Causes, Extent, and Consequences of Lead-Pellet Ingestion by Chukars (Alectoris Chukar) in Western Utah: Examining Habitat, Search Images, and Toxicology

R. Justin Bingham

Utah State University

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CAUSES, EXTENT, AND CONSEQUENCES OF LEAD-PELLET INGESTION BY
CHUKARS (ALECTORIS CHUKAR) IN WESTERN UTAH: EXAMINING
HABITAT, SEARCH IMAGES, AND TOXICOLOGY

by

R. Justin Bingham

A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Ecology

Approved:

John A. Bissonette
Major Professor

Jeffery O. Hall
Committee Member

Frank P. Howe
Committee Member

Mark R. McClellan
Vice President for Research and
Dean of the School of Graduate Studies

UTAH STATE UNIVERSITY
Logan, Utah
2011
ABSTRACT

Causes, Extent, and Consequences of Lead-pellet Ingestion by Chukars (*Alectoris chukar*) in Western Utah: Examining Habitat, Search Images, and Toxicology

by

R. Justin Bingham, Master of Science

Utah State University, 2010

Major Professor: Dr. John A. Bissonette
Department: Wildland Resources

Lead ingestion adversely affects humans and over 130 species of wildlife. Wild chukars (*Alectoris chukar*) are documented to ingest lead, but the causes and consequences of this ingestion are poorly understood. The objectives of this research were to 1) examine the influence of habitat use, the hunting season, and seasonal climate on the extent and severity of lead ingestion by chukars in western Utah, 2) assess the effects of habitat use, feeding behaviors, and lead density on the causes of lead-pellet ingestion in captive and wild chukars, and 3) investigate the consequences of lead-pellet ingestion in captive chukars as a function of lead weathering, diet, and wild onion (*Allium spp.*) supplementation. I documented that 11.5% (n=54) of my sample of wild-harvested chukars contained an ingested lead pellet or increased liver lead (≥ 0.5 ppm). In conjunction with data from captive chukars dosed with lead, I was able to differentiate between bone-lead concentrations resulting from chronic or acute exposure to lead. I documented individuals from seven different mountain ranges with an ingested lead pellet or increased liver lead. I recorded 19 instances of ingested lead during June-
October (n=221) and 20 during November-January (n=193). I observed 14 events of increased liver lead for June-October (n=97), but did not find a single occurrence during November-January (n=24). The frequency of lead-pellet ingestion by captive chukars increased significantly when given a greater density of lead pellets with food and when fed a diet with seeds and grit pebbles that were similar visually to lead pellets. I estimated a density of 1,712,134 pellets/Ha in soils at an area used for target shooting. I found significantly more lead pellets in soils near springs than near guzzlers or reference points. I calculated that as many as 58,600 pellets/Ha may be present in soils near springs, and up to 2,445 pellets/Ha in soils surrounding guzzlers and reference points. One #6 lead pellet was able to induce morbidity and mortality in captive chukars. A mixed-seed diet and lead weathering exacerbated the effects of lead ingestion, whereas wild onion supplementation alleviated them.
ACKNOWLEDGMENTS

The culmination of research I present in this thesis would have been unattainable and infeasible without the aide, generosity, and selflessness of innumerable friends, family, advisors, and volunteers. I thank Travis Proctor and many other volunteers from the Utah Chukar and Wildlife Foundation for donating countless hours of volunteer time to enable the successful completion of my research. I thank Dave Olsen and Ernie Perkins for their unique help, information, and advice regarding chukars and water developments. Roger Stringham donated many hours and much energy helping me complete multiple stages of data collection, during which time he also willingly provided his opinion to my seemingly incessant inquiries.

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I thank Frank Howe and Jeffery Hall, my committee members, for providing much needed direction, insight, and oversight, which kept me properly focused and permitted my ideas to evolve into useful contributions to science. Meylena, my daughter, provided unconditional love and comic-relief to counteract the monotony of daily life during the writing process. I will be forever indebted to my parents for their support, counsel, and direction, which have shaped my mind and molded me into the person I am.
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R. Justin Bingham
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CHAPTER 1
INTRODUCTION

LEAD IN GENERAL

Lead contamination of the environment and the biological organisms within it is a pervasive and controversial issue. Lead adversely affects humans and wildlife (Pokras and Kneeland 2009), and instances of ingested lead pellets, bullet fragments, and fishing weights have been documented in humans and over 130 wildlife species (Tranel and Kimmel 2009). Lead is a heavy metal that occurs naturally in four stable isotopes (Pain 1996). Ratios of these isotopes can be used in conjunction with ancillary information to determine probable sources of lead contamination (Pokras and Kneeland 2009). In alkaline media (pH>7), lead is mostly present in non-soluble forms (Casas and Sordo 2006), but in acidic media (pH1-2.5) lead compounds become more soluble (Rattner et al. 2009). As the pH of a given medium decreases, so does the rate of lead dissolution.

Water with a pH of <6.5 begins to have a much more corrosive effect on lead than water with a pH ≥7 (Casas and Sordo 2006). However, the somewhat inert nature of lead allows lead shot to persist even in moderately acidic soils (pH 3.5-6.99) for an estimated 300 years (Jorgensen and Willems 1987, De Francisco et al. 2003). Environments with a pH of 1-2, such as those consistent with the stomach of humans and wildlife, can rapidly dissolve lead into various salts with high solubility (Casas and Sordo 2006). A specialized stomach like the avian muscular grinding gizzard, complimented with ingested grit pebbles, is an ideal environment for lead dissolution because it combines a low pH with abrasion and contraction forces (Trainer 1982, Pain 1996, Pain et al. 2009).
Lead possesses many desirable metallurgic characteristics such as high density, malleability, low melting point, and fairly inert chemical nature (Pokras and Kneeland 2009). Due to its ubiquity and utility, lead has been extracted from ore and widely used in human societies for about 4 millennia (Rodricks 2007). As a result of this prolonged and pervasive use of lead by humans, it is no longer possible to determine a pre-settlement concentration of lead in the environment (Pain 1996, Pain et al. 2009). Consequently, lead concentration can only be discussed in terms of background versus increased exposure, which are both relative terms and can be highly variable among study areas and populations (Pain et al. 2009).

Due to the molecular similarities between lead and other heavy metals, the body often uses lead in place of essential metals including iron, calcium, and zinc (Pokras and Kneeland 2009). Lead can bind to enzymes and inactivate them (Needleman 1991, Casas and Sordo 2006), as it does with those involved with nerve transmission, heme synthesis in the blood, and heme dependent detoxification processes in the liver (Sassa et al. 1975, Dieter and Finley 1979, Massaro 1997). Lead has an antagonistic relationship with beneficial heavy metals. Lead will actually compete with other divalent ions like calcium, iron, magnesium, and zinc and will substitute for these metals leading to deleterious effects (Mahaffey 1981, Massaro 1997, Pokras and Kneeland 2009). Additionally, lead plays a role in multiple diseases and ailments that involve iron and calcium such as: anemia (Pokras and Kneeland 2009); osteoporosis; and inferior growth and development of enamel and bone. Lead can also adversely affect the metabolism of vitamin D (Massaro 1997).
Despite the abundance and utility of lead, it is unfortunately quite toxic and has no known beneficial function in biological organisms (Pain 1996, Scheuhammer et al. 1998, Pokras and Kneeland 2009, Rattner et al. 2009). Nevertheless, lead has a high economic value. Consequently, its health risks have been largely overlooked or at least accepted as necessary or tolerable drawbacks (Pokras and Kneeland 2009). Additionally, the accepted threshold for a safe level of exposure to lead for human children has progressively decreased from a blood concentration of 80µg/dL in 1968 to 2µg/dL (Lanphear et al. 2000, Canfield et al. 2003). A safe concentration of ingested lead, other than zero, cannot be defined because lead can inhibit biological processes at the lowest measurable concentrations (Pain 1996, Pokras and Kneeland 2009).

**LEAD REGULATION AND ALTERNATIVES**

Although the toxic properties of lead have been apparent to humans for over 2 millennia (Nriagu 1983; 1998), only in the last 40-50 years has there been a marked effort to understand the effects of lead in the environment and subsequently reduce the use of lead in manufacturing. Western societies have recently made an attempt to reduce or eliminate the use of lead in many products like: leaded gasoline, which was phased out from 1973 to 1995; leaded paints, which were banned for residential use in 1978; and lead solders. These programs have aimed to minimize lead exposure to humans and lead deposition throughout the environment (Pokras and Kneeland 2009). As a result of these regulations on lead, the mean concentration of blood lead in humans in North America has decreased over the last few decades (Pirkle et al. 1994). However, lead is still widely used and emitted or deposited into the environment at an alarming rate. With lead
ammunition and fishing sinkers alone, an estimated 6-10 thousand tons of lead are
deposited annually into the environment solely in the U.S. (Pokras and Kneeland 2009).

Disconnected and contradictory paradigms currently govern the use and toxic
tolerability of lead in human societies. For example, the Occupational Safety and Health
Administration (OSHA) recommends on their website (http://www.osha.gov/SLTC/lead/) that humans should “avoid purchasing or using products known to contain lead.”
Additionally, industrial processes that involve exposure to lead—such as mining and
recycling of lead products—require federal permits to regulate the release of lead into the
environment (Pokras and Kneeland 2009). Nevertheless, anyone can legally purchase
lead sinkers for fishing purposes, and if ≥ 18 years old, one can purchase lead
ammunition. Moreover, most leaded projectiles, when used as intended, are destined to
be deposited into the environment, and many fishing sinkers are released into aquatic
ecosystems under conditions of normal use. Pokras and Kneeland (2009) present another
curious example, also involving sporting equipment, of conflicting and counterintuitive
standards regarding lead regulation. The authors explained that in April 2005, the U.S.
Consumer Product Safety Commission (USCPSC) announced a nationwide recall of 1.5
million youth-style fishing rods because they discovered that the paint in the rods
exceeded the 0.06% threshold for lead. The USCPSC advised parents to discontinue use
of the product immediately. Contrastingly, an online retailer that markets fishing gear for
children was concurrently selling a product called, “The Ultimate Fishing Kit for Kids,”
which included a tackle box complete with 78 large fishing sinkers made entirely of lead.

The key to understanding the controversy and contradictory regulation of lead
apparently lies in the disparity between its utility and healthiness. Schulz et al. (2009)
presented two opposing paradigms that are applicable to the lead ammunition controversy: 1) The Risk Paradigm, which affirms that environmental risks are manageable and uses risk management as the primary tool; and 2) The Ecological Paradigm, which is based on the precautionary principle (reviewed by Matsuda 2003) and suggests that scientific knowledge of complex systems is incomplete and imprecise, so precautionary measures are warranted to manage the lead dilemma. Schulz et al. (2009) interpreted the ecological paradigm to mean that substances that can be objectively and scientifically judged as having the potential to promote or influence widespread, long-term, and severe types of environmental damage—such as the issue with lead-based ammunition—should be replaced, if feasible, with safer alternatives rather than continuing use with acceptable amounts of environmental risk. The evidence concerning the adverse effects of lead on both biotic and abiotic portions of the environment is sufficient to warrant serious contemplation, debate, and resolution of the lead controversy using the input of all applicable stakeholders.

Nontoxic alternatives to lead are available, affordable, economically feasible, effective, and ethical (Cade 2007, Sullivan et al. 2007, Oltrogge 2009). Currently, lead-free rimfire and centerfire bullets, and lead-free shot are widely available (Oltrogge 2009). Although there is no candidate to replace lead pellets on a density to price basis, small modifications in shooting habits, shot sizes, and velocities can help account for the inferior ballistics of most lead-free alternatives (Oltrogge 2009). Despite widespread concern about the efficacy of steel shot, crippling rates from the lead-shot era and during the 5-year-phase-in period were actually greater than the analogous rates during the nontoxic-shot era for both ducks and geese (Schulz et al. 2006). The U.S. government
provided educational videos to inform hunters of the differences for the behavior of lead versus steel shot to help hunters adapt their shooting techniques (Friend et al. 2009).

Due to regulations in areas like Southern California, nontoxic shotgun loads are now available for upland-game applications (Thomas 2009). Thomas (2009) also suggested that competition should decrease the costs of nontoxic ammunition. Production of nontoxic projectiles by the ammunition industry will require assurance of sustained market demand. Despite the views of many stakeholders in the debate on lead ammunition, the anti-lead campaign is not analogous to and should not be viewed as an anti-hunting initiative (Thomas 2009). According to Thomas (2009 pg. 352), “The real quest with lead is to improve the apparent sustainability of [hunting and fishing] by finding and approving lead substitutes that leave no toxic legacy in the environment.” Nevertheless, lead-free alternatives must be accurate, effective, and affordable to gain popularity (Stroud and Hunt 2009) with hunters. Educating hunters on the impacts of lead on wildlife and the environment will also be essential to resolving the lead controversy (Friend et al. 2009, Stroud and Hunt 2009).

**TOXICITY OF LEAD TO HUMANS AND WILDLIFE**

Lead can cause immediate or somewhat prolonged declines in physical condition and mortality (Pokras and Kneeland 2009). Conversely, many sub-lethal effects also stem from ingestion of both large and small concentrations of lead (Demayo et al. 1982, Pokras and Kneeland 2009) and affect the entire body, including the central nervous, circulatory, digestive, immune, reproductive, and skeletal systems (Massaro 1997, Pokras and Kneeland 2009). Effects of lead on the central nervous system include: aggressive behavior (Burright et al. 1989, Sciarillo et al. 1992, Wright et al. 2008) in humans and
wildlife; learning difficulties; impaired nerve transmission; lowered IQ (Canfield et al. 2003); encephalopathy, peripheral neuropathy, memory loss, seizures, hyperactivity, headache, and coma (Pain 1996, Massaro 1997). Digestive ailments consist of loss of appetite; inability to digest food; severe abdominal pain, which is a clinical sign common to nearly all lead-poisoned animals (Osweiler 1996); and diarrhea (Pokras and Kneeland 2009); all of which combine to produce severe weight loss. Reproductive consequences include spontaneous abortion, reduced fertility, reduced lactation, and altered sperm morphology in mammals (Patrick 2006, Pokras and Kneeland 2009); reduced sperm production (Kendall et al. 1981); lowered weight of testes (Kendall and Scanlon 1981); decreased clutch sizes in birds (Edens and Garlich 1983); impaired eggshell formation in birds (Edens et al. 1976); and diminished hatchability of eggs (Buerger et al. 1986) in birds. Lead can also be transferred through the blood and milk of mammalian mothers to their offspring (ATSDR 2007). Complications from lead that negatively influence the skeletal system include: weakened bone structure, and impaired bone and enamel growth, repair, and development (Pain et al. 2009, Pokras and Kneeland et al. 2009).

Lead exposure is commonly described as acute, i.e., exposure to large concentrations of lead over a short period of time, or chronic, i.e., prolonged exposure to lead in smaller concentrations (Pain 1996) consistent with background exposure. Similarly, lead poisoning is divided into three distinct categories: 1) subclinical, which means physiological effects exist, but are not sufficiently severe to inhibit most biological functions, 2) clinical, which implies that normal biological functions are severely impaired or even ceased, and 3) severe clinical, which suggests that the effects of lead are life threatening (Pain 1996).
After ingestion, lead follows a general pathway in the body: dissolution in the stomach, absorption into the bloodstream through the intestinal wall, distribution to soft tissues such as liver and kidneys, immobilization and storage in bone tissue, and finally infrequent mobilization into bone tissue (Pain 1996, Pain et al. 2009, Pokras and Kneeland 2009). Lead in avian blood has a half-life of about two weeks (Fry et al. 2009). Lead concentrations in blood and soft tissues are useful for information on recent exposure, because these concentrations begin to increase almost immediately post-ingestion and remain increased for up to several months (Pain 1996, Pain et al. 2009, Pokras and Kneeland 2009), whereas bone concentrations of lead more accurately describe lifetime exposure (Pain 1996).

