PATTERNS AND PATHWAYS OF LEAD CONTAMINATION IN MOTTLED DUCKS (ANAS FULVIGULA) AND THEIR HABITAT

by

BRIAN VANCE KEARNS

B.A., Whitman College, 2010
M.S., University of Southern California, 2012

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

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Abstract

Mottled ducks (*Anas fulvigula*) are dabbling waterfowl species native to coastal wetlands of the Gulf of Mexico of the United States and Mexico. Although closely related to common waterfowl species such as the mallard (*A. platyrhynchos*) and American black duck (*A. rubripes*), the mottled duck exhibits unique behavior, mainly in its life history as a non-migratory species. As such, because of population declines caused by predation, habitat destruction, and environmental contaminants, this species requires specialized conservation concerns and species-specific management to protect population numbers. The goal of this study was to assess ongoing effect of observed lead (Pb) contamination and exposure issues in mottled ducks and their habitats, which I achieved by conducting assessments that will provide managers habitat and organism level metrics to detect and mitigate lead in mottled ducks and their environments.

My field study was conducted at the Texas Chenier Plain National Wildlife Refuge Complex (TCPC), which was the area of greatest mottled duck density on the Texas Coast. I first created a body condition index to provide managers a tool to monitor population health, and a proxy for lead exposure and avian health without destructively sampling individuals. I then used presence-only maximum entropy (MaxENT) and multivariate statistical modeling procedures in conjunction with mottled duck movement data to elucidate sets of habitat conditions that were conducive to predicting the occurrence of mottled ducks and environmental lead “hot spots”. MaxENT analyses suggested that lead in the top portion of the soil column is similarly related to all environmental variables considered, may be increasingly available after large-scale environmental disturbances. Lack of variation in coarse-scale habitat use

between breeding and non-breeding seasons may further point to a food-based exposure pathway for lead as mottled ducks switch from an invertebrate to plant diet, either as a result of changing age classes or normal adult phenology, during the period of increased lead exposure. Using stable isotope ratio analysis, I then tested environmental samples of soil and vegetation as well as mottled duck blood to determine isotopic signatures that were consistent with particular sources of lead deposition (e.g., lead shot pellets, leaded fossil fuel combustion, industrial effluents). Comparisons suggested a great deal of similarity to lead shot reference values in vegetation and blood samples, especially in blood samples with higher concentrations of lead present. Last, I conducted a formal Ecological Risk Assessment (ERA) procedure to quantify the risk to mottled ducks from lead exposure in their current habitat and direct managers towards effective mitigation and habitat management strategies to reduce exposure in the future. One scenario suggested that mottled ducks were at greatest risk from eating an invertebrate-based diet, but lead content values at the TCPC suggest that a plant-based diet may provide a higher lead exposure risk for mottled ducks, depending on true levels of bioavailability in environmental media.

Overall, I determined that mottled ducks experience greatest lead exposure risk from lead shot pellets on the TCPC or in nearby habitat, while potentially also experiencing low levels of exposure from several other sources. Additionally, management efforts that focus on plants that do not provide food resources for mottled ducks as a potential environmental sink for lead contamination, such as phytoremediation, may prove effective in reducing the overall lead load from historical activities that likely deposited much of the lead in this ecosystem.

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Introduction

Wetlands persist as some of the most essential ecosystems in the world, supporting water resources and providing essential ecosystem functions, goods, and services for humans and innumerable species of flora and fauna. A meta-analysis suggested that the approximate value of wetlands at 14,785 \$ ha^{-1} yr^{-1} was among the highest of any ecosystem service (Costanza 1997). Equal to their value is their fragility, as coastal wetland ecosystems suffer some of the greatest impacts from human development and associated activities (Kennish 2002). A great deal of research has consequently sought to quantify the direct impact of human activities on wetland ecosystems. Plant and wildlife species remain some of the best indicators of ecosystem health due to their differential short- and long-term responses to changes in their habitats, as well as their importance in shaping their abiotic surroundings (McGeoch 1998). Certain species, therefore, may be selected as bioindicators of ecosystem health due to a particular response to ecosystem change or an important biotic interaction. As managers, however, we must take care to assess the efficacy of managing for all species while using only one as a bioindicator as the needs of species may vary widely in an ecosystem (Simberloff 1998, Caro and O'Doherty 1999). Waterbirds are of particular importance for study because of their variable life history strategies and consequent use of wetland habitats for a wide range of activities during different stages of their life history (Baldassarre et al. 2006). Waterbirds have been used to assess habitat change, especially in response to global climate change (Sorenson et al. 1998, Johnson et al. 2005), effects of large scale stochastic events such as hurricanes (O'Connell and Nyman 2011), and ecological community interactions (Sondergaard et al. 1996).

The mottled duck (*Anas fulvigula)*, as a close phylogenetic relative of the mallard (*A. platyrhynchos*) and American black duck (*A. rubripes*), shares general characteristics and some life history traits with other, more common, game species. Unlike other members of the family *Anatidae*, however, mottled ducks have a unique life history as they are non-migratory and reside year-round in the coastal wetlands of the Gulf of Mexico (Stutzenbaker 1988). This unique life history characteristic of mottled ducks has many other implications on breeding, molting, and movement behaviors. As such, there are particular conservation concerns that may affect this species due to its sedentary nature that may require the implementation of directly tailored management tactics.

Mottled ducks have experienced sharp population declines during the past two decades. The only continuous breeding survey of mottled ducks includes large portions of Texas' National Wildlife Refuges (NWRs), where estimates indicate that breeding pairs, based on a visually-corrected aerial survey on NWR complexes, have experienced a 95% decline since 1986 and remained at relatively low levels since 2000. The 2012 estimate of 1.04 breeding pairs/km^2 across the mottled duck range represented a 27% decrease from 2011, a 69.2% decrease below the long-term average (3.37 pairs/km^2), and an 86.4% decrease since 1993-1994. The breeding pair estimates on NWRs specifically have been relatively constant since 2002 (average = 1.13 pairs/km^2) (Haukos 2012). Other nonbreeding indices indicate recent declines of varying intensity for mottled ducks throughout the remainder of the western portion of the species' range in Texas and Louisiana (GCJV 2007).

Factors identified as contributors to the population decline include loss or degradation of reproductive habitat (e.g., pair ponds or breeding territories with good resource access, suitable nesting cover, and brood-rearing habitats); loss and degradation of required non-breeding habitats (i.e., winter, molt); increases in predation (Elsey et al. 2004); hybridization with migratory congenerics (namely wild and feral mallards) (Williams et al. 2005); and ongoing exposure to lead in the environment, notably through the ingestion of spent lead shot pellets from historical hunting activities (Merendino et al. 2005). For mottled ducks and other potentially susceptible species, awareness has recently increased on the part of managers on the issue of heavy metal exposure; this exposure may potentially originate from sources other than lead shot (Motto et al. 1970, Aberg et al. 1999, Bollhöfer and Rosman 2001). Although ingestion of lead shot pellets is the most likely form of lead exposure for dabbling waterfowl such as the mottled duck, other avenues of exposure from atmospheric, terrestrial, or food sources cannot be ruled out.

Additional avian species on the Upper Texas Coast have demonstrated elevated levels of exposure to environmental lead. For instance, black-necked stilts (*Himantopus mexicanus*), a species known to bioaccumulate heavy metals and ingest shot in much the same way as mottled ducks (Eagles-Smith et al. 2009), have demonstrated exposure. Black-necked stilts on the Texas Chenier Plain NWR complex have exhibited notably high levels of lead, with 74.6% showing signs of lead exposure (2 ug/L $\leq$ blood lead $\leq$ 5 ug/L) and 4.8% showing signs of toxic lead exposure (blood lead $\geq$ 5 ug/L)

(Riecke 2013). These preliminary results suggest elevated exposure risks for other species on the Upper Texas Coast with similar food sources and habitat uses. Although differences in life history necessitate caution when considering differences between species, waterfowl, and specifically the mottled duck, are likely to also experience risk from these pathways in this ecosystem.

Environmental lead contamination remains a contentious political issue, and is an issue of great importance for environmental management (Needleman et al. 1990, Graney et al. 1995, Kennish 2002, Fisher et al. 2006). Perhaps the most widely publicized case of lead contamination in an avian species is that of the California condor (*Gymnogyps californianus*). Once near the brink of extinction, this species has been the focus of intensive reintroduction efforts and now persists in the wild. One of the chief issues causing declines in this species continues to be lead exposure (Church et al. 2006). Condors and other avian scavengers experience exposure chiefly through the ingestion of lead bullet fragments in carcasses resulting from upland hunting activities, accumulating lead in their bone and soft tissues, which leads to health problems (Hunt et al. 2006, Haig et al. 2014). The state of California, whose geographic boundaries constitute a major part of the condor's native range, banned the use of lead ammunition in the pursuit of all game species (Anonymous 2014); activist groups in other states seek similar action to protect birds of prey and avian scavengers as well.

Lead also presents a highly relevant conservation issue in conjunction with waterfowl. Before the development of steel and other non-toxic shot types, lead was used almost exclusively in hunter ammunition for both upland and wetland game species. Bellrose, researching lead toxicity in the 1950's, addressed a growing concern surrounding observed increased mortality in lead-exposed waterfowl discovered increased band return rates for ducks dosed with lead in captivity and released into the wild (Bellrose 1955, Bellrose 1959). Although lead began to get phased out for the pursuit of waterfowl in the 1970's and was eventually banned federally at a national level in 1991, lead shot still remains a potential threat for waterfowl because they can continue to ingest it as grit (Anderson et al. 1987, USGS 2012). Lead fishing sinkers also present an issue in many parts of the country where sport fishing areas and waterfowl habitat overlap (Haig et al. 2014). Furthermore, lead shot is still used over agricultural fields, an important food source for waterfowl, in the pursuit of both upland and webless migratory game species such as mourning dove (*Zenaida macroura*). This includes use on private lands nearby NWR's and some state lands which vary in their regulations from site to site. It was estimated that, over the course of the early and mid-twentieth century, hunters deposited roughly 700 tons of lead shot per year into coastal Texas wetlands (Stutzenbaker 1988). Fischer et al. (1986) estimated >1.5 million shot per acre to be present in a Texas coastal marsh, and, because lead ions do not deteriorate, this lead source may still provide a risk for dabbling duck species such as the mottled duck.

Excessive lead exposure has been shown to have a number of detrimental effects on bird species, including atrophy of organs, dysfunction in the nervous and digestive systems, reduced disease resistance, weight loss, lowered survival, and potentially increased susceptibility to harvest and predation (Bellrose 1959, Irwin and Karstad 1972, Rocke and Samuel 1991, Sanderson et al. 1992, Wobeser 1997, McCracken et al. 2000). After ingestion or exposure, lead is typically measureable in blood, liver, and bone tissues (Pain 1996). While blood and liver lead levels typically indicate recent exposure, bone tissue lead levels tend to indicate long-term or lifetime exposure and bioaccumulation because calcium chelates lead causing the heavy metal to be deposited into and stored in bone tissue. Storage of lead particles in conjunction with calcium can potentially lead to re-exposure when calcium stores are accessed for egg laying or feather production (Karasov and del Rio 2007).

Historically, surveys have shown mottled ducks to have the greatest ingestion rate of lead shot for waterfowl, with one pre-shot ban survey showing 25.6% of birds having ingested shot, 98.5% of which was lead (Anderson et al. 1987). Mottled ducks have also displayed high concentrations of wing-bone lead, with an average concentration of 16.62 parts per million (ppm) during the 1998-1999 hunting season, and 28% and 22% of after-hatch-year (AHY) and hatch-year (HY) birds, respectively, demonstrating concentrations ≥20 ppm between 1987-2002, considered to be severe clinical poisoning that could be life threatening (Merendino et al. 2005). Although these contamination values do show a reduction in average values from those observed in 1987-1988 of 74.1 ppm and 40.0 ppm for AHY and HY birds in this area, respectively (Merchant et al. 1991), it is still cause for concern as even the mean value approaches the 20 ppm threshold for toxic exposure. Repeated surveys suggest mottled ducks are still being exposed to environmental lead, despite an assumed reduction in lead input into the ecosystem since previous studies due to the lead shot ban for waterfowl hunting.

Mottled ducks of the West Gulf Coast population occur at their greatest density on the Chenier Plain of Texas and Louisiana, making this region critical habitat for this species (see following "Study Area" section). Because the density and distribution of lead shot is still largely unknown on the Chenier Plain, public lands being managed for waterbirds may still contain large quantities of available lead; this may be leading to the presence of an ecological trap, or a situation in which a normal life history activity is leading to population declines for a population (Kokko and Sutherland 2001). Results from a previous study, based on 232 soil samples from within the Chenier Plain NWR complex and surrounding areas suggest that upwards of 1.9 billion total lead shot pellets could be present and available for ingestion and leaching. In addition, McDowell (2014) reported soil lead levels ranging from 7.35 to 88.28 ppm on the Chenier Plain, demonstrating great spatial variability of lead concentrations.

Avenues of exposure to lead have been one of the chief research questions for this species in recent years, largely due to the unknown absolute availability and potential continued deposition of anthropogenic lead in wetland ecosystems on the Texas Gulf Coast. In addition to lead deposition through the use of lead in ammunition and fishing sinkers in waterbird habitats, several other important pathways have been elucidated through climate and atmospheric research. Leaded fossil fuel combustion, for instance, provides an input to the atmospheric pool of lead, which can then be assimilated into sediments and plant tissues through abiotic and biotic processes (Motto et al. 1970, Sharma and Dubey 2005). The atmospheric pathway also exists as a ramification of industrial processes, a common land use on the Texas coast (Vivian and Massie 1977), and suggests a large pool of potential lead input into the environment. Lead does not break down in ecosystems, but little is understood about temporal variation in environmental hotspots of lead, and those sources most bioavailable.

The general goal of my dissertation was to achieve an understanding of how environmental lead contamination is affecting waterfowl and waterbirds on the Upper Texas Gulf Coast. More specifically, I sought to develop information regarding spatial patterns and general pathways both of exposure and deposition of lead to work towards effective management and mitigation plans to improve important habitats for species at risk. I accomplished this by establishing spatial and temporal patterns in lead availability in the environment, making connections between exposure risk in the environment and at an organism level, and quantifying future risk for wildlife in the ecosystem in question. Additionally, my research efforts will inform managers and conservationists to help mitigate any negative effects that biota may be experiencing as a result of lead exposure.

Study Site

Data collection for these analyses occurred on the Texas Chenier Plain National Wildlife Refuge Complex (TCPC), which included four refuges: Anahuac, McFaddin, Texas Point, and Moody National Wildlife Refuges (Figure i). The TCPC comprised a cumulative area of 42,762 ha. Approximately 40% of Anahuac, McFaddin, and Texas Point NWR's were open to waterfowl hunting. The refuges imposed a non-toxic shot requirement in conjunction with the banning of lead ammunition on the Texas Gulf Coast initiated during the 1978 hunting season and finalized by 1981 (Moulton et al. 1988). Land acquisition to form the TCPC began in 1954, with ongoing rigorous management for waterfowl production via various land management methods (USFWS 2008b). Land use history and change on and around the TCPC has largely been driven by agricultural and industrial development. Historical land uses in the region included rice agriculture and cattle ranching and, as the technologies became available, petrochemical and other industry such as marine commerce and chemical production. As coastal marsh habitats were converted to provide for an increasing land demand, the U.S. Fish and Wildlife Service (USFWS) set aside large tracts of land in response to declining waterfowl populations (USFWS 2008a). Much of the habitat surrounding the NWRs remained in rice agriculture or cattle ranching, with industrial development prevalent in the Houston/Galveston/Beaumont, Texas area. Areas of Anahuac NWR included agricultural fields (~890 ha) cultivated by cooperative farmers producing rice on refuge properties (USFWS 2008a), which can provide important food sources for mottled ducks (Stutzenbaker 1988).

The landscape of the TCPC was largely influenced by the hydrology and climate of the region which is characterized by sub-tropical weather patterns. The TCPC receives, on average, 144 cm of rain per year, with annual values ranging from 52 cm - 218 cm. The Upper Texas Gulf Coast is also prone to hydrologic and other effects stemming from the landfall of hurricanes, which can have devestating effects both on land forms and vegetation communities due to changes in salinity, sedimentation, and other effects (Stone et al. 1997, Turner et al. 2006, Howes et al. 2010, O'Connell and Nyman 2011). Dominant marsh types on both Anahuac and McFaddin NWR's include fresh marsh, intermediate marsh, and brackish marsh (USFWS 2008a). Vegetation communities in wetlands vary greatly based on water depth, salinity level, and amount of tidal force. Intermediate and brackish marshes were dominated by marshhay cordgrass (*Spartina patens*), with other species such as *Scirpus* spp., *Typha* spp., *Distichlis* spp., *Juncus* spp., and *Paspalum* spp. intermixed (Rigby 2008). Freshwater marshes were more diverse, and included *Alternanthera philoxeroides*, *Sesbania* spp., *Ludwigia* spp., *Nymphaea* spp., *Sagittaria* spp., *Eleocharis* spp., *Typha* spp., *Cyperus* spp., *Papsalum urvillei*, and *Panicum hemitonum* (Rigby 2008). Upland habitats persist on the refuge as well and are mainly characterized by tallgrass prairie vegetation such as grasses (*Schizachyrium scoparium*, *Paspalum plicatulum*, *Tripsacum dactyloides, Panicum virgatum, Paspalum livium*), forbs (*Liatris pynostachya*, *Rudbeckia hirta*, *Cacalia* spp., *Eryngium yuccifolium*), and woody shrubs (*Baccharis halimfolia*, *Myrica cerifera*). Large variations in topography are minimal due to the geologic nature of the area (USFWS 2008a).

The five most abundant migratory waterfowl species included green-winged teal (*A. crecca*), gadwall (*A. strepera*), Northern shoveler (*A. clypeata*), blue-winged teal (*A. discors*), and Northern pintail (*A. acuta*) (USFWS 2008a). Mottled ducks were also prevalent on the TCPC and represented the principal resident waterfowl species (USFWS 2008a). As with much of the Gulf Coast region, the refuge demonstrated a high degree of bird diversity that varied temporally but included species of shorebird, songbird, waterbird, and other terrestrial migrants. The TCPC was also an important location for avian species of concern. As of 2008, 37 out of 48 species defined as species of conservation concern in the U.S. portion of the Gulf Coast region used habitat on the TCPC (USFWS 2008a;b). The TCPC was also home to several federally threatened or endangered species including several species of sea turtle (e.g., *Caretta caretta*, *Chelonia mydas*, *Eretmochelys imbricata*, *Lepidochelys kempii*), the brown pelican (*Pelecanus occidentalis*), and more.

Objectives and Hypotheses

Objective 1: Develop body condition and fat indices for Texas Gulf Coast mottled ducks

Hypotheses:

1. Decreased fat stores will indicate poorer body condition in mottled ducks.
2. Increased levels of lead in tissue and environment will adversely affect mottled duck body condition index (BCI) (see objective 2).

Objective 2**:** Determine ratios of lead isotopes in bone and blood tissue from mottled ducks, and examine environmental ratios of lead isotopes from vegetation and soil samples using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) to determine contamination sources, potential bioavailability, and exposure pathways.

Hypotheses:

1. Lead sources in the Texas Chenier Plain NWR complexes will be largely anthropogenic, originating from hunter-deposited lead shot before the toxic shot ban, upland game bird hunting that still allows the use of lead shot in nearby habitats, and fossil fuel combustion from nearby industrial sites (Stutzenbaker 1988).
2. Higher lead levels in mottled duck tissues will be correlated with a decrease in BCI (see objective 1).

Objective 3: Create a predictive surface for high risk areas of lead contamination in the Texas Chenier Plain and Midcoast NWR Complexes using spatial interpolation techniques.

Hypothesis:

1. Areas associated with higher levels of environmental lead will provide a higher risk of contamination for resident mottled ducks and contribute to ongoing negative population trends.

Objective 4: Use Species Distribution Modeling (SDM) technology to create distribution maps of mottled ducks at an ecosystem scale for the Texas Chenier Plain NWR complex, and assess potential effects lead contamination on habitat use.

***Hypothesis**:*

1. Mottled ducks will avoid areas with higher concentrations of environmental lead

Objective 5: Develop a formal Environmental Risk Assessment report for risk of exposure to environmental lead for mottled ducks and other waterbirds on the Upper Texas Gulf Coast.

***Hypothesis**:*

1. High risk values for lead exposure in mottled ducks will be presented by soil and food sources in the mottled ducks environment, but risk will vary from site to site.

Figure i. Map depicting the Texas Chenier Plain National Wildlife Refuge Complex and the Texas Midcoast National Wildlife Refuge Complex on the Upper Texas Gulf Coast. Field data collection for this project took place chiefly on the Texas Chenier Plain Complex, which provides some of the highest mottled duck breeding pair densities on federal lands in Texas.

Chapter 1 – Factors Affecting Fat Content in Mottled Ducks on the Upper Texas Gulf Coast

Body condition, or an individual's ability to address present and future metabolic needs and stresses, is an important measure of organism health in birds (Owen and Cook 1977). In many waterfowl species, knowledge of relative body condition is needed to give managers an understanding of potential responses to increasing anthropogenic changes in local habitats and the environment at large (Austin et al. 2000). For species in decline, it is especially important that estimates of body condition can be made easily and quickly so that negative impacts of these changes can be detected *in situ*. For migratory species, body condition can also have wide-reaching implications on breeding success. For instance, a primary hypothesis for significant population declines of lesser scaup (*Aythya affinis*) is that females are arriving on breeding grounds in poor condition after the substantial energetic cost of migration and relative lack of available forage at stop-over sites; thus, birds are unable to allocate necessary resources to produce successful clutches (Afton and Anderson 2001, Anteau and Afton 2004).

Much debate continues regarding appropriate methods to represent body condition in avian species. For many waterfowl species, it is commonly considered that estimates of body fat content provide a suitable proxy for organism health (Whyte et al. 1986). Although other measures exist, such as observing metabolic products in the blood (Brown 1996), collection of usable data for these analyses frequently proves costly and labor intensive. Fat content, however, is often directly related to mass adjusted for body size, although the appropriate way to characterize this relationship and the efficacy with which morphometric indices predict condition varies greatly. Many waterfowl studies seek to use a ratio model consisting of body mass corrected for a length metric, which generally provides fairly good predictive power (Whyte and Bolen 1984, Miller 1989). Some studies have, however, discouraged the use of these ratio models. Green (2001) identified several assumptions used in creating ratio models of condition including: (1) mass is linearly related to size, (2) condition is independent of length, (3) length accurately indicates size, (4) no colinearity between length metrics, (5) length is independent of mass, and (6) length is not subject to error. Green posits that many of these assumptions may be violated in biological reality, and suggests cases where ratio indices cause type I or type II statistical error, essentially suggesting that analysis of this kind produces statistical artifacts. Labocha and Hayes (2012) confirm many of these concerns in their review, especially noting cases where a lack of size independence can confound condition results. They further caution that ratio models of condition do not always represent fat mass well, and that any ratio index should be validated before use, as I do in this study. However, for wildlife managers operating hunter-check stations or engaged in other field operations, the ability to estimate the condition of a bird from morphometric measurements alone can prove invaluable in assessing long-term effects of factors such as environmental disturbance, changes in trophic factors such as competition, or availability of food resources. The precedent in the literature for the use of ratio indices of condition is present, and is arguably quite viable provided validation with *in vivo* measures of condition (fat stores) is conducted.

Because lipids have been so closely linked with health and the dynamics of different life history periods in these and other avian species, lipid reserves in waterfowl have been an important focus of research (Budeau et al. 1991) largely because lipid stores fluctuate during the course of a year based on abiotic factors, species in question, and energetic needs of the individual. In a typical waterfowl species, the life history periods of concern are typically: wintering, breeding/reproduction, molt, and migration (Baldassarre et al. 2006). Life history transitions demonstrate different energetic needs, and suggest periods where resources become critical and habitat use is dynamic. As such, the degree of energy store usage that occurs during different parts of the year varies widely, and is usually supplemented by adjusting foraging behavior to avoid complete depletion of corporeal energy stores. During remigial molt of mottled ducks (*Anas fulvigula*), for instance, body lipid reserves were only able to satisfy about 33% of the overall energetic need of the nearly month-long flightless period (Moorman et al. 1993). During reproduction in Northern pintails (*A. acuta*), lipid reserves at the start of the breeding season were positively linked to timing of nest initiation, suggesting that birds wait until a resource surplus is reached before breeding. Changes in lipid reserves also relate to the spring condition hypothesis in scaup, wherein lower lipid reserves on arrival at the breeding grounds potentially indicate reduced breeding success that may be linked to species declines (Anteau and Afton 2004). The largest direct energetic pressure most waterfowl species face, however, is likely migration itself, as energy available for other life history related activities hinges on management of stores during migration periods.

