceae	C3	Forb	Non-legume	-28.41	3.16	40.7	4.72
Vegetation	1	4/5/2013	April	2013	Spring	RER	Crowpoison	*Nothoscordum bivalve*	Liliaceae	C3	Forb	Non-legume	-28.23	0.19	31.18	1.98
Vegetation	1	4/23/2012	April	2012	Spring	CAG	Cutleaf evening primrose	*Oenothera laciniata*	Onagraceae	C3	Forb	Non-legume	-31.56	2.16	41.48	1.34
Vegetation	1	2/3/2012	February	2012	Winter	CAG	Woodsorrel	*Oxalis*	Oxalidaceae	C3	Forb	Non-legume	-30.33	5.34	35.96	2.81
Vegetation	1	2/3/2012	February	2012	Winter	CAG	Woodsorrel	*Oxalis*	Oxalidaceae	C3	Forb	Non-legume	-30.18	5.97	43.13	3.62
Vegetation	1	2/3/2012	February	2012	Winter	CAG	Woodsorrel	*Oxalis*	Oxalidaceae	C3	Forb	Non-legume	-30.32	6.56	44.12	3.66
Vegetation	1	4/23/2012	April	2012	Spring	CAG	Woodsorrel	*Oxalis*	Oxalidaceae	C3	Forb	Non-legume	-30.08	0.6	43.82	1.58
Vegetation	1	9/25/2013	September	2013	Fall	CN2	Oxalis	*Oxalis stricta/corniculata*	Oxalidaceae	C3	Forb	Non-legume	-30.13	3.79	45.16	2.89
Vegetation	1	9/25/2013	September	2013	Fall	CN2	Oxalis	*Oxalis stricta/corniculata*	Oxalidaceae	C3	Forb	Non-legume	-30.19	3.82	45.26	2.9
Vegetation	1	4/23/2012	April	2012	Spring	CAG	Canarygrass	*Phalaris*	Poaceae	C3	Grass	Non-legume	-29.28	0.59	39.67	1.08
Vegetation	1	4/23/2012	April	2012	Spring	CAG	Phlox	*Phlox*	Polemoniaceae	C3	Forb	Non-legume	-30.85	0	43	1.1
Vegetation	1	4/23/2012	April	2012	Spring	CAG	Plantain	*Plantago*	Plantaginaceae	C3	Forb	Non-legume	-23	2.36	42.61	1.14
Vegetation	1	4/5/2013	April	2013	Spring	RN4	Macartney rose	*Rosa bracteata*	Rosaceae	C3	Forb	Non-legume	-27.99	-6.2	43.34	2.33
Vegetation	1	4/23/2012	April	2012	Spring	CAG	Dewberry	*Rubus spp.*	Rosaceae	C3	Forb	Non-legume	-28.96	1.88	45.24	1.19
Vegetation	1	9/25/2013	September	2013	Fall	CN1	Coneflower/Prairie coneflower	*Rudbeckia spp.*	Asteraceae	C3	Forb	Non-legume	-30.06	1.69	45.91	1.19
Vegetation	1	6/26/2012	June	2012	Summer	CAG	Wild petunia	*Ruellia*	Acanthaceae	C3	Forb	Non-legume	-28.11	7.8	41.48	1.72
Vegetation	1	7/18/2013	July	2013	Spring	DN4	Wild petunia	*Ruellia*	Acanthaceae	C3	Forb	Non-legume	-29.3	-0.77	41.16	1.07
Vegetation	1	4/23/2012	April	2012	Spring	CAG	Fringeleaf wild petunia	*Ruellia humilis*	Acanthaceae	C3	Forb	Non-legume	-29.68	0.26	39.62	2.24
Vegetation	1	9/25/2013	September	2013	Fall	CN1	Wild petunia	*Ruellia spp.*	Acanthaceae	C3	Forb	Non-legume	-29.42	-0.7	41.35	1.96
Vegetation	1	4/23/2012	April	2012	Spring	CAG	Dock	*Rumex spp.*	Polygonaceae	C3	Forb	Non-legume	-29.84	1.08	43.08	0.95
Vegetation	1	4/23/2012	April	2012	Spring	CAG	Meadow pink	*Sabatia campestris*	Gentianaceae	C3	Forb	Non-legume	-29.58	2.52	44.71	1.42
Vegetation	1	4/23/2012	April	2012	Spring	CAG	Meadow pink	*Sabatia campestris*	Gentianaceae	C3	Forb	Non-legume	-29.57	2.84	44.82	1.44
Vegetation	1	4/23/2012	April	2012	Spring	CAG	Blue-eyed grass	*Sisyrinchium spp.*	Iridaceae	C3	Forb	Non-legume	-29.97	2.68	42.15	1.53
Vegetation	1	4/23/2012	April	2012	Spring	CAG	Blue-eyed grass	*Sisyrinchium spp.*	Iridaceae	C3	Forb	Non-legume	-29.52	4.37	45.05	1.22
Vegetation	1	4/23/2012	April	2012	Spring	CAG	Multibloom tephrosia	*Tephrosia onobrychoides*	Fabaceae	C3	Forb	Legume	-28.38	-0.58	45.41	2.68
Vegetation	1	2/3/2012	February	2012	Winter	CAG	Spiderwort	*Tradescantia*	Commelinaceae	C3	Forb	Non-legume	-29.47	7.9	39.04	6.09
Vegetation	1	2/3/2012	February	2012	Winter	CAG	Spiderwort	*Tradescantia*	Commelinaceae	C3	Forb	Non-legume	-29.34	8.06	43.44	6.94
Vegetation	1	4/23/2012	April	2012	Spring	CAG	Venus looking glass	*Triodanis perfoliata*	Campanulaceae	C3	Forb	Non-legume	-29.91	-0.31	34.34	1.05
Vegetation	1	4/23/2012	April	2012	Spring	CAG	Venus look glass (white)	*Triodanis perfoliata*	Campanulaceae	C3	Forb	Non-legume	-29.04	4.26	45.03	1.56
Vegetation	1	4/23/2012	April	2012	Spring	CAG	Venus look glass (white)	*Triodanis perfoliata*	Campanulaceae	C3	Forb	Non-legume	-29.04	4.36	41.61	1.46
Vegetation	1	2/3/2012	February	2012	Winter	CAG	Vetch flowering	*Vicia*	Fabaceae	C3	Forb	Legume	-29.97	0.14	42.37	2.69
Vegetation	1	2/3/2012	February	2012	Winter	CAG	Vetch	*Vicia*	Fabaceae	C3	Forb	Legume	-30.81	0.26	44.68	5.44
Vegetation	1	2/3/2012	February	2012	Winter	CAG	Deer pea	*Vicia ludoviciana*	Fabaceae	C3	Forb	Legume	-31.1	1.98	40.79	3.92

Appendix D

All information for arthropods collected at the APCNWR in 2012–2013.