The amount of lead that is absorbed in tissues or required to cause sub-lethal effects or mortality is highly variable and difficult to predict (Pain et al. 2009). It is affected by 1) species, 2) age, 3) gender, 4) stressful situations like disease, pregnancy, and injury, 5) diet, 6) idiosyncrasies within individuals, 7) lead pellet or fragment retention-time, and 8) multiple abiotic factors (Pain 1996). Sometimes lead shot can be voided very quickly, which leads to little or no effect on the organism (Pain 1996). However, lead shot is commonly retained much longer and is eroded entirely in an average of 42 days (Pain 1996). A study of Japanese quail (Coturnix coturnix) showed complete erosion of lead in only 22 days (Yamamoto et al. 1993). Average retention time for lead shot in waterfowl is 18-21 days (Jordan and Bellrose 1951). In humans and other mammals, juveniles usually absorb 40-50% of ingested lead; whereas adults may only extract 5-15% of the lead they ingest (Casas and Sordo 2006, ATSDR 2007, Rodricks 2007, Pokras and Kneeland 2009). Children are also at a greater risk to the
effects of lead exposure before their central nervous system is fully developed (Verbrugge et al. 2009). The most important factor that influences lead absorption may be the quality and quantity of diet (Jordan and Bellrose 1951, Longcore et al. 1974, Sanderson and Bellrose 1986). As a result, diets deficient in calcium, iron, and phosphorus can increase the absorption of lead and exacerbate its effects (Trainer 1982, Massaro 1997, Casas and Sordo 2006). Certain types of food, e.g., those high in phosphorus, can produce what is known as a ligand effect, whereby lead is tied up in an insoluble form in the intestines, making it less available for absorption (Pain 1996). This binding process is the same principle at work in chelation treatments designed to alleviate the effects of lead ingestion (Casas and Sordo 2006). Consequently, diets high in these binding nutrients and in protein, as well as those diets made up of soft, easily digestible foods tend to decrease lead absorption and ameliorate its negative effects (Pain 1996).

Although lead is a natural element, most lead exposure to biological organisms stems from anthropogenic sources related to industrial processes like mining and manufacturing of consumer products such as leaded gasoline, paint, and ammunition (Casas and Sordo 2006). Small concentrations of atmospheric lead can accumulate in plants as a result of uptake from roots followed by distribution and absorption throughout additional plant materials including leaves and seeds. Most lead deposited near roads through the burning of leaded gasoline is present in insoluble forms, particularly in alkaline soils (Quarles et al. 1974), and is thus unavailable for plant uptake. Additionally, of the total amount of lead available to plants, only a small portion will reach the fruits and foliage. Some research has demonstrated that plant uptake of soluble lead can result in concentrations of 4-9% of those in corresponding soils (Mellor and McCartney 1994):
0.05-7.0% in leaves (Manninen and Tanskanen 1993) and 1.4-3.0% in seeds (Mellor and McCartney 1994). However, these studies were conducted at shooting ranges with acidic soils and very high concentrations of lead. Notwithstanding these potential sources of lead exposure, the greatest and most widespread risk for exposure of toxic amounts of lead to avian wildlife is through ingestion of lead ammunition. Lead ammunition is present in two common forms: 1) lead-cored or all-lead bullets, and 2) lead pellets, which are also referred to as lead shot. Avian species ingest and are subsequently poisoned by both types of lead ammunition (Franson 1996, Pain 1996, Fisher et al. 2006). Lead shot that is deposited through hunting and shooting activities is available to birds that can mistake these pellets for food and grit items (Gionfriddo and Best 1999, Schulz et al. 2002, Mateo 2009). Lead pellets and bullets can fragment on impact, especially when striking bone, and remain embedded in edible tissues of wildlife (Frank 1986, Scheuhammer and Norris 1995, Scheuhammer et al. 1998). Many animals that are killed or wounded by hunters are irretrievable (Scheuhammer et al. 1998) and can be struck with lead pellets or bullet fragments without being mortally wounded or crippled (Scheuhammer and Norris 1995). Hunters generally remove the entrails of larger wildlife species in the field, and these vital organs can contain lead pellets or bullet fragments (Hunt et al. 2006, Knopper et al. 2006, Cade 2007, Stroud and Hunt 2009). Consequently, predators and scavengers risk exposure to increased concentrations of lead by consuming prey items that were wounded or harvested with lead. These exposure pathways of lead to upland birds have been corroborated by isotopic analysis (Scheuhammer and Templeton 1998, Meharg et al. 2002, Church et al. 2006, Pain et al. 2007).
Research has shown that even if lead pellets or bullet fragments are removed from harvested wildlife, particles of lead can remain undetected. They range in size from dust to about 1mm and are visible through radiography. Hence this is a pathway for lead exposure to humans (Frank 1986, Scheuhammer et al. 1998, Hunt et al. 2006, Knopper et al. 2006, Pain et al. 2007). Emergency food assistance is sought by 2.8% of all households in the U.S. annually, and a portion of this need is satisfied by organizations and individual hunters who donate meat from wild game (Avery and Watson 2009). Recent research has demonstrated that lead fragments are present in meat donated to food banks (Cornatzer et al. 2009). As a result, humans, particularly subsistence hunters (Tsuji and Nieboer 1997) and other individuals who use cooking techniques or recipes of low pH (Mateo et al. 2007), risk exposure to increased concentrations of lead and its concomitant effects by consuming wildlife that is harvested with lead, even if visible pellets and fragments are removed (Tsuji et al. 1999). Accordingly, Tsuji et al. (1999 pg. 183) reported that “people who consume any species harvested with lead shot risk exposure to this metal by way of ingestion of tissue-embedded lead pellets and fragments.” These small fragments of lead are especially of concern both physically and chemically, because they are both hard to detect, and they possess a greater surface area in relation to mass, which facilitates and accelerates their dissolution in the digestive system (ATSDR 2007, Stroud and Hunt 2009).

As a result of extensive research (Bellrose 1959, Longcore et al. 1974, Sanderson and Bellrose 1986, Anderson et al. 1987), a regulatory ban against the use of lead shot for hunting waterfowl species was implemented by the U.S. Government in 1991. Trainer (1982) estimated that before the discontinuance of lead for waterfowl hunting, 3,000 tons
of spent lead was introduced annually into the environment by waterfowl hunters alone. Concerning the effects of lead ingestion on waterfowl, Bellrose (1959) estimated that 4% (approximately 700,000 individuals) of the annual Mallard (*Anas platyrhynchos*) population succumbed to mortality due to lead poisoning, and that 2-3 million non-harvested waterfowl in general died of lead poisoning annually in North America. Additionally, lead ingestion has caused mortality in waterfowl on multiple continents for over a century (Bellrose 1959, Sanderson and Bellrose 1986, Pain 1992).

Even though a ban on leaded ammunition is in place for waterfowl in the U.S., hunting of sympatric upland species within and nearby waterfowl habitat continues to deposit lead in wetlands and fields used by waterfowl (Tranel and Kimmel 2009). The ban on lead for waterfowl hunting was aimed largely at protecting the bald eagle (*Haliaeetus leucocephalus*) (Anderson 1992). After bald eagles were decimated by pesticides like DDT, scientists also discovered that bald eagles were negatively affected by lead poisoning concomitant the ingestion of lead-projectile fragments (Pattee and Hennes 1983, Elliott et al. 1992, Scheuhammer and Norris 1996, Wayland and Bollinger 1999). Multiple countries have similar bans in place for hunting waterfowl with lead, but lead shot and bullets are still used in the US and many other countries worldwide for hunting upland game and for various other hunting and shooting activities. A few countries are exceptions, namely Denmark (ban on all lead), The Netherlands (ban on lead shot only), Norway (shot only), and Sweden (all lead) (Avery and Watson 2009). The disconnection in lead regulation for hunting in the U.S. provides another example of a counterproductive measure—Although the U.S. banned lead ammunition for waterfowl hunting because bald eagles hunt and scavenge waterfowl species, lead ammunition is
still permitted in the U.S. in most areas for all other types of hunting and shooting, even though bald eagles are known to scavenge species of upland game (Tranel and Kimmel 2009) and are still hampered by lead poisoning (Bedrosian and Craighead 2009, Neumann 2009, Redig et al. 2009).

In addition to the havoc wreaked on bald eagles, lead has played a role in reducing numbers and hampering recovery efforts for various threatened and endangered species including: Egyptian vultures (*Neophron percnopterus*) (Donazar et al. 2002), red kites (*Milvus milvus*) (Mateo et al. 2001, Pain et al. 2007), Spanish imperial eagles (*Aquila adalberti*) (Mateo et al. 2001, Pain et al. 2005), Stellar’s sea eagle (*Haliaeetus pelagicus*) (Saito 2009), and white-tailed sea eagles (*Haliaeetus albicilla*) (Krone et al. 2009, Saito 2009). In the U.S., lead has been implicated in undermining the reintroduction effort for California Condors (*Gymnogyps californianus*) (Snyder and Snyder 2000, Fry 2003, Cade 2007, Parish et al. 2009) and is the most frequently diagnosed cause of death among the Grand Canyon population despite intensive monitoring and treatment efforts to mitigate consequences (Green et al. 2008). To further advance these mitigation efforts, the Arizona Game and Fish Department and the Utah Division of Wildlife Resources have implemented programs to offer lead-free ammunition to hunters in applicable areas (Sieg et al. 2009). Additionally, lead poisoning was a major factor in the original decline to endangered status for California condors (Janssen et al. 1986, Wiemeyer et al. 1988, Snyder and Snyder 1989, Pattee et al. 1990).

Many species of upland birds are adversely impacted by lead ingestion. As of 2008, there were 16 species of upland game-birds and 29 species of raptors that had been
reported in the literature as being adversely affected by lead ammunition (Tranel and Kimmel 2009). Lead ingestion and its resulting toxicosis are well-documented for most waterfowl species, and some species of raptors and doves (reviewed in Kendall et al. 1996). However, ingestion of lead shot by other avian taxa and its negative effects are quite poorly understood, but recent research demonstrates that lead-pellet ingestion can be a significant source of sub-lethal detriments and mortality for many additional species of birds (Keymer and Stebbings 1987, Lewis and Schweitzer 2000, Vyas et al. 2000, Butler et al. 2005). Available literature for upland bird species is often simply a documentation of ingested lead shot (Walter and Reese 2003, Butler 2005, Larsen et al. 2007b) or a report of individuals of a given species that have succumbed to lead poisoning as a result of lead ingestion (Keymer and Stebbings 1987, Lewis and Schweitzer 2000).

**LEAD AND CHUKARS**

Chukars (*Alectoris chukar*) are a species of upland bird for which ingestion has been recently documented (Walter and Reese 2003, Larsen et al. 2007b, Kreager et al. 2008), but the concomitant causes and consequences of this ingestion are poorly understood. Chukars are medium-sized, gallinaceous game-birds native to semi-arid, mountainous regions in portions of Eurasia (Dement'ev and Gladkov 1952, Cramp and Simmons 1980, Ali and Ripley 2001). Because chukars are a popular hunting quarry, they have been widely introduced across the world. The most extensive and successful introduction efforts for chukars have occurred in North America (Long 1981) and have continued from 1893 (Lever 1987) to the present. Currently, self-sustaining populations of
chukars occur in 11 western states (U.S.) and one Canadian Province with populations occupying approximately 252,800 km² (Christensen 1996).

Although chukars are largely granivorous (Cole et al. 1995) and eat mostly cheatgrass seeds (*Bromus tectorum*) (Christensen 1996, Larsen et al. 2007a), they are opportunistic feeders consuming a wide variety of foods including many other seeds, bulbs, flowers, grass shoots, and insects (Walter and Reese 2003, Larsen et al. 2007a). One of the bulbs consumed by chukars—apparently for its high water content—is wild onion (*Allium spp.*) (Larsen et al. 2010). Garlic (*Allium sativum*), which is very similar to wild onion, has been shown to contain beneficial properties against lead absorption and toxicosis when consumed in substantial amounts over extended time periods (Craig 1999, Tandon et al. 2001). Additionally, vitamins like thiamine and minerals such as calcium, which are found in allium species, have been documented to reduce lead absorption (Bratton et al. 1991, Coppock et al. 1991, Olkowski et al. 1991). Onion-bulb consumption by wild chukars may reduce their risk of developing lead poisoning or augment their ability to survive it.

Lead-pellet ingestion by chukars is likely related specifically to 1) the arid climate, rocky topography (Walter and Reese 2003), and alkaline soils of chukar habitat, which reduce the rate of pellet settlement (Schranck and Dollahon 1975) and dissolution (Stansley et al. 1992), 2) similarities in appearance between lead pellets and grit and food sources used by chukars (Gionfriddo and Best 1999, Schulz et al. 2002), and 3) density of lead pellets in soils, particularly as it relates to the use of areas of concentration for lead deposition like water sources (Best et al. 1992), roads, and sites used for target shooting. Additional and equally important factors that increase the frequency or exacerbate the
effects of lead ingestion by many avian species include: 1) hunting pressure for the species in question or any sympatric species hunted with lead shot, 2) presence of a well-developed muscular grinding gizzard with a low pH (Pain 1996, Pain et al. 2009), 3) use of pebbles to aide in food digestion (Trainer 1982, Pain 1996, Pain et al. 2009), 4) extreme daily and seasonal variability in climate and food availability and other stressful situations like predator avoidance, and 5) the propensity to intensely probe soils for food (Gionfriddo and Best 1999).

Chukars must obtain grit pebbles to aid in food digestion. Some chukars use grit that looks similar to lead pellets. Additionally, chukars commonly consume Indian ricegrass seeds (*Stipa hymenoides*) as part of their diet. These seeds have a strong resemblance in size, color, and shape to lead pellets. We hypothesized that if chukars consume food or grit items that approximate lead pellets, the affinity to mistake lead pellets for food or grit will increase, thereby leading to an increased probability of lead-pellet ingestion.

Sources of free water, both natural springs and types of man-made water catchments (guzzlers), are used by chukars during the hot, dry months of summer, particularly during years of above average temperatures and below average precipitation (Larsen et al. 2007a, Larsen et al. 2010). The density of lead pellets in soils may be indirectly increased by presence of water due to concomitant shooting activities. A stock tank in New Mexico that was used for both livestock watering and hunting of mourning doves (*Zenaida macroura*) was estimated to contain densities of 167,593 pellets/ha in August prior to the dove-hunting season and 860,185 pellets/ha in October after the season had concluded (Best et al. 1992).
Many desert springs in Utah are used by both chukars and mourning doves, which means chukars may be exposed to lead pellets in soils near springs that are heavily shot over for mourning doves. Additionally, because some chukars are often still congregated near water sources after the beginning of the hunting season, more lead may be deposited near these water sources through chukar hunting and could be subsequently ingested by chukars.

Larsen et al. (2007b) documented the ingestion of lead pellets by multiple populations of chukars within four counties of western Utah. Of 106 crops, the authors found 2 (1.9%) with ingested lead pellets. After examining 75 gizzards, they discovered 8 (10.7%) containing ingested lead pellets. We documented that this dataset has now increased to 466 individuals with 43 (9.2%) containing lead pellets with no sign of penetration wounds. Walter and Reese (2003) also documented ingestion of lead pellets by chukars in nearby Eastern Oregon. Kreager et al. (2008) confirmed ingestion of lead pellets by chukars in southern Canada. The discovery that multiple chukars from independent populations in Utah and elsewhere have ingested lead pellets warrants investigation of the causes, extent, and consequences of lead-pellet ingestion by chukars. These specific objectives attempt to fulfill the research needs concerning chukars and lead-pellet ingestion that were outlined by Larsen et al. (2007b).

Chapter 2 examines the influence of the interaction among habitat use, the hunting season, and seasonal climate on the extent and severity of lead ingestion by chukars in western Utah. Chapter 3 focuses on assessing the effects of habitat use, feeding behaviors, and lead density on the causes of lead-pellet ingestion in captive and wild chukars. Chapter 4 investigates the consequences of lead-pellet ingestion in captive
chukars as a function of lead weathering, diet, and wild onion (*Allium spp.*) supplementation.