I examined body condition variables in the mottled duck, a non-migratory waterfowl species native to the coastal marshes of the Gulf of Mexico. The mottled duck resides year-round chiefly in the coastal marshes of Texas and Louisiana as well as peninsular Florida (Bielefeld et al. 2010). Mottled ducks demonstrate unique energetic considerations, mainly because of their adaptations to avoid the migratory life history period (Stutzenbaker 1988). Where other waterfowl species require an increase in foraging to create surplus energy stores before the substantial energetic cost of migration, mottled ducks are able to otherwise allocate surplus energy. Moorman et al. (1992, 1993) provided some of the only studies to examine variables related to the unique features of mottled duck energetics. In a study examining lipid dynamics over-winter for mottled duck all age and sex combinations, Moorman et al. (1992) discovered an increase in lipid reserves and overall body mass after molt and during wintering, an observation that did not include fluctuations in body mass due to resource expenditure related to long distance movements or wintering in cold climates seen in other migratory species. Additionally, because mottled ducks have lower breeding propensity than many other species and move only short distances within their year-round habitat, they face different ecophysiological challenges than many other species (Stutzenbaker 1988, Rigby and Haukos 2012). In essence, mottled ducks will delay breeding until habitat conditions are suitable, and will not execute large movements to find other suitable habitat. These factors confirm that mottled ducks are unique in their energy expenditure and warrant the development of a species-specific condition index that describes their particular life history. Trends in body condition of mottled ducks are of particular interest in their management because their life-history and energetic demands differ substantially from their migratory phylogenetic relatives. The mottled duck has been designated as a focal species by the U.S. Fish and Wildlife Service, making conservation issues related to this species priorities in management of regional wetland habitats (Haukos 2012).

The Texas Chenier Plain National Wildlife Refuge (NWR) Complex and Texas Midcoast NWR Complex have historically accounted for >80% of mottled ducks that reside on federal lands in Texas due to the central location of these sites in its range and abundance of suitable waterfowl habitats (Ballard et al. 2001, Finger et al. 2003). Mottled ducks have been declining on Texas NWR's since the mid 1990's, with the only continuous breeding survey effort indicating a 95% reduction in breeding pair densities in the Chenier Plain of Texas (Haukos 2012). Factors potentially contributing to mottled duck decline may be numerous, and include increasing predator populations (Elsey et al. 2004), loss of coastal prairie and marsh habitats (Varner et al. 2013), conversion of native habitat to agriculture (Durham and Afton 2003), saltwater intrusion (Moorman et al. 1991), and ingestion of lead shot pellets from historical hunting activities or ongoing hunting for mourning doves (*Zenaida macroura*) (Merendino et al. 2005).

My primary goal was to create a nonbreeding season (~October - January) body condition index for mottled ducks that would predict fat content in mottled ducks without the need for destructive sampling. A predictive equation using external body metrics to predict fat content should provide managers on the upper Texas Coast with the ability to conduct field estimation of abdominal fat content, which represents the most variable body fat depot and correlates with total fat content (Thomas et al. 1983). In the course of field operations such as banding or running hunter check stations, ease and speed of condition estimation is paramount as resources (financial or otherwise) are often not available for more precise forms of condition estimation. Condition estimates for mottled ducks can also be used comparatively with those collected via similar means for other waterfowl species to assess energetic differences inferred by variation in life-history strategies. Such analysis has not yet been conducted for this species, although similar equations are available for mallard (Owen and Cook 1977), northern pintail (Smith et al. 1992), and American wigeon (*A. americana*) (DeVault et al. 2003). Additionally, I applied the developed model to check-station data from the upper Texas Coast to evaluate variations in predicted fat content in mottled ducks relative to precipitation and potential resulting annual variation in food resources or available cover. Once a predictive equation is developed, fluctuations in predicted fat could potentially also be linked to blood or wing bone lead content in collected birds to assess ongoing effects of heavy metal contamination on condtion.

Methods

Study Site

Data were collected on Anahuac and McFaddin NWRs, which comprise part of the Texas Chenier Plain NWR complex on the Upper Texas Gulf Coast. Other refuges in this complex include Texas Point and Moody NWR's. This complex had a cumulative area of ~42,762 ha, and included a mix of coastal wetland habitats, including intermediate, brackish, saline, and freshwater marshes (USFWS 2007, Haukos et al. 2010). Much of the surrounding land was used for agriculture, specifically rice (*Oryza sativa*), which is an important food source for mottled ducks (Stutzenbaker 1988). Approximately 40% of the complex was open to waterfowl hunting activities, and so provided a suitable location for collecting morphometric data from hunter-bag birds.

Condition Data Collection

Mottled ducks were collected between 1 October and 31 January at hunter-check stations and from confiscations from law enforcement efforts during 2005-2007. Collected birds were frozen and transported to a laboratory at Stephen F. Austin State University for compositional analysis. In the lab, body mass (g) was measured using an electronic scale, and rulers or calipers were used to measure flattened wing chord (mm), culmen (mm), keel (mm), tarsus (mm), and total body length (mm). Abdominal fat mass (g) (omental, mesentery, and visceral fat) was determined by removing and weighing these fat depots. Total percent fat content was determined through ether extraction (Schemnitz 1980) for a subset of adult birds ($n = 11$) to provide a correlation with measured abdominal fat mass (W. Conway, Stephen F. Austin State University, unpublished data). Exploratory analysis using simple linear regression showed a suitable correlation between abdominal fat and total percent fat ($r = 0.69$, $P=0.02$), suggesting that abdominal fat content provides a useful proxy to total percent fat in mottled ducks, similar to other waterfowl species (Thomas et al. 1983).

Condition Model Development

We ranked linear regression models based on various combinations of field-measurable metrics as listed below for their utility in providing an *in-situ* measure of abdominal fat content such as mass (M), wing chord (WC), body length (L), and keel (K). In addition to primary morphometric variables, I tested ratio indices for body condition (i.e., adjusting body mass for body size) by dividing total body mass by various length metrics including wing chord, body, and keel length (Owen and Cook 1977, DeVault et al. 2003). Because the different morphometric measurements were related, I also reduced the morphometric measures using a Principal Components Analysis (PCA) with the resulting score from the first principal component as an additional independent variable in the regression model set (Alisaukas and Ankney 1990).

Energetic requirements and behavioral demands were hypothesized to differ between age (juvenile and adult) and sex classes (male and female) due to changes in diet (invertebrate vs. plant) or differences breeding investment or foraging behavior (Baldassarre et al. 2006). Interactive model terms were used to address potential differences in the relationship between external metrics and fat content due to sex and age. I assessed model fit using Akaike's Information Criterion corrected for small sample size (AIC_c) (Akaike 1974). Models with $\Delta\ AIC_c \leq 2$ were considered to have adequate support (Burnham and Anderson 2002). In addition to AIC_c, the correlation coefficient (r) and coefficient of determination (r^2 or R^2) were used to assess the strength of the relationship between external metrics and abdominal fat content provided by each model. All statistical analyses were conducted using JMP 11 (SAS Institute Inc. 2014).

Model Application

After development of an equation to predict fat content based on morphometric measurements, historical check station data of mottled duck morphometrics were used to assess annual variation in population-level fat content since 1986. Check station data (total field M [g] and WC length [mm] by age and sex) were available from years 1986-1999, 2004, 2006, 2007, 2010, and 2011. Birds were aged and sexed in the field using tail and wing feather characteristics (Carney 1992). Hurricane Rita precluded check-station operations in 2005, and Hurricane Ike precluded check-station operation in 2008 and destroyed data from 2000-2003. Data from 2009 and 2012-2013 were excluded as data was determined to be of low quality resulting from poorly trained check-station personnel.

Estimated abdominal fat mass was compared among years using a factorial analysis of variance including an age by year interaction using JMP 11 ($\alpha = 0.05$). Average annual estimated abdominal fat mass was compared against measures of growing season precipitation of the associated year to determine whether this variable would impact food availability (Bhattacharjee et al. 2009) and consequently a change in observed fat stores. Precipitation data for years addressed in this study were sourced from the Texas Water Development Board Precipitation and Lake Evaporation Database (TWDB, 2014), which provided monthly average precipitation values. Precipitation values were grouped into six-month (April - September) and twelve-month (October - September) periods to capture variation in precipitation leading up to the start of the hunting season. Pearson's correlation was used to determine the relationship between measures of precipitation and annual variation in estimated percent fat.

Results

Abdominal fat content was compared against body metrics for 24 mottled ducks: three adult females, seven adult males, five juvenile females, and nine juvenile males (Table 1.1). Predicted fat content values were estimated from historical data for 690 adult birds and 472 juvenile birds (Table 1.2).

Three models showed nearly equal support using AIC values, all of which were based on ratio models of mass and an external body length metric (Table 1.3). The condition model based around PC1 also showed a high level of support from its AIC_c value, but did not demonstrate any improvement in model fit for its added complexity. Although age class showed some potential importance in determining fat content in sampled birds, the top model with an age interaction was not well supported (Δ $AIC_c > 2$). Sex was not a factor in determining fat content in the non-breeding season.

Based on the ratio model of M/WC, which showed nearly identical support to the top model and has been commonly used in the field of waterfowl biology as a condition index, abdominal fat can be predicted for mottled ducks using the following equation:

AbFat = (-24.3276) + 9.0497(M/WC) [Figure 1.1]

Predicted fat values from historical check station data differed among years ($F_{18, 1143} = 26.40$, $P < 0.0001$; Figure 1.2). Essentially, there was little variation among years with the exception of 2004 and 2006. Measures of precipitation did not have an effect on predicted abdominal fat content. Linear model fits were poor for both 6-month ($r = 0.22$, $F_{1, 36} = 2.08$, $P = 0.16$) and 12-month ($r = 0.08$, $F_{1, 36} = 0.28$, $P = 0.60$; Figure 1.3), suggesting that precipitation during the previous growing season or entire year did not directly affect fat content in mottled ducks at this study site.

Discussion

This analysis has yielded a model that has utility, based on r-squared values with a relatively small sample size, in predicting changes in fat content using abdominal fat deposits for mottled ducks using morphometric field measurements. Mottled ducks appear to follow the trend of other waterfowl species in that their fat content appears to be reasonably well-represented by a ratio model adjusting body mass for structural size. Mallards showed a similar relationship between total fat stores and a ratio index of M/WC (r^2 = 0.73), and age and sex also provided no additional information in this relationship for this species (Whyte and Bolen 1984). The comparatively low r^2 values for top-ranked ratio models in this study (r^2 = 0.29) are likely attributable to small sample size requiring merging of available data and resulting sampling variation. Northern pintails in California demonstrated a difference in predicting fat content based on sex, but their condition predictions were also based on a ratio model (Miller 1989). Sample sizes in my study for individual sexes and age classes were too small to establish meaningful model interactions for the different groups, so I was unable to predict variation in fat content between groups for mottled ducks from these data. This was confirmed by the low level of support for these models in my model set. Additionally, although I believe that examination of hunter-collected mottled ducks provides a reasonable proxy to the overall population in this area, there is some concern that there may be a condition bias in hunter-shot birds where birds in poorer condition (less fat) are more likely to be harvested (McCracken et al. 2000). Additional analyses would be necessary to substantiate a difference between these two categories.

The top-ranked models in our set, however, confirms that a ratio model based on M and a length metric, which I selected to be WC for simplicity in data collection and analysis, is a reasonably good approximation of condition for this species on this study site, and provides at least an initial insight for managers into organism health. Although it has been acknowledged in the literature that there are some potential factors in ratio models that may generate spurious statistical results (Green 2001, Peig and Green 2010, Labocha and Hayes 2012), the model developed herein uses correlation with collected fat data to show a reasonable estimate of condition. The lowest observed mass value for a mottled duck in this study was measured at 544 g with 1.1 g abdominal fat. As such, we warn that this model will be ineffective at predicting fat content for birds below these mass values. Additionally, fat store usage would likely vary during different life history periods (e.g., egg laying, molt) when energetic needs differ, so this model should be used only to track nonbreeding season condition over time.

Overall, when the regression model was applied to historic check station data, fat content remained relatively constant for mottled ducks across years with the exception of 2004 and 2006. Although standard error values were relatively large for mean values, the predictive equation tracked major fluctuations in predicted fat content over time. The decreased condition for both age classes in 2004 and 2006 can likely be explained by the occurrence of large-scale landscape environmental disturbances. Surveys in 2004 took place following a substantial drought in 2003; conditions similar to this drought were not experienced again until 2011, at which time precipitation levels still remained higher (TWDB, 2014). Drought would likely reduce food availability and, consequently, fat content (Bhattacharjee et al. 2009). The drop in estimated fat in 2006 can likely be attributed to the occurrence of Hurricane Rita, which passed over the Chenier Plain of Texas in 2005. Hurricanes, as a major ecological disturbance (Michener et al. 1997), have several impacts that could influence the condition of animals living in affected habitats. First, mottled ducks, because of their non-migratory life history strategy, do not relocate to distant habitats to escape immediate and resultant hurricane impacts (Stutzenbaker 1988); this was corroborated by similar population counts of year-round resident waterbirds in wetlands before and after Hurricane Rita (O'Connell and Nyman 2011). Additionally, hurricanes have major effects at a landscape level and many environmental ramifications such as greatly increased sedimentation (Turner et al. 2006), rapid erosion of coastal land forms such as barrier islands (Stone et al. 1997), and drastic changes in salinity due to oceanic storm surges and sedimentation (Blood et al. 1991). One of the results of these changes is also physical destruction of plant communities. On a smaller scale, plant communities in a coastal marsh took up to 10 years to recover from removal by muskrats (*Ondatra zibethicus*) (Bhattacharjee et al. 2007); a hurricane would have similar effects on a landscape scale, significantly limiting food resources and potentially causing reductions in condition on a short-term basis. Although these disturbances would intuitively suggest an impact on organism success in an affected habitat, this dataset is admittedly small and correlation values generated from regression analyses are relatively weak even for the top ranked models ($r = 0.54$). Concrete scientific support for these concepts would require further body composition analysis of mottled ducks to determine fat content in relation to measured environmental conditions, which was not feasible as part of the current study.

Trends in precipitation effects on fat content, although correlations were not present given the current dataset, provide an interesting initial result. Intuitively, a relationship might be expected between precipitation and mottled duck fat reserve levels. Fat content would be expected to increase with increasing precipitation, as increased precipitation would translate in many ecosystems to an increase in plant biomass and food availability. In mottled ducks, however, increased precipitation and resource availability is typically associated with increased breeding effort, because the species does not face temporal pressure to breed like many migratory species (Stutzenbaker 1988, Rigby and Haukos 2012). As such, an increase in adult breeding effort during years of increased precipitation might manifest as a reduction in fat reserves because of greater energetic input into reproduction. Sex partitioning of analyses would be required to determine whether this effect is sex-specific; if not, factors such as molt may also play a role in reduced condition. Reproductive variation in condition is a potentially complex issue for this species and warrants further investigation.

Climate may have other impacts on mottled duck condition as well in the form of their unique breeding dynamics. Because mottled ducks live year-round in a mild climate region, they have the opportunity to breed earlier than many other species of waterfowl. Additionally, because any surplus reserves are not reduced or eliminated during the effort of migration, mottled ducks likely have another advantage for early season breeding because of they do not need to re-establish energetic stores after movements and before a breeding effort. These factors also provide mottled ducks with the opportunity to be more selective about their breeding effort and avoid breeding during unfavorable times, while other similar species have a shorter breeding window and face pressure to reproduce quickly (Stutzenbaker 1988). Mottled ducks, if breeding is forgone, may thus go into molt and wintering with very different body condition than other species of migratory waterfowl.

In conclusion, this study provides a first effort to describe body condition in mottled ducks and an equation to estimate condition in the field. As landscape changes continue to become more frequent and drastic, managers may desire to track changes in relevant metrics of focal species, such as mottled ducks. Especially in the context of lead exposure, recent surveys have shown that, despite the national ban on the use of lead shot for waterfowl hunting, individual mottled ducks continue to experience lead exposure at occasionally toxic levels (Chapter 3, 4). Body condition measures in the field may provide rough insights on this issue given the numerous documented negative physiological effects of lead on waterfowl (Pain 1996) and results indicating that heavy metal concentrations may be directly and inversely related to body condition measures in at-risk species (Takekawa et al. 2002). Although destructive sampling of this species is not advisable because of its current population status, having a condition index that effectively and easily predicts fat content from normal check station or banding operation during the non-breeding season may allow managers to track responses to habitat change and observe the effects of anthropogenic impacts in this heavily impacted region.

Table 1.1 Summary morphometric statistics for 24 mottled ducks collected for body condition analyses from the Texas Chenier Plain National Wildlife Refuge Complex during 2005-2007.

	AF (n = 3)		AM (n = 7)		JF (n = 5)		JM (n = 9)	
	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE
Mass (g)	810.6	19.32	974.0	17.30	871.6	8.56	951.6	10.68
Wing Chord (mm)	240	0.84	251	1.66	242	2.46	247	0.85
Tarsus (mm)	51	0.88	51	0.67	51	0.55	52	0.28
Keel (mm)	94	1.90	99	1.06	95	0.75	98	0.56
Body Length (mm)	505	5.49	535	3.58	523	3.28	538	1.83
Ab. Fat (g)	4.58	0.97	11.62	0.97	7.80	0.79	10.68	0.63

[1]AF = adult female, AM = adult male, JF = juvenile female, JM = juvenile male

Table 1.2 Measures of average mass, wing chord, and estimated abnominal fat for 1,162 mottled ducks from historic check-station data (1986-2011) at Anahuac National Wildlife Refuge on the Texas Chenier Plain National Wildlife Complex.

	Adult (n = 690)		Juvenile (n = 472)	
	$\bar{x}$	SE	$\bar{x}$	SE
Mass (g)	1034.6	3.7	925.6	4.77
Wing Chord (mm)	253	0.37	246	0.46
Estimated Abdominal Fat (g)	14.27	0.22	10.36	0.15

Table 1.3 Top ranked models describing the relationship between external morphometric measurements and abdominal fat content in nonbreeding mottled ducks sampled from the Texas Chenier Plain National Wildlife Refuge Complex during 2005-2007.

Model	R^2	Adj. R^2	AIC_c[1]	ΔAIC_c	K
M/K [(2)]	0.3008	-	145.5275	0.0000	2
M/WC	0.2980	-	145.6202	0.0927	2
PC1	0.2942	-	145.7435	0.2160	2
M	0.2554	-	146.9710	1.4435	2
M/L	0.2202	-	148.0351	2.5076	2
M/WC, M/WC*Age, Age	0.3594	0.2583	149.7795	4.2520	4
L	0.1206	-	150.7996	5.2721	2
Null	-	-	151.0935	5.5660	1
M/WC, M/WC*Sex, Sex	0.3076	0.1982	151.5698	6.0423	4
PC1, PC1*Age, Age	0.2726	0.1578	152.7014	7.1739	4

[1]AICc= Akaike's Information Criterion, correction for small sample size
[2]M = mass, WC = wing chord, K = keel, PC1 = 1st principal component, L = body length

Figure 1.1 Sampled abdominal fat values regressed against a mass/wing chord ratio model for fat prediction in mottled ducks (n=24) on the Upper Texas Gulf Coast. A best fit line for this relationship is displayed with corresponding equation and R^2 value.

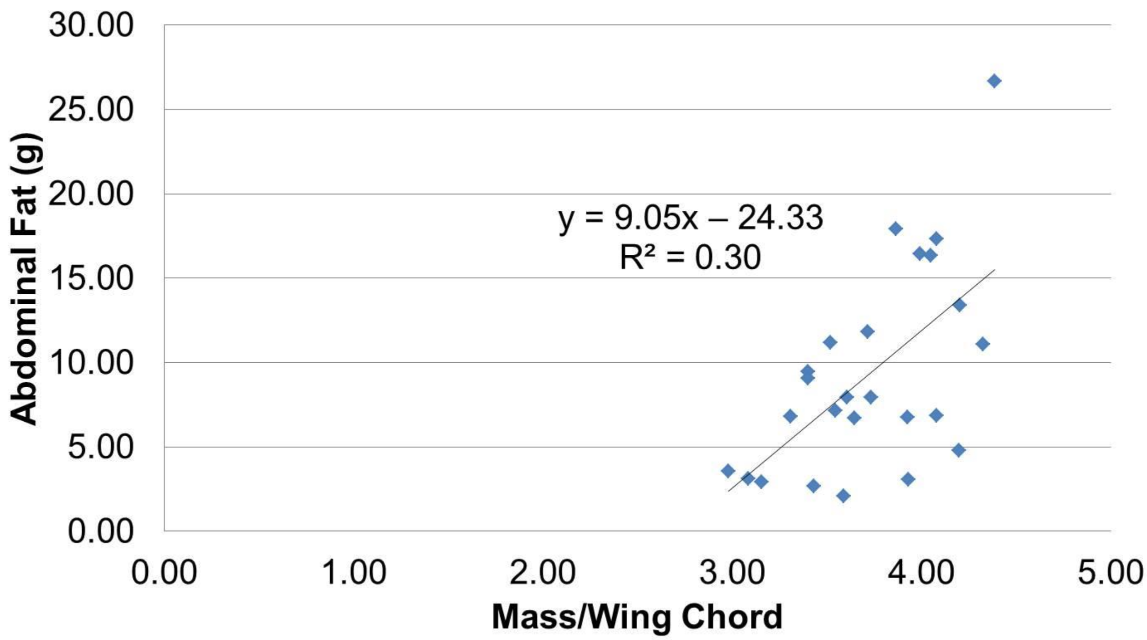


Figure 1.2 Mean estimated abdominal fat (±SE) by year from morphometric measurements of mottled ducks presented at hunter-check stations at the Texas Chenier Plain National Wildlife Refuge Complex during 1986-2011. Years with the same letter are not different (P < 0.05).