Material	*n*	Collection Date	Month	Year	Season	Collection Site	Order	Suborder	Family	Species	Adult/ Immature	$\delta^{13}C$	$\delta^{15}N$	%C	%N
Arthropod	40	4/23/2012	April	2012	Spring	CAG	Araneae					-24	6.87	46.46	12.35
Arthropod	6	6/26/2012	April	2012	Spring	CAG	Araneae					-25.34	6.09		
Arthropod	27	6/26/2012	April	2012	Spring	CAG	Araneae					-25.24	5.67		
Arthropod	1	5/16/2012	July	2012	Summer	CN1	Araneae					-24.69	7.3	50.04	11.67
Arthropod	1	7/24/2012	June	2012	Summer	CN2	Araneae					-25.3	8.97	48.65	11.55
Arthropod	11	7/24/2012	July	2012	Summer	CN2	Araneae					-22.17	6.65	46.45	11.81
Arthropod	2	8/16/2012	March	2012	Spring	CN6	Araneae					-20.44	6.39	46.41	12.63
Arthropod	1	4/23/2012	May	2012	Spring	CN8	Araneae					-24.43	6.83	47.32	11.74
Arthropod	1	11/2/2012	May	2012	Spring	CN8	Araneae					-24.38	6.83	48.98	11.38
Arthropod	2	7/18/2013	June	2012	Summer	CN9	Araneae					-25.24	8.43	49.54	12.15
Arthropod	1	7/17/2012	June	2012	Summer	DN1	Araneae					-22.3	7.19	49.68	11.25
Arthropod	2	7/24/2012	June	2012	Summer	DN1	Araneae					-23	7.33	48.81	11.57
Arthropod	3	7/17/2012	June	2012	Summer	DN1	Araneae					-22.4	6.74	48.48	11.43
Arthropod	4	7/17/2012	June	2012	Summer	DN1	Araneae					-26.48	7.48	48.9	11.26
Arthropod	1	7/17/2012	April	2012	Spring	GAC	Araneae					-21.88	7.83	46.82	12.05
Arthropod	1	5/24/2012	May	2012	Spring	PWR	Araneae					-19.61	5.02	49.23	10.83
Arthropod	1	5/16/2012	May	2012	Spring	RN2	Araneae					-28	7.25	51.52	10.61
Arthropod	1	5/16/2012	May	2012	Spring	RN3	Araneae					-22.79	6.6	50.84	10.81
Arthropod	15	8/16/2012	June	2012	Summer	RN7	Araneae					-17.14	6.99	53.27	9.82
Arthropod	1	4/23/2012	April	2012	Spring	ZIG	Araneae					-25.24	7.84	47.8	12.39
Arthropod	1	4/23/2012	April	2012	Spring	ZIG	Araneae					-24.99	6.51	48.96	11.42
Arthropod	1	4/23/2012	April	2012	Spring	ZIG	Araneae					-26.05	7.54	49.44	10.96
Arthropod	8	4/23/2012	June	2012	Summer	AM50	Coleoptera					-27.1	4.8	52.35	10.31
Arthropod	55	6/26/2012	April	2012	Spring	CAG	Coleoptera		chrysomelidae	colaspis favosa		-22.46	3.81	46.39	11.14
Arthropod	1	5/16/2012	July	2012	Summer	CN1	Coleoptera		curculionidea			-26.64	11.37	50.95	10.64
Arthropod	2	6/4/2012	August	2012	Summer	CN12	Coleoptera		curculionidea			-26.76	4.16	49.67	10.65
Arthropod	1	4/23/2012	August	2012	Summer	CN12	Coleoptera					-25.88	3.83	53.08	9.77
Arthropod	8	7/17/2012	November	2012	Fall	CN2	Coleoptera		curculionidea			-26.49	2.78	49.85	9.83
Arthropod	1	4/23/2012	November	2012	Fall	CN2	Coleoptera					-26.21	5.29	52.16	9.67
Arthropod	62	4/23/2012	November	2012	Fall	CN2	Coleoptera					-27.27	7.95	47.67	10.72
Arthropod	5	4/23/2012	November	2012	Fall	CN2	Coleoptera		chrysomelidae			-24.22	6.31	54.98	8.81
Arthropod	1	3/3/2012	July	2012	Summer	CN2	Coleoptera		elateridae			-21.09	9.2	49.76	10.82
Arthropod	4	11/2/2012	July	2012	Summer	CN2	Coleoptera		chrysomelidae	griburius		-25.26	7.35	49.69	11.49
Arthropod	1	11/2/2012	May	2012	Spring	CN8	Coleoptera		elateridae			-20.6	8.85	47.33	10.67
Arthropod	1	7/17/2012	June	2012	Summer	CN9	Coleoptera		chrysomelidae	cryptocephalus		-25.95	3.68	49.84	10.75
Arthropod	1	5/16/2012	May	2012	Spring	PWR	Coleoptera		curculionidea			-26.86	4.41	49.82	10.01
Arthropod	1	5/16/2012	May	2012	Spring	PWR	Coleoptera		chrysomelidae			-17.93	2.37	47.13	10.26
Arthropod	1	5/16/2012	August	2012	Summer	RN1	Coleoptera					-27.87	4.52	50.9	10.49
Arthropod	1	5/16/2012	May	2012	Spring	RN3	Coleoptera		chrysomelidae			-22.17	7.84	50.01	10.06
Arthropod	1	6/26/2012	May	2012	Spring	RN3	Coleoptera		chrysomelidae			-25.2	5.56	58.18	13.46

Appendix D Continued

Material	*n*	Collection Date	Month	Year	Season	Collection Site	Order	Suborder	Family	Species	Adult/ Immature	$\delta^{13}C$	$\delta^{15}N$	%C	%N
Arthropod	1	4/23/2012	August	2012	Summer	RWR	Coleoptera					-29.19	4.02	51.86	10.25
Arthropod	1	4/23/2012	April	2012	Spring	ZIG	Coleoptera		chrysomelidae	diabrotica undecimpunctata		-23.42	6.42	49.79	10.68
Arthropod	1	4/23/2012	April	2012	Spring	ZIG	Coleoptera		curculionidea	hypera sp.		-23.81	6.9	50.25	10.33
Arthropod	1	4/23/2012	April	2012	Spring	ZIG	Coleoptera		curculionidea	sitona sp.		-29.76	4.4	51.23	9.8
Arthropod	1	4/23/2012	April	2012	Spring	ZIG	Coleoptera		curculionidea			-29.03	6.32	48.9	8.74
Arthropod	3	6/4/2012	April	2012	Spring	ZIG	Coleoptera		carabidae	notioba sp.		-21.5	4.97	53.53	8.95
Arthropod	4	11/2/2012	July	2012	Summer	CN1	Diptera					-20.33	5.46	48.72	11.01
Arthropod	14	11/2/2012	July	2012	Summer	CN2	Diptera					-24.13	9.14	48.42	11.49
Arthropod	3	7/17/2012	April	2012	Spring	GAC	Diptera		syrphidae			-26.94	5.26	47.63	9.63
Arthropod	1	4/23/2012	April	2012	Spring	ZIG	Diptera		syrphidae			-26.51	5.96	48.23	9.84
Arthropod	9	9/25/2013	November	2012	Fall	CN1	Hemiptera	Heteroptera				-25.85	3.75	50.81	10.93
Arthropod	1	6/26/2012	July	2012	Summer	CN1	Hemiptera	Heteroptera	pentatomidae			-23.03	3.36	49.94	11.07
Arthropod	1	4/23/2012	August	2012	Summer	CN12	Hemiptera	Heteroptera	pentatomidae			-26.67	1.52	52.52	10.28
Arthropod	1	4/23/2012	August	2012	Summer	CN12	Hemiptera	Heteroptera	pentatomidae			-26.25	1.55	49.78	11.61
Arthropod	1	4/23/2012	August	2012	Summer	CN12	Hemiptera	Heteroptera	pentatomidae			-25.37	0.58	43.55	12.61
Arthropod	2	7/18/2013	August	2012	Summer	CN12	Hemiptera	Heteroptera	pentatomidae			-25.37	0.54	48.34	11.46
Arthropod	5	9/25/2013	August	2012	Summer	CN12	Hemiptera	Heteroptera				-27.81	0.94	48.91	11.59
Arthropod	1	9/25/2013	August	2012	Summer	CN12	Hemiptera	Heteroptera	pentatomidae			-25.9	0.56	49.19	12.09
Arthropod	1	11/2/2012	November	2012	Fall	CN2	Hemiptera	Heteroptera				-26.41	2.01	49.22	12.04
Arthropod	1	5/16/2012	November	2012	Fall	CN2	Hemiptera	Heteroptera				-25.58	2.94	51.49	11.15
Arthropod	1	5/16/2012	November	2012	Fall	CN2	Hemiptera	Auchenorrhyncha				-27.33	5.77	52.8	9.56
Arthropod	1	11/2/2012	November	2012	Fall	CN2	Hemiptera	Heteroptera				-27.46	3.15	51.03	10.92
Arthropod	1	5/16/2012	November	2012	Fall	CN2	Hemiptera	Heteroptera	pentatomidae			-12.74	2.14	49.48	12.16
Arthropod	1	5/16/2012	November	2012	Fall	CN2	Hemiptera	Heteroptera	pentatomidae			-12.18	0.96	51.39	10.87
Arthropod	1	7/24/2012	June	2012	Summer	CN2	Hemiptera	Heteroptera	alydibae			-26.6	2.09	49.64	11.71
Arthropod	1	11/2/2012	July	2012	Summer	CN2	Hemiptera	Heteroptera	pentatomidae	euchistus		-25.6	3.26	50.68	11.89
Arthropod	5	11/2/2012	July	2012	Summer	CN2	Hemiptera	Heteroptera	pentatomidae	euchistus		-25.36	2.3	50.99	11.58
Arthropod	1	11/2/2012	July	2012	Summer	CN2	Hemiptera	Heteroptera	pentatomidae			-27.56	1.84	49.74	11.66
Arthropod	1	4/23/2012	July	2012	Summer	CN2	Hemiptera	Heteroptera	pentatomidae		immature	-26.83	0.59	49.47	12.36
Arthropod	1	11/2/2012	May	2012	Spring	CN8	Hemiptera	Auchenorrhyncha	cicadidae			-25.57	4.24	47.25	10.92
Arthropod	1	8/23/2012	May	2012	Spring	CN8	Hemiptera	Auchenorrhyncha	cicadidae			-27.7	0.65	50.96	8.97
Arthropod	6	7/17/2012	August	2012	Summer	DN1	Hemiptera	Heteroptera	pentatomidae			-27.07	3.45	53.11	10.29
Arthropod	1	7/17/2012	June	2012	Summer	DN1	Hemiptera	Heteroptera	pentatomidae		immature	-26.66	1.81	47.29	10.7
Arthropod	1	7/17/2012	June	2012	Summer	DN1	Hemiptera	Auchenorrhyncha				-27.5	1.5	50.35	10.05
Arthropod	2	6/26/2012	June	2012	Summer	DNR	Hemiptera	Heteroptera	lygaeidae			-26.98	1.87		
Arthropod	4	7/17/2012	June	2012	Summer	DNR	Hemiptera	Auchenorrhyncha	cicadellidae			-13.43	1.63		
Arthropod	1	7/17/2012	April	2012	Spring	GAC	Hemiptera	Auchenorrhyncha	membracidae			-28.16	0.09	49.89	9.98
Arthropod	1	7/24/2012	April	2012	Spring	GAC	Hemiptera	Heteroptera	miridae			-27.14	5.98	50.88	11.5