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CHAPTER 2

EXTENT AND SEVERITY OF LEAD-PELLET INGESTION BY WILD
CHUKARS (ALECTORIS CHUKAR) IN WESTERN UTAH

ABSTRACT

Lead ingestion is ubiquitous and controversial, negatively affecting humans and at least 130 species of wildlife worldwide. Although chukars (Alectoris chukar) in Utah have been documented to ingest lead, the extent and severity are largely unknown. Our objectives were to: 1) determine the influence of hunting on lead ingestion, and 2) investigate the extent and severity—both spatially and temporally—of lead ingestion by chukars throughout western Utah. Of 466 chukar gizzards, we found 43 (9.2%) containing ingested lead shot and 54 (11.5%) containing either ingested lead shot or increased liver lead (≥0.5ppm). A Mann-Whitney U test was insignificant for ranked values of liver-lead concentration from hunted vs. non-hunted sites (W=934, P=0.269). Birds from the Cedar, Deep Creek, Gilson/Canyon, and Stansbury Mountains had above average frequency of ingested and increased liver lead. A Kruskal-Wallis test (KW=27.44, P<0.001) with individual Bonferroni corrections (α=0.0018) showed that ranked liver-lead concentrations for chukars were statistically greater from Deep Creek (W=19.5, P=0.0018) and Stansbury Mountains (W=158, P<0.001) vs. those from Lakeside Mountains. The ranked values of tibia-lead concentration for Antelope Island were significantly greater than Cedar (W=234, P<0.001), Gilson-Canyon (W=91, P<0.001), and Stansbury Mountains (W=195, P<0.001). Stansbury was statistically higher than Cedar (W=29, P<0.001) and Gilson-Canyon (W=93, P<0.001). Ranked values for liver lead did not vary statistically by season or harvest year. Nevertheless, we

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1Coauthored by R. Justin Bingham, Randy T. Larsen, John A. Bissonette, and Jeffery O. Hall
observed 19 individuals with ingested lead and 14 with increased liver lead during June-October vs. 20 with ingested lead from November-January, without documenting a single individual with increased liver lead from November-January. Lead ingestion by chukars in western Utah is widespread both spatially and temporally. Individuals from Antelope Island and Stansbury likely ingest more atmospheric lead. Birds ingesting lead November through January may quickly succumb to lead poisoning due to increased stress. Chukars and sympatric mourning doves (*Zenaida macroura*) and raptors are at high risk for lead ingestion and poisoning due to their habitat use, physiology, and behavior. If managers desire to avoid increased lead exposure to these avian species, lead-pellet ingestion will be part of the discussion.

**INTRODUCTION**

Lead ingestion adversely affects humans and wildlife (Pokras and Kneeland 2009), and instances of ingested lead pellets, bullet fragments, and fishing weights have been documented in at least 130 wildlife species (Tranel and Kimmel 2009). Lead is a naturally occurring toxic metal found in four stable isotopes (Pain 1996). Ratios of these isotopes can be analyzed and used in conjunction with separate lines of evidence to determine sources of lead contamination (Pokras and Kneeland 2009). In alkaline media (pH>7), lead is mostly present in non-soluble forms (Casas and Sordo 2006). The somewhat inert nature of lead allows its shot pellets to persist even in moderately acidic soils (pH 3.5-6.99) for many decades (Jorgensen and Willems 1987, De Francisco et al. 2003). Nevertheless, in highly acidic media (pH 1-2.5) lead becomes increasingly soluble (Rattner et al. 2009). A specialized stomach like the avian muscular grinding gizzard, complete with ingested grit pebbles, is an ideal environment for lead dissolution.
because it combines a low pH with grinding and contraction (Trainer 1982, Pain 1996, Pain et al. 2009). Although lead is extremely abundant and useful, it is highly toxic and has no known benefit in biotic organisms (Pain 1996, Scheuhammer et al. 1998). Lead shot deposited through hunting and shooting activities is available to birds that can mistake these pellets for food and grit items (Gionfriddo and Best 1999, Schulz et al. 2002, Mateo 2009). Trainer (1982) estimated that before the discontinuance of lead for waterfowl hunting, 3,000 tons of spent lead was introduced annually into the environment by waterfowl hunters alone. Concerning the effects of lead ingestion on waterfowl, Bellrose (1959) estimated that 4% (approximately 700,000 individuals) of the annual Mallard (*Anas platyrhynchos*) population succumbed to mortality due to lead poisoning, and that 2-3 million waterfowl in general died of lead poisoning annually in North America. As a result of extensive research (Bellrose 1959, Longcore et al. 1974, Sanderson and Bellrose 1986, Anderson et al. 1987), a regulatory ban against the use of lead shot for hunting waterfowl species was implemented by the U.S. Government in 1991.

Despite the volumes of research conducted with lead and waterfowl, lead ingestion and its adverse effects in avian taxa from upland habitats are poorly understood. Nevertheless, recent research shows that lead-pellet ingestion can result in sub-lethal detriments and mortality in many species of birds other than waterfowl (Kendall et al. 1996, Fisher et al. 2006). Available literature for upland-bird species is often simply a documentation of ingested lead shot (Walter and Reese 2003, Butler 2005, Larsen et al. 2007b), or a report of individuals of a given species that have succumbed to lead poisoning as a result of lead ingestion (Keymer and Stebbings 1987, Lewis and
Schweitzer 2000, Vyas et al. 2000). Chukars are an upland bird for which lead-pellet ingestion has been documented (Walter and Reese 2003, Larsen et al. 2007b, Kreager et al. 2008), but the severity and extent of this ingestion are mostly unclear.

Chukars are medium-sized, gallinaceous game-birds native to semi-arid, mountainous regions in portions of Eurasia (Cramp and Simmons 1980, Ali and Ripley 2001). Although chukars are largely granivorous (Cole et al. 1995) and eat mostly cheatgrass seeds (*Bromus tectorum*) (Christensen 1996, Larsen et al. 2007a), they are opportunistic feeders and consume a wide variety of foods including bulbs, flowers, grass shoots, insects, and other seeds (Walter and Reese 2003, Larsen et al. 2007b). Because chukars are a popular hunting quarry, they have been widely introduced across the world. Currently, self-sustaining populations of chukars occur in 11 western states (U.S.), and one Canadian Province, occupying approximately 252,800 km² (Christensen 1996) in North America.

Due to anthropogenic sources of lead emission and dispersal, small concentrations of lead can accumulate in soils and plants and be subsequently ingested by chukars. However, the greatest and most widespread risk for increased lead exposure to avian wildlife is through ingestion of lead ammunition; this risk has been validated with multiple avian species (Thomas 1982, Sanderson and Bellrose 1986, Kendall et al. 1996, Fisher et al. 2006) and corroborated by isotopic analyses (Scheuhammer and Templeton 1998, Meharg et al. 2002, Church et al. 2006, Pain et al. 2007).

Lead-pellet ingestion by chukars is likely related specifically to 1) the arid climate, rocky topography (Walter and Reese 2003), and alkaline soils, which reduce the rate of pellet settlement (Schranck and Dollahon 1975) and dissolution (Stansley et al. 
1992); 2) hunting pressure for chukars and other sympatric species such as mourning doves (*Zenaida macroura*); and 3) habitat use, particularly as it relates to areas with concentrated lead deposition like water sources (Best et al. 1992), roads, and sites used for target shooting.

The principal objectives of this chapter were to: 1) determine the influence of the hunting season on lead ingestion, and 2) investigate the extent and severity—both spatially and temporally—of lead ingestion by chukars throughout western Utah. We hypothesized that birds from hunted areas would have a greater ranked values for liver and tibia lead vs. those from non-hunted areas, and that burgeoning population growth during a successful reproductive year would increase lead ingestion by chukars. We also predicted that frequency of lead ingestion, increased liver lead, and mean liver-lead would be greater during the seasons of water use and hunting because water sources and hunting activities concentrate lead-pellet deposition and subsequent ingestion.

**METHODS**

*Study Area*

The study area we used to conduct the sampling of wild chukars consists of portions of Box Elder, Davis, Juab, Tooele, Millard, and Utah counties located within western Utah. A detailed description some of the locations comprising our study sites is available in Larsen et al. (2007a) and Robinson et al. (2009). Additional specific sites of investigation were centered at the following coordinates: Blue Spring/West Hills, Box Elder County (41° 50’ 13” N 112° 19’ 58” W); Canyon/Gilson Mountains, Juab and Millard Counties (39° 36’ 34” N 112° 13’ 20” W); Deep Creek Mountains, Juab and Tooele Counties (39° 50’ 42” N 113° 54’ 38” W); East Tintic Mountains, Juab, Tooele, and Utah Counties (39° 53’ 58” N 112° 3’
40

Wild-harvested Chukars: Collection, Processing, and Analysis

To assess lead ingestion in wild chukars, we utilized hunter-harvested chukars previously collected for diet analyses. A detailed overview of our methods for wild chukar collection is presented in Larsen et al. (2007b). We took care to confirm that the pellets we discovered were lead and not steel by using pliers to apply pressure in an attempt to deform the pellets. We determined that all pellets found in harvested chukars were lead. We divided this sample according to the presence or absence of lead pellets in the gizzard. For presence of lead, we only included gizzards that lacked entry wounds.

We removed the right tibia and right liver lobe and stored, processed, and analyzed the samples for lead residue. A detailed overview of our protocol for analyses to acquire tissue-lead concentration can be found in Chapter 4, this volume. To reduce biases in lead concentration in liver or bone, we did not use livers or tibia that had evidence of trauma, which could have been caused by lead shot. We compared these data for lead concentration in liver tissue and tibia tissue with those for presence vs. absence of ingested lead pellets to check for any potential relationships. As a control for hunting in the analyses of tissue lead, we harvested 13 chukars with steel shot on
Antelope Island State Park in the Great Salt Lake, Utah. This State Park has been closed to hunting since its inception in 1981.

From wild chukars collected in hunted areas, we obtained 466 undamaged and 4 damaged gizzards with corresponding liver samples and had a total of 121 livers from birds in hunted areas analyzed for lead residue. Although the 4 damaged gizzards could not be used to assess presence of ingested lead pellets, we were able to use the 4 corresponding liver samples of these damaged gizzards for liver-lead analyses. Consequently, for analyses involving the combination of ingested lead in gizzards or increased liver lead, we had a sample size of 470 for hunted areas. Birds were only counted once for ingested lead or increased liver lead. For example, if an individual contained lead in its gizzard and increased liver lead, we only used presence of ingested lead in the gizzard. We analyzed 13 livers and gizzards from birds harvested at Antelope Island for a total of 134 livers and 479 gizzards analyzed overall.

We used our conservative estimate (Bingham unpublished data) of 0.5 ppm liver lead as the threshold value for background vs. increased lead exposure in chukars from our study area because we found no instances of liver lead ≥0.5 ppm in any control birds from our dosing trials (Chapter 4, this volume). For our sample of wild chukars, we sorted the data for lead concentration in livers and presence of ingested lead pellets by hunted vs. non-hunted areas, mountain range of harvest, and year of harvest. We had a total of 134 (for liver analyses), 397, and 421 harvested chukars from our overall sample of 134, 470, and 470 birds for which hunted vs. non-hunted location, mountain range of harvest, and year of harvest were recorded, respectively. We examined these data to determine if lead ingestion by chukars was a localized or widespread problem. We also
checked for a possible legacy effect of spent lead that could occur if burgeoning chukar populations resulted in improved hunting opportunity and more frequent lead deposition into soils. We compared our data for lead concentration in liver from wild-harvested birds from hunted areas to the analogous data for our control sample from Antelope Island State Park where hunting is not allowed.

We used individuals from our sample of wild-harvested chukars to investigate if lead concentrations in liver tissue and rates of lead ingestion are influenced by the hunting season as demonstrated in research with raptors (Elliott et al. 1992, Pain et al. 1997, Mateo et al. 1998). We separated our sample of 415 chukars with an associated harvest date into two groups: 1) during the hunting season, and 2) outside the hunting season. We included a time buffer of six weeks, starting from the beginning and after the end of the period of water use for ingested lead, and an analogous buffer—one 10 weeks in length—for increased liver lead to avoid biases arising from including or excluding individuals that potentially ingested lead prior to the beginning of this period or after its termination.

We also assessed the relation between the season of water use and lead ingestion. In accordance with previous research involving chukars in western Utah (Larsen et al. 2007a), we defined the season of water use as 1 June-30 September, which approximates the period of highest use. We included a time buffer for the season of water use that was analogous to the buffer that we employed for lead exposure vs. the hunting season.

**Statistical Analysis**

We calculated the frequencies of ingested lead and ingested lead plus non-corresponding events of increased liver lead according to our distinctions of interest.
Next we calculated the overall frequencies of interest for the entire sample and compared them to those for our categories of interest. Because we were unable to assume approximate normality for our data concerning lead concentration in liver or tibia tissue, we used non-parametric rank tests instead of mean comparisons for our formal statistical analyses. We employed Mann-Whitney U tests in place of traditional t-tests, and Kruskal-Wallis tests instead of analyses of variance (ANOVA). We set $\alpha$ at 0.05 for all formal analyses and used a classic Bonferroni correction (Holm 1979) for cases of multiple comparisons.

**RESULTS**

We confirmed the presence of ingested lead pellets in chukars from five different counties and eight separate mountain ranges in hunted areas of western Utah. We found that of 466 chukar gizzards from hunted areas, 43 (9.2%) contained ingested lead shot. When we combined our total sample of 470 gizzards and liver samples, 54 (11.5%) non-corresponding individuals contained ingested shot or increased liver lead (>0.5 ppm). We observed zero (n=13) chukars from non-hunted areas with ingested lead.

We determined that chukars from hunted areas did not have statistically higher ranked values for liver-lead concentrations than those from our non-hunted site, given that our Mann-Whitney U test returned insignificant results between the two groups (W=934, P=0.269).

We found high variability in lead-pellet ingestion and mean liver-lead concentration according to harvest area. We observed that the Cedar, Gilson-Canyon, and Stansbury Mountains exhibited a frequency of lead ingestion and a mean liver-lead that was higher than average. The Deep Creek Range was below average for ingestion,
but when we factored increased liver lead, these mountains also had an above average frequency. The mountain ranges of west Box Elder County, along with West Mountain, and the East Tintic and Lakeside Mountains all had lower than average frequency of lead ingestion and mean liver-lead. The Blue Spring, Thomas-Dugway, Hogup, Keg, Promontory, Sheprock, and Simpson Mountains all had insufficient sample size to adequately assess the extent and severity of ingestion (Table 2.1).

Through a Kruskal-Wallis test (KW=27.44, P<0.001) with individual Bonferroni corrections (α=0.0018), we determined that ranked concentrations of liver lead were greatest for chukars from the Deep Creek Mountains, which were statistically greater than Lakeside (W=19.5, P=0.0018), but not significantly higher than Antelope Island, Cedar, East Tintic, Gilson-Canyon, Lakeside, Stansbury or West Box Elder mountain ranges. Stansbury was significantly higher than Lakeside (W=158, P<0.0001). Additionally, Antelope Island, East Tintic, Lakeside, and West Box Elder contained the median values for liver-lead concentration. We observed no other significant differences for ranked concentrations of liver lead between any two mountain ranges according to all 28 individual comparisons.

We also analyzed a suite of tibia samples from wild chukars with respect to concentration of lead and mountain range of harvest. Our overall model was significant for the Kruskal-Wallis test (KW=38.49, P<0.001). Antelope Island had the highest ranked value for tibia-lead concentration, which was significantly greater than Cedar (W=234, P<0.001), Gilson-Canyon (W=91, P<0.001), and Stansbury Mountains (W=195, P<0.001). The value for Stansbury was significantly higher than that of Cedar
(W=29, P<0.001) and Gilson-Canyon (W=93, P<0.001), but these last two sites were statistically the same (W=62, P=0.976).

We observed that presence of ingested lead and liver-lead concentrations fluctuated not only spatially, but temporally as well. However, we encountered chukars with ingested lead in all calendar years 2003-2007 and with increased lead in liver tissue from 2005-2007. Lead-pellet ingestion was below average for chukars that we harvested in 2003-2004 and 2005-2006 (Table 2.2), whereas such ingestion was above average during 2004-2005, 2006-2007, and 2007-2008. For the harvest years 2006-2007 and 2007-2008, events of increased liver lead were greater than average, whereas those from 2005-2006 were less than average.

We detected no significant differences for ranked values of liver lead among harvest year. Because we were unable to analyze any chukar livers from 2003-2004 and only one for 2004-2005, we acquired no concomitant information for increased liver lead and harvest year with regards to these hunting seasons. The Kruskal-Wallis test (KW=0.38, P=0.87) showed that ranked values for liver-lead concentration exhibited no significant differences overall. All three years were highly similar: 2005-2006 vs. 2006-2007 (W=1423, P=0.559), 2005-2006 vs. 2007-2008 (W=429, P=0.941), and 2006-2007 vs. 2007-2008 (W=247, P=0.701).

We found no significant difference in ranked values for liver lead pre vs. post the boom-reproduction-year of 2005. The Mann-Whitney U test showed pre-boom ranked values were slightly higher, but not markedly (W=1270, P=0.1473). Of the individuals analyzed for liver-lead concentration, 14.6% (n=6) of pre-boom and 14.8% (n=8) of post-boom samples contained increased liver lead.
The water use and hunting seasons both showed temporal variation for ingested lead and events of increased liver lead (Table 2.3). Lead ingestion was slightly higher outside the hunting season, but much greater when we combined it with occurrences of increased liver lead. Similarly, ingested lead was more common during the period of water use, especially when we joined it with instances of increased liver lead.

We observed no statistically significant differences in ranked values for lead concentration according to our seasonal distinctions. Ranked values for liver lead were greater outside vs. during the hunting season, but our Mann-Whitney U test presented no statistical difference (W=577, P=0.096). We also documented that ranked values for liver-lead were not significantly greater during vs. outside the season of water use (W=855, P=0.2289).