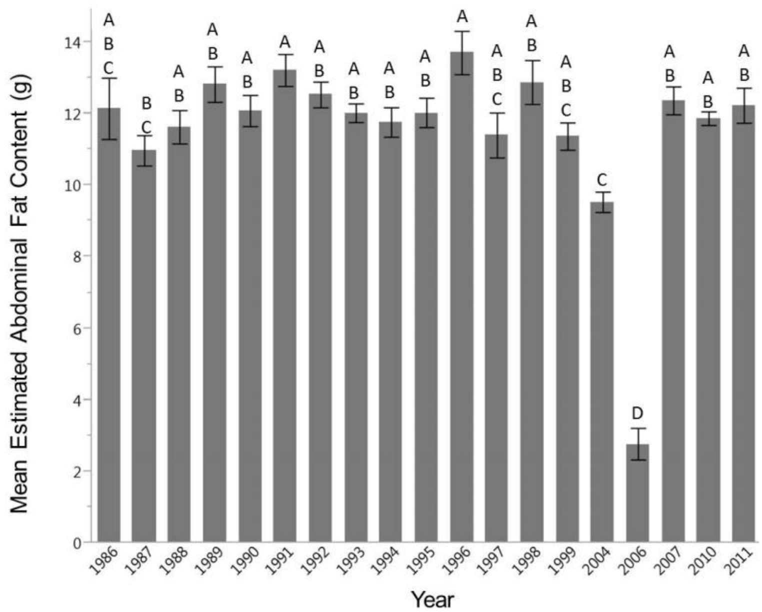
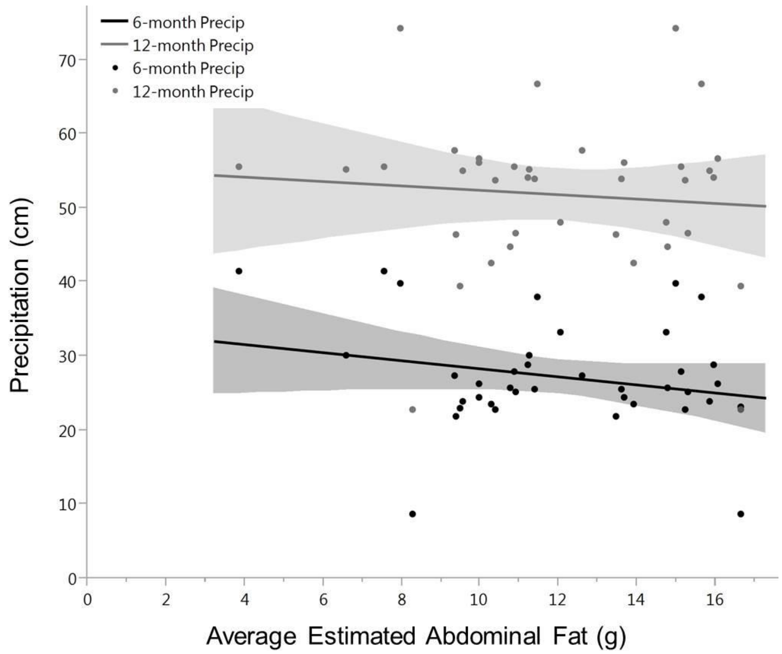


Figure 1.3 Relationships between annual average estimated abdominal fat content and cumulative 6-month and 12-month precipitation values from April 1 - September 30 and October 1 – September 30, respectively, for mottled ducks presented at hunter-check stations stations at the Texas Chenier Plain National Wildlife Refuge Complex during 1986-2011.



Chapter 2 – Using Species Distribution Models to Assess Habitat Use by Mottled Ducks and the Potential Presence of a Lead-Related Ecological Trap on the Upper Texas Coast

Lead contamination is an issue of paramount importance in wildlife and habitat management despite significant efforts to reduce input and mitigate extant environmental pools (Haig et al. 2014). Texas is no exception to this point, as historic environmental surveys in coastal marshes have shown large quantities of residual lead in the form of lead shot pellets (Fisher et al. 1986). Lead ammunition continues to be used in the pursuit of upland and webless migratory game species in many parts of the country, creating an increased risk for deposition in wetlands despite substantial management efforts to counter these risks. As a result, lead contamination has become a prevalent conservation concern on the Upper Texas Gulf Coast. Despite a 1983 ban on lead shot on the Upper Texas Coast for waterfowl hunting (Moulton et al. 1988) and regional bans that followed shortly thereafter, migratory birds continue to experience negative health impacts and population effects from lead exposure (McCracken et al. 2000, Fisher et al. 2006, Haig et al. 2014). As one of the United States' most important migratory bird wintering zones, continued lead contamination and consequent exposure risk for wildlife on the Texas Gulf Coast could have far reaching effects if not managed properly.

The mottled duck (*Anas fulvigula*), a non-migratory relative of the mallard (*A. platyrhynchos*) and the American black duck (*A. rubripes*), has experienced particularly strong effects of lead contamination on the Texas Coast. Because they do not migrate, mottled ducks use this impacted habitat during all stages of their life history without a life history mechanism for dispersal (Stutzenbaker 1988). Mottled ducks have exhibited some of the highest lead shot ingestion and lead exposure rates recorded for waterfowl, which is of particular concern to managers of mottled duck populations (Anderson et al. 1987). More recent studies have examined lead levels in mottled duck wing bones and reported an average concentration of 16.62 parts per million (ppm) during the 1998-1999 hunting season. In addition, 28% and 22% of after-hatch-year (AHY) and hatch-year (HY) birds, respectively, demonstrated concentrations ≥ 20 ppm between 1987-2002. Lead concentrations above this threshold are considered to be severe clinical poisoning that could be life threatening (Pain 1996). Although corporeal lead values showed a reduction in average values from those observed in 1987-1988 of 74.1 ppm and 40.0 ppm for AHY and HY birds in this area, respectively (Merchant et al. 1991), it is still cause for concern as even the mean value approaches the 20 ppm threshold for toxic exposure. Recent surveys of blood lead levels from mottled ducks on the Upper Texas Gulf Coast continue to indicate the presence of toxic levels of exposure in many individuals (McDowell et al. 2015). Despite this, lead exposure pathways in mottled ducks have been poorly studied (with the exception of lead shot presence in gizzards) and could be related to a number of environmental factors.

Understanding current spatial patterns of environmental lead availability is a critical first step in mitigating lead issues related to mottled ducks and directing management efforts focused on their habitat. Need for information on spatial lead is made more important in a conservation setting with limited resources. However, obtaining this information can be difficult, especially for environmental variables that cannot be surveyed using modern remote sensing technologies and must be directly measured. Due to cost and workforce related limitations, large scale sampling regimes are often not viable when measuring environmental variables. As a result, studies often obtain estimates of overall values for a region by collecting point data and interpolating to estimate values at points that were not directly sampled (Cambardella et al. 1994, Zhang 2006, Janssen et al. 2008). Interpolation allows researchers and managers to identify areas that contain contaminant levels of concern and efficiently direct resources and management efforts towards these problem areas.

To translate the results of an individual study to a larger scale or to a different ecosystem, however, it is helpful to understand whether certain sets of environmental factors are typically linked to a particular level of contamination. Although knowledge of the spatial patterns of contaminants in the environment is helpful on its own, modern analytical approaches make it possible to determine the environmental factors linked to contamination to draw connections to animal space use and movement. The first step in these analyses is to obtain information on local habitat use of a particular population of a species that can, arguably, be considered as the species' niche. Although the concept of the niche has taken many different forms as the ecological sciences have progressed and spatio-temporal concerns and factors have taken a more prominent position in the mind of the researcher, a niche is generally considered to be some description of the set of environmental conditions that suggest species occurrence (Grinnell 1917, Elton 1927, Hutchinson 1965, MacArthur 1968).

The niche, depending on spatial extent and theoretical definition, can be modeled by determining important environmental factors to a species *a priori* and consequently building predictive models. Ecologists developed Species Distribution Models (SDMs) to evaluate relative importance of environmental factors. SDMs were first conceived in the 1970s and developed into their current form during the 1990s, and provide a rigorous approach for biologists to determine the effects of disturbances or generalized environmental changes on the resource and space use of affected species (Guisan and Zimmermann 2000). SDMs are optimized to provide information about the three ecological factors deemed to be of high importance in predicting species range: (1) limiting factors for a given species, (2) occurrence of disturbances in environmental systems of natural or human origin, and (3) available resources (Guisan et al. 2002). All these factors, including biotic and abiotic aspects, are prominent in general ecological theory, as each plays an important role in determining usable habitat patches for a given organism or a species' niche. In essence, the goal of these analyses is to create a spatio-temporal snapshot of the environment, determine how a given species uses their habitat, and attempt to gain information about which landscape-level factors influence the movements and distribution of a species.

One of the more common approaches in recent studies has been to use maximum entropy statistical modeling, often referred to as MaxENT, as a way of determining species occurrence probabilities. This modeling approach has been particularly valuable as it is well-suited for use with either presence-only or presence-absence data (Elith et al. 2011). MaxENT can therefore be used with data from different sampling techniques such as radio/satellite telemetry or avian point counts. MaxENT attempts to predict occurrence probabilities by generating the most uniform possible distribution in space from a given set of environmental predictor variables and species locations (Elith et al. 2011). The standalone software works by taking a user-defined set of environmental input variables (in geospatial raster format) and providing several useful metrics as output (Schapire 2012). First, models provide percent contributions to species occurrence of each selected environmental variable in a ranking table, which allows the determination of the most important factors contributing to species occurrence. Second, these models identify particular values of each environmental variable that have the most influence on species occurrence. It does this both by examining effects on the model when the variable is removed and the amount of information contained in individual variables. As such, one can quickly determine the most salient factors influencing species occurrence.

Although MaxENT will run with location data that is either presence-absence or presence-only in nature, MaxENT is often used in ecological studies related to surveys that use presence-absence data (e.g. plant distribution where the plant is present at a site or not) (Padonou et al. 2015). This is generally considered to be a more statistically robust approach, as MaxENT is not forced to create pseudoabsences to run models. Using presence-only data in MaxENT can have statistical ramifications. It can be argued that although we lose some information in presence-only data, it alleviates the problem of non-detection in presence-absence surveys. However, presence data can be subjected to many of the same things that might cause a non-detect in a presence-absence survey such as ecological disturbance, species interactions, or local extinctions. Using presence-only models additionally assumes that we have perfect detectability, which in many biological surveys may or may not be accurate, especially when considering motile animals. Telemetry studies such as this one, however, which allow for surveyors to obtain positions regardless of observation point, eliminate many of the issues associated with running presence-only models and allow for statistically robust conclusions to be drawn from model results (Elith et al. 2011).

Although MaxENT provides information on environmental factors that determine species occurrence, it can also be useful in certain analytical scenarios to understand how abiotic factors relate not only to the species in question but also to each other. In a contaminant study, for instance, one would ideally like to understand if, how, and why spatial hotspots of contamination exist. In conjunction with species occurrence predictions, relationships between habitat variables are crucial in a management setting because it allows managers to link important variables for determining occurrence with environmental factors typically indicative of contamination. The novel component of my methodology, described below, was to simultaneously interpret the results of a multivariate ordination of habitat variables considered important to mottled duck occurrence and soil survey points with results from MaxENT SDM models. The approach of using SDM's in conjunction with ordination, by linking probabilities of species occurrence to associations in different habitat variables, will allow managers to assess landscape level indicators of lead contamination while also describing how environmental factors drive mottled duck occurrence. MaxENT modeling combined with ordination has great overall value in the field of environmental contaminant management that has perhaps not yet been realized. By including raster surfaces interpolated from data collected via environmental surveys for contaminants (in this case lead) as input variables in MaxENT models, I can determine both whether or not the presence of contaminants is effectively predicting species occurrence in mottled ducks (i.e., there is a correlation between animal space use and contaminant presence) and what levels of contaminants are typically predictive of animal presence. Although other environmental factors may be present that are driving species occurrence, the method used here can provide a good baseline for assessing and determining mechanisms for exposure risk in this species (Chapter 4). Given the high level of historical lead exposure exhibited in mottled ducks even long after the lead shot ban (Merchant et al. 1991, Merendino et al. 2005), it is likely that there is a habitat use-related pathway whereby mottled ducks are exposed to lead.

The goal of my study was to use species occurrence information to determine whether mottled ducks are at risk of being caught in an “ecological trap” connected with lead contamination in high-use habitat areas (Kokko and Sutherland 2001). The concept of an ecological trap connects the concept of a niche and environmental impacts experienced by a species in a given ecosystem. Ecological traps occur when a species selects a habitat that would normally be favorable, but instead the population suffers a loss of fitness, reproductive success, or (in the case of contaminant studies) organism health (Robertson and Hutto 2006). This behavior has been observed to result in an “allee effect”, wherein a population with low numbers exhibits further density-related reduction in individuals and eventually becomes extinct (Courchamp et al. 1999). Ecological traps often originate from human-caused changes in the environment that result in highly selected habitats becoming unfavorable without organisms perceiving this change (Robertson and Hutto 2007). Over time, if mottled ducks are experiencing an ecological trap related to lead deposited by human activities and the resulting exposure, population declines may worsen and negative health impacts in this species could become severe. Especially if high species occurrence is predicted in areas where lead hot spots are present, negative impacts on the species would likely be more severe. Furthermore, because mottled ducks spend their entire life cycle in the coastal marshes of Texas, temporal variations may exist in lead exposure due to life history-related habitat use shifts or changes over longer time periods due to changes in habitat itself. By determining environmental variables linked both with mottled duck habitat use and the presence of lead suggested by soil surveys, I hope to inform management efforts that will have the greatest efficacy in preventing ongoing exposure in this species at risk.

Methods

Study site

Data collection for these analyses occurred on the Texas Chenier Plain National Wildlife Refuge Complex (TCPC), which included Anahuac, McFaddin, Texas Point, and Moody National Wildlife Refuges. The TCPC comprised a cumulative area of 42,762 ha. Approximately 40% of Anahuac, McFaddin, and Texas Point NWR’s were open to waterfowl hunting. The refuges imposed a non-toxic shot requirement in conjunction with the banning of lead ammunition on the Texas Gulf Coast, which was implemented during the 1978 hunting season and finalized by 1981 (Moulton et al. 1988). Land acquisition to form the TCPC began in 1954, and much of the TCPC has since been rigorously managed for waterfowl via various land management methods.

The landscape of the TCPC was largely influenced by the hydrology and climate of the Gulf of Mexico, which was in turn influenced by sub-tropical weather patterns.

The TCPC receives, on average, 144 cm of rain per year with values ranging from 52 cm – 218 cm. This region was also prone to hydrologic and other effects stemming from the landfall of hurricanes, which can have devestating effects both on land forms and vegetation communities due to changes in salinity, sedimentation, and others (Stone et al. 1997, Turner et al. 2006, Howes et al. 2010, O'Connell and Nyman 2011). Dominant marsh types on both Anahuac and McFaddin NWR's included fresh, intermediate, and brackish marsh (USFWS 2008). Vegetation communities in wetlands varied greatly based on water depth, salinity level, and amount of tidal force.

Mottled duck locations

Movement data were collected from female mottled ducks from 2006 - 2012 marked with either very-high-frequency (VHF) or satellite telemetry tags. Satellite telemetry data were collected from 2009 - 2012 (Moon 2014), with VHF data collected from 2006 -2008 (Rigby and Haukos 2012). Satellite locations were collected using Model 100 solar/satellite platform transmitter terminal (PTT) transmitters attached via backpack harnesses to hens weighing >740 g, deemed to be a body mass above which the 18 g transmitter would not have substantial negative effect. The PTT units indicated hen survival by using measures of unit temperature and bird body motion to detect mortality. The VHF radio tags were equipped with a mortality signal, which occurred when the transmitter was stationary for >8 hours. Analyses on movement were largely limited to the breeding season; although satellite data provided mottled duck locations year round, more labor intensive VHF telemetry studies only collected locations from ~May-September. MaxENT analyses for VHF data were confined to Anahuac NWR, as locations were only collected there. Satellite locations were available for the entire

TCPC and were used in larger scale analyses. Satellite locations were additionally subset into breeding (May-September) and non-breeding (October-April) seasons, so changes in habitat use between life history periods could be documented. Additionally, duckling movements were monitored from 2006-2008 using ATS radio transmitters attached using sutures or cyanoacrylate glue (Rigby 2008). Locations were taken either from radio signals or from visual observation of ducklings. Duckling locations were used in an additional MaxENT model to attempt to determine differences in space use between age classes.

Soil sample collection and lead content sampling

Level of stable lead isotopes in soil/vegetation samples were determined from samples taken across the Chenier Plain NWR complex. Soil samples were collected on NWRs by stratifying habitat type within coastal marsh by salinity level (McDowell 2014). Within each habitat stratification category, a grid of 40 ha was overlaid and 20% of corresponding grid cells were randomly selected for sample collection (Figure 2.1). Soil was collected as 30 cm deep by 48 mm circumference soil cores, and separated by depth at intervals of 0-5 cm (stratum A), >5-10 cm (stratum B), and >10-20 cm (stratum C). Depth partitioning allowed for the determination of lead availability at various soil depths and, consequently, inferences regarding the mobility of lead in the soil column and availability to wetland plants as well as other biota. The nearest perennial and annual plant to each randomly selected soil sampling site was also collected. In the lab, soil samples were sieved to remove any whole lead shot pellets, which were then counted and weighed, and soil cores were radiographed to determine the presence of lead shot pellets. Only two lead shot pellets were identified and removed during data collection. Lead concentrations (ppm) in environmental samples were estimated using AAnalyst 600 and 800 atomic absorption systems that read within ranges set by the Center for Disease Control (CDC) and Occupational Health and Safety Administration (OSHA) (McDowell et al. 2015).

Interpolation of environmental lead

Once lead concentration values were determined for each point (n=175), I used ArcGIS (ESRI 2012) to interpolate values to create a surface demonstrating lead values across space. The ArcGIS Geostatistical Wizard was used to assess relevant spatial statistics and determine the best method for interpolation. Due to the sampling design for soil and vegetation surveys, certain interpolation methods were immediately ruled out because they failed to capture variation in spatial patterns at smaller scales (e.g., clustering). Namely, I excluded kriging from my model development, despite the fact that it is typically considered the most statistically robust interpolation approach under some modeling circumstances. Studies specifically designed for eventual kriging interpolation typically use a uniform sampling distribution, while this study used a random sampling distribution (ESRI 2011). Simple Inverse Distance Weighting (IDW) was determined to be a suitable method for representing these data in fitting a surface, which had the additional advantage of methodological simplicity. Lead content rasters were created for each of the three soil strata across the TCPC.

Species distribution modeling

SDM was conducted by overlaying mottled duck movement data with lead contamination raster surfaces and other environmental variables. I employed the computer program MaxENT (Schapire 2012), an open source software that allows the user to input a set of species locations (in latitude-longitude units) along with various environmental covariates to obtain an estimate of species distribution. Although this software has the capability to model the entire range of species if locations are available, animal locations and ancillary data for this study were only consistently available on the Texas Chenier Plain NWR complex (specifically Anahuac and McFaddin NWR's), so outputs were limited to this region. Ancillary data used to build SDMs included measures of soil permeability derived from the SSURGO (soil survey geographic) database and local documentation (UDSA 2014) to describe particulate penetration in the soil column, a digital elevation model from the National Elevation Dataset (NED), and a landcover dataset derived by a member of our research group (Moon 2014), and interpolated lead values. Variables were defined as categorical or continuous within the modeling framework, depending on their characteristics (Table 2.1). All raster datasets were converted from the ESRI grid raster files to the ASCII file type to provide suitable input for the MaxENT program, and clipped to the exact extent of the boundary of Anahuac and McFaddin NWRs. Model parameters, for the most part, were left as default as the datasets in use did not necessitate any special considerations. I did, however, use a built in subsampling procedure whereby a portion of location data was withheld from subsequent model runs to account for random variation. Each model was subsampled and re-tested 15 times. Additionally, a random test percentage of 25% was defined; this setting allows data not used to train model iterations to test the models.

Models were evaluated using several criteria. First, model fit was assessed using the area under a Receiving Operator Characteristic (ROC) curve (AUC), which described how far the model was from making completely random predictions; AUC values closer to 1 indicate the most non-random possible fit. Second, I examined MaxENT response curves, which indicated values of each environmental variable used in the model that were most important for predicting mottled duck occurrence. Third, I examined variable percent contribution values, which indicated the extent to which each variable contributed to creating the model for occurrence. Last, I examined the results of a jackknife test of variable importance, whereby MaxENT established the importance of variables; this was accomplished by removing the variable from the overall model and quantifying the effect on predictive strength, and also by using the variable individually to predict occurrence. This procedure effectively informs analysts of the variables that have the most information not present in other variables and those variables that contain the most information by themselves (Schapire 2012).

Multivariate statistical analysis

Once SDM models were completed, all habitat and location data were subjected to a correspondence analysis in Program R to assess relationships in habitat variables and their potential suggestion of lead presence in the environment (R Development Core Team 2014). This type of ordination was well suited for combining categorical and continuous variables, and was deemed to be best suited for our analyses due to the dynamic nature of the habitat input variables (Keith Gido, *personal communication*). Variables included in the correspondence analysis were the same as those included in the MaxENT models, and classified in code as either categorical or continuous.

Results

Interpolated surfaces to represent refuge-wide lead concentrations demonstrate a large degree of variability across the TCPC (Figure 2.2). Lead in stratum A demonstrated the greatest variability, with values ranging from ~4 – 86 ppm. Lead in strata B and C had lower ranges, with values spanning ~8 – 37 ppm and ~2 – 44 ppm respectively. Qualitatively, when mottled duck locations collected during all years of this study were overlaid with interpolated lead surfaces, mottled duck locations appear to be more associated with high lead values in upper portions of the soil column (stratum A) (Figure 2.2). When mottled duck survey locations are examined in conjunction with lead in lower portions of the soil column, lead hot spots appear to be in areas used less frequently by mottled ducks on the TCPC (Figure 2.2).

Space use predictions from MaxENT models varied across years and categories (e.g., season, age class), with ranges and environmental variables shifting in importance. All models demonstrated AUC values ≥ 0.75, and were considered to predict mottled duck occurrence non-randomly. Although each model had different quantitative values for assessment criteria, Perm_A and Lead_C came out as important in many of the models (Table 2.2). Other variables that consistently ranked high included NWI and Landcover. This remained true when considering both percent contribution and jackknife test results (Table 2.3).

MaxENT models for this species and study site do not suggest that areas with high probabilities of species occurrence for mottled ducks have high concentrations of lead in all years. Of the models that included lead layers as important environmental predictor variables, the lead values that best predicted species occurrence were typically < 20 ppm. This could partially be due to spatial resolution used in MaxENT models, where close proximity high-risk habitat may not be accounted for if locations did not overlap. Notable exceptions where Lead_A ranked high in percent contribution were years 2006, 2009, and 2011. When comparing breeding and non-breeding season models using satellite data, MaxENT models did not suggest a substantial shift in habitat usage, at least on the scale considered in this study as dictated by percent contributions and jackknife test of variable importance. Duckling occurrence was best predicted by permeability in soil stratum A and landcover classification.

The correspondence analysis including mottled duck location and the associated combinations of environmental variables suggested several trends in habitat variable relationships. First, lead A, or lead in the top soil stratum, appears in the center of the ordination space (Figure 2.3). Central orientation in ordination space indicates that Lead A is associated to a degree with all combinations of environmental variables associated with mottled duck locations, and suggests at least low grade contamination in the upper soil stratum at all surveyed locations. Lead B and Lead C, however, although they are correlated and appear associated with a subset of mottled duck locations, do not show any significant linkages to other habitat variables. One possible exception is Perm_4 (very slow soil permeability), which could suggest greater retention of lead in soils that drain more slowly. Last, NWI and landcover values related to agricultural practices on-refuge [LC3 and LC10 (agriculture)], wetland types with fast hydrology or unsuitable mottled duck habitat [NWI4 (Freshwater forested/shrub wetlands) and NWI8 (riverine wetlands)], and moderate soil permeability (PERM_3) were clustered and oriented orthogonally from any vectors for lead content. Low association of agriculture with lead content at any level suggests that agricultural areas on-refuge as well as the aforementioned wetland types have little relation to environmental lead contamination. This claim was corroborated by relatively low lead levels on agricultural fields located in the northern portion of Anahuac NWR (Figure 2.2, Table 2.4).

Discussion

By using MaxENT SDM analysis in concert with multivariate statistics, I have been able to determine that mottled ducks are likely experiencing an ecological trap in some of their most important habitats on the TCPC. Lead that is accessible to mottled ducks in surficial soils appears to exist in concert with many different environmental variables in this ecosystem. Among these variables are habitat factors that could easily be argued to make up the mottled duck's niche such as open water, sufficient emergent vegetation cover, agricultural development that provide food for mottled ducks, and more (Stutzenbaker 1988). As such, it can be concluded, to a degree, that mottled ducks are exposed to lead particles from soil, and potentially through other pathways, through habitat they normally select during different life history periods. Furthermore, they continue to use that habitat despite negative effects stemming from lead contamination and exposure, some of which may already be observable. These factors together describe an ecological trap, one which could be even more acute given the use of this habitat by mottled ducks year round (Stutzenbaker 1988).

Interpolation analyses suggested that the spatial distribution of lead on the TCPC was variable depending on the soil stratum and spatial location considered. Maximum lead content value measured in soil samples during this study was greatest in the top portion of the soil column, which provides sediments most accessible to mottled ducks.