Appendix D Continued

Material	*n*	Collection Date	Month	Year	Season	Collection Site	Order	Suborder	Family	Species	Adult/ Immature	$\delta^{13}C$	$\delta^{15}N$	%C	%N
Arthropod	1	4/23/2012	March	2012	Spring	KN2	Hemiptera	Auchenorrhyncha	cicadellidae			-26.93	-0.46	50.51	9.85
Arthropod	1	9/25/2013	November	2012	Fall	KWR	Hemiptera	Heteroptera				-13.67	1.63	53.97	9.21
Arthropod	1	11/2/2012	May	2012	Spring	KWR	Hemiptera	Heteroptera	pentatomidae	oebalus		-11.43	5.45	53.5	10.07
Arthropod	1	3/3/2012	May	2012	Spring	RN3	Hemiptera	Heteroptera	pentatomidae			-24.28	3.44	51.57	9.42
Arthropod	1	6/26/2012	May	2012	Spring	RN3	Hemiptera	Heteroptera	miridae			-25.78	6.97	60.7	12.61
Arthropod	1	6/26/2012	March	2012	Spring	RN4	Hemiptera	Heteroptera	pentatomidae	oebalus sp.		-21.39	3.62	51.31	10.03
Arthropod	1	11/2/2012	November	2012	Fall	RN7	Hemiptera	Heteroptera	pentatomidae			-24.25	3.19	55.29	8.77
Arthropod	1	7/24/2012	November	2012	Fall	RN7	Hemiptera	Heteroptera	coreidae			-25.43	2.39	52.77	9.08
Arthropod	1	7/24/2012	November	2012	Fall	RN7	Hemiptera	Heteroptera				-25.55	6.37	50.34	11.53
Arthropod	1	4/23/2012	November	2012	Fall	RWR	Hemiptera	Heteroptera				-25.27	2.53	49	11.34
Arthropod	7	6/4/2012	April	2012	Spring	ZIG	Hemiptera	Heteroptera	miridae			-28.38	7.41		
Arthropod	1	4/23/2012	April	2012	Spring	ZIG	Hemiptera	Heteroptera	cydnidae			-30.84	5.14	55.96	8.76
Arthropod	10+	6/4/2012	April	2012	Spring	ZIG	Hemiptera	Auchenorrhyncha	membracidae			-28.06	-1.36	50.29	9.75
Arthropod	5	6/4/2012	April	2012	Spring	ZIG	Hemiptera	Heteroptera	pentatomidae		immature	-26.06	2.31	49.54	11.15
Arthropod	1	7/17/2012	July	2012	Summer	CN1	Hymenoptera		chalcididae			-26.22	7.2	49.2	13.14
Arthropod	1	11/2/2012	July	2012	Summer	CN2	Hymenoptera		vespidae	polistes sp.		-23.94	7.26	48.77	12.52
Arthropod	1	11/2/2012	July	2012	Summer	CN2	Hymenoptera		apidae	apis melifera		-24.04	1.95	49	11.84
Arthropod	3	8/16/2012	July	2012	Summer	CN2	Hymenoptera		braconidae			-29.79	9.27	49.46	12.79
Arthropod	5	8/16/2012	July	2012	Summer	CN2	Hymenoptera		mutilliade			-23.34	13.5	49.13	13.43
Arthropod	2	8/16/2012	July	2012	Summer	CN2	Hymenoptera		mutilliade			-12.73	9.19	49.05	13.71
Arthropod	10	8/16/2012	July	2012	Summer	CN2	Hymenoptera		mutilliade			-25.25	9.47	49.41	12.53
Arthropod	1	8/16/2012	July	2012	Summer	CN2	Hymenoptera		megachilidae	megachile sp.		-25.8	3.09	49.71	13.23
Arthropod	1	6/26/2012	May	2012	Spring	KN4	Hymenoptera					-27.13	3.26	48.03	12.35
Arthropod	1	7/24/2012	July	2012	Summer	RN7	Hymenoptera		halictidae			-24.43	2.1	49.3	12.59
Arthropod	1	7/24/2012	July	2012	Summer	RN7	Hymenoptera		sphecoidea			-25.75	8.92	46.81	8.88
Arthropod	28	5/16/2012	June	2012	Summer	AM150	Lepidoptera				immature	-17.14	2.86	44.67	7.98
Arthropod	2	7/17/2012	July	2012	Summer	CN1	Lepidoptera		pieridae			-27.56	1.7	48.06	13.84
Arthropod	1	7/24/2012	July	2012	Summer	CN1	Lepidoptera		pieridae			-27.84	2.26	47.89	13.83
Arthropod	7	11/2/2012	July	2012	Summer	CN1	Lepidoptera		nymphcelidae			-13.57	4.66	49.1	13.24
Arthropod	2	4/23/2012	July	2012	Summer	CN1	Lepidoptera		pieridae			-27.21	5.19	48.34	13.03
Arthropod	3	8/16/2012	July	2012	Summer	CN1	Lepidoptera		nymphcelidae			-29.07	7.67		
Arthropod	1	11/2/2012	July	2012	Summer	CN1	Lepidoptera		arctiidae			-23.24	6.41	51.34	11.77
Arthropod	1	8/16/2012	July	2012	Summer	CN1	Lepidoptera				immature	-20.62	1.27	45.81	7.09
Arthropod	1	5/16/2012	November	2012	Fall	CN2	Lepidoptera					-27.96	10.34	54.18	9.58
Arthropod	2	7/17/2012	June	2012	Summer	DNR	Lepidoptera				immature	-30.07	0	51.32	9.47
Arthropod	1	6/4/2012	May	2012	Spring	KWR	Lepidoptera				moths-poor quality	-28.91	5.17	51.98	11.03
Arthropod	1	5/16/2012	August	2012	Summer	RN1	Lepidoptera					-28.48	5.16	50.59	11.19
Arthropod	1	5/16/2012	May	2012	Spring	RN2	Lepidoptera					-29.92	9.04	41.74	11.79