The frequency of lead ingestion and increased liver lead were dynamic from month to month (Fig. 2.1). Through analysis of lead ingestion according to month of harvest, we encountered birds with ingested lead in June and August-January, and we obtained values for increased liver lead in chukars from July-October. We found that presence of ingested lead in a gizzard from a harvested chukar was common summer-winter: 19 events during June-October and 20 from November-January. Nevertheless, occurrences of increased liver lead were observed during July-October (n=14), but non-existent from November-January.

DISCUSSION

The frequency of ingested lead (9.2%) for our complete sample of wild-harvested chukars—particularly when combined with increased liver lead (11.5%)—is greater than any other that we found in the documented literature for upland game-birds (Castrale
1989, Schulz et al. 2002, Butler et al. 2005, Ferrandis et al. 2008, Franson et al. 2009), with the exception of 19.9% for 221 mourning doves from the Gila Valley, Arizona, USA (Franson et al. 2009). We combined the two frequencies in non-corresponding individuals because together we consider they more accurately represent the true frequency of lead-pellet ingestion for our sample population; because presence of ingested lead alone can underestimate ingestion frequency (Pain 1996, Schulz et al. 2002). Before the ban on lead shot for waterfowl was implemented in the U.S., The International Association of Fish and Wildlife Agencies (IAFWA) proposed that if liver-lead concentration of ≥2.0ppm was found in at least 5% of tissue samples from a particular population, then recommending a conversion to nontoxic shot for waterfowl hunting was justified (USFWS 1986). Of the 121 livers from our sample of wild chukars, 4 (3.3%) were ≥2ppm. However, this value of 2 ppm was considered by the IAFWA to be the threshold of background vs. increased lead in waterfowl. Using our estimated threshold of 0.5 ppm for increased lead exposure, 11.5% of our sample contained increased lead, which is higher than the 5% threshold used to recommend nontoxic shot for waterfowl.

Ranked values for liver-lead did not differ significantly between hunted and non-hunted sites. Ingestion events are infrequent, so analyses based on means may be problematic. These rare ingestion events skew the distribution (Pain 1996, Pain et al. 2009). Using simple analyses based on ingested and increased lead frequency appear better for assessing extent and severity of lead ingestion.

Chukars harvested in the Cedar, Deep Creek, Gilson, and Stansbury Mountains had the highest mean liver-lead and above average frequencies for ingested plus
increased liver lead. Lead ingestion is apparently more common at these sites, which may indicate that these mountain ranges have the greatest hunting pressure. When combining ingested lead pellets and increased liver lead, all these populations had at least a 12% lead-ingestion rate (range = 12.0-23.8%). If wild chukars typically succumb to lead poisoning as a result of lead-pellet ingestion, populations such as these could be negatively affected. Additional research is needed to clarify these issues.

Multiple mountain ranges exhibited low frequencies of ingested and increased lead. Unfortunately, we acquired insufficient data from many mountain ranges within our study area, which means there is still need for additional sampling on these mountain ranges to determine their frequency and extent of lead ingestion. Nevertheless, we do have sufficient data overall to gain a general understanding for the problem of lead-pellet ingestion by chukars in western Utah.

Our tibia samples from Antelope Island are an interesting anomaly, being significantly greater than all tibia samples from chukars in hunted areas, which demonstrates that chukars from Antelope Island accumulate more tibia lead over their lifetime. Tibia lead is accumulative and correlated with age (Pain 1996, Pain et al. 2009). Although we observed significant differences in tibia lead among the 4 mountain ranges we sampled, we consider all values—including those from Antelope Island—to coincide with background exposure to lead because these concentrations were significantly less than those in captive chukars dosed with 1 lead pellet and fed a mixed seed diet (Chapter 4). Additionally, liver-lead values from Antelope Island birds were among the lowest we observed; whereas their corresponding tibia-lead values were by far the greatest encountered. Accordingly, chukars on Antelope Island likely ingest more atmospheric
lead due to proximity to major metropolitan areas and longer lifespan. Additional sampling of atmospheric lead in soil and water will help elucidate the potential sources of higher concentrations of tibia lead in samples from Antelope Island.

We documented lead ingestion in all calendar years 2003-2007, which indicates that lead ingestion by chukars occurred under a variety of climatic regimes. With respect to ingested and increased lead and harvest year, our results suggest that ingested lead may become slightly more common as a legacy effect of a very successful reproductive year, because it can be followed by increased hunter opportunity and subsequently higher lead deposition in soils. However, because ranked values for liver-lead were statistically the same among harvest years and pre vs. post boom year, we consider that lead is more likely persistent and accumulative in soils (Jorgensen and Willems 1987, De Francisco et al. 2003, Casas and Sordo 2006), particularly those of a rocky, dry, and alkaline consistency as found in chukar habitat (Schranck and Dollahon 1975, Walter and Reese 2003, Rattner et al. 2009). Additionally, 2 of 3 recent diet studies with chukars have identified individuals with ingested lead pellets (Walter and Reese 2003, Larsen et al. 2007b), whereas lead-pellet ingestion was not reported pre-1980 despite multiple diet studies with chukars (Zembel 1977, Knight et al. 1979). Although it is possible that lead ingestion occurred in chukars during this period, but failed to be reported, the inert nature of lead in basic media promotes persistence and accumulation.

We intended to determine if the hunting season had a similar effect on lead ingestion by chukars as it does in that by raptors (Pain et al. 1997, Hunt et al. 2006, Craighead and Bedrosian 2008). Although our results are the inverse of the examples from avian predators and scavengers, we did see an effect of the hunting season on
frequency of ingested plus increased lead, although ranked values for liver lead were not significantly different. Higher frequencies of increased liver lead outside the hunting season and within the season of water use correspond with our evidence that more chukars succumb to the adverse effects of lead ingestion in November-January and are thus unavailable for harvest.

Although lead ingestion occurred in most months that we collected samples, we discovered a huge disparity between the frequencies of ingested lead and instances of increased liver lead for the period of November-January. These data suggest that chukars ingesting lead pellets during the harsher climate of November through January may die quickly as a result of lead poisoning. The dataset of chukars available to us had no specific information for physical condition or behavior at harvest. Individuals that tolerated increased liver lead during the summer months were possibly more prone to harvest because of a weakened condition, or could have subsequently worsened and succumbed to mortality. The effects of lead are exacerbated by stressful situations such as predator evasion, disease, and over-winter survival (Pain 1996, Pain et al. 2009). Chukars from our study area ingesting lead in autumn must confront peak raptor migration and pursuit by humans simultaneously with multiple sub-lethal effects such as severe weight loss, lethargy, and peripheral neuropathy (Redig et al. 1980, Sanderson and Bellrose 1986, Mateo et al. 1998), all of which increase the probability of mortality.

Additionally, quantity and quality of diet may be the most important factor influencing lead absorption (Loncore et al. 1974, Sanderson and Bellrose 1986). Mineral deficiencies in diets—such as those that could occur in chukars from eating leached plant materials
during the winter months—tend to increase lead absorption and exacerbate its negative effects (Trainer 1982, Casas and Sordo 2006).

Aside from dangers to primary consumers of lead, there is also risk to avian and mammalian predators and scavengers that feed on lead-poisoned individuals or those that potentially carry embedded lead shot in edible tissues (Johansen et al. 2001, Merkel et al. 2006, Mateo 2009). In the literature, we found multiple examples of raptors having increased levels of blood lead during the hunting season (Pain et al. 1997, Hunt et al. 2006, Craighead and Bedrosian 2008). Robinson et al. (2009) reported raptors as the greatest probable cause of mortality for chukars in our study area. There is a pressing need for information concerning raptors and lead ingestion.

We documented that widespread lead ingestion occurs in chukar populations from western Utah. We also found that as few as one lead pellet can result in mortality for captive-dosed chukars (Chapter 4). These findings corroborate those of other authors who have investigated lead ingestion by waterfowl, upland birds, and raptors. We add our data to the consensus of the literature. Thomas (2009, pp. 353-354) stated the consensus succinctly, “A huge body of independently replicated research reveals consistently that there is a single syndrome of ingested lead toxicosis, whose collective scientific credibility exceeds the burden of proof used in other forms of environmental chemical regulation.”

**MANAGEMENT IMPLICATIONS**

Chukars are at high risk for lead-pellet ingestion and its negative effects because they: 1) use dry, rocky habitat with alkaline soils, 2) use habitat near water and roads, 3) are a popular game-bird, 4) inhabit areas occupied by sympatric species that are
dependent on free water and are popular for hunting, 5) are present in habitat used for
target shooting, 6) use habitat that harbors many raptors (a source of additional stress)
particularly during raptor migration, 7) probe soils for food and grit items, 8) live year-
round in habitats with extreme variability in daily and seasonal temperatures and food
and water availability, 9) possess search images for food and grit items similar to lead
pellets, and 10) contain a muscular, grinding gizzard with low pH.

Chukars in our study area likely die from lead poisoning. If this mortality is
additive, applicable populations—particularly those that are localized or isolated—will be
adversely affected because these birds are heavily r-selected and inhabit harsh
environments. We make no comments regarding the privilege of hunting itself because
of its demonstrated economic, management, and conservation value. If the goal of
wildlife managers is to reduce unnecessary illness and mortality in wildlife that ingest
lead, the problem of lead-pellet ingestion will be part of the discussion.

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Table 2.1. Wild chukars with ingested lead pellets (n=33) or increased lead in liver tissue (n=11), assorted by mountain range of harvest in western Utah, USA, collected 2 August 2003-12 January 2008. For the West Box Elder group, we combined a few small mountainous areas together.

<table>
<thead>
<tr>
<th>Mountain Range</th>
<th>No. individuals collected</th>
<th>No. with ingested lead</th>
<th>No. without ingested lead but with increased lead in liver</th>
<th>% ingested lead (plus % increased lead in liver)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blue</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0.0 (0.0)</td>
</tr>
<tr>
<td>Cedar</td>
<td>117</td>
<td>11</td>
<td>3</td>
<td>9.6 (12.0)</td>
</tr>
<tr>
<td>Deep Creek</td>
<td>21</td>
<td>1</td>
<td>4</td>
<td>4.8 (23.8)</td>
</tr>
<tr>
<td>Gilson/Canyon</td>
<td>79</td>
<td>10</td>
<td>2</td>
<td>12.8 (15.2)</td>
</tr>
<tr>
<td>Hogup</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0.0 (0.0)</td>
</tr>
<tr>
<td>Keg</td>
<td>5</td>
<td>1</td>
<td>0</td>
<td>20.0 (20.0)</td>
</tr>
<tr>
<td>Lakeside</td>
<td>18</td>
<td>0</td>
<td>0</td>
<td>0.0 (0.0)</td>
</tr>
<tr>
<td>Promontory</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0.0 (0.0)</td>
</tr>
<tr>
<td>Sheeprock</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>0.0 (0.0)</td>
</tr>
<tr>
<td>Simpson</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0.0 (0.0)</td>
</tr>
<tr>
<td>Stansbury</td>
<td>28</td>
<td>4</td>
<td>2</td>
<td>14.3 (21.4)</td>
</tr>
<tr>
<td>Thomas/Dugway</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0.0 (0.0)</td>
</tr>
<tr>
<td>Tintic</td>
<td>15</td>
<td>1</td>
<td>0</td>
<td>6.7 (6.7)</td>
</tr>
<tr>
<td>West</td>
<td>13</td>
<td>1</td>
<td>0</td>
<td>7.7 (7.7)</td>
</tr>
<tr>
<td>West Box Elder</td>
<td>85</td>
<td>4</td>
<td>0</td>
<td>4.8 (4.7)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>397</strong></td>
<td><strong>33</strong></td>
<td><strong>11</strong></td>
<td><strong>8.4 (11.1)</strong></td>
</tr>
</tbody>
</table>
Table 2.2. Chukars containing ingested lead pellets (n=39) or increased liver lead (n=11) sorted by harvest year. We did not have any livers analyzed for individuals from 2003-2004, and only one from the 2004-2005 hunting season. We collected chukars in western Utah, USA, 2 August 2003–12 January 2008.

<table>
<thead>
<tr>
<th>Hunting season</th>
<th>No. individuals collected</th>
<th>No. with ingested lead</th>
<th>No. without ingested lead but with increased lead in liver</th>
<th>% ingested lead (plus % increased lead in liver)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2003-2004</td>
<td>19</td>
<td>1</td>
<td>N/A</td>
<td>5.3 (N/A)</td>
</tr>
<tr>
<td>2004-2005</td>
<td>46</td>
<td>5</td>
<td>N/A</td>
<td>10.9 (N/A)</td>
</tr>
<tr>
<td>2005-2006</td>
<td>234</td>
<td>18</td>
<td>5</td>
<td>7.8 (9.8)</td>
</tr>
<tr>
<td>2006-2007</td>
<td>104</td>
<td>12</td>
<td>4</td>
<td>11.7 (15.4)</td>
</tr>
<tr>
<td>2007-2008</td>
<td>18</td>
<td>3</td>
<td>2</td>
<td>16.7 (27.8)</td>
</tr>
<tr>
<td>Total</td>
<td>421</td>
<td>39</td>
<td>11</td>
<td>9.3 (12.0)</td>
</tr>
</tbody>
</table>
Table 2.3. Wild chukars from western Utah with ingested lead pellets (n = 28) or increased liver lead (n=8 or 6), categorized temporally by harvest date according to the hunting season and period of water use. We collected chukars in western Utah, USA, 8 August 2003–12 January 2008.

<table>
<thead>
<tr>
<th>Season of Collection</th>
<th>No. individuals collected</th>
<th>No. with ingested lead</th>
<th>No. without ingested lead but with increased lead in liver</th>
<th>% ingested lead (plus % increased lead in liver)</th>
</tr>
</thead>
<tbody>
<tr>
<td>During Hunting</td>
<td>213</td>
<td>20</td>
<td>0</td>
<td>9.4 (9.4)</td>
</tr>
<tr>
<td>Outside Hunting</td>
<td>99</td>
<td>8</td>
<td>8</td>
<td>8.1 (16.2)</td>
</tr>
<tr>
<td>Total</td>
<td>312</td>
<td>28</td>
<td>8</td>
<td>9.0 (11.5)</td>
</tr>
<tr>
<td>During Water Use</td>
<td>119</td>
<td>10</td>
<td>6</td>
<td>8.4 (13.4)</td>
</tr>
<tr>
<td>Outside Water Use</td>
<td>193</td>
<td>18</td>
<td>0</td>
<td>9.3 (9.3)</td>
</tr>
<tr>
<td>Total</td>
<td>312</td>
<td>28</td>
<td>6</td>
<td>9.0 (10.9)</td>
</tr>
</tbody>
</table>
Figure 2.1. Number of ingested lead pellets and events of increased lead in liver tissue for wild chukars harvested in western Utah, USA grouped by month of collection. Our sample size for ingested lead and increased liver lead was: May, 1 and 0; June, 1 and 0; July, 24 and 10; August, 47 and 24; September, 103 and 51; October, 49 and 12; November, 26 and 1; December, 73 and 8; and January, 95 and 15.
CHAPTER 3
CAUSES OF LEAD-PELLET INGESTION BY CHUKARS (ALECTORIS CHUKAR): EXAMINING SEARCH IMAGES, HABITAT CHARACTERISTICS, AND DENSITY

ABSTRACT

Lead is ubiquitous throughout the environment and is toxic to humans and wildlife. Although chukars (Alectoris chukar) in western Utah ingest lead, the causes are poorly understood. The specific objectives of this chapter were to assess the influence of feeding behaviors, lead density, and habitat use on the frequency of lead-pellet ingestion in captive and wild chukars. Search images for food or grit items that resemble lead pellets, combined with increased lead-pellet density, resulted in captive chukars voluntarily ingesting significantly more lead. All Indian ricegrass seeds (Stipa hymenoides) and nearly 1/3 of grit pebbles removed from the digestive tract of wild chukars were consistent with lead-shot sizes 4-9, which are commonly used for hunting and target shooting. We estimated a density of >1.7 million lead pellets/ha at a popular site for target shooting within occupied mourning dove (Zenaida macroura) and chukar habitat. We found more lead pellets in soils near springs (z = -4.236, P < 0.001) vs. man-made water sources or reference points. Our negative-binomial linear analyses showed a significant negative relationship (higher lead closer to sites) between liver-lead concentrations in collected chukars and linear distance of harvest location from springs (z = -3.844, P <0.001) or guzzlers (z = -2.003, P = 0.045), but not roads (z = 1.089, P = 0.276). The corresponding analyses for tibia lead concentrations in birds resulted in a
similarly significant negative relationship for springs (t = -2.292, P = 0.03), but not roads (t = 1.04, P = 0.307). A Mann-Whitney U test revealed that ranked values for liver lead were significantly greater for individuals collected near vs. far from springs (W = 707, P = 0.038). Additionally, chukars that were harvested closest to springs vs. guzzlers had higher rates of lead ingestion and increased liver lead. Chukars and sympatric mourning doves in western Utah are at high risk for increased lead exposure and its concomitant effects. Although hunting is a viable management and conservation tool, preventable poisoning of protected wildlife species by lead ammunition is an unnecessary form of mortality.