Dabbling ducks that feed mostly on aquatic vegetation and benthic invertebrates do not search far down in the soil column for food but rather eat what is available at soil surface or above (Baldassarre et al. 2006). As such, if soil is going to be ingested either incidentally or intentionally as grit, it will likely be soil from the top of the soil column, which was observed to have a greater potential contamination level. Given that lead hot spots, or areas with consistently high soil lead concentrations, are indeed occurring, it is further concerning to observe high mottled duck location densities near areas that demonstrate high concentrations of lead. Additionally, shallow-rooted marsh plants, which provide a food source for mottled ducks, likely obtain most of their nutrients from the top portion of the soil column. If natural phytoextraction of lead is occurring in these plants, mottled ducks could additionally become exposed to lead by consuming contaminated vegetation as well as experiencing the effects of biomagnification. Vegetation contained greater lead concentrations than invertebrates at the TCPC (McDowell 2014), and my work suggests that vegetation may provide high lead exposure risk (Chapter 4). While ducklings consume almost exclusively invertebrates and adults increase invertebrate consumption during breeding-related life-history periods, invertebrates compose <2% of the diet during many months (Stuzenbaker 1988). Should vegetation present lead exposure risk, this could present a significant management concern for mottled ducks in contaminated areas. Lead in the lower portions of the soil column had a narrower range, smaller maximum contamination values, and noticeably less observable overlap with mottled duck locations.

Although lead in the lower portion of the soil column appeared to pose less of a concern based on interpolated values and location overlap, it was highly ranked in several of the MaxENT models along with soil permeability in the various portions of the soil column. Use of lead in the lower soil column to predict mottled duck occurrence can likely be attributed to its relatively uniform distribution across areas of high use by mottled ducks. With many locations at a similar value of lead contamination, the modeling procedure may assume that value predicts bird location well because it is common despite this value being incidental. Soil permeability, also a strong predictor in several models, may be indicative of other environmental features such as vegetation community (Rusanov 2011) or the presence of standing water, features that would likely be important in determining mottled duck occurrence. In fact, in many of the models where soil permeability showed a high percent contribution, landcover also ranked somewhat highly, suggesting potential co-linearity between these two environmental factors, although this was not specifically addressed. Co-linearity does not necessarily weaken predictions made by MaxENT models, but must be identified by researchers so models can be properly interpreted (Elith et al. 2011). Land cover became an important variable with respect to duckling occurrence predictions as well, as it was ranked most highly in that model, most likely because ducklings require specific habitat for foraging, occupying a small geographic region around the nest before size increase and fledging allowed them to disperse, and are more vulnerable to predators so cover becomes a critical factor in space use (Stutzenbaker 1988, Mauser et al. 1994, Baldassarre et al. 2006).

My results also indicated few differences between breeding and non-breeding habitat use using satellite telemetry data which could be a result of few changes in behavior between life history periods because individuals do not make large movements in the interim as do migratory waterfowl (Stutzenbaker 1988). Even when seasonal habitat use is considered at multiple scales in mottled ducks, few substantial changes can be seen (Moon 2014). This result is somewhat interesting, however, when considered in conjunction with the fact that mottled ducks in this same population exhibited differences in lead exposure level between the breeding and non-breeding season, with increased blood lead levels being displayed in fall and winter compared to summer (McDowell et al. 2015). As such, there may be a culprit other than habitat shifts responsible for increased lead exposure during the non-breeding season. I hypothesize that this increase may be a result of a shift in diet from invertebrates during the breeding season to vegetation during the non-breeding season. Although one might hypothesize that bioaccumulation could occur while eating an invertebrate diet resulting in higher blood lead levels (Valdes et al. 2014), the risk assessment I conducted in connection with this data suggest under certain scenarios that exposure risk may be greater from vegetation on the TCPC (Chapter 4). The switch to a high percentage vegetation diet may be the cause of elevated lead levels observed in mottled duck blood between breeding and wintering seasons (Stutzenbaker 1988).

Despite observed high lead levels, lead in the top part of the soil column did not provide a high percent contribution or jackknife test importance in many of the MaxENT models. Lead A did occasionally, however, rank highly in prediction models, namely in 2006, 2009, and 2011. This has an interesting implication, as all of these years came either directly after or during a major ecological disturbance: 2006 after Hurricane Rita in 2005, 2009 after Hurricane Ike in 2008, and 2011 during a major drought. Major disturbances such as hurricanes have substantial and lasting effects on the vegetation community (Bhattacharjee et al. 2007) and on various sediment and landscape dynamics that can directly impact animal species in affected ecosystems (Blood et al. 1991, Stone et al. 1997, Turner et al. 2006, Howes et al. 2010, O'Connell and Nyman 2011). Reductions in precipitation and marsh water levels from drought could additionally affect the plant productivity of the ecosystem reducing mottled duck food sources, as well as having direct effects on breeding success from deterioration of suitable habitat (Sorenson et al. 1998). Effectively, my results suggest that in the aftermath or in the midst of environmental disturbances there may be an increased exposure risk to lead for mottled ducks in their normally used habitat, especially in the top portion of the soil column. Given evidence for sediment transport during hurricanes, there is some chance that lead lower in the soil column may be accessed during these catastrophic events as well. This further suggests causation for decreases in body condition observed in post-hurricane and drought years (Chapter 1), since inorganic contaminant exposure has been linked to reduced body condition (Takekawa et al. 2002).

Although temporal or life history-related dynamics appear to be related to lead exposure in mottled ducks, this species appears to be subjected to an ecological trap in this ecosystem resulting from point source high levels of lead contamination. The trap is indicated by the presence of lead hotspots in surficial soils across the TCPC in areas of high space use, the periodic importance of surficial lead in predicting mottled duck occurrence and its ubiquitous relation to other environmental factors, and the apparent contamination of plant food sources. However, especially given that lead directly accessible to mottled ducks is not consistently indicated by a particular subset of environmental variables, the spatial component of managing for lead exposure on the TCPC is still somewhat elusive. Lead in hot spots can be mitigated through a variety of techniques such as dredging or intensive phytoremediation (Salt et al. 1998). Given the evidence I present here, there may be merit in seeking to mitigate the effects of this ecological trap by targeting contaminated food sources, chiefly vegetation, across the TCPC that may provide undue risk for this species (Chapter 4). Lead exposure in mottled ducks is a multi-faceted conservation problem, and one that will demand a diverse management approach to alleviate.

Figure 2.1 Points designated for soil and vegetation sampling based on a 40 ha sampling grid on Anahuac and McFaddin National Wildlife Refuges on the Upper Texas Gulf Coast. Approximately three data points were available within each grid cell. For each point, soil strata A (0 - 5 cm), B (>5 - 10 cm) , and C (>10 - 20) were sampled and subsequently tested for lead content (and later isotope ratio values). These points also provided the geographic basis for vegetation sample collection.

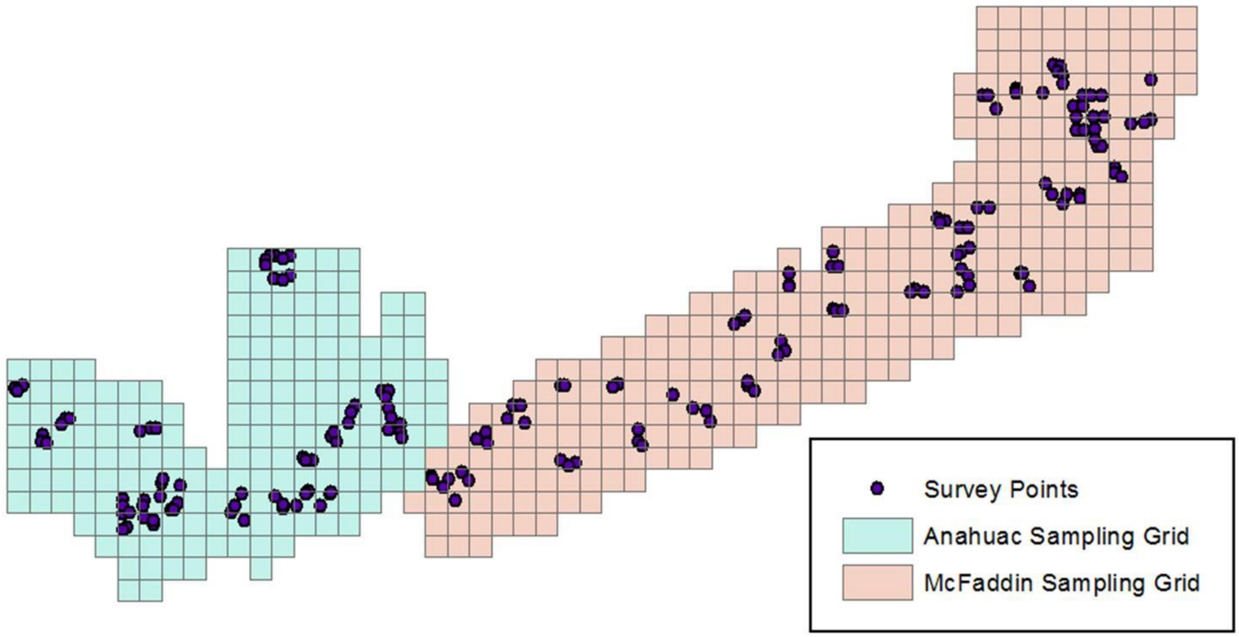


Figure 2.2 Maps demonstrating lead distributions on the Upper Texas Gulf Coast for soil strata A (0 - 5 cm), B (>5 - 10 cm) , and C (>10 - 20), labeled respectively, collected during 2010-2011. Content estimates were based on Inverse Distance Weighting interpolation of soil lead concentration levels in ArcGIS. Mottled duck locations from satellite telemetry for 2009-2011 are additionally overlaid on the right hand pane.

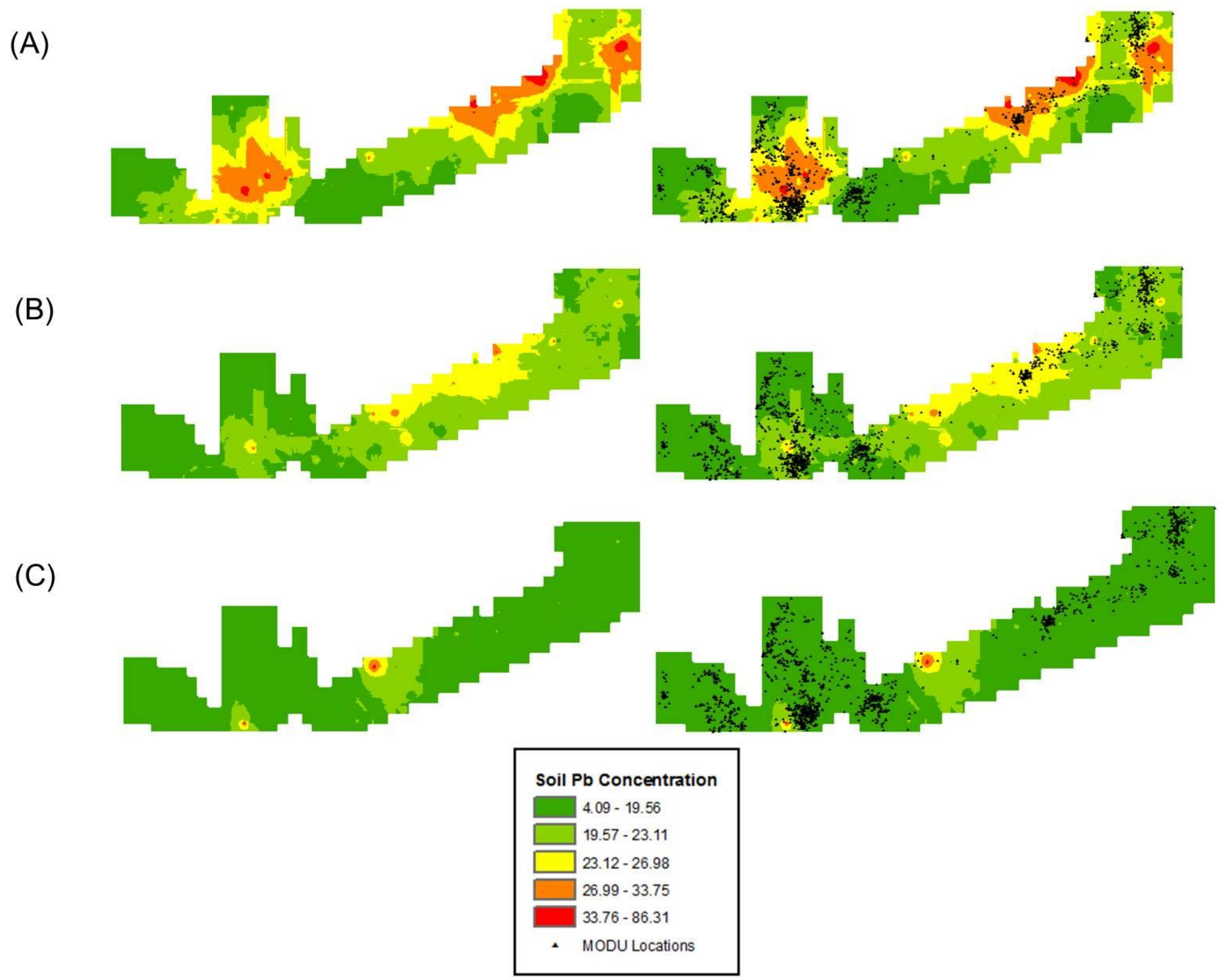


Table 2.1 Input variables (data type and source) used in MaxENT models constructed to predict occurrence of mottled ducks on the Upper Texas Gulf Coast. Variables affected by soil strata, namely soil permeability (PERM) and lead content (LEAD), are referred to first with the variable and then with soil strata referenced (e.g. Lead_A, Perm_A).

Variable	Type	Source	Abbreviations
Soil Permeability	Categorical	SSURGO	Perm A, B, C
Wetland Classification	Categorical	National Wetland Inventory	NWI
Elevation	Continuous	National Eleveation Dataset	DEM
Land Cover	Categorical	Moon 2014	LC
Lead Content	Continuous	McDowell 2014	Lead A, B, C

Table 2.2 Model results from MaxENT models based on all location data, specific years, breeding/non-breeding season, and duckling locations for mottled ducks on the Upper Texas Gulf Coast during 2006-2011. The Area Under the Curve (AUC) values are indicated to provide estimates of the relative distance of how far models are from random predictions (non-random closer to 1). Percent contributions (%) indicate the amount each variable contributes to building each prediction model and Permutation Importance (P.I.) gives the result of a permutation test of variable importance using model training presence data (both values are percentages averaged over all model runs). Highest values for percent contribution are highlighted in grey.

		NWI		Landcover		DEM		Perm_A		Perm_B	
Model	AUC	%	P.I.	%	P.I.	%	P.I.	%	P.I.	%	P.I.
All	0.75	8	2.8	19.5	6.1	8	5.1	29.4	43.8	3	14
2006	0.94	31.6	2.5	13.2	6.3	2.3	1.8	17.1	41.9	0.4	1.2
2007	0.94	11.6	1.1	20.4	1.7	5.1	3.1	30	50.4	0.1	0.6
2008	0.90	4.7	1.5	15.2	5.4	6.1	5.2	44.1	57.2	0.1	0.3
2009	0.78	6.6	1.6	15.2	8.3	7.5	2.9	16.2	32.2	1.6	10.1
2010	0.75	9.4	5	10.9	4.7	5.5	4.1	12.2	30.4	9.9	21.5
2011	0.76	15.5	9.3	4.2	2.8	4.5	5.6	2.6	14.8	1.2	7.2
Breed	0.74	11.2	5.4	5.9	2.8	12.7	6.8	6.7	26.4	4	16
NB	0.75	10.6	6.3	10.9	6.1	4.2	4	11	28	7.9	20.5
Duckling	0.93	0.9	0.3	20.5	4.8	6.9	5.4	33.7	56.3	0.2	0.2

		Perm_C		Lead_A		Lead_B		Lead_C	
Model	AUC	%	P.I.	%	P.I.	%	P.I.	%	P.I.
All	0.75	6.7	3	6.5	1.7	9	12.8	10	10.8
2006	0.94	0.2	0.7	23.5	25.3	8.5	16	3.3	4.3
2007	0.94	6.9	9.8	4.5	9.9	18.7	18.2	2.7	5.2
2008	0.90	9.7	6.8	4.5	5	10	12.9	5.7	5.8
2009	0.78	1.1	2	20.3	19.9	5.9	5.2	25.6	17.8
2010	0.75	6.9	1.9	4	2	15.2	14.5	26	16
2011	0.76	23.3	4.7	17.2	10	13.9	21.7	17.5	23.9
Breed	0.74	13.8	4.8	4.9	3.5	18.3	17.9	22.4	16.4
NB	0.75	11.4	2.1	4.2	3.7	16.6	15.8	23.2	13.6
Duckling	0.93	6.6	6.3	5.8	4.3	9.6	7.5	15.8	14.8

[1]NB = Non-breeding, AUC = Area under Receiver Operating Characteristic Curve, % = Variable Percent Contribution to model, P.I. = Permutation Importance, NWI = National Wetland Inventory, DEM = Digital Elevation Model, Perm = Soil Permeability, Lead = Soil Lead content

Table 2.3 Results of jackknife tests of variable importance for MaxENT models based on all location data, specific years, breeding/non-breeding season, and duckling locations for mottled ducks on the Upper Texas Gulf Coast from 2006-2011. Jackknife tests examine model performance by measuring the predictive power of variables both by testing them alone and then by running the model without them. For each variable deemed important from these two procedures, the high value from the respective response curve is indicated. The variable with the highest percent contribution value from each model is indicated for comparison.

Model	High % contribution	With only variable	Response Curve High	Without variable	Response Curve High
All	Perm_A	Perm_A	Very Slow	Perm_A	Very Slow
2006	NWI	NWI	Estuarine Wetland	Land_cov	Agriculture/ Water
2007	Perm_A	Perm_A	Moderate	Lead_B	21.5 ppm
2008	Perm_A	Perm_A	Moderate	Perm_A	Moderate
2009	Lead_C	Lead_C	16 ppm	Lead_A	17ppm
2010	Lead_C	Lead_C	14 ppm	Perm_A	Very Slow
2011	Perm_C	Lead_B	25 ppm	Perm_C	Slow
Breeding	Lead_C	Lead_C	15 ppm	Lead_C	15 ppm
Non-Breeding	Lead_C	Lead_C	14 ppm	Perm_A	Very Slow
Duckling	Perm_A	Perm_A	Moderate	Land_cov	Agriculture

[1]Perm = Soil permeability, Lead = Soil lead content, NWI = National Wetland Inventory, Land_cov = Landcover classification

Figure 2.3 Results from canonical correspondence analysis (CCA) using mottled duck locations and habitat variables selected *a priori* as important for determining mottled duck occurrence on the Upper Texas Gulf Coast for 2006-2011. Continuous variables are indicated as vectors while values for categorical variables are indicated in black text. See table 2.4 for definitions of variables from their acronyms.

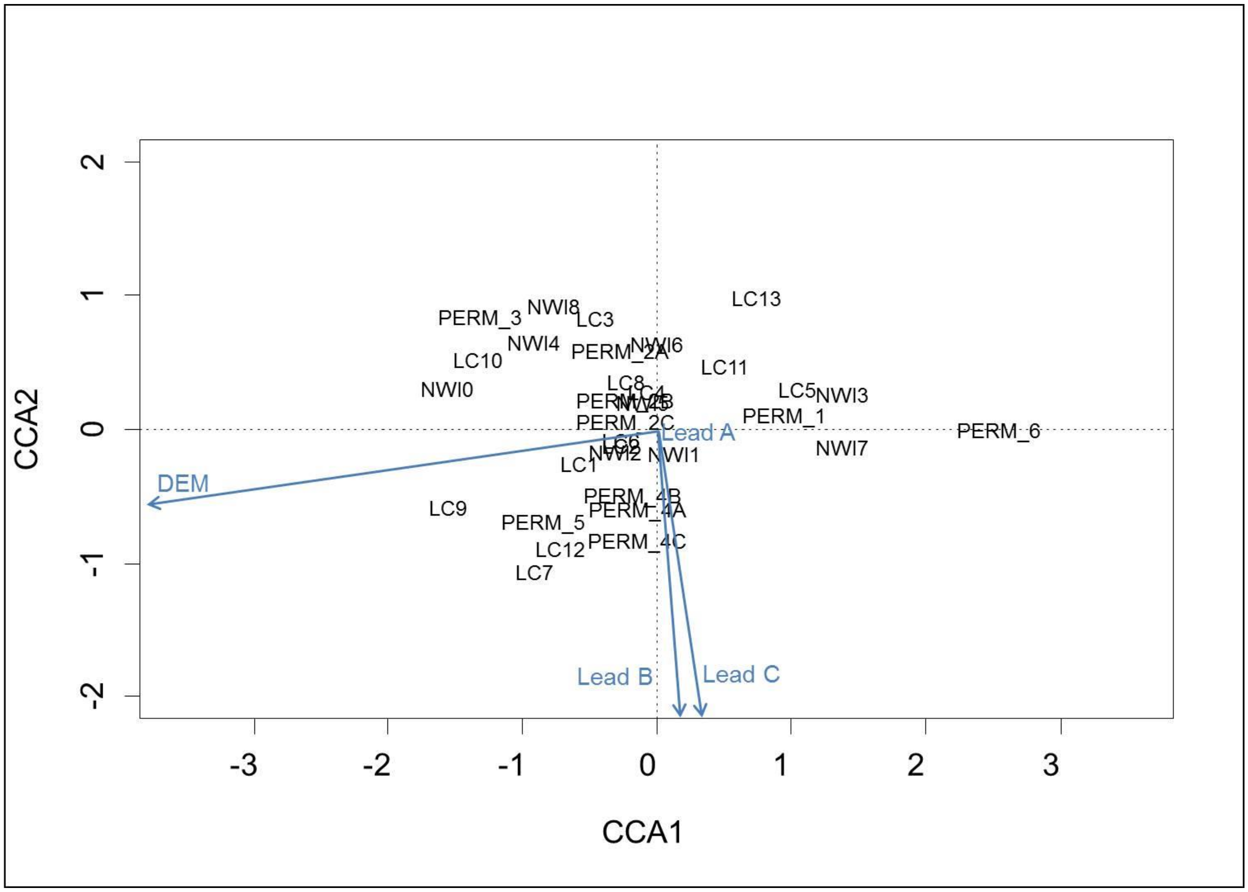


Table 2.4 Abbreviations and definitions for variables used in a correspondence analysis to establish relationships between mottled duck locations and habitat variables on the Upper Texas Gulf Coast from 2006 – 2012.

Variable	Descriptor	Variable	Descriptor
Perm_1	Water	LC1	Pasture
Perm_2	Very Slow	LC2	Grass
Perm_3	Moderate	LC3	Agriculture
Perm_4	Slow	LC4	Emergent Wetland
Perm_5	Rapid	LC5	Water
Perm_6	Very Rapid	LC6	Spartina patens
NWI1	Freshwater Pond	LC7	Beach
NWI2	Freshwater Emergent	LC8	Phragmites australis
NWI3	Estuarine/Deepwater Marine	LC9	Forest
NWI4	Fresh Forested/Shrub Wetland	LC10	Agriculture
NWI5	Other	LC11	No Classification
NWI6	Estuarine/Marine	LC12	Urban
NWI7	Lacustrine Wetland	LC13	No Data
NWI8	Riverine Wetland	DEM	Elevation
Lead	Lead		

Chapter 3 – Use of lead isotope ratios to determine sources of environmental lead deposition and mottled duck exposure

Lead contamination has been one of the most prevalent conservation and environmental issues of the twentieth century. From human health concerns in domestic settings (Pirkle et al. 1998), to many cases of documented wildlife hardship, decline, and negative health impacts (Heikens et al. 2001, USGS 2012, Haig et al. 2014), lead exposure has been a persistent management issue in many different ecosystems. Given the proliferation of industry during the last 200 years and the use of leaded gasoline in private automobiles during much of the 1900s (Nriagu 1990), concerns of heavy metal contamination near industrial sites and roadways have increased (Chow 1970, Blus et al. 1991). Of particular concern are wetland ecosystems, which provide valuable ecosystem services (Costanza 1997) but are prone to disturbance with consequent long recovery times (Bhattacharjee et al. 2007). Additionally, wetlands support rich biotic communities, many of which have been historically affected by lead exposure through hunting, industrial development, or environmental disasters (Graney et al. 1995, Kennish 2002, Haig et al. 2014). Efforts have been taken to reduce continued input of lead and other heavy metals into the environment, but surveys continue to demonstrate the presence of these compounds in important wildlife habitats in various forms, suggesting a contemporary conservation and management concern.