Appendix D Continued

Material	*n*	Collection Date	Month	Year	Season	Collection Site	Order	Suborder	Family	Species	Adult/ Immature	$\delta^{13}C$	$\delta^{15}N$	%C	%N
Arthropod	1	5/16/2012	May	2012	Spring	RN2	Lepidoptera					-30.05	8.99	44.4	10.84
Arthropod	5	11/2/2012	November	2012	Fall	RN7	Lepidoptera					-22.63	4.47	50.06	11.8
Arthropod	1	3/3/2012	November	2012	Fall	RN7	Lepidoptera					-28.22	5.8	49.65	11.46
Arthropod	15	8/23/2012	April	2012	Spring	ZIG	Lepidoptera				immature	-30.8	4.29	53.31	9.03
Arthropod	4	5/31/2012	April	2012	Spring	ZIG	Lepidoptera				moths-poor quality	-29.12	6.85	48.14	5.16
Arthropod	1	5/16/2012	June	2012	Summer	AM150	Mantodea					-19.38	6.26	47.8	12.39
Arthropod	1	5/16/2012	June	2012	Summer	AM150	Neuroptera					-21.29	3.96	48.35	7.54
Arthropod	1	6/26/2012	November	2012	Fall	CN2	Neuroptera					-25.49	4.05	50.03	10.45
Arthropod	1	4/23/2012	June	2012	Summer	AM150	Orthoptera		acrididae			-16.76	1.61	46.85	11.61
Arthropod	7	4/23/2012	May	2012	Spring	AM250	Orthoptera					-23.12	7.77	51.71	8.85
Arthropod	5	4/23/2012	May	2012	Spring	AM50	Orthoptera					-25.74	4.93	51.2	10.27
Arthropod	3	4/23/2012	June	2012	Summer	AM50	Orthoptera					-25.62	2.37	46.89	9.59
Arthropod	2	4/23/2012	June	2012	Summer	AM50	Orthoptera					-24.56	2.37	47.19	10.38
Arthropod	1	6/26/2012	August	2012	Summer	CER	Orthoptera					-26.66	3.77	50.23	10.57
Arthropod	1	6/26/2012	June	2012	Summer	CE-R	Orthoptera					-25.77	3.4	46.62	9.66
Arthropod	2	6/26/2012	June	2012	Summer	CE-R	Orthoptera					-25.75	3.49	49.92	10.88
Arthropod	1	11/2/2012	July	2012	Summer	CN1	Orthoptera		acrididae			-15.58	2.33	47.64	12.04
Arthropod	20	8/16/2012	July	2012	Summer	CN1	Orthoptera		acrididae			-26.66	3.48	45.7	9.34
Arthropod	2	8/16/2012	July	2012	Summer	CN1	Orthoptera		tettigoniidae			-23.25	3.67	46.95	10.57
Arthropod	15	7/18/2013	August	2012	Summer	CN12	Orthoptera					-23.91	1.09	46.37	10.78
Arthropod	2	9/25/2013	August	2012	Summer	CN12	Orthoptera					-22.62	2.1	50.44	10.42
Arthropod	1	6/26/2012	November	2012	Fall	CN2	Orthoptera					-21.88	4.57	50.96	11
Arthropod	1	6/26/2012	November	2012	Fall	CN2	Orthoptera					-16.54	2.51	50.78	10.45
Arthropod	11	6/26/2012	November	2012	Fall	CN2	Orthoptera					-27.48	9.76	47.49	11.26
Arthropod	1	7/24/2012	June	2012	Summer	CN2	Orthoptera		acrididae			-27.06	1.8	59.19	12.67
Arthropod	1	7/24/2012	June	2012	Summer	CN2	Orthoptera		acrididae			-19.72	5.42	43.93	11.81
Arthropod	1	4/23/2012	July	2012	Summer	CN2	Orthoptera		tettigoniidae			-15.47	5.55	59.24	9.39
Arthropod	2	4/23/2012	May	2012	Spring	CN8	Orthoptera		acrididae			-27.44	4.15	44.27	10.67
Arthropod	1	4/5/2013	May	2012	Spring	CN8	Orthoptera		acrididae			-28.25	4.44	50.34	11.73
Arthropod	2	7/18/2013	May	2012	Spring	CN8	Orthoptera					-25.35	4.15	48.06	10.85
Arthropod	1	7/17/2012	June	2012	Summer	DN1	Orthoptera		tettigoniidae			-26.81	4.82	57.09	11.92
Arthropod	1	5/29/2012	June	2012	Summer	DN1	Orthoptera		acrididae			-27.56	3.96	47.13	10.99
Arthropod	1	11/2/2012	June	2012	Summer	DN1	Orthoptera		acrididae			-28.61	4.44	55.25	11.47
Arthropod	1	5/16/2012	June	2012	Summer	DN1	Orthoptera					-28.31	4.24	46.79	10.83
Arthropod	1	5/16/2012	March	2012	Spring	DN4	Orthoptera		acrididae		immature	-26.81	1.91	49.65	10.46
Arthropod	2	6/26/2012	May	2012	Spring	DN5	Orthoptera					-28.64	8.96	45.09	9.74
Arthropod	3	7/24/2012	April	2012	Spring	GAC	Orthoptera		tettigoniidae		immature	-25.45	3.17	46.93	10.67
Arthropod	1	11/2/2012	April	2012	Spring	GAC	Orthoptera		gryllidae		immature	-26.99	4.52	46.86	9.37

Appendix D Continued

Material	*n*	Collection Date	Month	Year	Season	Collection Site	Order	Suborder	Family	Species	Adult/ Immature	$\delta^{13}C$	$\delta^{15}N$	%C	%N
Arthropod	1	11/2/2012	April	2012	Spring	GAC	Orthoptera		acrididae		immature	-23.17	2.48	45.69	10.37
Arthropod	1	8/16/2012	April	2012	Spring	GAC	Orthoptera		tettigoniidae		immature	-26.16	3.42	45.62	10.93
Arthropod	2	4/23/2012	April	2012	Spring	GAC	Orthoptera		tettigoniidae		immature	-25.8	3.73	47.54	10.86
Arthropod	1	9/25/2013	March	2012	Spring	KN2	Orthoptera		tettigoniidae		immature	-24.15	4.54	50.09	9.85
Arthropod	1	11/2/2012	May	2012	Spring	KWR	Orthoptera		tettigoniidae			-25.43	4.69	55.09	11.7
Arthropod	2	5/23/2012	May	2012	Spring	KWR	Orthoptera		tettigoniidae			-25.86	4.24	51.11	9.97
Arthropod	1	5/16/2012	May	2012	Spring	PWR	Orthoptera		tettigoniidae			-25.26	2.96	60.02	14.32
Arthropod	1	5/16/2012	May	2012	Spring	PWR	Orthoptera					-24.77	2.41	46.3	11.88
Arthropod	1	5/16/2012	May	2012	Spring	RN3	Orthoptera		tettigoniidae			-24.94	4.03	62.57	13.23
Arthropod	1	6/26/2012	May	2012	Spring	RN3	Orthoptera		tettigoniidae			-25.62	4.13	46.53	11.08
Arthropod	1	6/26/2012	May	2012	Spring	RN3	Orthoptera		acrididae			-28.67	11.29	44.93	10.54
Arthropod	10	11/2/2012	May	2012	Spring	RN3	Orthoptera					-28.42	1.98	52.2	9.17
Arthropod	1	6/26/2012	May	2012	Spring	RN3	Orthoptera		acrididae			-28.53	3.47	45.49	12.15
Arthropod	1	6/26/2012	May	2012	Spring	RN3	Orthoptera					-27.01	3.67	48.2	9.51
Arthropod	1	4/23/2012	July	2012	Summer	RN7	Orthoptera		acrididae			-26.9	2.95	31.84	7.36
Arthropod	1	4/23/2012	November	2012	Fall	RWR	Orthoptera					-15.82	3.19	49.6	11.9
Arthropod	4	8/16/2012	August	2012	Summer	RWR	Orthoptera					-26.55	2.46	48.49	10.67
Arthropod	6	4/5/2013	April	2012	Spring	ZIG	Orthoptera		acrididae			-29.01	7.57	84.75	23.05
Arthropod	1	4/5/2013	April	2012	Spring	ZIG	Orthoptera		acrididae		immature	-29.15	3.14	65.36	15.74
Arthropod	9	9/25/2013	April	2012	Spring	ZIG	Orthoptera		gryllidae		immature	-26.29	4.95	46.62	8.28
Arthropod	5	9/25/2013	April	2012	Spring	ZIG	Orthoptera		tettigoniidae		immature	-28.33	3.57	47.84	9.55
Arthropod	7	9/25/2013	April	2012	Spring	ZIG	Orthoptera		acrididae		immature	-28.87	5.2	44.76	10.37
Arthropod	7	7/18/2013	April	2012	Spring	ZIG	Orthoptera		gryllidae		immature	-25.78	4.94	48.36	8.83
Arthropod	2	7/18/2013	April	2012	Spring	ZIG	Orthoptera		tettigoniidae		immature	-26.97	3.74	47.57	9.88
Arthropod	4	6/26/2012	April	2012	Spring	ZIG	Orthoptera		acrididae		immature	-28.37	5.31	47.38	10.88
Arthropod	2	7/24/2012	April	2012	Spring	ZIG	Orthoptera		gryllidae		immature	-28.09	5.38	47.07	10.05
Arthropod	5	4/23/2012	July	2012	Summer	CN1	Phasmida					-26.59	3.55	46.23	10.2
Arthropod	1	7/17/2012	June	2012	Summer	DN1	Phasmida					-27.85	7.44	49.17	11.89
Arthropod	34	4/23/2014	April	2012	Spring	ZIG	Sternorrhyncha		aphididae			-28.93	-0.49	51.72	6.82
Arthropod	1	3/3/2012	July	2013	Summer	AM150	Araneae					-20.08	5.84	48.12	11.69
Arthropod	1	7/17/2012	September	2013	Fall	CN2	Araneae					-18.45	6.2	47.56	12.24
Arthropod	1	6/26/2012	September	2013	Fall	CN1	Coleoptera					-25.38	2.81	51.54	9.85
Arthropod	1	7/24/2012	September	2013	Fall	CN1	Coleoptera					-18.36	2.67	53.77	8.11
Arthropod	5	7/24/2012	September	2013	Fall	CN1	Coleoptera					-25.63	0.11	53.17	9.64
Arthropod	2	4/23/2012	July	2013	Summer	CN2	Coleoptera					-28.91	5.47	51.89	10.34
Arthropod	1	7/24/2012	July	2013	Summer	RN7	Coleoptera					-27.85	6.22	51.27	10.32
Arthropod	4	5/16/2012	July	2013	Summer	AM150	Hemiptera	Auchenorrhyncha				-27.61	0.74	51.96	9.84
Arthropod	1	3/3/2012	April	2013	Spring	CN6	Hemiptera					-23.8	10.31	47.51	12.11