INTRODUCTION

Lead ingestion is a ubiquitous and controversial issue that can adversely affect both biotic and abiotic components of the environment. Lead has deleterious effects on humans and wildlife (Pokras and Kneeland 2009), and instances of ingested lead pellets, bullet fragments, and fishing weights have been documented in at least 130 wildlife species (Tranel and Kimmel 2009). In alkaline media (pH>7), lead is mostly present in non-soluble forms (Casas and Sordo 2006), but in highly acidic media (pH 1-2.5) it becomes increasingly soluble (Rattner et al. 2009). However, the somewhat inert nature of lead allows its shot-pellets to persist even in moderately acidic soils (pH 3.5-6.99) for many decades (Jorgensen and Willems 1987, De Francisco et al. 2003).

Lead shot deposited in soils through hunting and shooting activities is available to birds that can mistake these pellets for food or grit items (Gionfriddo and Best 1999, Schulz et al. 2002). Trainer (1982) estimated that before the discontinuance of lead for waterfowl hunting, 3,000 tons of spent lead was introduced annually into the environment
by waterfowl hunters alone. Bellrose (1959) estimated that 4% (approximately 700,000 individuals) of the annual Mallard (Anas platyrhynchos) population succumbed to mortality due to lead poisoning, and that 2-3 million waterfowl in general died of lead poisoning annually.

Despite volumes of research conducted with lead and waterfowl, lead ingestion and its adverse effects in upland birds are poorly understood. Nevertheless, recent studies show that lead-pellet ingestion results in sub-lethal detriments and mortality in many species of upland birds (Kendall et al. 1996, Fisher et al. 2006). Lead-pellet ingestion has been documented for chukars (Walter and Reese 2003, Larsen et al. 2007b, Kreager et al. 2008), but the causes of this ingestion are largely unknown.

Chukars are gallinaceous game-birds native to semi-arid, mountainous regions in portions of Eurasia (Cramp and Simmons 1980, Ali and Ripley 2001). Although chukars are largely granivorous (Cole et al. 1995) and eat mostly cheatgrass seeds (Bromus tectorum) (Christensen 1996, Larsen et al. 2007a), they consume a wide variety of foods including many other seeds, bulbs, flowers, grass shoots, and insects (Walter and Reese 2003, Larsen et al. 2007a). Because chukars are a popular hunting quarry, they have been widely introduced across the world. Currently, self-sustaining populations of chukars occur in 11 western states (U.S.) and one Canadian Province with chukar populations occupying approximately 252,800 km² (Christensen 1996) in North America.

Potential reasons for an increased risk of lead exposure and absorption in chukars include: 1) the arid climate, rocky topography (Walter and Reese 2003), and alkaline soils of occupied habitat, which reduce the rate of pellet settlement (Schranck and Dollahon 1975) and dissolution (Stansley et al. 1992); 2) similarities in appearance
between lead pellets and grit or food sources used by Chukars (Gionfriddo and Best 1999, Schulz et al. 2002); 3) hunting pressure; 4) presence of a well-developed muscular grinding gizzard with grit pebbles and a low pH (Trainer 1982, Pain 1996, Pain et al. 2009); 5) the propensity to probe soils for food (Gionfriddo and Best 1999); 6) stressful situations such as predator avoidance and extreme daily and seasonal variability in climate and food availability and quality; and 7) density of lead pellets in soils, especially as related to use of water sources (Best et al. 1992), roads, and target-shooting sites.

Sources of free water are used by chukars during the hot, dry months of summer, particularly during years of above average temperatures and below average precipitation (Larsen et al. 2007a, Larsen et al. 2010). Lead-pellet density in soils can be increased by water sources due to concomitant lead deposition through shooting activities. Best et al. (1992) found densities of 167,593 lead pellets/Ha in soils near an in-ground water tank in New Mexico during August—prior to the dove-hunting season—and 860,185 pellets/Ha in October after the season had concluded. Many desert springs in Utah are used by chukars, mourning doves, and are visited by humans who hunt these species. Chukars that forage near water sources may confront a greater risk of increased lead exposure compared to those that do not feed near water.

The specific objectives of this chapter were to assess the influence of habitat use, feeding behaviors, and lead density on lead-pellet ingestion in captive and wild chukars. We hypothesized that search images for food and grit items similar to lead, and higher lead density would increase lead-pellet ingestion by chukars. We also predicted that soils near water sources would have greater lead densities than those near control points, and that chukars using habitat near water sources would ingest lead pellets more frequently.
METHODS

Study Area

We collected soil and chukars in portions of Box Elder, Davis, Juab, Tooele, Millard, and Utah counties located within western Utah. A detailed description for some of the locations comprising our study sites is available in Larsen et al. (2007a) and Robinson et al. (2009). Additional specific sites of investigation were centered at the following coordinates: Blue Spring/West Hills, Box Elder County (41° 50’ 13” N 112° 19’ 58” W); Canyon/Gilson Mountains, Juab and Millard Counties (39° 36’ 34” N 112° 13’ 20” W); Deep Creek Mountains, Juab and Tooele Counties (39° 50’ 42” N 113° 54’ 38” W); East Tintic Mountains, Juab, Tooele, and Utah Counties (39° 53’ 58” N 112° 3’ 37” W); Hogup Mountains, Box Elder County (41° 26’ 2” N 113° 10’ 47” W); Keg Mountains, Juab County (39° 47’ 8” N 112° 52’ 22” W); Lakeside Mountains, Box Elder and Tooele Counties (41° 6’ 19” N 112° 52’ 19” W); Promontory Mountains, Box Elder County (41° 24’ 60” N 112° 28’ 58” W); Stansbury Mountains/Island, Tooele County (40° 38’ 11” N 112° 34’ 6” W); and West Mountain, Utah County (40° 5’ 20” N 111° 49’ 20” W). Partial details of our collection protocol for wild chukars can be found in Larsen et al. (2007b). A detailed overview of our methods for processing and analyzing wild chukars for lead-ingestion data is available in Chapter 2.

Lead and Chukars: The Role of Search Images and Density

When animals repeatedly encounter a food type, they develop a representation or search image for that item, and this search image strengthens through continued exposure to the item, which allows the foraging animal to become more efficient at finding and
recognizing this food (Tinbergen 1960, cited in Dugatkin 2009). Chukars commonly consume grit items and Indian ricegrass (*Stipa hymenoides*) or bulbous bluegrass seeds (*Poa bulbosa*) that approximate lead pellets in size, shape, and color. We hypothesized that chukars would ingest more lead pellets if they had a well-developed search image for *S. hymenoides* seeds. Using soil sieves, we compared sizes of lead with grit and *S. hymenoides* seeds. We conducted this analysis using a series of soil sieves with mesh sizes of: 3.35, 1.91, and 0.98mm. Shot sizes 4-9, which are the most popular shot sizes for chukars, sympatric game species, and target shooting are between 3.35 and 1.91mm in diameter.

We conducted an experiment with 24 captive chukars purchased at 5 months old from H&R Gamebirds® in Tremonton, Utah, USA to assess our hypothesis that lead density and search images increase lead-pellet ingestion. For this experiment with captive chukars, we intended to induce a search image for foods and grit items similar to lead shot. This experiment was approved by the Institutional Animal Care and Use Committee (approval #1366) at Utah State University. We present a detailed overview of our methods of animal husbandry for captive chukars in Chapter 4, this volume.

Our experiment was set up as a 2×2 factorial with diet as factor 1 and lead density as factor 2. The diet factor had two levels: 1) light-colored food (light), which consisted of processed food pellets, and 2) dark-colored food with light and dark-colored grit (dark), which was composed of food pellets with *S. hymenoides* seeds and both light and dark-colored grit pebbles. We used the light group as a reference condition for diet. We modified the food portion of the dark diet over the course of the trial period as follows so that the birds receiving this diet would become habituated to eating *S. hymenoides* seeds:
for the first two weeks, the dark diet contained a 1:1 ratio of food pellets to *S. hymenoides* seed; at the beginning of the third week, we changed the ratio to 1 part food pellets to 3 parts *S. hymenoides*; and for the final week, the food portion of the dark diet was all *S. hymenoides* seeds. We altered the diet in this way to ensure that birds actually developed a search image for *S. hymenoides* rather than potentially selecting only food pellets. We did not alter the grit portion of the diet for this group during the trial period.

The density factor had two levels: 1) low (10 #8 lead pellets/food dish) and 2) high (100 #8 lead pellets/food dish). We did not have a control for the density factor. We organized the trial in this way because we were not interested in the difference between zero density and our two levels of density, but rather we intended to merely assess the possibility of a difference between low and high lead density. We obtained lead pellets by removing them from a shot shell. We varied the two levels by an order of magnitude to represent the wide spectrum of dissimilarity for lead-pellet density in soils (Castrale 1989, Best et al. 1992, Kendall et al. 1996).

We allowed each chukar to acclimate to its experimental diet for 4 weeks. At the close of the trial period, we administered the respective diet to each individual with the addition of either 10 or 100 size #8 lead pellets that we mixed in with food. We allowed each individual to feed for 30 minutes and then promptly removed all individuals from their cage and euthanized them in a portable CO₂ chamber. We placed each chukar in a plastic freezer bag and later processed all birds to remove their crop, esophagus, proventriculus, and gizzard. We emptied the contents of the digestive tract for each bird and counted the number of pellets ingested as our response variable.
To compare diameters of grit and *S. hymenoides* seeds from wild chukars with popular sizes for lead shot, we recorded the total weight (g) of grit pebbles and *S. hymenoides*, passed them through a series of sieves (3.35, 1.91, and 0.98 mm openings), and documented the proportion of each sample that remained in each sieve. We acquired the diameter of each popular lead-shot size from the Federal® 2010 Ammunition and Ballistics Catalog, available from www.federalcartridge.com, and compared these diameters to those of our grit pebbles and *S. hymenoides* seeds.

*Lead and Chukars: The Role of Habitat Use*

We estimated the size of the affected area for lead deposition near a water source or road using Journee’s Rule (Bussard and Wormley 2006), which posits that the approximate maximum horizontal range in yards for a round projectile is 2,200 times its diameter in inches. We assumed the affected area was circular solely for illustrative purposes, simulated a measurement from the water source to the maximum expected range of each shot size (6, 7.5, and 8) by using 3.14 for pi, multiplying 2,200 by the diameter of each shot size in inches to obtain distance in yards, converting yards$^2$ to meters$^2$, and dividing by 100 to present as Ha. For example, for shot size 6 the calculation was $(3.14\times2,200\times0.109\times0.914)/100 = 6.88$ Ha.

To assess the spatial influence of increased lead exposure with use of water sources or roads, we combined harvest locations of chukars by relative proximity to a water source or road. We accessed data from motion and heat-sensing cameras (Camtrakker Inc®), pellet counts from water sources (Larsen et al. 2007a), and live visual observations to document the use of particular water sources by chukars.
To determine the proper depth for sampling the abundance of lead pellets in soils, we excavated soil at varying depths, 1-4 cm depth in 1-cm intervals. We acquired these preliminary soil samples from a popular location for skeet shooting in the Lake Mountains west of Utah Lake, Utah, USA. We placed samples into plastic sealable bags and stored them for analysis. We then passed each sample through a series of sieves with openings of 3.35, 1.91, and 0.98 mm and tallied any pellets present according to sampling depth.

For our general soil sampling effort, we used a 30.5 x 30.5 cm (1 ft²) steel frame as a template and removed the approximate top 1 cm of soil, placed it in a plastic bag, and labeled it for storage. Next, we passed each sample through a series of sieves with openings of 3.35, 1.91, and 0.98 mm, to collect lead pellets. Our sampling design addressed three levels of comparison: 1) springs (n=11), 2) guzzlers (n=9), and 3) reference (n=7). For the reference plots, we randomly chose a water source, and traveled 1.5 km in one of the four cardinal directions from the water source. We chose the direction in which to travel from the water source by picking the cardinal direction that was the most directly accessible by road. We acted accordingly because 72.3% (n=154) of our wild-harvested chukars—with a Global Positioning Satellite location (UTM, NAD 83)—were taken < 1 km from a road and we wanted an accurate representation of our sample. We projected these harvest points in ArcGIS 9.3® to calculate linear distances to water sources or roads. We also used the spatial join function to link harvest points to the nearest water source and provide the linear distance between them.

To select water sources for soil sampling, we prepared a randomization code in Program R (2.9). We created a sampling plot by laying out a series of lines with a
measuring tape to form a grid. Using the water source or reference point as the center, we sampled in opposite directions that were parallel to the aspect of the site. We produced a 240 m line extending 120 m from the central point travelling in opposite directions. Along this 240 m line at 30, 60, and 90 m from the central point in both directions, we produced a line of 180 m, for a total of 6 lines (each with 6 sample points), stretching 90 m perpendicularly from the 240 m line in opposite directions. The 240-m line contained 8 sample points: 1 each at 30, 60, 90, and 120 m on either side of the central point. This sampling framework yielded a total of 44 sample points per plot.

Statistical Analysis

We fit each captive bird with an identification band and wrote a corresponding randomization code in Program R (2.9) to randomly assign treatments to experimental units. We used an Analysis of Variance (ANOVA) in Statistical Analysis System version 9.2, with the Least Squares Means Option and the Tukey Adjustment, to assess the results of our diet and lead-density experiment.

For our pilot sampling of soil lead, we added the number of pellets we found for each category and assessed the results using an ANOVA with Tukey’s Honest Significant Difference because we were able to assume approximate normality. We summed the results similarly for our general sampling effort of lead in soils. Nevertheless, to analyze the influence of water-source type on lead-pellet density in soils for our general sampling scheme, we used a negative-binomial linear model—using Program R (2.9)—because the data would not conform to the assumption of approximate normality. For both the pilot soil analysis and general sampling for soil lead, we extrapolated (or interpolated) the density of lead pellets found—according to the amount of soil collected—to obtain an
estimate for lead density in pellets/Ha and pellets/m² by: 1) Obtaining the number of lead pellets found, 2) acquiring the value for the number of m² of soil that we collected (2.97 m² for pilot soil sampling and 4.08 m² for general soil sampling), 3) converting this area to an hectare or leaving it alone for m², and 4) calculating the product for Ha (or dividend for m²) of extrapolated (or interpolated for m²) density of lead pellets in soils.

We computed the frequencies for presence of ingested lead and increased liver lead according to our distinctions of interest. Next, we calculated the overall frequencies of lead ingestion and increased liver lead for the overall sample and compared them to those of our individual categories of interest.

To test for a relation between the concentration of liver lead and distance from a road or water source, we used negative-binomial linear models; whereas to test this same relation with tibia-lead concentration, we employed normal linear models. We performed both types of analyses in Program R (2.9).

In conjunction with the aforementioned analyses related to lead concentration and distance from water sources and roads, we used a distance threshold of 1.5 km to separate use and nonuse of these sites by chukars. Our estimates of affected area for lead deposition surrounding a water source or road demonstrate that birds merely need to feed within this space to risk lead ingestion. Walter (2002) estimated chukar home-range to span between 406 m² and 785 m², depending on the method of estimation and inter-annual variability. However, both these authors and other investigators have observed occasionally greater movements in these birds (Lindbloom 1998, Walter 2002). Additionally, Dickens et al. (2009) assumed chukar home-range was <2 km² because homing behavior occurred—with 100% fidelity to original capture site—in translocated
individuals that returned to two water sources separated by <2 km distance. We combined these lines of evidence to produce an estimate for the size of chukar home-range combined with a buffer to account for the affected area of lead deposition surrounding a particular site. Accordingly, we assumed that birds harvested <1.5 km (near) from a water source or road were likely to use that site; whereas those harvested outside this distance (far) would not use the site.

Our liver-lead data with regards to previously mentioned use vs. nonuse investigations did not conform to normality, so we analyzed our datasets using Mann-Whitney U tests. Contrastingly, we were able to assume approximate normality with our tibia-lead concentrations. Consequently, we performed two-tailed t-tests assuming equal variances for questions of near vs. far with respect to tibia lead in harvested chukars and relative distance from water sources or roads. We had insufficient data to properly assess this relation between tibia lead and guzzlers, so we solely present our results concerning tibia lead with springs and roads.