The issue of lead contamination is one in which spatial and temporal environmental availability must be quantified; continued exposure to wildlife can only be mitigated through successful identification of sources of extant lead contamination, ongoing deposition of lead, and spatial extent of environmental hot spots. Various pathways of lead and other heavy metal exposure in waterfowl have been presented in the literature. Exposure can occur from residence in areas contaminated via mining or other industrial activities and subsequent exposure through ingestion (van der Merwe et al. 2011), bioaccumulation (the accumulation of lead in tissues of a species) or biomagnification (increase in contamination level with an increase in trophic level), especially in the case of higher trophic level species like diving ducks (Cohen et al. 2000), incidental ingestion of lead shot pellets during feeding efforts (Anderson et al. 1987, Moulton et al. 1988), or incidental ingestion of contaminated vegetation sediments (Valdes et al. 2014). Pathways of exposure vary greatly on a species and ecosystem level based on feeding strategy, habitat use, and life history. Without the ability to identify the pathways through which wildlife continue to be exposed, executing management that reduces lead exposure for waterfowl in particular becomes a challenge.

Of direct management concern since the 1940's has been the issue of direct ingestion of lead by waterfowl (Bellrose 1955, 1959). Lead ingestion may occur by ingesting contaminated sediments, lead shot pellets, or lead fishing sinkers, all of which persist in the environment because, although lead can erode, it does not radioactively decompose on a short time scale (Irwin and Karstad 1972). In addition to pellet ingestion, exposure may occur through the consumption of contaminated food sources and sediments. Lead deposition into ecological systems has been attributed to atmospheric deposition from combustion of leaded fossil fuels (Bollhöfer and Rosman 2001), byproducts of mining, smelting, and associated industrial processes (Blus et al.

1991, van der Merwe et al. 2011), oil and gas development, and other anthropogenic activities (Komarek et al. 2008). Historical studies, such as that of Chow (1970), show significant contribution of lead content to these environmental pools from aerosolized lead in the atmosphere produced by leaded fuel combustion. That study in particular documented atmosphere-related contamination near roads during a time period when leaded gasoline was still used in cars, but it stands to reason that wetland habitat currently extant near large urban centers (e.g. Houston, Texas), oil and gas, or other industrial development might have been subjected to similar contamination pressures historically or perhaps currently. Especially given that trans-oceanic lead transport has been observed, regional atmospheric contamination is of definite management concern (Bollhöfer and Rosman 2001).

I sought to identify linkages in two factors of concern for waterfowl conservation: lead contamination in mottled duck (*Anas fulvigula*) tissues and mottled duck habitats. Because of the occurrence of lead shot or fishing weight ingestion in mallards (*A. platyrhynchos*), pintails (*A. acuta*), common loons (*Gavia immer*), and notably mottled ducks, this particular exposure pathway has been closely studied in this group (Irwin and Karstad 1972, Scheuhammer and Norris 1996, Merendino et al. 2005). Mottled ducks have exhibited some of the highest lead shot ingestion and exposure rates recorded for waterfowl, which is of particular concern in relation to mottled duck populations (Anderson et al. 1987). More recent studies have examined mottled duck wing bone lead levels, demonstrating an average concentration of 16.62 parts per million (ppm) during the 1998-1999 hunting season, and 28% and 22% of after-hatch-year (AHY) and hatch-year (HY) birds, respectively, demonstrating concentrations ≥20 ppm between 1987-2002, considered to be severe clinical poisoning that could be life threatening (Merendino et al. 2005). Although this does show a reduction in average values from those observed in 1987-1988 of 74.1 ppm and 40.0 ppm for AHY and HY birds in this area, respectively (Merchant et al. 1991), it is still cause for concern as even the mean value approaches the 20 ppm threshold for toxic exposure (Pain 1996). The most recent surveys on my study site, which examined blood lead values, continue to indicate the presence of toxic levels of exposure in many individual mottled ducks (McDowell et al. 2015). While the current level of direct shot ingestion is unknown, this and other exposure pathways continue to be of interest in the ongoing health and persistence of this species.

Although lead shot ingestion has been identified as the most likely pathway for waterfowl lead exposure, of additional concern on the Gulf Coast of the United States is the deposition of lead in surficial soils, and subsequent uptake of lead by plants and invertebrates, from industrial byproducts and historical leaded fuel combustion. Lead deposited from the atmosphere can be assimilated into surface soils (Lead A, in previous chapters), especially near roads or in close proximity to development (Bollhöfer and Rosman 2001, Tomasevic et al. 2013). Additionally, in previous studies that have attempted to differentiate lead sources from one another, atmospheric sources have shown similar isotopic characteristics to lead shot in analysis (Komarek et al. 2008). Fortunately, different sources of lead can be differentiated by using their unique isotopic signatures. The most common isotopes of lead present in environmental samples are ^{206}Pb, ^{207}Pb, and ^{208}Pb, which are derived from ^{238}U, ^{235}U, and ^{232}Th respectively. The most common stable isotope of lead, ^{204}Pb, exists at a constant quantity on earth, and has also been used in several studies to differentiate lead sources. Researchers identify unique lead sources by comparing the ratios of ^{206}Pb : ^{207}Pb and ^{208}Pb : ^{207}Pb either to reference values of various sources or to one another (Graney et al. 1995, Aberg et al. 1999, Bollhöfer and Rosman 2001). Because isotopes are present in different quantities in different environments and degrade from their parent elements at different rates depending on time and origin, ratios of ^{206}Pb, ^{207}Pb and ^{208}Pb are effective at indicating the location and time point of smelting of lead particles that are present in environmental samples (Komarek et al. 2008). Additionally, although coarse identification of source can be achieved with a single ratio, using both ratios allows each sample to be described with greater degrees of accuracy and precision. This method has been used frequently in examining human-related contamination issues, often testing atmospheric or dust samples to determine both geographic and source origins of pollution events (Sturges and Barrie 1987, Nageotte and Day 1998, Lee et al. 2006, Tomasevic et al. 2013).

The approach of comparing isotope ratios in avian species and their habitats/food sources has been used in several previous studies, including work on marbled teal (*Marmaronetta angustirostris*) (Svanberg et al. 2006), American woodcock (*Scolopax minor*) and their food sources (Scheuhammer et al. 2003). At least one larger scale analysis assessed blood samples from many bird species from different clades and with different feeding habits (Scheuhammer and Templeton 1998). In this study, I sought to use isotope ratios to evaluate source linkages between lead present in the blood of mottled ducks and environmental lead available in their habitats. Blood is a useful tissue type for assessing exposure to lead on a short-term time scale (<30 days), as lead had not yet been either filtered out by normal kidney function or deposited in bone tissue (Pain 1996). Bone tissue, mentioned in relation to previous mottled duck studies, constitutes an indicator of long-term exposure, as lead chemically mimics calcium and, when chelated, can be deposited in bone tissues during life history periods involving calcium dynamics such as growth or egg production (Karasov and del Rio 2007). Obtaining wing bone samples (the most common bone analyzed in avian lead studies), however, requires destructive sampling which is unadvisable for mottled ducks given their current population trends.

By examining the ratio values of lead present in mottled duck habitat and food sources, comparing these to reference values, and finally comparing environmental samples to tissue samples collected from mottled ducks in contaminated habitats, my goal was to establish a link between sources of lead in the environment and those most directly affecting mottled ducks. Although this may not provide an immediate or simple solution to the issue of lead exposure in mottled ducks, I hope to determine the sources of environmental lead that are of greatest threat to mottled ducks in order to assist managers in creating mitigation plans that minimize risk exposure for this species.

Methods

Study site

Data collection for these analyses occurred on the Texas Chenier Plain National Wildlife Refuge Complex (TCPC), with efforts focused on Anahuac and McFaddin National Wildlife Refuges. The TCPC comprised a cumulative area of 42,762 ha, and included a mix of coastal wetland habitats including intermediate, brackish, saline, and freshwater marshes (USFWS 2007, Haukos et al. 2010). Much of the surrounding land was used for agriculture, specifically rice (*Oryza sativa*), which is an important food source for mottled ducks (Stutzenbaker 1988). Approximately 40% of Anahuac, McFaddin, and Texas Point NWR's were open to waterfowl hunting. The refuges imposed a non-toxic shot requirement in conjunction with the banning of lead ammunition on the Texas Gulf Coast, which was initially implemented during the 1978 hunting season and finalized by 1981 (Moulton et al. 1988).

Sample Collection Procedures

Levels of stable lead isotopes in soil and vegetation samples were determined from samples taken on the TCPC. Soil samples were collected on the TCPC using a stratified sampling method (McDowell 2014). Within refuge areas, a grid of 40 ha cells was overlaid and 20% of corresponding grid cells were randomly selected for sample collection (n=175) (Figure 2.1). Soil was collected at depths of 0-5 cm (stratum A), >5-10 cm (stratum B), and >10-20 cm (stratum C). This allowed for the determination of lead availability at various soil depths, and, consequently, inferences regarding the mobility of lead in the soil column and availability to wetland plants as well as biota. The nearest perennial and annual plants to each randomly selected soil sampling site was also collected. In the lab, soil samples were sieved to remove any whole lead shot pellets and vegetation samples were dried and ground to provide easier processing. All samples were then subsequently digested and filtered in an attempt to eliminate superfluous materials that could negatively affect isotope sampling (e.g., plant cellulose, silica in soil, or proteins in blood) that would potentially contaminate the ICP-MS or bias isotope readings (see following subsections for sample-specific methods).

Sample Preparation and Digestion

Blood Samples

Blood samples were obtained from mottled ducks both during breeding season banding efforts and at hunter check stations during 2010 and 2011. Given the timing of sampling, destructive sampling was not possible and, as such, wing bones could not be collected from sampled individuals to assess long term lead exposure. Blood (up to 3.0 mL) was collected via brachial venipuncture in live birds and from the thoracic cavity in hunter-shot individuals. Samples were stored in 3.0 mL vessels coated with ethylenediaminetetraacetic acid (EDTA) to prevent coagulation and stored at -20°C until analysis (McDowell 2015). Blood samples were transferred to Kansas State University and kept frozen until processing. Samples were thawed at room temperature immediately before digestion.

To begin digesting blood samples, I mixed approximately 1 mL of blood/EDTA solution with 3 mL of distilled-deionized water (DDI water) and 4 mL of 70% trace metal grade nitric acid, and heated the mixture on an Environmental Express Hot Block for three hours at ~90°C to break down organic materials in samples. Not all samples achieved the full blood volume of 1 mL, as previous analyses consumed large proportions of some samples. In these cases, the maximum obtainable volume was taken from storage vials; samples were later assessed for their viability based on raw isotope counts. After samples were allowed to cool, samples were diluted with an additional 18 mL of DDI water, bringing total sample volumes to ~25 mL. Environmental Express micro-filters were then used to remove any remaining biological components of samples. I transferred 1 mL of the solution into an ICP-MS (Inductively Coupled Plasma

Mass Spectrometer) sample tube with 4 mL of DDI water and covered with Parafilm until blood samples could be tested for isotope ratio values at a later time.

Soil Samples

Dried soil samples collected on the TCPC were transported in paper bags to Kansas State University where they were stored until digestion procedures began. Soil samples were digested using a similar protocol to EPA method 3050, which describes a procedure for processing soil samples for environmental analysis (Edgell 1989). I initially digested samples using only nitric acid, but given the sand- and clay-rich soils present on the study site (USFWS 2008), the samples quickly contaminated critical components of the ICP-MS and produced erroneous isotope values. I modified my extraction procedures and used 70% nitric acid and 30% hydrogen peroxide in a multi-stage digestion process that was more successful in eliminating or reducing organic and solid material in the samples. Approximately 0.5 g of soil was measured out on a high-precision scale and placed in Environmental Express 50 mL ICP-MS sample tubes. Tubes were labeled with the mass of soil and the unique identifier relating to the sampling grid location and the appropriate soil stratum from which the sample was taken. Samples were then mixed with 3 mL of DDI water and 4 mL of trace metal grade 70% nitric acid and heated on an Environmental Express Hot Block for three hours at ~90°C. After samples were allowed to cool, samples were diluted with an additional 18 mL of DDI water, bringing total sample volumes to ~25 mL. Environmental Express micro-filters were then used to remove small particulates from the solution of sample and dilute nitric acid. Samples were then stored in plastic-lined containers until the next step in digestion, which was conducted immediately before measuring isotope values in the ICP-MS.

The second step in the digestion of the soil samples consisted of further digestion using 30% hydrogen peroxide. I centrifuged the 50 mL tubes at 3000 rpm for 5 minutes to push any remaining large sediments to the bottom of the supernatant. Following this, 0.5 mL of supernatant was placed in a Midwest Scientific 2 mL graduated microcentrifuge tube with 1.5 mL of 20% hydrogen peroxide to complete the second digestion step. I heated the vials at 75°C on an Eppendorf Thermomixer for approximately one hour or whenever chemical reactions in the microcentrifuge tubes appeared to have ceased, or when reactants had stopped producing bubbles. Tubes were then capped and spun at 10,000 rpm in a microcentrifuge to once more remove any particulate from the supernatant. I placed 1 mL of the supernatant in an ICP-MS sample tube with 4 mL DDI water and stored samples covered in Parafilm until testing.

Vegetation Samples

Root samples from the nearest plants to soil cores were collected during survey efforts, and subsequently used to test for isotope ratios to attempt to link lead sources between different potential environmental contaminant pools. As vegetation would likely obtain lead from soil nutrient absorption, roots were considered to be representative of aboveground biomass. After collection, samples were dried and ground at Stephen F. Austin State University. Digestion for ICP-MS analysis used only the nitric acid step also used for soil and blood samples (see above sections). After digestion, samples were filtered using Environmental Express plunger microfilters. I mixed 1 mL of the resulting solution with 4 mL of DDI water in an ICP-MS sample tube and stored samples covered with Parafilm until testing.

Lead Shot Pellets: Establishing Reference Values

Geographically and otherwise specific reference values were challenging to find in the literature, creating a need to test various lead shot pellets to establish reference values for this source in particular. This was particularly relevant because this lead source has been hypothesized to be an important contributor to ongoing exposure in mottled ducks (Merendino et al. 2005). Shot pellets from Winchester (~1960 and ~2010), Federal (~1980), Peters (~1960), and Remington (~1980 and ~2010) brands were tested to assess variability in isotope ratio values across time and brands. Shotgun shells were disassembled and pellets were placed in Ziploc bags until sampling occurred. I tested three shot pellets from each shot type (brand and year) and averaged values to ensure obtained isotopic signatures, or specific isotope ratios, were representative of this particular lead source. Additionally, soil sampling on the Texas Chenier Plain NWR complex yielded two pieces of lead shot that were identified as present in soil cores by the use of x-ray. Samples of lead shot pellets were stored in plastic vials after extraction from soil cores.

Lead shot samples were prepared for testing in the ICP-MS by placing 1 shot pellet of each brand and year into a preparation vial with 3 mL of DDI water and 4 mL of trace metal grade 70% nitric acid and heated on an Environmental Express Hot Block for three hours at ~90°C. Samples were diluted substantially to achieve a lead isotope concentration low enough so as not to present a contamination risk for the sampling instrument. Samples were first brought up to a total volume of 25 mL (4x dilution). I added 50 µL of this solution to 50 mL of DDI water to achieve a 1000x dilution. I then added 1 mL of this solution to 4 mL of DDI water for a total volume of 5 mL, which was then added to 5 mL of 1% Nitric Acid. This achieved a total dilution of approximately 32,000x, providing isotopic counts similar to those in heavily contaminated soil samples.

Isotope Analysis

All isotope analyses were conducted on an Agilent Technologies 7500 series ICP-MS at the Kansas State University Veterinary Diagnostics Toxicology Laboratory in Manhattan, KS. The ICP-MS used argon gas to create a plasma that suspended biological samples so heavy isotopic values could be observed. For each sample tested, data were recorded for values of ^{206}Pb, ^{207}Pb, and ^{208}Pb; ^{209}Pb was additionally read as an internal standard with which to monitor changes in the detection properties of the instrument and become alerted to potential contamination or drift in readings. Standards read, prepared by the veterinary diagnostics lab staff, were 0 ppb, 1 ppb, 5 ppb, 20 ppb, 100 ppb, 500 ppb, and 1000 ppb. Although many studies also use ^{204}Pb as the most stable isotope with constant abundance over time (Komarek et al. 2008), the small geographic scale and resulting reduced variability of ^{204}Pb in this study make it suitable to compare only the other isotopes decayed from isotopes of Uranium and Thorium. Because I was only interested in ratios between isotopes present in samples, knowledge of dilution factors and precise internal standard readings were not critical; regardless of values between samples, relative values of the different isotopes provided the information necessary to determine unique ratio values. Raw isotope counts were measured as they passed over the sensor in the ICP-MS, and these values were used for each isotope to calculate appropriate ratios.

Samples were introduced to the ICP-MS either manually or via a sample auto-loader. Samples that were expected to have higher levels of undigested material or greater raw counts of lead isotopes (mainly soil and shot reference samples) were introduced manually to avoid creating blockages in the instrument, either in the lines or internally. Once the instrument began detecting isotope values from each sample, ≥20 points were taken and the mean of these points was recorded for each isotopic signature of interest. I strove to achieve minimal sampling error possible between data points, which in most cases amounted to <10% variation from reading to reading within a single sample; this metric was displayed during data collection, and can thus be monitored as samples are analyzed. The ICP-MS was flushed between samples using a 2% nitric acid solution, and external copper components of the ICP-MS were cleaned periodically to prevent contamination. Throughout testing, standards were read at the beginning of each sampling session, and approximately every 15 samples thereafter.

Once sample data were compiled, samples with lead isotope counts ≤500 for any values of interest were removed from the dataset because ratios between isotopic values would likely be too heavily influenced by even minor stochasticity in the detection parameters of the ICP-MS. Isotope ratios were calculated for $^{206}Pb : ^{207}Pb$ and $^{208}Pb : ^{207}Pb$ for all samples. These ratio values were then compared against Pb concentrations in soil and vegetation samples, and ratios between different sample types were compared to assess the similarity in lead sources present in different environmental pools. Isotope ratio values for various other environmental sources, including those from atmospheric pools, were compared to literature reference values when available.

I calculated ratios from raw isotope counts and generated descriptive statistics and graphics in the JMP statistical software package (SAS 2007). Ratios of ^{206}Pb : ^{207}Pb and ^{208}Pb : ^{207}Pb were compared in soil (strata A, B, C), vegetation, blood, and shot reference values using box plots and *t*-tests to examine differences in means. Overlap of value ranges also proved effective to determine connections between environmental pools of lead, as considering ranges in two dimensions allows a more specific comparison than simply using one ratio.

Results

Reference values established for lead shot samples ranged from 1.10 to 1.21 with a mean value of 1.16 (SE = 0.008) for the ^{206}Pb : ^{207}Pb ratio and from 2.41 to 2.64 with a mean value of 2.48 (SE = 0.060) for the ^{208}Pb : ^{207}Pb ratio (Figure 3.1, Table 3.1). Samples from shot pellets taken from circa 1980 and later were fairly constant across the ^{208}Pb : ^{207}Pb ratio, with some variation in the ^{206}Pb : ^{207}Pb ratio. Shot pellets taken from Peters brand shells from the 1960's showed more variation among shot, which was most likely attributable to isotopic degradation over time. The two pieces of lead shot obtained from soil cores yielded values for (^{206}Pb : ^{207}Pb , ^{208}Pb : ^{207}Pb) of (1.11, 2.44) and (1.18, 2.46), both of which fell within the range of more modern shot samples from the area (Figure 3.1).

Vegetation, soil, and blood samples, when compared to reference shot values, showed variable results. Soil values for ^{206}Pb : ^{207}Pb ranged from 1.11 to 1.37and values for ^{208}Pb : ^{207}Pb ranged from 2.30 to 2.92. Vegetation values for ^{206}Pb : ^{207}Pb ranged from 1.14 to 1.17 and values for ^{208}Pb : ^{207}Pb ranged from 2.38 to 2.47 (Figure

3.2). Blood values for $^{206}Pb : ^{207}Pb$ ranged from 1.01 to 1.43, and values for ^{208}Pb : ^{207}Pb ranged from 2.21 to 2.(Table 3.1, Figure 3.3). All values for vegetation samples fell within the range of isotopic signature values produced for lead shot, while about 50% of soil sample values fell within that range (Figure 3.2). Most blood samples fell within the range established for lead shot ratio values (Figure 3.3).

When I compared the lead isotope ratios soil strata to lead shot reference values using a *t*-test, soil samples in the top of the soil column, sediments most directly available to dabbling waterfowl looking for gizzard grit or invertebrate food sources, showed isotopic values more similar than other strata to lead shot for both $^{206}Pb : ^{207}Pb$ and $^{208}Pb : ^{207}Pb$. Soil strata, however, all demonstrated isotope values that were significantly different from lead shot (Stratum A t = -2.25, p = 0.025; Stratum B t = -4.54, p = <0.0001; Stratum C t = -3.54, p = 0.0005) (Figure 3.4). When examining blood using *t*-tests, samples showed consistency with lead shot isotopic signatures as well as with vegetation lead isotope ratio values for $^{206}Pb : ^{207}Pb$ but samples showed little consistency in $^{208}Pb : ^{207}Pb$ (Figure 3.5). Blood was, however, the only sample type to not demonstrate a significant difference to shot in both $^{206}Pb : ^{207}Pb$ (t = -1.36, p = 0.182) and $^{208}Pb : ^{207}Pb$ (t = -0.5, p = 0.617). Soil was the only sample type that demonstrated significant difference with lead shot reference values and all other sample types for $^{206}Pb : ^{207}Pb$ (Blood t = 6.60 p < 0.0001; Shot t = 4.44, p < 0.0001; Vegetation t = -4.42, p < 0.0001). Additionally, when blood sample $^{206}Pb : ^{207}Pb$ and $^{208}Pb : ^{207}Pb$ ratios were compared against blood lead concentrations, individuals with higher concentrations of blood lead showed isotopic ratio values consistent with those I developed as references for lead shot (Figure 3.6).

When lead isotope ratio values in this study were compared against lead ratio value ranges for other sources of lead contamination as sourced from the Komarek et al. (2008) review as well as the Sturges and Barrie (1987) study, values also came out within the range suggested for USA automobile sources of lead of approximately 1.18 and 1.2 for the ^{206}Pb : ^{207}Pb ratio in these articles, respectively (Table 3.2). Neither of these studies present values for the ^{208}Pb : ^{207}Pb ratio. Industrial slag from lead ore sources in Mexico, likely to be responsible for any industrial contamination along the lower Texas Gulf Coast, also show ^{206}Pb : ^{207}Pb values near 1.20, although it is unlikely that lead from these sources would travel as far as the Upper Texas Coast (Komarek et al. 2008). Isotope ratio values for environmental and blood samples outside of the range I developed for lead shot pellets could be attributable to lead from coal, industrial slag, or leaded gasoline from other locations such as Mexico (Table 3.2).

Discussion

Our major findings were that lead isotope ratio values present in mottled duck blood are statistically consistent with values both in the literature and developed by this study for lead shot. Therefore, lead shot cannot be ruled out as an ongoing source of lead exposure and contamination for this species, despite management efforts to reduce input of lead shot pellets into wetland ecosystems across the United States. The ratio signatures consistent with lead shot additionally overlap partially with those of other lead sources, making absolute discrimination difficult; overall, this study provides strong evidence for lead shot. Unfortunately, I was largely unable to test isotope ratios of lead sources other than shot. I thus made comparisons to some geographically specific reference values present in the literature.