Appendix D Continued

Material	*n*	Collection Date	Month	Year	Season	Collection Site	Order	Suborder	Family	Species	Adult/ Immature	$\delta^{13}C$	$\delta^{15}N$	%C	%N
Arthropod	1	4/23/2012	July	2013	Summer	RWR	Hemiptera	Heteroptera				-18.28	6.62	51.08	10.79
Arthropod	15	8/16/2012	September	2013	Fall	RN7	Lepidoptera					-27.17	4.91	50.8	11.86
Arthropod	11	7/18/2013	September	2013	Fall	CN1	Mantodea					-17.38	4.69	51.71	10.79
Arthropod	4	5/16/2012	July	2013	Summer	AM150	Orthoptera					-16.75	0.95	47.87	11.93
Arthropod	1	5/16/2012	April	2013	Spring	CN1	Orthoptera					-28.18	1.53	46.84	11.49
Arthropod	1	5/16/2012	September	2013	Fall	CN1	Orthoptera					-19.91	0.99	48.72	11.62
Arthropod	1	5/16/2012	September	2013	Fall	CN1	Orthoptera					-14.97	0.56	51.76	10.37
Arthropod	1	6/26/2012	July	2013	Summer	CN2	Orthoptera					-26.78	0.87	50	10.26
Arthropod	3	5/16/2012	September	2013	Fall	DN4	Orthoptera					-25.09	-1.4	51.4	10.01
Arthropod	1	5/16/2012	April	2013	Spring	RER	Orthoptera					-25.86	1.79	46.47	11.44

Appendix E

All information for Attwater's prairie-chicken blood, feather, and fecal samples collected.

Material	*n*	Year	Month	Season	Location	Sex	Age at time of collection (Months)	$\delta^{13}C$	$\delta^{15}N$	Historic/ Contemporary
Blood	1	2011	November	Fall	APCNWR	F	6	-24.87	5.99	
Blood	1	2011	August	Summer	APCNWR	F	15	-27.42	3.78	
Blood	1	2011	November	Fall	APCNWR	F	17	-18.35	6.68	
Blood	1	2011	November	Fall	APCNWR	M	17	-22.58	6.14	
Blood	1	2011	November	Fall	APCNWR	M	17	-20.21	6.15	
Blood	1	2011	November	Fall	APCNWR	M	17	-19.56	6.62	
Blood	1	2011	November	Fall	APCNWR	M	18	-24.55	6.13	
Blood	1	2011	November	Fall	APCNWR	M	18	-24.83	5.66	
Blood	1	2012	June	Summer	Goliad - Papaloti	F	1	-25.03	7.14	
Blood	1	2012	October	Fall	APCNWR	F	5	-24.13	6.9	
Blood	1	2012	January	Winter	APCNWR	F	7	-28.35	7.07	
Blood	1	2012	January	Winter	APCNWR	F	7	-28.41	6.48	
Blood	1	2012	January	Winter	APCNWR	F	7	-24.22	6.9	
Blood	1	2012	January	Winter	APCNWR	F	7	-24.66	6.54	
Blood	1	2012	January	Winter	APCNWR	F	7	-23.02	7	
Blood	1	2012	January	Winter	APCNWR	F	7	-28.09	6.04	
Blood	1	2012	January	Winter	APCNWR	F	7	-27.75	6.65	
Blood	1	2012	January	Winter	APCNWR	F	7	-24.53	6.58	
Blood	1	2012	January	Winter	APCNWR	F	8	-27.96	6.41	
Blood	1	2012	January	Winter	APCNWR	F	8	-28.3	6.98	
Blood	1	2012	January	Winter	APCNWR	F	8	-28.42	7.05	
Blood	1	2012	January	Winter	APCNWR	F	8	-24.99	6.49	
Blood	1	2012	January	Winter	APCNWR	F	8	-22.15	6.98	

Appendix E Continued

Material	*n*	Year	Month	Season	Location	Sex	Age at time of collection (Months)	$\delta^{13}C$	$\delta^{15}N$	Historic/ Contemporary
Blood	1	2012	January	Winter	APCNWR	F	8	-25.72	6.58	
Blood	1	2012	October	Fall	APCNWR	F	16	-23.39	6.65	
Blood	1	2012	November	Fall	APCNWR	F	17	-18.59	6.83	
Blood	1	2012	November	Fall	APCNWR	F	17	-20.99	6.8	
Blood	1	2012	January	Winter	APCNWR	F	19	-25.13	7.25	
Blood	1	2012	January	Winter	APCNWR	F	19	-28.24	6.32	
Blood	1	2012	January	Winter	APCNWR	F	20	-25.81	6.42	
Blood	1	2012	January	Winter	APCNWR	F	20	-23.96	7.01	
Blood	1	2012	November	Fall	APCNWR	F	30	-19.3	6.98	
Blood	1	2012	June	Summer	Goliad - Papaloti	M	1	-24.37	8.66	
Blood	1	2012	July	Summer	APCNWR	M	2	-23.93	7.36	
Blood	1	2012	October	Fall	APCNWR	M	5	-19.96	6.77	
Blood	1	2012	January	Winter	APCNWR	M	7	-23.9	6.79	
Blood	1	2012	January	Winter	APCNWR	M	7	-24.54	6.47	
Blood	1	2012	January	Winter	APCNWR	M	8	-24.07	6.81	
Blood	1	2012	January	Winter	APCNWR	M	8	-26.87	6.74	
Blood	1	2012	January	Winter	APCNWR	M	8	-25.56	6.67	
Blood	1	2012	January	Winter	APCNWR	M	8	-26.59	6.7	
Blood	1	2012	July	Summer	APCNWR	M	14	-25.49	8.59	
Blood	1	2012	July	Summer	APCNWR	M	14	-24.58	7.32	
Blood	1	2012	January	Winter	APCNWR	M	19	-26.9	6.23	
Blood	1	2012	January	Winter	APCNWR	M	19	-23.97	6.85	