RESULTS

The ANOVA revealed significant interaction between diet and lead density (P=0.002, F=13.56) on voluntary lead ingestion by captive chukars. Using the Tukey adjustment, we observed that the diet×lead interaction showed a significant difference (P<0.001) between the dark diet×high density (LS MEAN=37.83) and the dark diet×low density (LS MEAN=5.17) groups, and between the dark diet×high density and both the light diet×low density (LS MEAN=0.17) (P<0.001) and light diet×high density (LS MEAN=0.00) (P<0.001) groups. We did not observe any other significant differences among remaining treatment combinations (Fig. 3.1).
We determined that 100% of *S. hymenoides* seeds and 32.1% of grit pebbles that were voluntarily ingested by wild chukars coincided with lead-shot sizes 4-9 (Fig. 3.2). *S. hymenoides* seeds and grit pebbles also approximate lead pellets in shape and color.

Through our pilot soil-sampling effort, we determined that the optimal depth to sample soil in chukar habitat for lead shot is approximately 1 cm from the soil surface. The results from our ANOVA revealed that there was no significant difference (P=0.99, F=0.01) among the four comparison depths. We sampled a total of 32 ft² (2.97 m²) in this pilot effort for soil lead and found 509 lead pellets, which translates to an estimated density of just over 1.7 million lead pellets/Ha (171/m²) for our pilot-sampling site.

Analyzing our general soil-sampling data, we encountered more lead pellets in soils near springs (69, n=11) than those near guzzlers (4, n=9) or random reference sites (1, n=7) (Fig. 3.3). We found significantly more lead pellets in soils near springs (z = -4.236, P < 0.001), but guzzlers and reference sites were statistically equal. We made calculations for lead-pellet density in soils for these sites according to the 4.08 m² of soil we sampled per site. As a result, we determined that as many as 6 pellets/m² and >58,000 pellets/Ha are likely present in soils at springs in our sample (Table 3.1). We estimated an affected area for lead deposition near a water source or road of 6.88 Ha, 5.94 Ha, and 5.62 Ha for shot sizes 6, 7.5, and 8, respectively.

We computed a significant negative relationship (higher lead closer to sites) between liver-lead concentrations in harvested chukars and linear distance from springs (z = -3.844, P <0.001) and guzzlers (z = -2.003, P = 0.045), but not roads (z = 1.089, P = 0.276). The corresponding analyses for tibia lead produced a similarly significant negative relationship for springs (t = -2.292, P = 0.03), but not roads (t = 1.04, P = 0.307).
We found a significant difference for liver-lead concentrations in harvested birds and relative distance from springs (near vs. far). With regards to liver lead, ranked values were significantly greater near springs \((W = 707, P = 0.038)\). However, the analogous results were statistically identical for guzzlers \((W = 192, P = 0.265)\) and roads \((W = 822, P = 0.201)\). Corresponding results for tibia lead showed markedly greater mean concentration near springs \((t = 3.604, P < 0.001)\), but not roads \((t = -1.792, P = 0.075)\).

Frequency of ingested lead plus increased liver lead in harvested chukars was much higher in birds harvested near springs vs. far from them. The frequency of both ingested lead pellets and instances of increased liver lead were nearly identical in birds collected near vs. far from guzzlers and roads (Table 3.2). Overall, 9 of the 14 individuals with increased liver lead (>0.5 ppm) were harvested near springs, as opposed to only 3 near guzzlers, and 2 near roads (without the presence of a spring or guzzler).

Frequency of ingested lead and increased liver lead also varied according to the type of closest water source (Table 3.2). The birds harvested closest to springs had more ingested lead than average; whereas those closest to guzzlers had less than average ingested lead. After we factored in increased liver lead, birds closest to guzzlers had much less ingested lead than average; whereas with this addition the frequency greatly increased to much more than average for individuals harvested closest to springs.

**DISCUSSION**

Our experimental results for lead ingestion by diet type and lead density provided some intriguing insights into the causes of lead ingestion in chukars. Diet and lead density combined to influence the number of pellets voluntarily ingested. Of the birds that we presented with *S. hymenoides* seeds and dark-colored grit in their diet, 91.7%
(n=12) ingested at least one lead pellet, but individuals in the high density group ingested significantly more lead than those in the low density group. Density alone had no effect on lead ingestion in captive chukars unless they had developed a search image for *S. hymenoides* seeds and dark-colored grit.

Several studies have found no significant relation between lead ingestion and lead availability (Castrale 1989, Best et al. 1992, Schulz et al. 2007). However, by combining lead density with search images for food and grit items that were similar in appearance to lead pellets, we documented a significant relation among diet type, lead density, and voluntary lead ingestion by chukars. Our soil-sieving results demonstrate that all of the *S. hymenoides* sample and nearly one-third of the grit sample taken from the digestive tract of wild chukars were consistent in diameter with popular lead-shot sizes used in hunting and shooting activities. Wild chukars that ingest *S. hymenoides* or other food and grit items that mimic lead are highly at risk for lead ingestion and poisoning. Additionally, we expect that when greater lead availability is combined with these search images, the frequency of lead ingestion and poisoning in wild chukars will further increase. These findings add to the body of evidence (Gionfriddo and Best 1999, Schulz et al. 2002) suggesting that similarities among grit, food items, and lead shot can increase the probability of a given individual or species ingesting lead pellets.

Our soil-sampling results helped us further quantify the role of lead density in lead-pellet ingestion by chukars. We found that public lands in mourning dove and chukar habitat that are used for recreational shooting can accumulate large quantities of lead shot.
Particularly for our investigations, soils near springs on public lands can accumulate lead shot because game animals congregating near water are fired at frequently, subsequently leaving many lead pellets in surrounding soils (Best et al. 1992). Our results suggest that springs appear to be more heavily hunted than guzzlers and control sites. We found a higher frequency of ingested plus increased liver lead and much higher overall concentrations of liver-lead in chukars harvested close to springs. Although the corresponding relationships with tibia lead in harvested birds and distance of harvest from springs was significant, the specific observation values we observed were consistent with background concentrations. The observed mean concentrations were statistically equal to captive control chukars, but significantly less than those we documented in captive-dosed chukars 15 post ingestion of one #6 lead pellet (Chapter 4). Tibia-lead concentrations rise more slowly because lead is absorbed lastly into bone (Pain 1996, Pain et al. 2009). Contrastingly, liver-lead can begin to increase beyond background levels in less than 24 hours (Pain 1996, Pain et al. 2009). Consequently, we hypothesize that these tibia-lead concentrations were still consistent with background because although the individuals with ingested lead had increased amounts in liver tissue, they had not yet absorbed a sufficient amount into bone due to recentness since ingestion. Our evidence strongly suggests that chukars feeding near hunted springs risk increased exposure to and ingestion of lead pellets.

Increased lead exposure appears to occur most often up to 1.5 km from springs. Although most estimates of the average size for seasonal home range of chukars are <1 km² (Lindbloom 1998, Walter 2002), movements of much greater distances have been documented (Lindbloom 1998, Walter 2002, Larsen et al. 2010). Great variability in size
for seasonal home range inter-annually has also been observed (Larsen et al. 2010). Additionally, when we factor in the affected area of lead deposition near springs, the distance from a spring at which a chukar would potentially find lead could easily increase to 1.5 km. Chukars merely need to feed in the relative vicinity of springs to increase the probability of ingesting lead shot that was fired from or close to them. Consequently, even though chukars may not use a particular (or any) water source or road, if their home range overlaps the affected area of lead deposition associated with such a site, lead ingestion can still occur. Nevertheless, we documented occasional lead ingestion and increased lead exposure at linear distances from water sources and roads that are much greater than most estimates for home range of chukars even with the buffer of the affected area for lead deposition. Therefore, although more lead is present near springs, lead-pellet deposition is possibly widespread throughout chukar habitat and chukars apparently find and ingest lead pellets even if densities in soils are low.

In contrast to the results we obtained for an influence of springs on lead ingestion by chukars, we found little evidence of an analogous relation for guzzlers and lead ingestion. Although we observed a significant relation between distance from a guzzler and liver-lead concentration, the signal was much weaker than with springs. Otherwise, we saw little evidence for an influence of guzzlers on increased lead ingestion and liver lead, which may be because chukars are less likely to feed in the immediate vicinity of guzzlers. Guzzlers lack the lush vegetation common to springs. Additionally, guzzlers likely receive less hunting pressure, because their location is not public information as with springs. Also, guzzlers and reference sites were statistically identical for number of lead pellets in surrounding soils. Consequently, guzzlers seem to be a viable option for
providing water for wildlife, while mitigating the effects of the increased lead deposition that occurs near springs. Nevertheless, finding one lead pellet at four guzzlers and one reference site again demonstrates that lead-pellet deposition is potentially widespread throughout chukar habitat.

We expect that much of the widespread lead deposition via hunting activities in chukar habitat is likely fired at mourning doves (*Zenaida macruora*). Mourning doves are usually hunted differently than chukars, by waiting for them to visit and return frequently to water—as opposed to covering large areas in search of a covey—and are arguably harder to harvest. Sometimes hunters require 5-6 shots per dove bagged (Missouri Dept. of Conservation, Unpublished Report, cited in Kendall et al. 1996). Finally, presumably more doves congregate at springs than guzzlers due to vegetation cues and migration traditions, and these birds are likely hunted more often at springs vs. guzzlers because guzzler locations are unknown to the public. Additional research to assess the role of mourning dove hunting on lead-pellet ingestion by chukars is essential to managing the problem, as addressing nontoxics for chukars only may have little effect on reducing lead ingestion.

**MANAGEMENT IMPLICATIONS**

Chukars in western Utah are ingesting lead pellets in a widespread manner. Use of natural waters sources, foods and grit that mimic lead-pellet morphology, and high density of lead shot in soils all contribute to an increased probability of lead ingestion and poisoning. Mourning dove hunting likely contributes to lead-pellet ingestion by chukars in our study area.
Guzzlers appear to be a viable option for minimizing the deposition of lead shot compared to that which occurs at hunted springs. Assuming guzzlers are inherently beneficial to wildlife for the primary purpose of providing free water—which depends on the population in question (Larsen et al. 2010)—we encourage the continued use of these constructed water sources to mitigate lead deposition, ingestion, and poisoning.

Of special concern is that our evidence regarding lead and chukars may be applicable to native and sympatric mourning doves. Additional research is warranted to understand and address lead ingestion by mourning doves in western Utah. Although hunting is a viable management and conservation tool, preventable poisoning of protected wildlife species by lead shot is an unnecessary form of mortality (Neumann 2009).

LITERATURE CITED


ammunition: implications for wildlife and humans. The Peregrine Fund, Boise, Idaho, USA.


Table 3.1. Locations for soil sampling, according to our designated categories: Springs (n=12), Guzzlers, which are man-made water sources (n=9), and Reference points (n=7). Each of our reference points was paired to a randomly selected water source at a distance of 1.5km. We collected soil samples in western Utah, USA, June 2007–May 2010 during Spring-Summer of each year.

<table>
<thead>
<tr>
<th>Location</th>
<th>Category</th>
<th>Mountain Range</th>
<th>No. of lead pellets</th>
<th>% of samples with lead pellets</th>
<th>Estimate of Pellets/Ha (m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brown's</td>
<td>Spring</td>
<td>Cedar</td>
<td>5</td>
<td>11.4</td>
<td>12,225 (1)</td>
</tr>
<tr>
<td>Cedar</td>
<td>Spring</td>
<td>Cedar</td>
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<td>0.0</td>
<td>N/A (0)</td>
</tr>
<tr>
<td>Eightmile</td>
<td>Spring</td>
<td>Cedar</td>
<td>5</td>
<td>9.1</td>
<td>12,225 (1)</td>
</tr>
<tr>
<td>Flint</td>
<td>Spring</td>
<td>Keg</td>
<td>7</td>
<td>13.6</td>
<td>17,115 (2)</td>
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<tr>
<td>Henry</td>
<td>Spring</td>
<td>Cedar</td>
<td>0</td>
<td>0.0</td>
<td>N/A (0)</td>
</tr>
<tr>
<td>Kane</td>
<td>Spring</td>
<td>Keg</td>
<td>7</td>
<td>13.6</td>
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</tr>
<tr>
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<td>0.0</td>
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<tr>
<td>Redlam</td>
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<td>Whiterocks</td>
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<td>Cedar</td>
<td>20</td>
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<td>Spring</td>
<td>Thomas/Dugway</td>
<td>1</td>
<td>2.3</td>
<td>2,445 (&lt;1)</td>
</tr>
<tr>
<td>CM 8</td>
<td>Guzzer</td>
<td>Cedar</td>
<td>0</td>
<td>0.0</td>
<td>N/A (0)</td>
</tr>
<tr>
<td>CK 3W</td>
<td>Guzzer</td>
<td>West Box Elder</td>
<td>1</td>
<td>2.8</td>
<td>2,445 (&lt;1)</td>
</tr>
<tr>
<td>DC 2</td>
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<td>0.0</td>
<td>N/A (0)</td>
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<td>Thomas/Dugway</td>
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<td>1.8</td>
<td>2,445 (&lt;1)</td>
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<td>Random 1</td>
<td>Control</td>
<td>Cedar</td>
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<td>2.3</td>
<td>2,445 (&lt;1)</td>
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<td>Control</td>
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<td>0.0</td>
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<td>Control</td>
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<td>0.0</td>
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<td>Random 6</td>
<td>Control</td>
<td>Hogup</td>
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<td>0.0</td>
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<td>Control</td>
<td>Keg</td>
<td>0</td>
<td>0.0</td>
<td>N/A (0)</td>
</tr>
</tbody>
</table>

Total — — 74 6.2 —
Table 3.2. Wild chukars with ingested lead pellets (n=19) or increased lead in liver tissue (n=11), grouped by closest type of water source (guzzler, spring) and relative proximity to a water source or road (near, far). We collected these chukars in western Utah, USA, 8 August 2003–12 January 2008.

<table>
<thead>
<tr>
<th>Harvest Location</th>
<th>No. individuals collected</th>
<th>No. with ingested lead</th>
<th>No. without ingested lead but with increased lead in liver</th>
<th>% collected with ingested lead or increased lead in liver</th>
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</thead>
<tbody>
<tr>
<td>Closest to Spring</td>
<td>104</td>
<td>11</td>
<td>10</td>
<td>10.7 (20.2)</td>
</tr>
<tr>
<td>Closest to Guzzler</td>
<td>108</td>
<td>8</td>
<td>1</td>
<td>7.5 (8.3)</td>
</tr>
<tr>
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<td>19</td>
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Figure 3.1. Median number of lead pellets that captive chukars ingested as part of an experimental trial, sorted according to the type of diet we provided (Dark, Light) and the density of lead pellets we presented with food (10/dish, 100/dish).
Figure 3.2. Spectrum of diameter for common sizes of lead shot, and that for grit pebbles and Indian ricegrass seeds taken from the crops of chukars harvested in western Utah, USA. We determined size categories by sifting grit or seeds through soil sieves with opening sizes of 3.35, 1.91, and 0.98mm.
Figure 3.3. Median number of lead pellets we found in soil according to our three types of sampling locations (Control n=7, Guzzler n=9, and Spring n=11).
CHAPTER 4

CONSEQUENCES OF LEAD PELLET INGESTION IN CAPTIVE CHUKARS

(ALECTORIS CHUKAR)³

ABSTRACT

Lead is toxic, ubiquitous, and adversely affects humans and at least 130 species of wildlife. Although wild chukars (Alectoris chukar) are documented to ingest lead pellets, the consequences of this ingestion are purely speculative. The objectives of this chapter were to assess the consequences of ingested lead pellets on captive chukars as a function of lead weathering, diet type, and wild onion (Allium spp.) supplementation. We documented 7 mortalities and 10 separate morbidities in captive chukars dosed with size # 6 lead pellets. As few as one lead pellet induced morbidity and death in captive chukars. Tibia-lead concentrations shared a positive relationship with lead retention (z = 5.59, P < 0.001) and a negative correlation with age (z = -7.038, P < 0.001). Approximately 70% of all lead-dosed birds retained ≥ 1 lead pellet for the trial duration. Weathered lead pellets were more frequently retained for the trial duration (80% vs. 52%). Lead-dosed individuals fed a mixed seed diet perished more frequently and gained significantly less weight (F = 8.51, P < 0.001) than those fed a commercial pellet diet. Breast-lead concentrations had a significantly negative relationship with weight gain (t = -2.586, P = 0.01). Wild onion supplementation had positive benefits on weight gain (F = 4.41, P = 0.046) and survival (F = 4.37, P = 0.047) in juveniles and on weight gain in adults from two separate trials (F = 3.16, P = 0.038 and F = 4.89, P = 0.01). Zero chukars succumbed to death when dosed with lead and given wild onion. Lead was toxic to captive chukars, producing severe weight loss, peripheral neuropathy, and death. A

³Coauthored by R. Justin Bingham, Randy T. Larsen, John A. Bissonette, and Jeffery O. Hall
mixed-seed diet and lead weathering exacerbated the effects of lead ingestion. Wild chukars that consume onion bulbs may be benefitted against the consequences of lead toxicosis. Additional research is needed to analyze the effects of wild onion on lead poisoning, assess the outcome of dosing juvenile chukars with weathered lead, and document the consequences of ingested lead in wild chukars.