Of note was that lead ratio signatures in soil were not as consistent with those of lead shot as were plants and mottled duck blood, which demonstrated ratios that showed influence of lead shot or a source with a similar ratio signature. Further incriminating evidence for lead shot contamination in mottled ducks was demonstrated by an observed, although not quantitative, relationship of high blood lead concentrations with lead shot ratio signatures. Lower mottled duck blood lead concentration values were much more variable, suggesting potential other sources of exposure for the general population in comparison to highly exposed individuals. In general, it seems likely given this evidence that elevated levels of lead contamination in coastal marshes and mottled ducks of the TCPC was a result of historical deposition of spent lead shot rather than current or ongoing deposition.

Regardless of the origin of the lead contamination, the connection between extant lead in blood lead ratio signatures and the ratio signatures of plants and soil suggests that these food and/or digestive pathways may be important in determining the level of exposure experienced by mottled ducks. Mottled ducks obtain sediment as part of their normal feeding process from the top part of the soil column (Baldassarre et al. 2006) and may ingest lead shot mistakenly for grit (Mateo et al. 2000). Additionally, the shallow rooted plants that dominate the estuarine marshes of the Upper Texas Coast likely draw most of their nutrients, and therefore contaminants, from the top portion of the soil column, which should give consistency in ratio values between these two environmental pools (USFWS, 2008a). Interestingly, isotope ratios of lead in the top portion of the soil column demonstrated less consistency with mottled duck lead ratio values than did plants. Vegetation, however, makes up a much greater portion of the mottled duck diet during most of the year, with soil largely ingested incidentally during foraging efforts or as grit (Stutzenbaker 1988). The fact that mottled ducks appear to be obtaining at least some of their blood lead from their chief food sources indicates a pressing management concern that should be dealt with quickly to mitigate bioaccumulation and ecological trap effects on this species.

Because of its apparent large contribution to lead contamination in this region, management of lead shot and associated contamination hot spots (Chapter 2) is of primary management concern on the TCPC. Although ratios found in my study site could potentially indicate other sources of contamination, chiefly leaded fossil fuel combustion or industrial lead, consideration of historical and modern land use on and near the TCPC specifically suggests that spent lead shot is a more likely contamination source. These other industrial or combustion related sources may perhaps account for some of the higher values of the ^{206}Pb : ^{207}Pb ratio present in soil samples, many of which were not necessarily consistent with lead shot reference values developed herein. Furthermore, given that atmospheric lead likely results in more uniform and large scale deposition, it is likely that background lead levels on the TCPC may stem from this source while environmental hot spots stem from lead shot deposition.

It should be noted that leaded gasoline combustion has posed its share of problems: at least one study in Europe suggested that even despite the phase out of lead gasoline during the 1980's that atmospheric lead values had not substantially decreased after many years (Aberg et al. 1999). Additionally, with leaded fossil fuels largely phased out, there may be greater contribution to the atmospheric lead pool from industry, and additional contribution because of long distance transport from industrialized countries with more lenient environmental regulations such as China or Russia (Bollhöfer and Rosman 2001). Bollhöfer and Rosman (2011) additionally suggest a $^{206}Pb : ^{207}Pb$ ratio range in atmospheric lead (1.17-1.23) which is similar to that of shot reference values developed in this study (1.10-1.21), which also shows similarity to ratio values from leaded fuel combustion (~1.20, (Sturges and Barrie 1987)). Atmospheric lead deposition would likely lead to a more spatially uniform contamination pattern; it therefore may be responsible for some of the widespread low level contamination across the TCPC. Roads and commercial waterways traverse the TCPC; however, much of the complex remains unaffected by these disturbances and is likely less impacted by contamination sources like historical leaded gasoline combustion, which tends to be localized around heavily trafficked roadways (Chow 1970). Additionally, with the observance of several lead "hot spots" on the TCPC, one must consider that point source contamination from lead shot or other sources is a more likely culprit for contamination. Hot spots could potentially also result from geographic location, as many of the hot spots occur near the inland water way which runs from SW to NE along the northern border of McFaddin NWR and on the SE portion of Anahuac NWR where oil and gas development persists (Figure 2.2).

Several factors could be responsible for the persistence of lead shot-related contamination in these wetland ecosystems. First, the continued presence of contaminated sediments is not likely to decrease without the intervention of managers because of the long radioactive half life of lead (Pain 1996). Areas with historically high inputs of lead shot, such as hunt clubs or shooting ranges that were acquired by the USFWS as part of the TCPC and other refuge complexes, might provide long-term lead hotspots even after lead shot pellets themselves were mechanically broken down by hydraulic forces (Irwin and Karstad 1972). Because of sediment transport in these coastal marshes, one could additionally expect that contaminated sediments from shooting ranges or hunting areas could easily shift to other portions of the refuge complex. This transport is likely to become even more pronounced after large-scale ecological disturbances such as hurricanes, where lead shot pellets that had descended in the soil column due to their density may resurface (Larson and Kraus 1995). Second, a potentially large portion of lead contamination in wetland ecosystems in Texas originates from the mourning dove (*Zenaida macroura*) harvest, for which lead shot is still commonly used (Pierce et al. 2014). Although NWR properties prohibit the use of lead ammunition, neighboring private-land rice fields where mottled ducks are likely to feed (and which were used by satellite marked birds in this study) (Stutzenbaker 1988) may be regularly hunted for doves, providing an input of ingestible lead shot into the environment. Studies conducted in Mediterranean waterfowl species also suggested that species whose diet contains large quantities of rice are more likely to ingest lead shot because of the need for larger diameter grit in digestion (Mateo et al. 2000, Figuerola et al. 2005). This association potentially creates another management concern, as controlling land use practices on private land in the United States has proven challenging in many conservation efforts. Last, although ingestion levels and soil lead concentrations seem to indicate that some level of extant lead shot pellets exists on refuges (McDowell 2014), surveys to assess lead shot pellet abundance on

NWR's in this area have been infrequent and estimates are varied (e.g. Fisher et al. 1986). Knowledge of areas that still provide large quantities of intact and extant lead shot pellets might suggest initial targets for directed management efforts, although areas with high soil lead concentrations may provide a suitable proxy for lead shot presence.

The several sources that I hypothesize to be responsible for lead contamination on the TCPC are consistent with other studies that examine similar problems in other ecosystems. The ratio values developed for environment, shot, and mottled ducks in the present study are further confirmed, to some degree, by the Svanberg et al. (2006) study in marbled teal (*Marmaronetta angustirostris*). Although their study took place in Spain, and ostensibly differences may be present in lead isotope signatures due to differences in geographic location, they demonstrated similar values of lead isotope ratios in affected teal as in mottled ducks. Because feeding and life history habits are most likely similar (short of the phonological differences resulting from a migration life history period), this is likely a useful comparison to demonstrate that our data reflect contamination issues that might be considered even at a global scale. Interestingly, Svanberg's study, which also examined lead isotope ratios in wing bones from collected teal, demonstrated slightly different ratio values for this tissue type (1.17, 2.46), which may suggest that further analysis is warranted to determine whether mottled ducks might sequester a slightly different type of lead in wing bones than in blood.

Management solutions are challenging, and depend largely on how lead exists in the environment. Atmospheric lead removal on a small scale is not likely feasible, so the best practice regarding this lead pool is to monitor it in connection with other environmental pools. Lead in pellet form is often removed via dredging of soils, which is a costly procedure with many environmental implications, particularly to plant communities. Strategies for removing particulate lead from soils are relatively few, but phtyoremediation appears to have a degree of promise, and might be particularly effective on an NWR where anthropogenic disturbance is carefully controlled and could be avoided altogether given a particular management decision. Phytoremediation involves either planting an area with plants that have a high metal uptake rate or applying a chelate such as EDTA to an area to increase uptake rate in native plants (Salt et al. 1998, Evangelou et al. 2007). Plant species effective for use with this management practice vary, although studies have demonstrated that marsh grasses in the genus *Spartina*, a prevalent genus on the TCPC, take up lead (Weis and Weis 2004). After a predetermined period, the contaminated vegetation is removed along with a much of the lead from the contaminated area that was planted. Studies examining the efficacy of this method have demonstrated as much as 28% reduction in contamination in the managed area (Salt et al. 1998). Given that naturally occurring plants in this area also contain a reasonably high concentration of lead, I suggest that removal of highly contaminated plants in impacted areas may also be a viable technique for removing lead from the ecosystem. Especially with the addition of a chelate, this management practice may show promise in removing particulate lead that would not be affected by dredging.

One of the largest political barriers to regulating or banning lead shot, for instance, has been the desire by hunters to continue to use lead ammunition based on its metallurgical properties. A recent study in Texas, however, showed non-toxic shot types to be equally lethal to lead shot in many hunting situations, and that shot pattern mattered more than the metallurgic properties of the ammunition used (Pierce et al. 2014). Additionally, the higher price of ammunition containing non-toxic shot, especially those that purport to mimic the metallurgic qualities of lead, is a perceived deterrent for many hunters for making the switch to non-toxic shot for upland hunting in areas where it is not already mandated. For mitigation efforts on refuges to be effective for long-term management of lead exposure in mottled ducks and similar species, off-refuge regulations must also work towards reducing exposure. On the Upper Texas Coast, that may include embracing a nascent national trend of working towards eliminating lead shot entirely for both upland and wetland hunting. An ever growing body of scientific work suggests the myriad negative environmental impacts of using lead shot in hunting pursuits (e.g., Scheuhammer and Norris 1996, USGS 2012, Haig et al. 2014), and with non-toxic shot becoming more available, reasons to avoid the further deposition of heavy metals into the environment are beginning to heavily outweigh the few benefits of using lead ammunition in hunting efforts. Although efforts along these lines will likely face a great deal of resistance from sportsmen and private landowners, the well-being of natural resources must be placed first.

Table 3.1 Means and standard errors for ^{206}Pb : ^{207}Pb and ^{208}Pb : ^{207}Pb ratios for lead shot pellet reference samples; mottled duck blood collected during 2010-2012 on the Upper Texas Gulf Coast; and soil and vegetation collected during 2010-2011 from coastal marsh on the Upper Texas Gulf Coast.

	Shot (N = 21)		Blood (N = 143)		Soil (N = 246)		Veg. (N = 24)	
	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE
Pb206:Pb207	1.155	0.008	1.169	0.006	1.201	0.002	1.159	0.002
Pb208:Pb207	2.476	0.060	2.484	0.105	2.508	0.037	2.423	0.018

Figure 3.1 ^{206}Pb : ^{207}Pb and ^{208}Pb : ^{207}Pb ratio values for lead shot reference samples tested using an Inductively Couple Plasma Mass Spectrometer (ICP-MS).

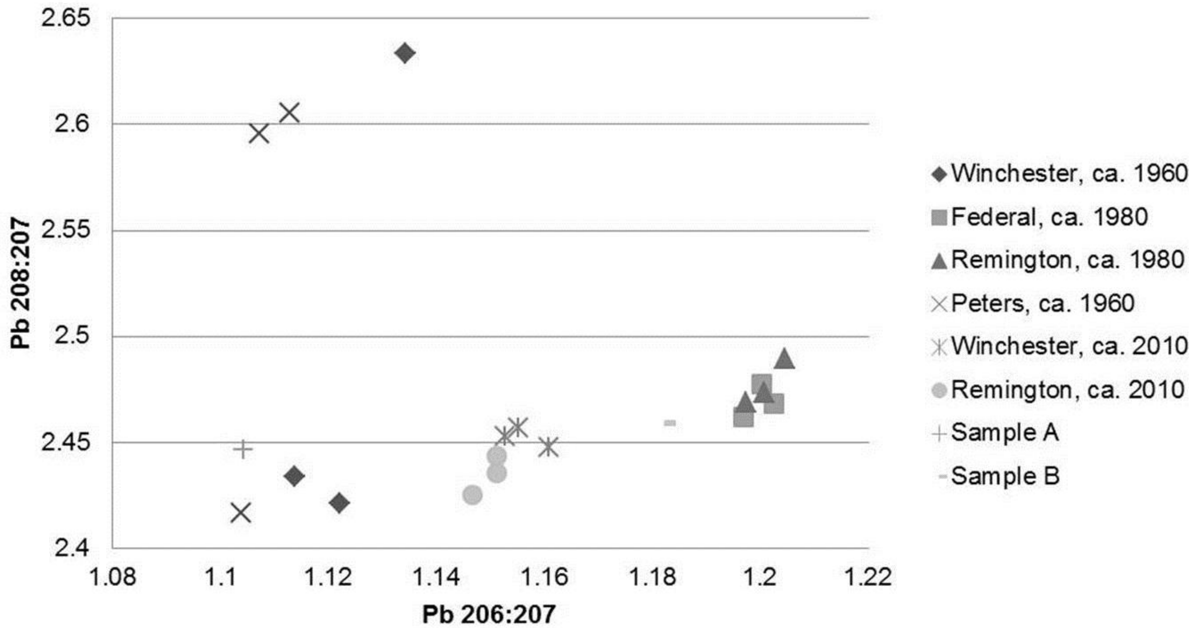


Figure 3.2 $^{206}Pb : ^{207}Pb$ and $^{208}Pb : ^{207}Pb$ ratio values for (A) Vegetation values, all of which fell within the range of lead shot reference values, and (B) Soil samples, the portion of which fell within the lead shot reference value range are highlighted within the grey box.

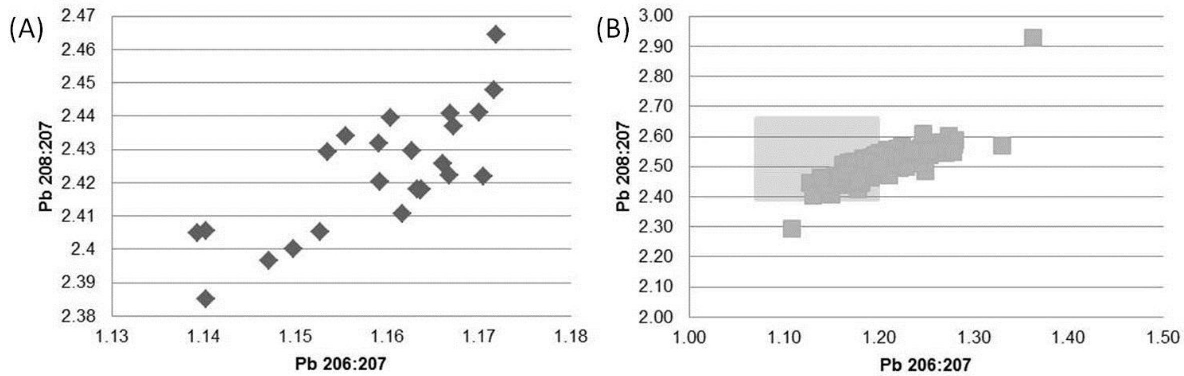


Figure 3.3 ^{206}Pb : ^{207}Pb and ^{208}Pb : ^{207}Pb ratio values of blood collected from mottled ducks during 2010-2012 on the Upper Texas Gulf Coast with lead shot reference values overlaid.

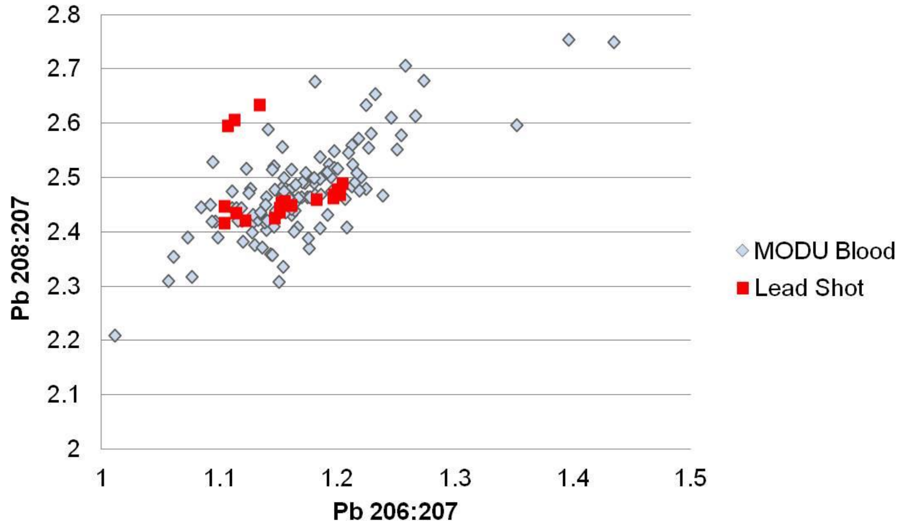


Figure 3.4 Box plots showing (A) ^{206}Pb : ^{207}Pb and (B) ^{208}Pb : ^{207}Pb ratio distributions for soil strata A (0 - 5 cm), B (>5 - 10 cm) , and C (>10 - 20) collected on the Upper Texas Gulf Coast during 2010-2011. Values are also included for developed lead shot reference results. Horizontal line indicates grand mean of all data points.

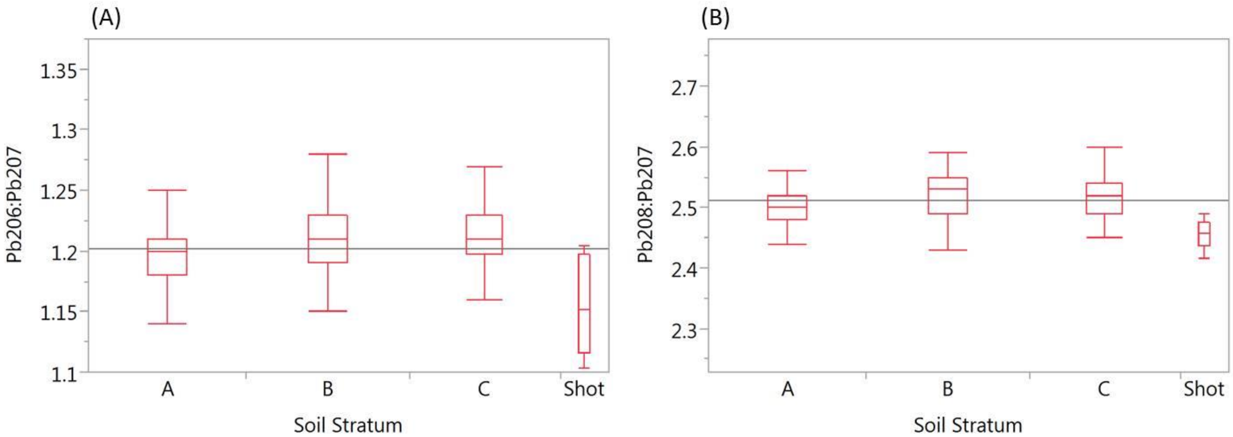


Figure 3.5 Box plots showing variation in (A) ^{206}Pb : ^{207}Pb and (B) ^{208}Pb : ^{207}Pb ratio distributions for all sample types (mottled duck blood, reference lead shot, soil, and vegetation) collected on the Upper Texas Gulf Coast during 2010-2012. Horizontal line indicates grand mean of all data points.

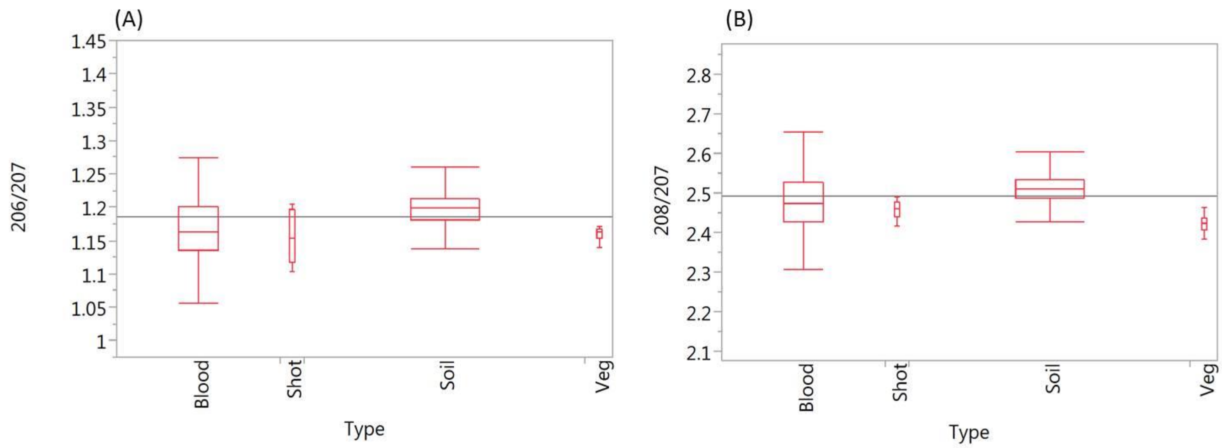


Figure 3.6 Blood lead concentration values from McDowell et al. (2015) for mottled ducks on the Upper Texas Gulf Coast during 2010-2012 compared against their respective ^{206}Pb : ^{207}Pb and ^{208}Pb : ^{207}Pb ratio values, with the range of lead shot reference values highlighted on each chart.

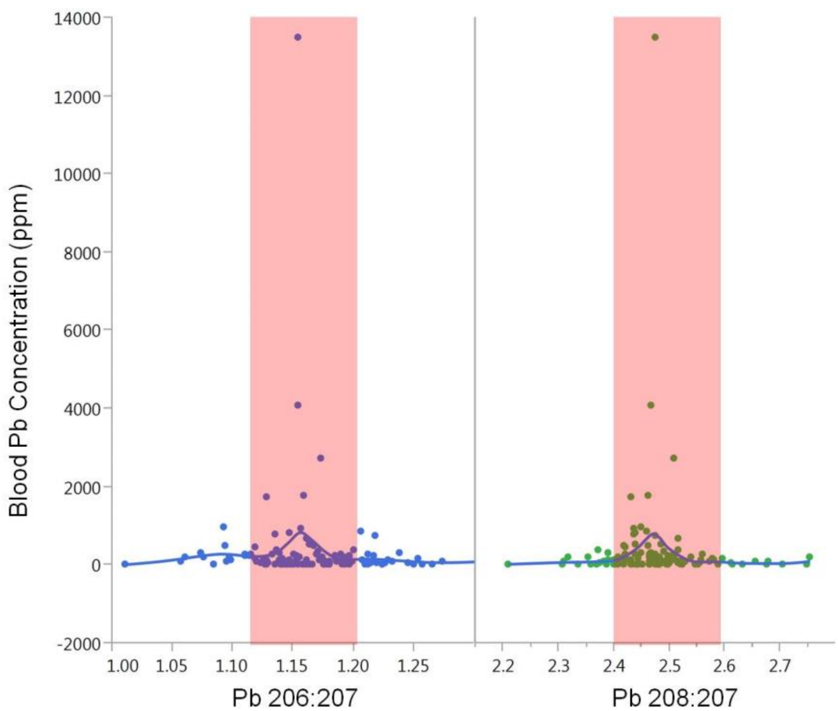


Table 3.2 $^{206}Pb : ^{207}Pb$ Lead isotope ratio reference values from Sturges and Barrie (1987) and Komarek et al (2008) as well as lead shot reference values developed as part of this study. Though other geographically specific values were available, ratios for sources considered relevant to the TCPC are included here.

Source	Pb Ratio Range
Leaded Gasoline (US)	1.040-1.390
Lead bearing ores (US)	1.190-1.200
Leaded Gasoline (MEX)	1.202-1.204
Coal	1.126-1.252
Slag (KS, MO, OK)	1.210-1.360
Ingots (MO)	1.310-1.340
Lead Shot	1.100-1.210

Chapter 4 – Population Level Ecological Risk Assessment for Lead Exposure in Mottled Ducks on the Upper Texas Gulf Coast

Lead contamination is an acknowledged environmental issue in a critical portion of mottled duck (*Anas fulvigula*) habitat on the Upper Texas Gulf Coast (Chapters 2, 3). Understanding levels of exposure risk at a quantitative level is critical for effectively directing management efforts in a conservation climate of limited available resources. Previous results indicate that use of contaminated areas varies based on year and life history period (e.g., breeding versus non-breeding); lead contamination and exposure likely results from historical or ongoing deposition from lead shot or atmospheric lead sources; and that plants and mottled ducks contain lead isotope ratios consistent with those of lead shot (Chapters 2, 3). The final step to determine the potential impact of lead exposure to mottled ducks is to attempt to link environmental factors to mottled duck habitat usage to describe meaningful connections that may aid managers in decision making. By quantifying risk and targeting high risk areas preferentially during remediation and management efforts, managers can increase the precision and efficacy of their actions both in regard to habitat and the species dependent upon critical landscapes. Understanding risk dynamics on a landscape allows the determination of what levels of a contaminant can be considered “acceptable” in an ecosystem, and those levels that might cause undue and avoidable harm can be strategically assessed. Mottled ducks specifically, with their unique non-migratory life history, sensitivity to environmental changes in their year-round habitat, and declining population abundance, require an in-depth understanding of exposure risk to create effective and efficient management plans to prevent further negative population effects.