Appendix E Continued

Material	*n*	Year	Month	Season	Location	Sex	Age at time of collection (Months)	$\delta^{13}C$	$\delta^{15}N$	Historic/ Contemporary
Blood	1	2012	January	Winter	APCNWR	M	20	-25.22	7.26	
Blood	1	2012	January	Winter	APCNWR	M	20	-28.6	6.83	
Blood	1	2012	July	Summer	APCNWR	M	26	-25.23	7.44	
Blood	1	2012	October	Fall	APCNWR	M	29	-23.91	7.54	
Blood	1	2012	January	Winter	APCNWR	M	31	-25.49	6.43	
Blood	1	2012	July	Summer	APCNWR	M	38	-25.52	8.43	
Blood	1	2012	January	Winter	APCNWR	M	43	-27.92	6.21	
Blood	1	2013	January	Winter	APCNWR	F	7	-24.01	6.01	
Blood	1	2013	January	Winter	APCNWR	F	7	-24.74	5.74	
Blood	1	2013	January	Winter	APCNWR	F	7	-23.51	5.78	
Blood	1	2013	January	Winter	APCNWR	F	7	-23.85	5.8	
Blood	1	2013	January	Winter	APCNWR	F	7	-22.44	5.79	
Blood	1	2013	January	Winter	APCNWR	F	8	-22.98	6.03	
Blood	1	2013	January	Winter	APCNWR	F	8	-22.9	6.35	
Blood	1	2013	January	Winter	APCNWR	F	8	-23.28	5.64	
Blood	1	2013	January	Winter	APCNWR	F	8	-22.63	5.87	
Blood	1	2013	January	Winter	APCNWR	F	8	-27.6	8.2	
Blood	1	2013	January	Winter	APCNWR	F	8	-22.56	5.88	
Blood	1	2013	January	Winter	APCNWR	F	8	-24.81	5.93	
Blood	1	2013	January	Winter	APCNWR	F	8	-24	7.22	
Blood	1	2013	January	Winter	APCNWR	F	8	-22.85	5.66	
Blood	1	2013	January	Winter	APCNWR	F	8	-22.78	6.12	
Blood	1	2013	January	Winter	APCNWR	F	8	-23.55	5.69	

Appendix E Continued

Material	*n*	Year	Month	Season	Location	Sex	Age at time of collection (Months)	$\delta^{13}C$	$\delta^{15}N$	Historic/ Contemporary
Blood	1	2013	January	Winter	APCNWR	F	8	-24.64	5.63	
Blood	1	2013	January	Winter	APCNWR	F	19	-24.83	6.92	
Blood	1	2013	January	Winter	APCNWR	F	19	-23.02	5.75	
Blood	1	2013	January	Winter	APCNWR	F	20	-22.02	6.21	
Blood	1	2013	January	Winter	APCNWR	F	20	-24.38	6.67	
Blood	1	2013	January	Winter	APCNWR	F	20	-23.8	5.59	
Blood	1	2013	January	Winter	APCNWR	F	32	-23.33	5.94	
Blood	1	2013	January	Winter	APCNWR	F	32	-23.11	5.68	
Blood	1	2013	January	Winter	APCNWR	F	32	-22.39	6.23	
Blood	1	2013	January	Winter	APCNWR	F	32	-22	7.32	
Blood	1	2013	January	Winter	APCNWR	F	44	-24.38	5.97	
Blood	1	2013	January	Winter	APCNWR	M	7	-23.59	6.63	
Blood	1	2013	January	Winter	APCNWR	M	7	-24.08	6.22	
Blood	1	2013	January	Winter	APCNWR	M	7	-24.19	6.09	
Blood	1	2013	January	Winter	APCNWR	M	8	-24.01	6.08	
Blood	1	2013	January	Winter	APCNWR	M	19	-24.09	5.17	
Blood	1	2013	January	Winter	APCNWR	M	19	-21.66	6.87	
Blood	1	2013	January	Winter	APCNWR	M	56	-23.52	5.95	
Feather	1	1894	May	Spring	Placedo, Tx	F		-21.7	9.04	Historic
Feather	1	1894	April	Spring	Placedo, Tx	M		-21.02	7.79	Historic
Feather	1	1894	April	Spring	Placedo, Tx	M		-20.2	8.85	Historic
Feather	1	1894	May	Spring	Placedo, Tx	M		-23.16	8.22	Historic
Feather	1	1910	October	Fall	Victoria, Tx	F		-22.72	9.24	Historic

Appendix E Continued

Material	*n*	Year	Month	Season	Location	Sex	Age at time of collection (Months)	$\delta^{13}C$	$\delta^{15}N$	Historic/ Contemporary
Feather	1	1910	October	Fall	Victoria, Tx	F		-19.62	9.5	Historic
Feather	1	1911	January	Winter	Victoria, Tx	F		-22.63	8.9	Historic
Feather	1	1911	January	Winter	Victoria, Tx	F		-21.66	9.88	Historic
Feather	1	1911	January	Winter	Victoria, Tx	M		-23.34	7.67	Historic
Feather	1	1911	January	Winter	Victoria, Tx	M		-21.06	9.77	Historic
Feather	1	1936	September	Fall	Eagle Lake, Tx	F		-19.83	11.62	Historic
Feather	1	1936	September	Fall	Eagle Lake, Tx	M		-18.65	9.26	Historic
Feather	1	1937	July	Summer	Callahan Ranch	M		-22.06	8.15	Historic
Feather	1	1937	October	Fall	Colorado, Co Tx	M		-21.02	10.18	Historic
Feather	1	1937	September	Fall	Eagle Lake, Tx	M		-19.56	9.12	Historic
Feather	1	1938	February	Winter	Hallahan Ranch	F		-21.9	8.88	Historic
Feather	1	1938	January	Winter	Colorado, Co Tx	F		-21.71	10.37	Historic
Feather	1	1938	April	Spring	Sealy, Tx	M		-21.98	10.27	Historic
Feather	1	1938	July	Summer	Eagle Lake, Tx	M		-18.95	8.87	Historic
Feather	1	1938	March	Spring	Colorado, Co Tx	M		-20.92	10.15	Historic
Feather	1	1939	July	Summer	Eagle Lake, Tx	M		-20.61	8.91	Historic
Feather	1	1940	March	Spring	Tivoli, Tx	F		-21.85	9.28	Historic
Feather	1	1941	April	Spring	Eagle Lake, Tx	M		-20.11	8.21	Historic
Feather	1	1951	August	Summer	Eagle Lake, Tx	M		-21.07	9.97	Historic

Appendix E Continued

Material	*n*	Year	Month	Season	Location	Sex	Age at time of collection (Months)	$\delta^{13}C$	$\delta^{15}N$	Historic/ Contemporary
Feather	1	1953	April	Spring	Eagle Lake, Tx	M		-22.3	10.58	Historic
Feather	1	1954	May	Spring	Refugio, Tx	M		-21.08	9.98	Historic
Feather	1	1956	December	Winter	Lissie, Tx	F		-20.61	11.37	Historic
Feather	1	1965	March	Spring	Houston, Tx	M		-23.49	8.66	Historic
Feather	1	1965	March	Spring	Houston, Tx	M		-22.96	8.07	Historic
Feather	1	1965	March	Spring	Houston, Tx	M		-23.8	8.91	Historic
Feather	1	2004	August	Summer	APCNWR	M	27	-23.3	9.48	Contemporary
Feather	1	2005	September	Fall	TNC	F	28	-26.05	6.95	Contemporary
Feather	1	2005	September	Fall	TNC	M	27	-24.23	7.22	Contemporary
Feather	1	2006	January	Winter	APCNWR	F	33	-17.38	8.17	Contemporary
Feather	1	2006	June	Summer	TNC	F	37	-22.77	7	Contemporary
Feather	1	2006	March	Spring	TNC	F	34	-25.24	7.89	Contemporary
Feather	1	2006	February	Winter	APCNWR	M	32	-24.8	7.31	Contemporary
Feather	1	2006	February	Winter	APCNWR	M	32	-21.75	7.53	Contemporary
Feather	1	2006	June	Summer	APCNWR	M	25	-26.41	5.28	Contemporary
Feather	1	2007	September	Fall	APCNWR	F	39	-22.7	8.24	Contemporary
Feather	1	2007	March	Spring	TNC	M	46	-26.01	7.22	Contemporary
Feather	1	2007	March	Spring	TNC	M	46	-25.97	8.49	Contemporary
Feather	1	2008	August	Summer	APCNWR	F	27	-22.61	10.85	Contemporary
Feather	1	2008	October	Fall	APCNWR	F	29	-15.48	9.03	Contemporary
Feather	1	2008	November	Fall	APCNWR	M	53	-14.25	8.24	Contemporary
Feather	1	2008	October	Fall	APCNWR	M	41	-14.62	8.46	Contemporary