INTRODUCTION

Lead is a toxic heavy metal that adversely affects humans and wildlife (Pokras and Kneeland 2009). Instances of ingested lead ammunition or fishing weights have been documented in at least 130 wildlife species (Tranel and Kimmel 2009). In alkaline media (pH>7), lead is mostly present in non-soluble forms (Casas and Sordo 2006), but in highly acidic media (pH 1-2.5) it becomes increasingly more soluble (Rattner et al. 2009). A specialized stomach like the avian muscular grinding gizzard, complemented with grit pebbles, is an ideal environment for dissolving lead because it combines a low pH with abrasion and contraction (Trainer 1982, Pain 1996, Pain et al. 2009).

Lead can cause immediate or somewhat prolonged sub-lethal effects and mortality (Pokras and Kneeland 2009). The consequences of lead poisoning can affect the entire body, including the central nervous, digestive, and skeletal systems (Massaro 1997, Pokras and Kneeland 2009). Effects of lead on the central nervous system include impaired nerve transmission, encephalopathy, and peripheral neuropathy (Pain 1996, Massaro 1997). Digestive ailments consist of: 1) loss of appetite, 2) inability to digest food, 3) severe abdominal pain and swelling, which is a clinical sign common to nearly all lead-poisoned animals (Osweiler 1996), and 4) diarrhea (Pokras and Kneeland 2009). Complications from lead that negatively influence the skeletal system include weakened

After ingestion, lead is: 1) dissolved in the stomach, 2) absorbed into the bloodstream through the intestinal wall, 3) distributed to soft tissues such as liver and kidneys, and 4) stored in bone tissue (Pain 1996, Pain et al. 2009, Pokras and Kneeland 2009). The amount of lead absorbed in tissues or required to cause sub-lethal effects or mortality is highly variable and difficult to predict (Pain et al. 2009). This amount is mostly affected by 1) idiosyncrasies among species and individuals, 2) age, 3) stressful situations like disease, pregnancy, and injury, 4) diet, 5) grit presence and type, 6) retention time in the digestive tract, 7) particle size, shape, and surface condition, and 8) multiple abiotic factors (Barltrop and Meek 1975, Pain 1996, Vyas et al. 2001).

One important factor in lead absorption is the condition of the ingested pellet. When ingested, deformed lead pellets have a greater surface area exposed to stomach acids. Weathered lead becomes oxidized and develops a crust of lead salts (Jorgensen and Willems 1987, Lin et al. 1995). Some of these salts are more soluble and more rapidly deposited into tissues than metallic lead (Barltrop and Meek 1975).

The most important factor influencing lead absorption may be the quality and quantity of diet (Jordan and Bellrose 1951, Longcore et al. 1974, Sanderson and Bellrose 1986). Diets deficient in calcium, iron, and phosphorus—particularly those made up of hard-to-digest foods—can increase lead absorption and exacerbate its effects (Trainer 1982, Massaro 1997, Casas and Sordo 2006). Contrastingly, diets high in these nutrients and protein, especially those diets made up of soft, easily digestible foods tend to decrease lead absorption and ameliorate lead toxicosis (Pain 1996).
As a result of extensive research with waterfowl (Bellrose 1959, Longcore et al. 1974, Sanderson and Bellrose 1986), the use of lead shot for hunting wetland species was banned in 1991 by the U.S. Government. Despite the heavy emphasis on lead ingestion in waterfowl, its consequences for upland birds remain poorly understood. However, recent research shows that lead-pellet ingestion can be a source of sub-lethal detriments and mortality to many species of birds other than waterfowl (Keymer and Stebbings 1987, Vyas et al. 2000, Butler 2005). Chukars (Alectoris chukar) are an upland game-bird for which lead-pellet ingestion by wild individuals has been documented (Walter and Reese 2003, Larsen et al. 2007b), but the consequences of this ingestion are purely speculative.

Chukars are gallinaceous birds native to semi-arid, mountainous regions in portions of Eurasia (Dement'ev and Gladkov 1952, Cramp and Simmons 1980, Ali and Ripley 2001). Although chukars are largely granivorous (Cole et al. 1995) and eat mostly cheatgrass seeds (Bromus tectorum) (Christensen 1996, Larsen et al. 2007a), they are opportunistic feeders and consume many other seeds, bulbs, flowers, grass shoots, and insects (Walter and Reese 2003, Larsen et al. 2007a). One of the bulbs consumed by chukars—apparently for its high water content—is wild onion (Allium spp.) (Larsen et al. 2010). Garlic (Allium sativum), which is very similar to wild onion has been shown to contain beneficial properties against lead absorption and toxicosis when consumed in substantial amounts over extended time periods (Craig 1999, Tandon et al. 2001). Additionally, vitamins like thiamine and minerals such as calcium, which are found in allium species, have been documented to reduce lead absorption (Bratton et al. 1981,
Coppock et al. 1991, Olkowski et al. 1991). Onion-bulb consumption by wild chukars may reduce their risk of developing lead poisoning or augment their ability to survive it.

The objectives of this chapter were to assess the consequences of ingested lead pellets on captive chukars—as a function of lead weathering, diet type, and wild onion supplementation. We hypothesized that lead would be toxic to chukars, causing both sub-lethal and lethal effects. We also predicted that a mixed seed diet would exacerbate the effects of lead; whereas wild onion supplementation would alleviate them.

**METHODS**

*Dosing Trials*

Our dosing trials were approved by the Institutional Animal Care and Use Committee (approval #1366) at Utah State University. We housed chukars in climate-controlled sheds—maintained at 20°C—at the Veterinary Science Farm on the Utah State University campus in Logan, Utah, USA. Within these sheds, we kept chukars individually in 30.5cm³ cages built in rows of eight stacked three rows high. We fabricated cages with wire mesh bottoms to allow feces to fall through. Each row of cages had a removable tray below it to empty feces and discarded food. As needed, we cleaned and sanitized the water containers and replaced dirty water with fresh water. We randomly assigned all treatment levels to experimental units by fitting each bird with an identification band and writing a corresponding randomization code in Program R version 2.9.

For the purposes of this chapter, our response variables were lead concentration in liver, breast, and bone tissue; overall weight trend; lead-pellet retention; survival; and days survived. With respect to survival, our protocol included incapacitated individuals.
We observed cases of extended peripheral neuropathy accompanied by emaciation, severe weight loss, and complete lethargy, which we classified as morbidity. Because we assumed that moribund birds were essentially dead—particularly if used as an index for the effects of lead on wild chukars—we combined morbidities with mortalities when assessing survival or days survived.

To assess body condition, we weighed each individual to the nearest 10 g on days 0, 3, 6, 9, 12, and 15 of the trial period. On day 15 of the trial, we euthanized each bird. We classified maximum weight loss or gain as the greatest negative or positive value of weight change during the trial period.

For pellet retention, we recorded how many pellets were retained at the end of the trial by removing the gizzard of each individual after euthanization to check for lead pellets. We carefully opened each gizzard using a stainless steel blade and emptied the gizzard contents. Next, we used a hard plastic container to combine each sample of gizzard contents with water to agitate them and separate the lighter materials from the heavier lead pellets. Finally, we recorded the number of pellets present in each sample.

To obtain bone samples for lead analysis, we removed the right tibia of each bird. Next, we removed a cross-section of the bone near the mid-shaft point. We extracted the marrow from the bone and submerged each sample in acetone to remove any lipids and help dry the sample. Additionally, we cut out the right liver lobe, and removed a section of breast tissue on the right side of the three-way intersection of the wish bone. We stored tissue samples by freezing them until having them analyzed for lead at the Utah Veterinary Diagnostic Lab. To remove organic materials from our samples, we digested them in trace mineral grade nitric acid (HNO₃) under a heating block. We weighed each
tissue sample to a precision of 0.0001g. We diluted the digested samples with ultra-pure water to a final nitric acid concentration of 5% using pipettes of 1-1000 mL with corresponding disposable tips to standardize the sample for comparison with analytical benchmarks. For quality control in sample identification, we labeled separate digestion and dilution tubes in permanent marker. For our digestion and dilution tubes, we used clear 15 mL plastic centrifuge tubes. Next, we vortexed the tissue samples. We determined tissue-lead concentrations using Inductively Coupled Plasma/Mass Spectroscopy and compared acquired values with curves of known lead standards. After every fifth analytical sample, we analyzed standard curves and quality control samples. We double checked all of our results for accuracy. For all applicable analyses, we determined tissue lead concentration in parts per million wet weight (ppm) for liver or breast tissue and ppm dry weight for tibia tissue.

Our first dosing experiment used 30 ten-week-old (sub-adult) chukars. We purchased the birds at seven weeks old and allowed them to acclimate to captivity for three weeks. We provided birds with purified water ad libitum and fed them \( \frac{1}{4} \) of a cup of food daily and a \( \frac{1}{2} \) teaspoon of commercial grit once every third day. This trial had five replicates of each treatment combination. We assessed both the effects of lead ingestion and the ability of wild onion, i.e., allium (Allium spp.) to alleviate these effects in captive chukars. At the beginning of the trial period, we dosed birds with zero (control), one, or three # 6 non-weathered lead pellets. To introduce the lead into each chukar, we inserted a gavage tube down the esophagus into the proventriculus and dropped each pellet into the tube by hand. For reference individuals, we injected 2 ml of water down the gavage tube into the proventriculus using a syringe.
We collected allium in Utah at Antelope Island State Park. Next, we removed the skin from the bulbs, washed them in water, and allowed them to air-dry for 24 hours. To facilitate equal rations of allium, we created a solution by adding water to allium and mixing them in a blender. We combined the ingredients in a ratio of 2:1 (allium:water). We stored the concoction in a refrigerator until it was needed. We administered allium using the same method as for dosing birds with lead pellets. Each bird received 2 ml of the allium solution every other day for the length of the trial period. We gave control birds 2 ml of water using the same methods as those used for allium.

The second dosing experiment had a few minor differences. Chukars received either zero (control), two, four, or six #6 lead pellets. We ended the trial period after day 12. We acquired the chukars for this trial at 12 weeks old and allowed them to acclimate to their captive surroundings for four weeks.

The third dosing experiment had a few additional changes. The second factor was ‘Weathering’ instead of allium. This trial had 7 treatment levels: no lead (control), 1, 3, or 5 weathered shot, and 1, 3, or 5 non-weathered shot. To simulate surface weathering of the pellet, we placed new lead pellets and a few small pebbles into an electric cement mixer for one hour to roughen the surface of each pellet. We purchased the chukars for this experiment at 13 weeks of age and permitted them to acclimate to their captive habitat for 1 week.

In the fourth dosing experiment, we purchased 36 chukars at 18 weeks of age and allowed them to acclimate to their new surroundings for four weeks. We changed the lead levels so individuals received zero (control), three, or six #6 lead pellets. We
conducted this experiment using only weathered pellets. Additionally, we used only weathered pellets in all subsequent dosing experiments.

In the fifth dosing experiment, we used 48 29-week-old birds acclimated to their environment for two weeks. We organized this trial with eight replicates per treatment combination. We gave birds zero (control), one, or five #6 lead pellets. Factor two was ‘Diet’, which we divided into two groups: ‘Food Pellets’ or ‘Mixed Seeds’. For the mixed seed diet, we produced a mixture of seven seeds by combining commercial red wheat (*Triticum* spp.), basin wildrye (*Leymus cinereus*), bluebunch wheatgrass (*Elymus spicatus*), Cicer milkvetch (*Astragalus cicer*), Indian ricegrass (*Stipa hymenoides*), intermediate wheatgrass (*Elymus hispidus*), and small burnet (*Sanguisorba minor*) in a 2:1:1:1:1:1:1 ratio. We obtained all seeds through a donation from the UDWR Great Basin Research Center, except for the wheat and Indian ricegrass, which we purchased from a farm supply store. Contrary to previous dosing trials, we provided each bird with ½ TSP of grit daily instead of every third day. We also obtained the concentration of liver and breast lead as response variables for this investigation.

For the sixth and final dosing experiment, we obtained 20 individuals at 31 weeks of age and held them for 2 weeks prior to commencing the trial period. We dosed each bird with either zero (control) or 5 lead pellets and either gave them allium or no allium. We fed all birds in this trial a mixed seed diet and grit daily.

*Statistical Analysis*

We used analyses of variance (ANOVA) with the Tukey Adjustment to test for significant differences in means with respect to factors of interest. For certain analyses, we also used simple linear or negative binomial regression to assess correlations between
response and explanatory variables. For all applicable analyses involving the calculation of a mean with a corresponding 95% confidence interval (95% CI): we calculated the sample mean ($x$), standard deviation ($SD_x$) standard error of the sample mean ($SE_x$), and 95% CI.

**RESULTS**

*Pooled Results for all trials*

For descriptive purposes, we pooled our results for weight trend (Figure 4.1), and maximum percent weight-loss (Table 4.1) and gain (Table 4.2) for all trials with adult chukars. Our results show the positive effects of allium supplementation, with both diet types, and negative effects of lead dosage—particularly with a mixed seed diet—on the ability of captive chukars to maintain a healthy body condition.

We also combined results for tissue-lead concentrations (Table 4.3). We determined that of 17 symptomatic cases of lead poisoning, zero had ≤ 5 ppm, 2 (11.8%) had > 5 ≤ 10 ppm, 7 (41.2%) had > 10 ≤ 20 ppm, and 8 (47%) had > 20 ppm tibia lead. Mean concentrations of tibia lead for adult chukars receiving weathered (28.29 ± 4.47 ppm) vs. non-weathered (30.59 ± 6.27) shot were quite similar. We observed much greater mean concentrations of tibia lead in adult birds fed a commercial pellet (32.53 ± 4.24) vs. mixed seed (15.95 ± 3.22) diet. Nevertheless, we also documented that age of birds at sacrifice had an effect on tibia-lead concentration: 12 weeks (47.33 ± 10.06); 16 weeks (36.86 ± 10.09); 18 weeks (30.94 ± 6.68), 24 weeks (42.17 ± 5.38), 33 weeks (14.28 ± 3.05), and 35 weeks (16.48 ± 5.93). Negative binomial regression showed a significantly negative relation between tibia-lead concentrations and age at sacrifice ($z = -7.038, P < 0.001$). Mean tibia-lead was greater in lead-dosed adult chukars given allium
(35.2 ± 6.38) vs. not (27.04 ± 4.3). Overall, we found no significant relation between weight trend and tibia or liver-lead concentration. However, breast-lead concentrations had a significantly negative relation with weight gain (t = -2.586, P = 0.01).

Negative binomial regression showed that tibia-lead concentrations and number of shot retained to necropsy (z = 5.59, P < 0.001) shared a significantly positive correlation. Of 146 birds dosed with lead, 68% (n=99) retained a lead pellet to necropsy. Of the 42 total birds given allium and lead shot, 71% (n=30) retained lead, which approximates the value of 66% (n=69) for the 104 chukars dosed with lead but denied allium. With respect to diet type, 73% (n=19) of the 26 individuals dosed with lead and fed mixed seeds retained lead, as compared with 67% (n=80) of the 120 birds dosed with lead but fed commercial pellet food. Of the 81 chukars receiving weathered lead, 80% (n=65) retained a lead pellet; whereas only 52% (n=34) of the 65 individuals given non-weathered shot retained any of it until being sacrificed.

Only 2.2% (n=1) of adult birds receiving non-weathered lead succumbed to morbidity, as opposed to 12.3% (n=10) of those dosed with weathered lead. Similarly, only 4.8% (n=2) of all birds dosed with lead and given allium became moribund; whereas 14.4% (n=15) of those given lead but denied allium were incapacitated. Of 7 actual mortalities, zero occurred in birds supplemented with allium.