Ecological Risk Assessment (ERA) offers a framework for understanding potentially dangerous situations in relation to humans, habitat, or wildlife that stem from changes in the environment (Norton et al. 1992). Use of ERA often seeks to specifically track the consequences of environmental changes caused by anthropogenic activities such as industrialization, urbanization, or effluent input into water resources. In general, the process associated with ERA constitutes estimating the quantitative value of risk related to a concrete situation for a recognized environmental threat or hazard (Suter 2007). Most ERAs also include an objective evaluation of risk in which assumptions and uncertainties are clearly considered and presented. Part of the difficulty of risk management is the measurement of two quantities in which risk assessment is concerned: potential loss suffered by the species/ecosystem in question and probability of risk occurrence (Suter 2007).

Risk assessment as a larger discipline, although it uses science as its foundation and is based around the scientific method, is somewhat distinct from science in that it is a preset methodology for determining the outcomes of various sociopolitical, governmental, or other actions based on many possible options. ERA is typically conducted in three steps: problem formulation, analysis, and risk characterization. Problem formulation is the beginning stage when assessors gather available information, define ecological endpoints, and create an analysis plan; analysis consists of measuring levels of contamination/exposure in environmental media of concern; and risk characterization is the final step in which results of analyses are gathered and risk is quantified (Suter 2007). Steps may be repeated as further information becomes available. When applied to ecological problems, risk assessment becomes a way for managers to track many different types of environmental problems such as the input of effluents into different habitats as a result of industrial development (Hernando et al. 2006), presence of historical contamination still affecting human or wildlife populations (Steenland et al. 1998), or the potential impacts of environmental disasters (Tsai and Chen 2010). On the Upper Texas Coast, a risk assessment based on lead exposure in mottled ducks will allow the determination of habitat types or life history periods that constitute greatest risk and the greatest need for conservation.

The Texas Chenier Plain NWR complex (TCPC) provides an excellent site for quantifying risk as it directly relates to mottled ducks because of high population densities of ducks on TCPC properties and plentiful suitable habitat (USFWS 2008b). My goal was to conduct an in-depth ERA of lead exposure to mottled ducks on the TCPC. My objectives were to quantify risk of lead exposure for mottled ducks and additionally identify areas of habitat that provide above acceptable risk for plant and animal species in general. By applying a spatial component of the risk for lead exposure present in this ecosystem, I additionally assessed how risk related to space use by mottled ducks during certain ecological states of their life cycle.

Problem formulation

Lead contamination has been acknowledged as a critical environmental issue. It is considered a threat both to human safety (Davis et al. 1990), and to wildlife resources (Haig et al. 2014). Numerous culprits have been identified for lead content increases in various environmental media. Lead deposition into ecological systems has been attributed to atmospheric deposition from combustion of leaded fossil fuels (Bollhöfer and Rosman 2001), byproducts of mining, smelting, and associated industrial processes (Blus et al. 1991, van der Merwe et al. 2011), oil and gas development, and other anthropogenic activities (Komarek et al. 2008). Historical studies, such as Chow (1970), show significant contribution of lead content to these environmental pools from aerosolized lead in the atmosphere directly resulting from leaded fuel combustion, which was discontinued starting in 1973 (EPA 2011). Chow (1970) in particular documented this effect near roads during a time period when leaded gasoline was still used in cars, but wetlands and other ecosystems currently extant near large urban centers (e.g., Houston, Texas), oil and gas refineries, or other industrial development might have been subjected to similar contamination pressures and persistent environmental lead.

Avian species in general, particularly wetland-obligate species, carnivores, and scavengers, have suffered among the most noticeable effects from environmental lead contamination, often as a result of human activities. Of notable management concern since the 1940s has been the use of lead ammunition in hunting or target shooting and the consequent ingestion of lead shot pellets by waterfowl because of the similar size of shot pellets to commonly ingested natural sources of grit (Bellrose 1959, Mateo et al. 2000). Carnivores and scavengers have additionally been exposed through feeding on carrion that contains whole bullets or fragments of bullets that would subject them to high levels of exposure or mortality (Church et al. 2006, Hunt et al. 2006). Contamination in either of these groups may occur by ingesting pellets or lead fishing weights themselves, but also by ingesting contaminated sediments; these pools persist in the environment because lead does not radioactively decompose on a short time scale (Haig et al. 2014). In addition to pellet or soil ingestion, exposure may occur through a more habitat-oriented pathway by consumption of contaminated food sources.

Regardless of the exposure pathway of concern, some waterfowl species have demonstrated continued high levels of exposure to lead even after health and survival issues in affected species were noticed and activists and managers moved towards the enactment of a lead shot ban for waterfowl hunting. Efforts to phase out lead shot for waterfowl hunting began on the Texas coast in 1978 and were finalized in 1983 (Moulton et al. 1988); nationally, lead shot became illegal for waterfowl hunting in 1991 (Avery and Watson 2009, USFWS 2013). Despite this large effort to limit heavy metal input into sensitive ecosystems, waterfowl have continued to experience lead exposure, ostensibly still through the ingestion of lead shot or bioaccumulation from the consumption of filter feeder invertebrate prey consumed by carnivorous diving duck species such as scaup (*Aythya spp.*) (Mazak et al. 1997, Weegman and Weegman 2007). This is not entirely surprising given the aforementioned chemical and physical properties of lead that cause it to be persistent in contaminated ecosystems. For instance, although surveys have been sporadic, estimates for environmental lead shot density exist for the Texas Chenier Plain National Wildlife Refuge complex that fall between ~1.25 million shot per hectare (McDowell 2014) and >3.75 million shot per hectare (Fisher et al. 1986). Lack of information regarding exposure pathways and level of risk to wetland organisms have proved challenging for managers who seek to mitigate environmental pools of lead that seek to threaten the areas they protect.

Mottled ducks, a close non-migratory relative of the American black duck (*A. rubripes*) and Mallard (*A. platyrhynchos*), are potentially of increased concern in issues related to lead contamination. High levels of lead have previously been found in mottled ducks (Anderson et al. 1987, Merchant et al. 1991, Merendino et al. 2005). Preliminary contemporary results obtained by other researchers connected with this study point to highly variable blood lead levels in mottled ducks, ranging from 0 to 12,000 ppb (S. McDowell, Stephen F. Austin State University, unpublished data). These values varied among gender, age class, and spatial location within study sites. Interestingly, however, higher blood lead levels have been consistently observed during winter months between hunting and nesting periods, further emphasizing the need for research on environmental lead exposure, sources, and the subsequent interaction with periods of the annual cycle (McDowell et al. 2015).

The ecological endpoint for this ERA was to create an initial assessment of the level of lead exposure based on relevant thresholds for mottled ducks as a whole and their habitats on the TCPC of the Upper Texas Gulf Coast. Mottled ducks have experienced a 95% reduction in their breeding pair density since 1986 based on aerial surveys, and continue to exhibit declines in population density in more recent studies (GCJV 2007, Haukos 2012). Although many factors have been identified that could potentially be contributing to population declines including increased predation (Elsey et al. 2004), hybridization with wild and feral sympatric mallards (Williams et al. 2005), and habitat destruction related to all portions of life history, lead exposure has remained an important conservation issue for this species over an extended period of time. Mottled ducks have consistently demonstrated high levels of lead exposure in a number of different variables such as ingestion rates of lead shot pellets (Anderson et al. 1987) and levels of lead contamination in different tissue types like blood or bone (Merendino et al. 2005, Stendell 1979, Merchant et al. 1991). Early studies were conducted closer to the time of the lead shot ban and may ostensibly reflect environmental conditions that were more directly affected by direct lead shot deposition, more recent surveys have also suggested continued exposure at high frequency in mottled ducks (McDowell 2014). Probably the most vexing aspect of lead contamination as a contributor to mottled duck decline, however, is the relative lack of knowledge surrounding this issue. Because, for this species, historical management efforts appear to be only marginally effective, new methods must be sought to mitigate negative effects and prevent further declines from this factor. Conducting a spatially-explicit ERA procedure in conjunction with mottled duck movement data will allow managers to directly target areas of high use/risk. Additionally, mottled duck conservation efforts related to exposure to environmental lead stand to improve habitat conditions for many other waterbird species.

STUDY SITE

Field data on lead distribution and duck movements were collected for this study on the Texas Chenier Plain National Wildlife Refuge (NWR) Complex (TCPC) on the Upper Texas Gulf Coast, with survey procedures conducted on Anahuac and McFaddin NWR's specifically. The TCPC comprised a cumulative area of 42,762 ha in Chambers and Jefferson Counties, Texas. Approximately 40% of Anahuac and McFaddin NWR's were open to waterfowl hunting. These NWRs have consistently demonstrated among the highest population densities of mottled ducks in Texas (Haukos 2012). The refuges imposed a non-toxic shot requirement in conjunction with the banning of lead on all federal lands in 1991 (Avery and Watson 2009, USFWS 2013). The TCPC marshes were widely hunted before properties were purchased as refuges by the U.S. Fish and Wildlife Service (USFWS), so there is concern about lead contamination from years prior to the lead shot ban.

Land use history and change on and around the TCPC has largely been driven by agricultural and industrial development. Historical land uses in the region included rice agriculture and cattle ranching and, as the technologies became available, petrochemical and other related industry. As coastal marsh habitats were converted to provide for increasing land demand, the USFWS set aside large tracts of land in response to declining waterfowl populations (USFWS 2008a). Land cover on the TCPC is variable with several different wetland types, urban areas, beaches, and more (Figure 4.1). Much of the surrounding habitat remains in rice agriculture, cattle ranching, or industrial development, which is prevalent in the Houston/Galveston/Beaumont, Texas area. Land acquisition to form the TCPC began in 1954, and since then much of the area has been rigorously managed for waterfowl production via various land management methods such as prescribed burning, cattle grazing, water management, and mechanical disturbance (USFWS 2008a). Land leased as part of Anahuac NWR still includes agricultural fields (~890 hectares) used in cooperation with farmers still producing rice on refuge properties (USFWS 2008b), which can provide important food sources for mottled ducks (Stutzenbaker 1988).

The landscape of the TCPC was largely influenced by the hydrology and climate of the Gulf of Mexico, which in turn is influenced by sub-tropical weather patterns. The TCPC receives, on average, 144 cm of rain per year with values ranging from 52 cm - 218 cm. This region is also importantly prone to hydrologic and other effects stemming from the landfall of hurricanes, which can have devestating effects both on land forms and vegetation communities due to changes in salinity, sedimentation, and other effects (Stone et al. 1997, Turner et al. 2006, Howes et al. 2010, O'Connell and Nyman 2011). Dominant marsh types on both Anahuac and McFaddin NWR's include fresh, intermediate, and brackish marsh (USFWS 2008a). Vegetation communities in wetlands vary greatly based on water depth, salinity level, and amount of tidal force. Intermediate and brackish marshes exhibit large quantities of marshhay cordgrass (*Spartina patens*), with other species intermixed such as *Scirpus* spp., *Typha* spp., *Distichlis* spp., *Juncus* spp., and *Paspalum* spp. (Rigby 2008). Freshwater marshes were more diverse, and included *Alternanthera philoxeroides*, *Sesbania* spp., *Ludwigia* spp., *Nymphaea* spp., *Sagittaria* spp., *Eleocharis* spp., *Typha* spp., *Cyperus* spp., *Papsalum urvillei*, and *Panicum hemitonum* (Rigby 2008). Upland habitats persist on the refuge as well and are mainly characterized by tallgrass prairie vegetation such as C4 and C3 grasses (*Schizachyrium scoparium*, *Paspalum plicatulum*, *Tripsacum dactyloides, Panicum virgatum, Paspalum livium*), forbs (*Liatris pynostachya*, *Rudbeckia hirta*, *Cacalia* spp., *Eryngium yuccifolium*), and woody shrubs (*Baccharis halimfolia*, *Myrica cerifere*). Large variations in topography are minimal due to the geologic nature of the area (USFWS 2008a).

Given that the TCPC has been rigorously managed as waterfowl habitat, the five most abundant migratory and wintering species were green-winged teal (*A. crecca*), gadwall (*A. strepera*), northern shoveler (*A. clypeata*), blue-winged teal (*A. discors*), and northern pintail (*A. acuta*). Mottled ducks represent the only year-round resident waterfowl population (USFWS 2008b). As with much of the Gulf Coast region, the refuge demonstrates a high degree of bird diversity that varies temporally but includes species of shorebird, songbird, waterbird, and other terrestrial migrants (USFWS 2008a). The TCPC is also an important location for avian species of concern. As of 2008, 37 out of 48 species defined as species of conservation concern in the U.S. portion of the Gulf Coast region use habitat on the TCPC (USFWS 2008a;b). The TCPC is also home to several federally threatened or endangered species including several species of sea turtle (*Caretta caretta*, *Chelonia mydas*, *Eretmochelys imbricata*, *Lepidochelys kempii*) and the brown pelican (*Pelecanus occidentalis*). Many of these species may also experience the effects of lead exposure because contamination appears to occur in varying degrees across many habitat types in this region.

METHODS

Survey Methods

Mottled duck locations

Movement data were collected from female mottled ducks from 2006 - 2012 via both very- high-frequency (VHF) and satellite telemetry. Satellite telemetry data were collected between 2009 and 2012 (Moon 2014), with VHF data collected from 2006 - 2008 (Rigby and Haukos 2012). Satellite locations were collected using Model 100 solar/satellite platform transmitter terminal (PTT) transmitters attached to females weighing >740g. The PTT units indicated hen survival by using measures of unit temperature and bird body motion to detect mortality. The VHF radio tags were equipped with a mortality signal, which occurred when the transmitter was stationary for >8 hours.

Sample collection and lead content sampling

Lead content in soil/vegetation samples were determined from samples collected on the TCPC. Soil samples were collected on Anahuac and McFaddin NWRs (McDowell 2014), which were stratified by habitat type and within coastal marsh by salinity level. Within each habitat stratification category, a grid of 40 ha was overlaid and 20% of corresponding grid cells were randomly selected for sample collection. Soil was collected at depths of 0 - 5 cm (stratum A), >5 - 10 cm (stratum B), and >10 - 20 cm (stratum C). This allowed for the determination of lead availability at various soil depths, and consequently inferences regarding the availability to wetland plants as well as other biota. For the purposes of risk assessment, risk for lead exposure was only characterized for results from stratum A, as dabbling ducks would most likely experience direct exposure through ingestion from this portion of the soil column and most plant species and bethic invertebrates would be drawing nutrients and, potentially, contaminants from this part of the soil column. Following soil sample collection, the nearest perennial and annual plant to each randomly selected soil sampling site was also collected. In the lab, samples were sieved and radiographed to remove any whole lead shot pellets, which were then counted and weighed. Only two lead shot pellets were identified and removed during data collection. Samples of both soil and vegetation were both dried and ground. Invertebrates were also collected and sampled for lead content as part of this study. However, due to a shortage of biomass, samples were stratified by management area and average values were noted to provide more robust estimates of lead content in this trophic level.

To link lead content present in abiotic samples to waterfowl, mottled duck blood samples were also evaluated for their lead content. Blood samples were collected from the body cavity of hunter bag birds at hunter check station at various locations on Anahuac and McFaddin NWR's during the 2010 - 2011 and 2011 - 2012 hunting seasons. Blood lead concentrations (ug/L) were determined in the lab using AAnalyst 600 and 800 atomic absorption systems that read within ranges set by the Center for Disease Control (CDC) and Occupational Health and Safety Administration (OSHA) (see McDowell et al. 2015).

Risk Characterization

Risk was quantified for this area using a Hazard Quotient approach (Suter 2007). We related the present quantity of a contaminant in the environment to thresholds of toxicity for organisms (e.g. No Observed Adverse Effect Level or Lowest Observed Adverse Effect Level). A hazard quotient can be generally represented as:

$$HQ = \text{Dose} / \text{Screening Benchmark}$$

or

$$HQ = \text{Estimated maximum concentration at site} / \text{Screening Benchmark}$$

When $HQ > 1$, risk is assumed to be at a level where exposure is demonstrated beyond an acceptable threshold; when $HQ = 1$, contaminant levels may be approaching a harmful level, but are at threshold; when $HQ < 1$, risk to biota from the contaminant in question is likely not a cause for management action because the contaminant in the environment is present at quantities below the threshold level (Suter 2007). Risk was assessed in two different forms for this site. First, soil lead (Pb) concentration values were compared against Ecological Soil Screening Level (ECO-SSL) guidelines put forth in recent studies by the EPA (EPA 2005). These guidelines were developed for 13 different contaminants that are often discovered at Superfund sites, and represent thorough literature reviews that suggest soil contaminant concentration thresholds likely to cause toxic exposure to several phylogenetic groups including terrestrial plants, invertebrates, avian species (insectivorous, herbivorous, and carnivorous), and mammals. Because mottled ducks, like many waterfowl, exhibit insectivorous feeding habits during certain life history periods or age states (e.g., females and ducklings) but are herbivores or granivores during much of the year (Baldassarre et al. 2006), ECO-SSL thresholds were assessed for both of these diet categories. ECO-SSL thresholds for Pb are 1700 mg/kg dry weight (dw) for insects, 120 mg/kg dw for terrestrial plants, 46 mg/kg dw for herbivorous avian species, and 11 mg/kg dw for insectivorous avian species. Once HQ values were developed, values for both insectivorous and herbivorous feeding strategies were interpolated in ArcGIS using an Inverse Distance Weighting (IDW) approach. Due to the sampling design for soil and vegetation surveys, IDW was determined to be the best method for representing these data. While interpolation methods such as kriging may offer a more statistically robust result, studies specifically designed for eventual kriging interpolation typically use a uniform sampling distribution, whereas this study utilized a random sampling distribution (ESRI 2011).

In addition to assessing potential linkages between soil Pb concentrations and mottled ducks through various exposure pathways, I sought to link values obtained from invertebrate, plant, and soil samples collected on the TCPC to established Pb exposure thresholds. As such, HQs were developed for each of these habitat and diet components. Additionally, in an effort to account for differential bioavailability, lead content values in soil, vegetation, and invertebrates were adjusted to reflect that not all lead present in a given environmental sample would be absorbed during ingestion. Little information was available in the literature to directly answer the question of bioavailability of Pb in different sample types. Given widely variable estimates for the bioacessibility of lead to different organisms and from different sources based on factors such as soil composition, soil pH, diet, and physiology (Bennett et al. 2007, Soto-Jimenez et al. 2011), I developed three different exposure scenarios based on 5%, 10%, and 25% bioavailability of Pb based on relatively low estimates from previous studies and a general lack of consensus in the literature on specific bioavailability values.

Information on mottled duck blood Pb content and movement was used to develop information on risk exposure based on usage of high risk habitats. Mottled duck blood Pb levels collected from birds at hunter check station were stratified by the management unit of collection, and average blood Pb values were compared to HQ values across the corresponding management unit. To assess habitat usage, bird location point densities were calculated using the location class 3 signals (≤150m error) from the aforementioned satellite telemetry study at a pixel size of 1 km^2. Density values reflecting mottled duck habitat use were then compared against HQ values from the ECO-SSL feeding strategy group to assess risk.

Last, to assess the important issue of whether biomagnification was occurring in this ecosystem with respect to lead, I calculated transfer factors between different trophic levels (Valdes et al. 2014). The transfer factor (TF) is a basic measure of biomagnifications that is expressed as:

$$TF = \text{Concentration Predator} / \text{Concentration Prey}$$

where, for biomagnification to be occurring, TF is ≥ 1 (Gray 2002) for two or more trophic levels (Barwick and Maher 2003). For this study, I assessed transfer factors for soil to plants, vegetation to invertebrates, and for invertebrates to mottled ducks as well from plants to mottled ducks to account for multiple possible dietary pathways. TF calculations were made using an assumption of 10% bioavailability.

RESULTS

ECO-SSL Risk Scenarios

HQ responses to ECO-SSL thresholds varied across feeding strategies (insectivore or herbivore) (Table 4.1). Under this scenario, mottled ducks with a mainly herbivorous diet experienced little hazard from lead exposure based on values collected across this study site, although localized risk does exist based on certain HQ values exceeding 1 (3.4% of sites sampled). The highest risk from this procedure is represented in avian species with an insectivorous diet, where the mean HQ value greatly exceeded 1 ($\bar{x}$ = 2.06, STDDEV =0.999) and the maximum value of 8.64 represented a very high level of risk. Greater than 97% of soil samples in this study represent ECO-SSL values for insectivores that represent high risk (Table 4.1). Examining food sources directly, invertebrate species and vegetation experience little risk for Pb exposure from soil in this ecosystem with HQ values for all samples collected falling below 1.

Variable bioavailability hazard quotients

Risk for baseline exposure from vegetation samples remained relatively high with 75.7% of samples showing HQ > 1 even at the 5% bioavailability level (Tables 4.2, 4.3). At this same bioavailability level, some risk for clinical exposure remained present (39.4%), with little risk for severe clinical exposure (8.8%). Percentages of HQ's above one for higher assumed bioavailability (10% and 25%) values increased sequentially (Table 4.2). HQ values generated for soil from variable bioavailability scenarios showed high levels of risk across all scenarios for all levels of exposure, with the lowest risk being for severe clinical exposure with 5% bioavailability ($\bar{x}$ = 1.1349, STDDEV =0.5498), which still provided HQ ≥ 1 for 57.4% of samples (Table 4.2, 4.4). All other bioavailability scenarios presented risk >97%, even for severe clinical exposure (Table 4.2). Invertebrate samples taken from the TCPC, when stratified by management area due to the necessity to merge tissue samples during content testing, demonstrated a different risk profile (Table 4.5). No bioavailability estimates for invertebrates tested in this study showed risk for severe clinical poisoning, and only the 25% bioavailability scenario provided a risk for clinical exposure, which still provided HQ ≥ 1 for 18.2% of management areas (Table 4.1). When examined by management unit, clinical exposure risk was only present at 25% assumed bioavailability for one management unit (Deep Marsh on Anahuac) (Table 4.5). Risk for subclinical exposure was only present at 25% assumed bioavailability for most management units as well, with the exception of North Unit on McFaddin and Deep Marsh on Anahuac (Table 4.5).

Transfer Factor biomagnification analysis

Transfer factor analysis did not indicate the occurrence of biomagnification in mottled ducks or their food sources. When analyzing the soil > vegetation > invertebrate > mottled duck trophic scheme, all transfer factors demonstrated values < 1, indicating that lead does not appear to be magnifying in this system as higher trophic levels are examined (Table 4.6). These analyses are based solely on average invertebrate lead values stratified by management unit on the TCPC, and may vary with additional spatial and analytical resolution.

RISK CHARACTERIZATION

This ecological risk assessment analysis confirms past research results suggesting that mottled ducks are indeed at risk for lead exposure from multiple environmental pathways (Bellrose 1959, Merchant et al. 1991, Merendino et al. 2005, Bielefeld and Cox 2006). Past studies have largely focused on ingestion of lead in the form of lead shot pellets, which potentially remains an issue for mottled ducks (Merendino et al. 2005), my results suggest risk from other digestive pathways including vegetation, soil grit, and, in some scenarios, invertebrates. In my analysis, ECO-SSL HQs and bioavailability-based HQs provided different interpretations for which factors determine risk for lead exposure in Upper Texas Gulf Coast mottled ducks. ECO-SSL suggests that mottled ducks may be at very high risk to lead exposure during insectivorous life history periods while bioavailability-based estimates suggest invertebrates may not present risk on the TCPC based on the parameters I chose. In contrast, although ECO-SSL approaches suggest that plants and invertebrates are at little risk for exposure themselves from soil lead levels, bioavailability-based HQs of risk suggest that vegetation may play an important role in lead exposure in mottled ducks, depending on true values of bioavailability and mottled duck ingestion rates.