Appendix E Continued

Material	*n*	Year	Month	Season	Location	Sex	Age at time of collection (Months)	$\delta^{13}C$	$\delta^{15}N$	Historic/ Contemporary
Feather	1	2009	August	Summer	APCNWR	F	39	-16.13	8.05	Contemporary
Feather	1	2009	July	Summer	APCNWR	F	97	-21.03	4.37	Contemporary
Feather	1	2009	November	Fall	APCNWR	F	30	-17.2	10.42	Contemporary
Feather	1	2009	November	Fall	APCNWR	F	30	-17.57	10.53	Contemporary
Feather	1	2009	September	Fall	APCNWR	F	28	-21.67	10.94	Contemporary
Feather	1	2009	January	Winter	APCNWR	M	104	-18.63	7.1	Contemporary
Feather	1	2009	November	Fall	APCNWR	M	66	-20.09	7.6	Contemporary
Feather	1	2009	September	Fall	APCNWR	M	28	-20.51	9.8	Contemporary
Feather	1	2010	September	Fall	APCNWR	M	28	-13.37	9.15	Contemporary
Feather	1	2010	September	Fall	APCNWR	M	40	-23.75	8.39	Contemporary
Feather	1	2011	August	Summer	APCNWR	F	27	-18.2	7.57	Contemporary
Feather	1	2011	August	Summer	APCNWR	F	27	-17.36	8.2	Contemporary
Feather	1	2011	January	Winter	Goliad	M	32	-22.11	9.95	Contemporary
Feather	1	2011	July	Summer	APCNWR	M	25	-23.98	7.23	Contemporary
Feather	1	2011	July	Summer	APCNWR	M	25	-23.99	7.26	Contemporary
Feather	1	2012	July	Summer	APCNWR	F	26	-23.27	8.22	Contemporary
Feather	1	2012	January	Winter	APCNWR	M	44	-15.13	7.44	Contemporary
Feather	1	2012	July	Summer	APCNWR	M	26	-23.52	8.19	Contemporary
Feather	1	2012	March	Spring	APCNWR	M	34	-17.29	7.37	Contemporary
Feather	1	2013	January	Winter	APCNWR	F	44	-21.41	8.01	Contemporary
Feather	1	2013	January	Winter	APCNWR	F	128	-19.23	7.02	Contemporary
Feather	1	2013	May	Spring	APCNWR	F	36	-19.44	7.61	Contemporary
Feather	1	2013	January	Winter	APCNWR	M	56	-21.3	9.43	Contemporary

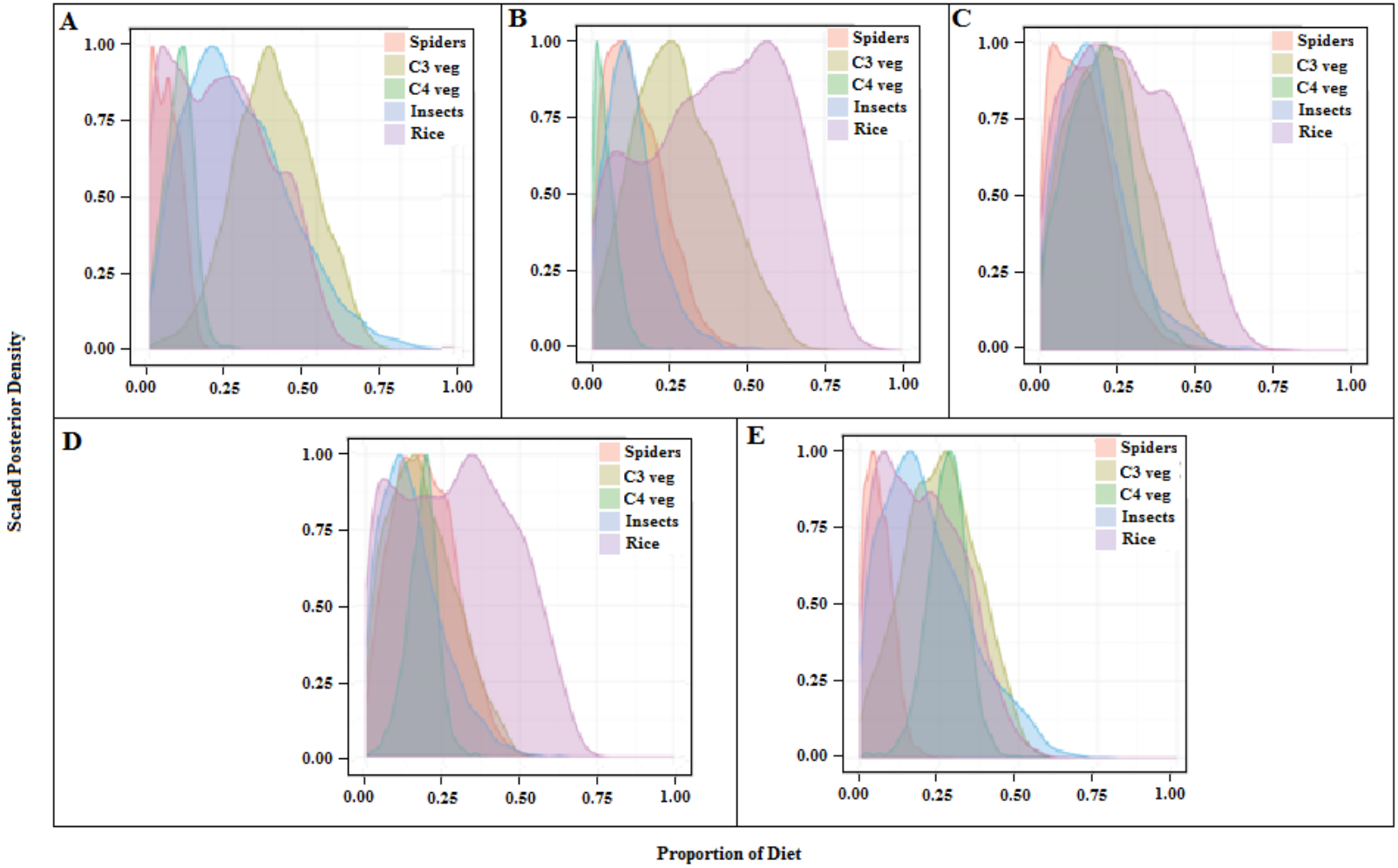
Appendix E Continued

Material	*n*	Year	Month	Season	Location	Sex	Age at time of collection (Months)	$\delta^{13}C$	$\delta^{15}N$	Historic/ Contemporary
Feather	1	2013	March	Spring	APCNWR	M	58	-20.04	7.77	Contemporary
Feces	1	2012	January	Winter	APCNWR	F	8	-29.32	3.93	
Feces	1	2012	January	Winter	APCNWR	F	19	-30.33	3.8	
Feces	1	2012	January	Winter	APCNWR	F	20	-29.67	4.21	
Feces	1	2012	January	Winter	APCNWR	F	7	-30.43	3.07	
Feces	1	2012	January	Winter	APCNWR	F	7	-30.56	2.63	
Feces	1	2012	January	Winter	APCNWR	F	8	-30.19	4.46	
Feces	1	2012	January	Winter	APCNWR	F	19	-30.33	6.4	
Feces	1	2012	January	Winter	APCNWR	F	7	-30.15	4.36	
Feces	1	2012	January	Winter	APCNWR	M	20	-30.58	3.28	
Feces	1	2012	January	Winter	APCNWR	M	43	-30.4	3.14	
Feces	1	2012	January	Winter	APCNWR	M	8	-30.18	3.72	
Feces	1	2012	January	Winter	APCNWR	M	20	-30.06	5.99	
Feces	1	2012	January	Winter	APCNWR	M	20	-30.29	3.76	
Feces	1	2012	January	Winter	APCNWR	M	8	-29.19	2.38	