The low threshold of exposure for insectivorous avian species suggested by the ECO-SSL framework is justified by a theoretically greater degree of biomagnification due to an increased number of trophic levels (Barwick and Maher 2003). During the pre-breeding period, invertebrates comprise a large proportion of the mottled duck diet, especially in females (Stutzenbaker 1988). Conversely, during late summer, fall, and winter, vegetation is the main food source, suggesting potential temporal shifts in risk. Given the thresholds put forth by the ECO-SSL, one might therefore expect exposure rates to be higher in the summer months. If ECO-SSL models were to hold true and risk estimates based on these thresholds reflect reality, mottled ducks may be at greater risk during insectivorous portions of their life history. The TCPC, however, demonstrates a somewhat counterintuitive pattern when compared to many other natural examples of systems where biomagnification may be occurring. My bioavailability-based HQs present a dynamic where invertebrate prey represent a lower exposure risk than would be suggested by the ECO-SSL model. This difference can likely be attributed to the apparent lack of biomagnification occurring in this ecosystem as demonstrated by all TF values being < 1. In this system, I observed decreasing lead concentrations as trophic level increased. Managers are thus presented with conflicting management suggestions. Based on the ECO-SSL thresholds, which were developed from a wide-ranging review of studies, mottled ducks are at risk from invertebrates and not from vegetation. Research connected with this study, however, suggests a higher degree of lead exposure in mottled ducks during the non-breeding season when diet is composed mostly of vegetation (McDowell 2014). The converse reduced exposure during the summer months equates to reduced exposure while eating a diet composed of a greater percentage of invertebrates (Stutzenbaker 1988). I suggest that based on much higher lead content in vegetation samples, little to no biomagnification occurring in this trophic scheme, and demonstrated higher risk from vegetation even at conservative bioavailabilty estimates, that ECO-SSL models may underestimate risk presented from these sources on the TCPC.

Although food sources are likely the largest avenue for exposure from ingestion, soil ingested either as grit or incidentally appears to represent a fairly significant risk for lead exposure based on HQ values in all three bioavailability scenarios tested in this study. Soil-based lead exposure may have particular importance due to the aforementioned life-history related diet shifts during spring and late summer. With relatively high content of lead in soil (disregarding the potential presence of lead shot in Texas coastal soils), exposure risk from this food source may increase during herbivorous life-history periods because grit ingestion tends to increase with a vegetation-based diet due to increased need for mechanical digestion of plant fibers (Figuerola et al. 2005). Should soil lead be even moderately bioavailable to mottled ducks, increased ingestion of contaminated soils in addition to ingestion of contaminated vegetation may present a large input of lead into mottled duck tissues. Additionally, during periods of increased soil ingestion, greater opportunity for lead shot ingestion may occur since dabbling ducks seem to have increased lead shot ingestion rates when also ingesting soil grit of similar size (Mateo et al. 2000).

Overall, mottled ducks appear to be largely at risk in this system from contaminated soil sediments and vegetation in their diet. Although some models, and perhaps biological theory, might suggest that biomagnification would cause invertebrates to be of greater risk to mottled ducks when considering heavy metal contamination issues, my analyses suggest that other pathways may be of greater concern than invertebrates in this particular ecosystem. The level of risk experienced, however, may depend greatly on environmental conditions and true values of bioavailability. Without intimate and site-specific knowledge of these parameters, it may be hard to accurately determine which of the risk estimates provides the most accurate portrayal of the risk landscape (see following section).

PROBLEM RE-FORMULATION AND MANAGEMENT SUGGESTIONS

One of the chief findings of this ERA was that mottled ducks on the TCPC may not conform to the conditions assumed by the ECO-SSL, necessitating the creation of measures of bioavailability specific to the complex. When considering bioavailability, variability is substantial given environmental conditions such as pH, sediment size, temperature, and more (Bennett et al. 2007, Soto-Jimenez et al. 2011). As such, although I assessed multiple scenarios for bioavailability, even the highest of these estimates may be conservative if soil pH was, for instance, lower in certain areas which may cause lead to become more bioavailable (Ruby et al. 1996). Thus, a laboratory study is warranted using conditions similar to both the environment and the digestive tract of the mottled duck to obtain accurate measures of bioavailability in ingestible materials. In concert with this, testing ingestion rates of different environmental materials will assist in providing more concrete risk estimates, as this would affect the total amount of lead consumed (e.g. Bennett et al. 2007).

The best solution for protecting mottled ducks from lead exposure, however, would be to mitigate lead first in known areas of high concentration. For apparent lead hotspots, dredging and phytoremediation are the two most feasible options for managing lead contamination in these ecosystems. Dredging is mainly effective in areas where contaminated sediments or high concentrations of intact lead shot pellets exist, as removing contaminated soil would provide relief for the surrounding biota. Phytoremediation involves using bioaccumulating vegetation species to leach lead from the soil; this is followed by removal of the contaminated plants (Salt et al. 1998). This method has been shown to be effective in use on urban brownfield sites and could be effective on the TCPC as well as a way to reduce lead contamination in surficial soils (Huang and Cunningham 1996). Additionally, if plants are indeed the chief lead exposure risk source for mottled ducks, then removing plants in contaminated areas may reduce exposure risk in and of itself. Targeting areas for the creation of waterfowl habitat in low risk locations may also be effective in altering behavior to reduce life-history related exposure. Depending on the outcome of laboratory procedures to determine actual bioavailability of lead and the ingestion rates of samples containing it, preference should be given to cultivating low risk habitat in the context of either vegetation or invertebrates. Given initial results presented here, however, it seems likely that vegetation-related remediation will be most effective.

Figure 4.1 Landcover classification of Anahuac and McFaddin National Wildlife Refuges based on remotely sensed data and ground referencing developed by Moon (2014).

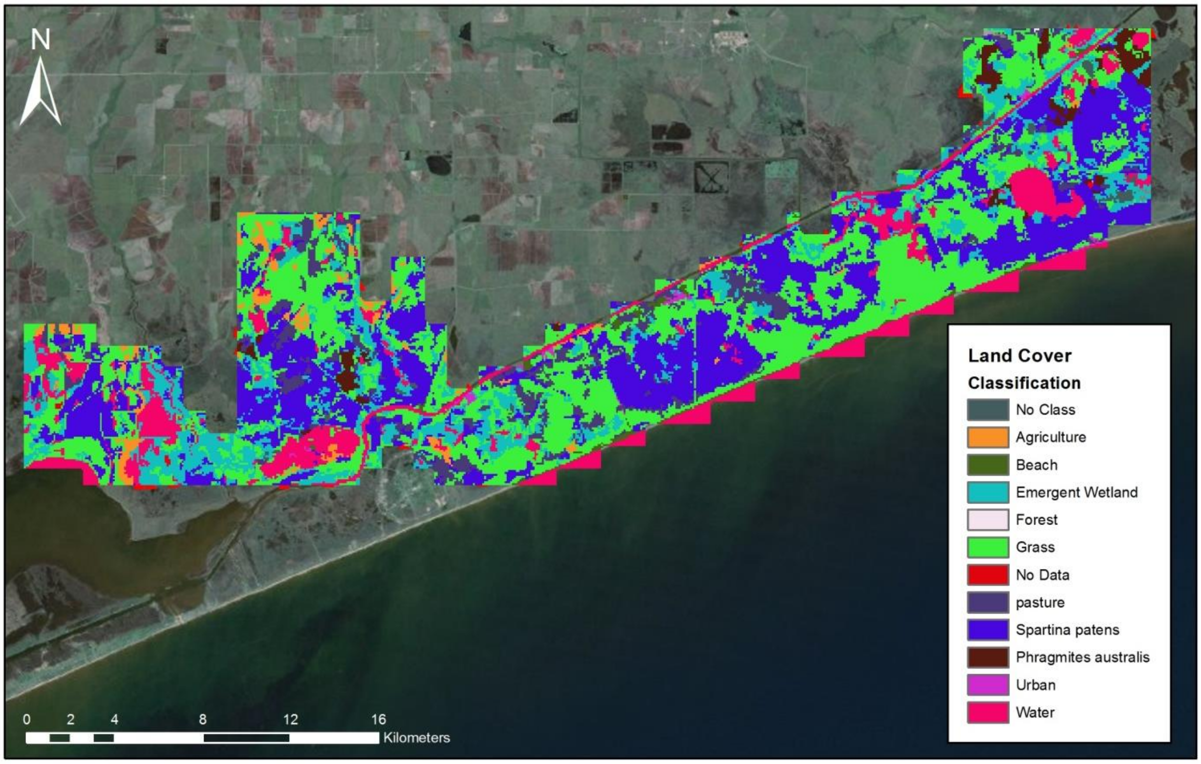


Table 4.1 Summary Statistics for ECO-SSL Hazard Quotient (HQ) values for herbivores, insectivores, and invertebrate and vegetative food sources in coastal marshes on the Upper Texas Gulf Coast during 2010 - 2011. ECO-SSL values were based on soil lead content, and suggest risk for different feeding strategies (herbivory/insectivory) and corresponding food sources. HQ values greater than 1 represent a high degree of lead exposure risk to mottled ducks. HQs were calculated using the soil lead content at each survey point on the TCPC and the corresponding ECO-SSL threshold. The percent of samples tested that indicate high risk (HQ > 1) for a given dietary pathway or food source is given in the last row.

	ECO-SSL Hazard Quotients			
	Herbivory	Insectivory	Invertebrates	Vegetation
Mean	0.493	2.0636	0.0124	0.189
Std Dev	0.239	0.999	0.007	0.0913
Min	0	0	0	0
Max	2.07	8.64	0.06	0.79
% High Risk	3.4	97.7	0	0

Table 4.2 Proportion of soil, vegetation, and invertebrate samples from the Upper Texas Gulf Coast during 2010 – 2011 that produced a Hazard Quotient (HQ) that suggested a high level of risk (HQ > 1). For example, at 5% bioavailability, 98.3% of soil samples present risk to mottled ducks of achieving a subclinical exposure level. Proportions are subdivided based on 5%, 10%, and 25% bioavailability and different levels of lead exposure (subclinical, clinical, or severe). Exposure thresholds were calculated based on thresholds of 2 ug/L and 5 ug/L (0.2 ppm and 0.5 ppm).

	Exposed (subclinical)			Clinical Exposure			Severe Clincal Exposure		
	HQ_5	HQ_10	HQ_25	HQ_5	HQ_10	HQ_25	HQ_5	HQ_10	HQ_25
Soil	0.982	0.982	0.982	0.977	0.982	0.982	0.573	0.977	0.982
Veg.	0.757	0.893	0.940	0.396	0.698	0.893	0.0887	0.396	0.757
Invert.	0	0.188	0.909	0	0	0.181	0	0	0

[1]HQ_5 = Hazard Quotient, 5% bioavailability, HQ_10 = Hazard Quotient, 10% bioavailability, HQ_25 = Hazard Quotient, 25% bioavailability

Table 4.3 Summary statistics for Hazard Quotient (HQ) values calculated from assumed 5%, 10%, and 25% bioavailability of lead to mottled ducks in vegetation samples collected on the Upper Texas Gulf Coast during 2010 - 2011 through ingestion. HQ values >1 indicate a high level of risk for exposure at a given level (subclinical, clinical, or severe). Exposure thresholds were calculated based on thresholds of 2 ug/L and 5 ug/L (0.2 ppm and 0.5 ppm).

	Exposed (subclinical)			Clinical Exposure			Severe Clincal Exposure		
	HQ_5	HQ_10	HQ_25	HQ_5	HQ_10	HQ_25	HQ_5	HQ_10	HQ_25
MEAN	2.335	4.671	11.67	0.934	1.868	4.671	0.467	0.934	2.335
STD DEV	1.836	3.672	9.181	0.734	1.468	3.672	0.367	0.734	1.836
MIN	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
MAX	10.255	20.51	51.275	4.102	8.204	20.51	2.051	4.102	10.255

[1]HQ_5 = Hazard Quotient, 5% bioavailability, HQ_10 = Hazard Quotient, 10% bioavailability, HQ_25 = Hazard Quotient, 25% bioavailability

Table 4.4 Summary statistics for Hazard Quotient (HQ) values calculated from assumed 5%, 10%, and 25% bioavailability of lead to mottled ducks in soil samples collected on the Upper Texas Gulf Coast during 2010 - 2011 through ingestion. HQ values >1 indicate a high level of risk for exposure at a given level (subclinical, clinical, or severe). Exposure thresholds were calculated based on thresholds of 2 ug/L and 5 ug/L (0.2 ppm and 0.5 ppm).

	Exposed (subclinical)			Clinical Exposure			Severe Clincal Exposure		
	HQ_5	HQ_10	HQ_25	HQ_5	HQ_10	HQ_25	HQ_5	HQ_10	HQ_25
Mean	5.674	11.349	28.373	2.269	4.539	11.349	1.134	2.269	5.674
Std Dev	2.749	5.498	13.745	1.099	2.199	5.498	0.549	1.099	2.749
Min	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Max	23.750	47.500	118.750	9.500	19.000	47.500	4.750	9.500	23.750

[1]HQ_5 = Hazard Quotient, 5% bioavailability, HQ_10 = Hazard Quotient, 10% bioavailability, HQ_25 = Hazard Quotient, 25% bioavailability

Table 4.5 Summary statistics for Hazard Quotient (HQ) values calculated from assumed 5%, 10%, and 25% bioavailability of lead to mottled ducks in invetebrate samples collected on the Upper Texas Gulf Coast through ingestion. Samples were stratified by management area because individual spatial information was not available for invertebrate samples due to the need to pool biomass for lead content testing. HQ values >1 indicate a high level of risk for exposure at a given level (subclinical, clinical, or severe). Exposure thresholds were calculated based on thresholds of 2 ug/L and 5 ug/L (0.2 ppm and 0.5 ppm).

			Exposed (subclinical)			Clinical Exposure			Severe Clincal Exposure		
Refuge	Mgmt. Unit	[Pb]	HQ_5	HQ_10	HQ_25	HQ_5	HQ_10	HQ_25	HQ_5	HQ_10	HQ_25
Anahuac	Roberts-Mueller	1.010	0.253	0.505	1.263	0.101	0.202	0.505	0.051	0.101	0.253
Anahuac	Jackson Ditch	1.367	0.342	0.684	1.709	0.137	0.273	0.684	0.068	0.137	0.342
Anahuac	West Lake	1.415	0.354	0.708	1.769	0.142	0.283	0.708	0.071	0.142	0.354
Anahuac	Deep Marsh	2.690	0.672	1.345	3.362	0.269	0.538	1.345	0.134	0.269	0.672
Anahuac	Pace	1.155	0.289	0.578	1.444	0.116	0.231	0.578	0.058	0.116	0.289
Anahuac	1985 Rice Fields	1.313	0.328	0.657	1.641	0.131	0.263	0.657	0.066	0.131	0.328
McFaddin	Star Lake	1.094	0.274	0.547	1.368	0.109	0.219	0.547	0.055	0.109	0.274
McFaddin	5 mile	1.077	0.269	0.539	1.346	0.108	0.215	0.539	0.054	0.108	0.269
McFaddin	Pay ponds	0.233	0.058	0.116	0.291	0.023	0.047	0.116	0.012	0.023	0.058
McFaddin	North Unit	2.935	0.734	1.468	3.669	0.294	0.587	1.468	0.147	0.294	0.734
McFaddin	Mud Bayou	0.838	0.209	0.419	1.047	0.084	0.168	0.419	0.042	0.084	0.209
	Mean	1.375	0.344	0.688	1.719	0.138	0.275	0.688	0.069	0.138	0.344
	SD	0.745	0.186	0.373	0.932	0.075	0.149	0.373	0.037	0.075	0.186
	Min		0.058	0.116	0.291	0.023	0.047	0.116	0.012	0.023	0.058
	Max		0.734	1.468	3.669	0.294	0.587	1.468	0.147	0.294	0.734

[1]HQ_5 = Hazard Quotient, 5% bioavailability, HQ_10 = Hazard Quotient, 10% bioavailability, HQ_25 = Hazard Quotient, 25% bioavailability

Table 4.6 Results for Transfer Factor (TF) analysis more tracking biomagnification in lead (Pb) in the trophic sequence soil > vegetation > invertebrates > mottled ducks (MODU) on the Upper Texas Gulf Coast during 2010 - 2011. TF values >1 indicate that biomagnification was occurring in a particular trophic scheme; in other words, lead must increase as the considered trophic level is higher.

	Average [Pb] (mg/kg)	TF
MODU	0.3566	0.2593
Invert	1.3750	0.1471
Veg	9.3429	0.4116
Soil	22.6985	

Conclusion

Although substantial efforts have been taken nationwide to attempt to mitigate the effect of environmental lead contamination and effects of its ongoing deposition on wildlife, my study confirms that mottled ducks (*Anas fulvigula*) are indeed still experiencing adverse effects from this contaminant. During various portions of their life history and in various portions of their habitat, mottled ducks continue to exhibit relatively high levels of lead even when surveyed on a study site such as the Texas Chenier Plain National Wildlife Refuge Complex (TCPC) where governmental protections are at their strictest. My study further suggests that ongoing exposure in mottled ducks and contamination at an ecosystem level are a result of historical deposition of lead, most likely from lead shot used in hunting efforts or also potentially from atmospheric lead contamination from the combustion of leaded fossil fuels or various industrial processes. Lead shot is also no longer legally used for hunting on TCPC properties and may not still exist in the amount it once did in its intact form. Particulate lead from lead pellets that have mechanically dissolved over time, however, appears to potentially still be quite prevalent on the TCPC judging by the values obtained in isotope ratio analysis that appear consistent with lead shot ratio values. Deposition from industrial sources, at least in the United States, has definitely slowed and the impact from automobiles is no longer of concern (Nriagu 1990, EPA 2011). Concerns remain for lead isotope transfer across long distances, for example from developing industrial countries like China and Russia with more lax environmental regulations (Bollhöfer and Rosman 2001), but not likely at a level that is of concern on the local scale considered here.

The problem of mottled duck ingestion of lead that originates from lead shot, however, is likely not localized to TCPC properties. Given the continued use of lead shot for many upland hunting pursuits on nearby private lands, deposition on these areas may still pose a serious threat to mottled ducks. Especially given that mourning doves (*Zenaida macroura*) are often hunted with lead ammunition over rice fields, which provide important food resources for mottled ducks (Stutzenbaker 1988), ingestion of whole shot could still be occurring at unknown levels when mottled ducks leave refuge habitats to feed. This could be an especially large problem given increased shot ingestion rates when waterfowl feed on larger food items like rice grains, which are of a similar size to lead shot pellets and/or require larger grit to process in the gizzard (Bellrose 1959, Mateo et al. 2000). Perhaps the most concerning piece of this potential exposure pathway is that deposition quantities are unknown, although current estimates for number of lead ammunition shots fired in the pursuit of mourning doves is astronomically high (Pierce et al. 2014).

One of the only ways to monitor direct lead exposure from lead shot and other sources, short of measuring gizzard shot pellet content or measuring blood lead levels during banding or check station efforts, is to monitor condition in handled birds. Given that condition bias for increased harvest of birds in poorer condition is observed in hunter shot waterfowl that were exposed to lead (McCracken et al. 2000), using the equation developed in this study to predict body fat content may allow for managers to additionally correlate condition with corporeal lead content (most likely blood values) in future studies and management efforts (Chapter 1). Given the numerous negative physiological effects observed in waterfowl species exposed to lead (Bellrose 1959, Irwin and Karstad 1972, Rocke and Samuel 1991, Pain 1996, van der Merwe et al. 2011), proportions of a population exposed to lead would be reflected as body condition using a mass/length based index and could be easily monitored.

Although tracking temporal exposure will continue to be of prime management importance in gleaning information about lead dynamics in mottled ducks, I have provided additional information about exposure pathways and conditions that present additional exposure risk through both risk assessment procedures and MaxENT species distribution modeling. The chief result of interest that connects these two analyses is the apparent role of vegetation in the mottled duck diet in exposing these birds to lead. McDowell et al. (2015) demonstrated higher lead concentrations in mottled ducks during the non-breeding season, potentially due to a diet shift from invertebrate to vegetation food sources, which maxent models did not indicate was a result of a shift in habitat or space use (Chapter 2). One of the only other plausible explanations is the diet shift that occurs between these two life history periods when mottled ducks shift to a chiefly plant-based diet after molt (Stutzenbaker 1988, Baldassarre et al. 2006). Risk assessment procedures conducted in this study corroborated this hypothesis by demonstrating higher risk from a vegetation based diet when risk was calculated on a bioavailability basis (Chapter 4). Although ECO-SSL procedures suggest greater risk from an invertebrate diet (EPA 2005), McDowell (2014) additionally discovered much lower average lead levels in invertebrates than in plants on the TCPC during his study years. This suggests lower exposure risk for mottled ducks from invertebrates that was confirmed by my transfer factor analysis demonstrating no evidence for biomagnification of lead on the TCPC based on this trophic scheme (Chapter 4). Furthermore, McDowell

(2014) showed some of the greatest mean lead concentration values in some of the mottled ducks main vegetative food sources such as bulrush (*Scirpus californicus*). Thus, in addition to direct ingestion of lead shot pellets or lead in contaminated sediments, managers should consider plants as the next possible culprit for mottled duck lead exposure.

Though modeling approaches used in this study have suggested pathways and environmental pools responsible for lead exposure in mottled ducks, further studies are warranted to gather more concrete evidence before management efforts are pursued. First, the nature of the temporal variation of lead in the environment should be evaluated. Given the body of research suggesting that sediments are highly mobile in estuarine ecosystems especially after stochastic events such as hurricanes (Larson and Kraus 1995, Kennish 2002, FitzGerald et al. 2008, Howes et al. 2010, O'Connell and Nyman 2011), research is warranted to determine the variability in soil lead concentrations over time in response to other environmental factors. Second, the risk assessment conducted herein would be greatly bolstered by knowledge of the ingestion rate and true bioavailability of lead in various mottled duck food sources. Laboratory analysis could be conducted with similar conditions both to the environment and to mottled duck digestive systems that would give better estimates of actual lead bioavailability and give evidence as to which risk scenario is most accurate. Last, the hypothesized plant-related exposure pathway could potentially be tested using a captive mallard (*A. platyrhynchos*) study, because of the mallard's close relationship with the mottled duck, where treatments consist of a controlled diet of invertebrates and vegetation with similar lead content values to those present on the TCPC naturally.

This would provide further evidence of the best risk scenario for determining management and mitigating environmental lead hotspots in relation to mottled ducks specifically.

The potential prevalence of plant food sources as an exposure pathway for mottled ducks, however, may have promising implications for future mitigation efforts on the TCPC and elsewhere. Given the physical mechanisms of phytoextraction as a management technique for reducing heavy metal concentrations in contaminated soils (Salt et al. 1998), removing plants in contaminated areas, with preference given to common mottled duck food sources, may have the effect of reducing the environmental lead pool while simultaneously reducing exposure risks to feeding mottled ducks and potentially encouraging them to feed elsewhere. Furthermore, when new seeds germinate from the seed bank, new plants can continue to take up lead particles from contaminated soil and be removed as necessary until contamination is sufficiently mitigated. Application of a chelating agent may additionally speed this process to more rapidly reduce exposure risk to mottled ducks and other wildlife (Evangelou et al. 2007). This tactic may be effective off-refuge as well, but the most effective method for mitigating lead contamination and further deposition would be to move towards a more wide-reaching lead shot ban for upland hunting both in Texas and across the country. Although lead may currently be a less expensive ammunition solution for hunters, non-toxic shot has recently been shown to be equally effective in the pursuit of mourning doves (Pierce et al. 2014) and could thus provide a suitable replacement for hunting this and other game species. Given observed long-term effects of lead input into the environment on several wildlife species (Church et al. 2006, Hunt et al. 2006, Haig et al.

2014), California has already created legislation to move towards a universal lead shot ban (Anonymous 2014). In the modern world where contaminants are becoming an increasing problem and our industrial and recreational legacy continues to demonstrate historical short-sightedness, a lead shot ban more and more appears to be common sense to reduce the negative impacts our recreation has on the natural resources that we also seek to protect.

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