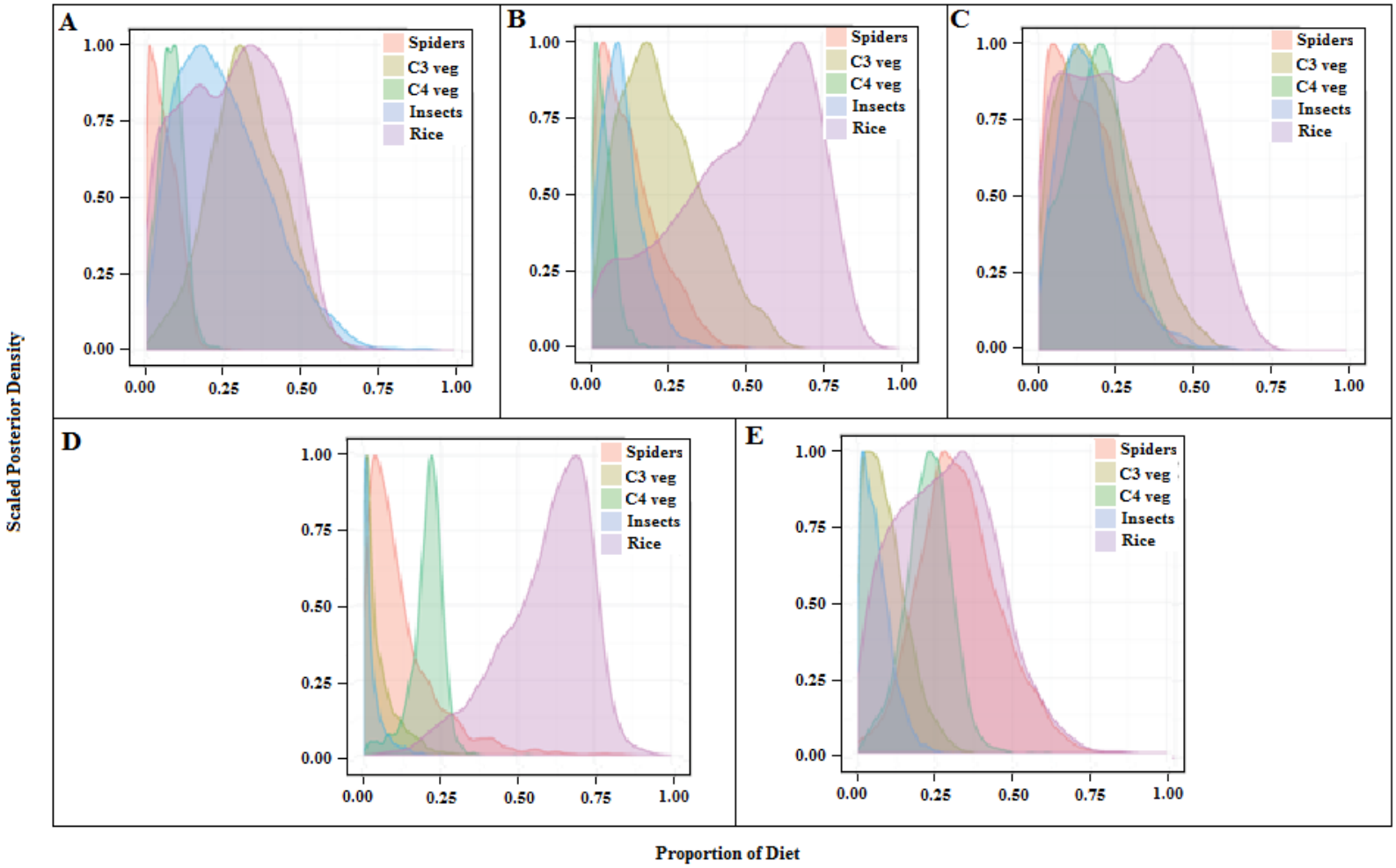
Appendix F

Results using proxy trophic discrimination values (TDF) from Caut et al. (2009), Hobson and Clark (1992), and Healy et al. (2017), and using diet sources from model three (C_3 and C_4 vegetation, spiders, Insects, and Rice)

Caut et al. TDF values - (Blood: $\delta^{13}C$= 1.26 ±1.1, $\delta^{15}N$= 2.37±0.6; Feathers: $\delta^{13}C$= 2.16±1.53, $\delta^{15}N$= 3.84±1.14). Combined blood (A), summer blood (B), fall blood (C), and historic (D) and contemporary (E) feathers

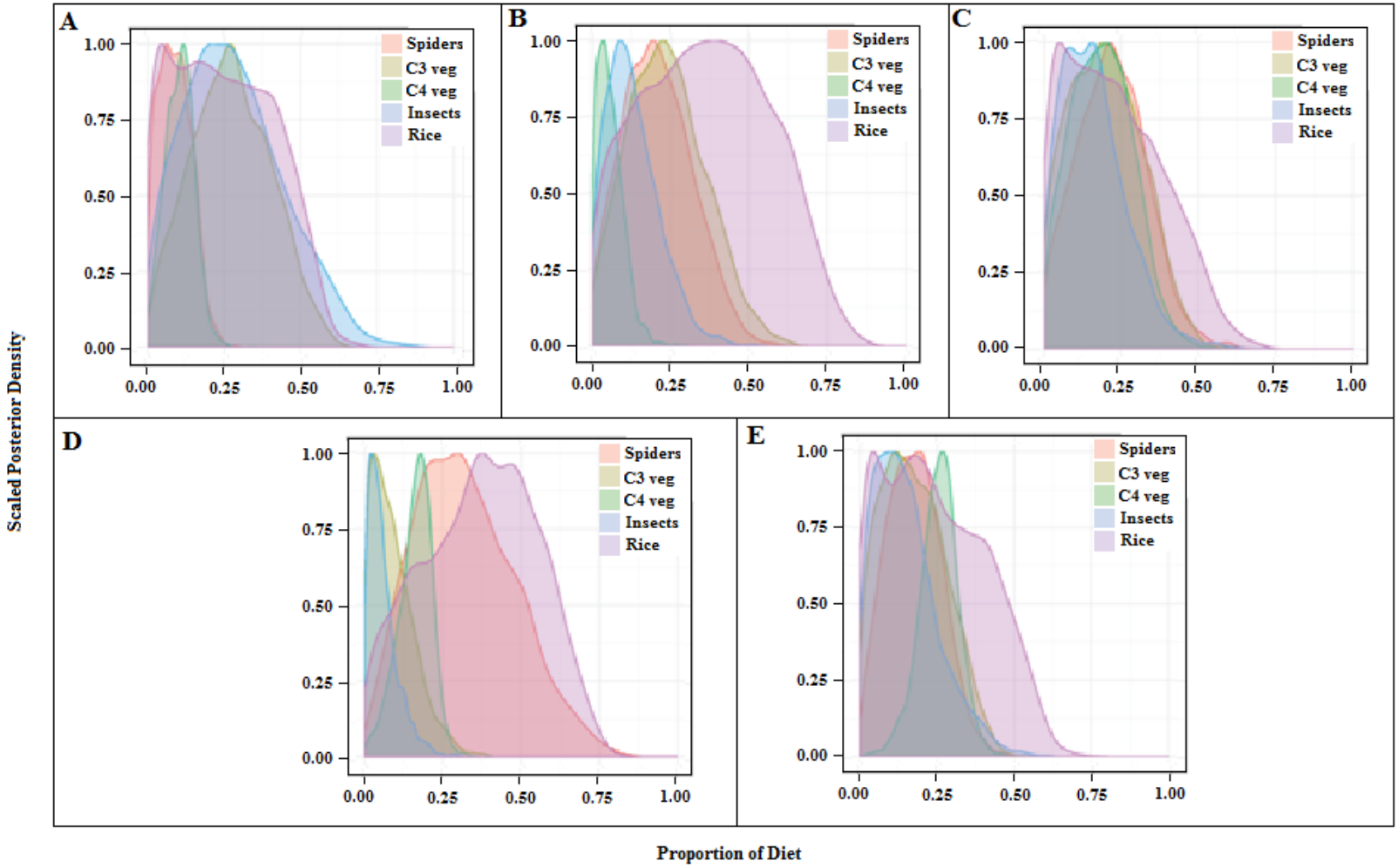

A
B
C
D
E
Spiders
C3 veg
C4 veg
Insects
Rice
1.00
0.75
0.50
0.25
0.00
0.00
0.25
0.50
0.75
1.00
Scaled Posterior Density
Proportion of Diet

Hobson and Clark TDF values - (Blood: $\delta^{13}C$= 1.2 ±0.6, $\delta^{15}N$= 2.2±0.2; Feathers: $\delta^{13}C$= 1.4±0.6, $\delta^{15}N$= 1.6±0.1). Combined blood (A), summer blood (B), fall blood (C), and historic (D) and contemporary (E) feathers


A
B
C
D
E
Spiders
C3 veg
C4 veg
Insects
Rice
1.00
0.75
0.50
0.25
0.00
0.00
0.25
0.50
0.75
1.00
Scaled Posterior Density
Proportion of Diet

Healy et al. (2017) TDF values - (Blood: $\delta^{13}C$= 0.61 ±1.33, $\delta^{15}N$= 1.99±1.44; Feathers: $\delta^{13}C$= 1.77±1.37, $\delta^{15}N$= 2.75±1.43).

Combined blood (A), summer blood (B), fall blood (C), and historic (D) and contemporary (E) feathers


A
B
C
D
E
Spiders
C3 veg
C4 veg
Insects
Rice
1.00
0.75
0.50
0.25
0.00
0.00
0.25
0.50
0.75
1.00
Scaled Posterior Density
Proportion of Diet