‘Juvenile Lead×Allium’ Dosing Trial

Our ANOVA analysis showed a significant effect of lead dosing on tibia-lead concentration in juvenile chukars (F = 42.1, P < 0.001). Tibia-lead concentration was significantly greater (p < 0.05) according to the following inequality describing lead pellets received: 3 > 1 > 0. Allium supplementation had no significant effect on tibia-
lead concentration. Using the overall trend in weight over the course of the trial, we observed that allium significantly improved body condition (F = 4.41, P = 0.046); whereas lead dosage had no statistical effect on body condition. We combined our results for overall weight trend in juvenile birds (Fig. 4.1).

We documented 6 cases of morbidity for juvenile chukars. Individuals receiving lead survived marginally statistically fewer days (F = 2.78, P = 0.08). Those birds dosed with 3 vs. 0 lead shot accounted for most of the difference. Individuals survived markedly longer if they were given allium (F = 4.37, P = 0.047), but we observed no significant interaction between lead and allium. Of the five chukars receiving one lead shot and no allium, 40% (n = 2) were moribund. Three (60%) of five birds dosed with three lead shot and given no allium experienced morbidity. One (20%) of five individuals given three lead shot and allium was found moribund; whereas no chukars dosed with one lead shot but given allium experienced morbidity.

‘Adult Lead×Allium (1)’ Dosing Trial

Through an ANOVA we determined that tibia-lead concentration was significantly increased in lead-dosed individuals (F = 15.55, P < 0.001). Birds receiving 6 vs. 0, 6 vs. 2, 4 vs. 0, and 2 vs. 0 shot all had significantly more tibia lead at sacrifice (p < 0.05). Chukars given allium and lead shot gained significantly more weight than birds receiving no allium and dosed with lead (F = 3.16, P = 0.038).

‘Weathered vs. Non-weathered Lead’ Dosing Trial

Lead dosage had a significant effect on tibia-lead concentration (F = 4.73, P = 0.009). Those birds given five lead shot had significantly more tibia lead than control
individuals (P = 0.005). Neither lead dosage nor lead weathering had a significant effect on the overall weight trend during the trial.

Chukars receiving weathered lead shot survived marginally significantly fewer days (F = 3.01, P = 0.09). We observed 2 mortalities and 2 separate cases of morbidity in this trial. One (20%) of five individuals given 3 and 5 weathered shot died, respectively. One (20%) of five additional individuals receiving 1 weathered shot became moribund. Finally, 1 (20%) of 5 chukars dosed with 5 non-weathered shot also succumbed to morbidity.

‘Adult Lead×Allium (2)’ Dosing Trial

Tibia-lead concentration was significantly greater in birds dosed with lead shot (F = 65.7, P < 0.001). Tibia-lead was significantly greater (p < 0.05) according to the following inequality describing lead pellets received: 6 > 3 > 0. Birds given lead and allium had significantly better body condition than those dosed with lead but denied allium (F = 4.89, P = 0.01).

‘Lead×Diet’ Dosing Trial

Concentrations of tibia (F = 32.4, P < 0.001), liver (F = 25.4, P < 0.001), and breast lead (F = 3.86, P = 0.03) were all significantly greater in lead-dosed chukars. Tibia and liver-lead concentrations were significantly greater (p < 0.05) according to the following inequality describing lead pellets received: 5 > 1 > 0. Regarding breast-lead concentration, the only significant difference was between those birds given 5 lead shot and the control group. Mean concentrations of tibia, liver, and breast lead for lead-dosed birds were 14.28 ± 3.05 (n = 32), 5.52 ± 1.47 (n = 32), and 0.12 ± 0.06 (n = 32),
respectively, whereas those of control individuals were 0.22 ± 0.06 (n = 16), 0.069 ± 0.062 (n = 16), and 0.013 ± 0.004 (n = 16). In lead-dosed chukars, breast lead was ~2.2% of liver lead. Contrastingly, breast lead was ~18.8% of liver lead in control birds.

Negative binomial regression revealed that tibia (z = 3.63, P < 0.001) and liver-lead (z = 6.71, p < 0.001) concentrations were positively correlated with number of pellets retained to sacrifice. Simple linear regression showed a positive, but statistically insignificant relation between breast lead and number of lead shot retained to necropsy. Birds fed the mixed seed diet had marginally significantly more breast lead (F = 3.67, P = 0.06).

Overall weight trend during the trial was significantly affected by the interaction of lead ingestion and diet type. Birds receiving the mixed seed diet and lead shot lost markedly more weight (F = 8.51, P < 0.001). Specifically, chukars dosed with 5 lead shot and fed a mixed seed diet lost much more weight (P < 0.05) than those given 1 or 5 lead shot and a commercial pellet diet, those receiving one lead shot and a mixed seed diet, and both control groups (Fig. 4.2).

We documented 5 mortalities in this trial. Two (25%) of 8 chukars given 1 lead shot and fed a mixed seed diet perished. Three (38%) of 8 individuals dosed with 5 lead shot and fed mixed seeds also died. We observed zero mortalities for birds dosed with lead but fed a commercial pellet diet (n = 16). Number of days survived was not significantly less for lead-dosed birds.

‘Adult Lead×Allium (3)’ Dosing Trial

Tibia-lead concentration was significantly greater in lead-dosed chukars (F = 34.8, P < 0.001). Birds receiving lead shot lost marginally significantly more weight (F =
We observed 2 cases of morbidity. One individual from each of the lead-dosed groups (allium vs. no allium) became moribund, yielding a 20% morbidity rate for lead-dosed chukars in this trial. The number of days survived was not significantly affected by lead or allium. Contrary to our other dosing trials, allium supplementation did not reduce weight loss in lead-dosed birds from this trial (Fig. 4.1).

**DISCUSSION**

Our results demonstrate that toxic amounts of lead are absorbed shortly after chukars ingest lead shot. According to our findings, as few as one #6 lead pellet can induce morbidity and mortality in captive chukars, which is consistent with amounts of lead that have caused death in ducks (Jordan 1968), songbirds (Vyas et al. 2001), and doves (Buerger et al. 1986, Schulz et al. 2006). Nevertheless, lead concentrations and factors causing morbidity and mortality were highly variable. We documented a total of 7 mortalities and 10 separate morbidities during our 6 dosing trials. This frequency may seem low, but captive environments are benign and constant, causing individuals to experience minimal stress and expend little energy. Because: 1) most captive chukars retain lead pellets long enough to reach toxic tissue concentrations, 2) one lead pellet can kill a captive chukar, and 3) conditions are much more stressful in wildland habitats, mortality rates of lead-poisoned wild chukars are likely much higher. The frequency of mortality and morbidity we observed in a controlled setting demonstrates the importance for understanding the effects of lead on wild chukars.

Over two-thirds of captive individuals retained lead long enough to reach tissue concentrations consistent with acute lead exposure. Lead retention-time is directly related to mortality in various avian species (Loncore et al. 1974, Sanderson and Bellrose.
Approximately 12% (n = 54) of wild chukars from western Utah had an ingested lead pellet or increased liver lead (Chapter 2). If captive chukars are an accurate index of lead retention and its effects on tissue lead in wild birds, approximately 8% (n = 38) of our sample of wild chukars would be at risk of acute lead exposure and its consequences.

Despite the increased lead concentrations in liver and tibia that resulted from lead-pellet ingestion, corresponding concentrations in breast tissue were very low. This evidence suggests that human consumers need not worry about bio-accumulated lead when consuming avian breast tissue. Nevertheless, lead pellets and fragments can still be present in edible portions of gamebirds harvested with lead shot (Frank 1986, Scheuhammer et al. 1998). Contrastingly, soft tissues like liver accumulate large amounts of lead and may not be worth consuming.

Although the negative effects of lead on body condition were not pervasive across all dosing trials, we still documented poisonings and deaths without significant decreases in weight. Acutely lead-poisoned birds may die quickly in apparently good body condition (Gill and Langelier 1994). Weight loss of about 20% was indicative of morbidity or mortality in lead-dosed birds from our trials, which coincides with previous trials involving lead ingestion by ducks (Chasko et al. 1984) and ptarmigan (Gjerstad and Hanssen 1984). A few control chukars lost ≥ 20% of their body weight with no visible symptoms of sickness or lethargic behavior. Because lead interferes with the digestive process (Osweiler 1996, Pokras and Kneeland 2009), individuals we dosed with lead shot apparently lost weight due to sickness and their inability to absorb food and nutrients.
Contrastingly, control birds withstood significant weight loss because such reductions were likely due to diet acclimation.

One important factor influencing lead absorption and retention was lead weathering. Previous research has documented disparate effects of weathered vs. non-weathered lead on dosed individuals. Vyas et al. (2001) found that shot erosion in the gizzard and lead absorption into the bloodstream were significantly accelerated with weathered lead. Our results confirm that weathered vs. non-weathered lead is more toxic to captive chukars. Weathered lead shot was more commonly retained for the duration of the trial period. Although non-weathered lead had minimal negative effects on adult chukars, it caused multiple morbidities in juvenile birds. Juveniles are more susceptible to lead toxicosis because sub-adults absorb more of the lead they ingest (Casas and Sordo 2006, ATSDR 2007, Pokras and Kneeland 2009), and because the consequences of lead poisoning are likely worse during periods of growth and development. Additional research is needed to clarify the potential differences in lead absorption and toxicosis between weathered and non-weathered lead in juvenile chukars.

Although mean concentrations of tibia lead were similar in adult chukars receiving weathered vs. non-weathered lead, the frequency of symptomatic lead poisoning was very different between these 2 groups. This occurrence is likely due to the effects of diet and age on lead absorption and poisoning. Of the many factors that influence lead absorption, diet may be paramount (Jordan and Bellrose 1951, Longcore et al. 1974, Sanderson and Bellrose 1986). Vyas et al. (2001) found that a mixed seed diet produced mortality in a medium-sized passerine; whereas birds fed a commercial pellet diet failed to succumb to clinical lead poisoning. When combining our trials, chukars
given commercial pellet food were able to gain significantly more weight than those fed mixed seeds, but they also absorbed more lead into tibia tissue than seed-fed individuals. However, more birds were fed a commercial pellet diet, including all of the chukars receiving non-weathered lead. Additionally, birds given non-weathered lead and pellet food were younger than those fed mixed seeds. These conditions inflated the values for pooled results of tibia lead in birds dosed with non-weathered pellets because tibia-lead was negatively correlated with age. Consequently, observed overall tibia-lead concentrations from weathered vs. non-weathered lead were likely similar because of the interaction of diet and age rather than weathering itself. Nevertheless, the mixed seed diet with its calcareous grit may have contained sufficient minerals to regulate lead absorption, but provided too few calories to promote comparable weight gain. The amount and type of grit present in the avian gizzard also affects lead erosion and absorption (Longcore et al. 1974, Sanderson and Bellrose 1986, Marn et al. 1988). When birds on the pellet diet ingested grit, it may have been used more exclusively to erode lead because food pellets are soft and easily digestible. However, even though less lead was absorbed by seed-fed individuals, less lead was required to produce symptoms of poisoning in these birds, apparently because of their reduced body condition.

Wild chukars ingesting lead face myriad stressors that could magnify the negative effects of lead poisoning. The ability of poor diet quality to enhance lead absorption would likely be intensified when combined with the stress of acquiring food and water, evading predators, coping with disease and severe temperature fluctuations, and breeding. Just as diets rich in calcium, iron, and phosphorus can reduce lead absorption (Trainer 1982, Pain 1996, Casas and Sordo 2006), allium ingestion by wild chukars may serve to
alleviate or prevent lead toxicosis. Garlic has been shown to reduce lead absorption and decrease tissue damage caused by lead (Craig 1999, Tandon et al. 2001).

We found that adult and juvenile lead-dosed chukars benefitted from allium supplementation in terms of weight gain and survival. These observed advantages may be due to a combination of the nutrients, vitamins, and proteins found in allium species. Allicin and anti-oxidants (Craig 1999, Tandon et al. 2001), thiamine (Bratton et al. 1981, Olkowski et al. 1991), and calcium-containing compounds (Coppock et al. 1991, Olkowski et al. 1991) have been shown to reduce lead absorption in various animal species. Nevertheless, allium supplementation was not a cure-all, as birds receiving a mixed-seed diet and weathered lead showed no benefits with regards to weight loss or survival. Additional research concerning the benefits of allium on the effects of lead ingestion would be advantageous. Particularly, we lack specific information regarding the frequency, size, and duration of allium dose against lead toxicosis, and how diet might affect the ability of allium to alleviate lead poisoning. Craig (1999) commented that benefits of allium species against lead are likely realized when substantial amounts are ingested over extended time periods. Because some wild chukars consume allium regularly for its high water content (Larsen et al. 2010), this avian species is an excellent model for research assessing the benefits of allium bulbs against lead toxicosis.

**MANAGEMENT IMPLICATIONS**

Lead pellets are toxic to captive chukars. As few as one #6 lead pellet can cause morbidity and mortality in adult individuals. Most birds retained ingested pellets long enough to reach tissue-lead concentrations consistent with acute lead poisoning. Because wild chukars have been repeatedly documented to ingest lead pellets, they are likely at
risk for lead poisoning. The negative consequences of lead ingestion would likely be magnified when combined with the many stressors encountered by wild birds.

Diet affects the consequences of lead-pellet ingestion in captive chukars. We observed most mortalities in birds fed a mixed seed diet, which more closely approximates the natural diet of wild chukars. Allium bulbs may serve as a natural supplement to benefit chukars that ingest lead shot. Because chukars are a popular game-bird, they are also a source of management funds to benefit all wildlife. Perpetuation of sustainable chukar populations may be influenced by the issue of lead-pellet ingestion. Research on the consequences of lead-pellet ingestion by wild chukars will be an integral part of understanding and mitigating lead poisoning and its effects on wild chukars.

**LITERATURE CITED**


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Oregon and discovery of ingested lead pellets. Western North American
Table 4.1. Mean maximum weight loss (%) during the trial period for all lead dosing trials with captive chukars. The trials were: ‘J_L_A’ (Juvenile Lead×Allium); ‘L_A_1’ (Adult Lead×Allium 1); ‘W_L’ (Weathered×Unweathered Lead); ‘L_A_2’ (Adult Lead×Allium 2); ‘L_D’ (Lead×Diet), and ‘F_L_A’ (Final Lead×Allium). To classify lead, we used: ‘w’ (Weathered); ‘u’ (Unweathered); and ‘c’ (Control). We divided diet as ‘p’ (Pellet) or ‘s’ (Seed). We denoted Allium allotment with ‘a’ (Allium) or ‘n’ (No Allium). The ‘j’ signifies juvenile; whereas all other classes had adult birds (≥ 14 weeks old).

<table>
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<th>W_L</th>
<th>L_A_2</th>
<th>L_D</th>
<th>F_L_A</th>
<th>Totals</th>
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Table 4.2. Mean maximum weight gain (%) during the trial period for all lead dosing trials with captive chukars. The trials were: ‘J_L_A’ (Juvenile Lead×Allium); ‘L_A_1’ (Adult Lead×Allium 1); ‘W_L’ (Weathered×Unweathered Lead); ‘L_A_2’ (Adult Lead×Allium 2); ‘L_D’ (Lead×Diet), and ‘F_L_A’ (Final Lead×Allium). To classify lead, we used: ‘w’ (Weathered); ‘u’ (Unweathered); and ‘c’ (Control). We divided diet as ‘p’ (Pellet) or ‘s’ (Seed). We denoted Allium allotment with ‘a’ (Allium) or ‘n’ (No Allium). The ‘j’ signifies juvenile; whereas all other classes had adult birds (≥ 14 weeks old).

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Table 4.3. Mean [lead] for dosed captive chukars. Trials were: ‘J_L_A’ (Juv. Lead × Allium); ‘L_A_1’ (Adult Lead × Allium 1); ‘W_L’ (Weathered vs. New Lead); ‘L_A_2’ (Adult L × A 2); ‘L_D’ (Lead × Diet), and ‘F_L_A’ (Adult L × A 3). We used: ‘w’ (Weathered); ‘u’ (New); and ‘c’ (Control) to classify lead, divided diet as ‘p’ (Pellet) or ‘s’ (Seed), and denoted Allium with ‘a’ (yes) or ‘n’ (no). The ‘j’ signifies juvenile birds. The ‘l’ means liver-lead, and ‘b’ stands for breast-lead. Remaining values are tibia-lead.

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<th>L A_2</th>
<th>L D</th>
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<td>16.8±13.0 (n = 5)</td>
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<tr>
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</table>
Fig 4.1. Overall trend in mean weight (g) during the lead-dosing trial period for captive juvenile (top) and adult (bottom) chukars. To separate lead type, we used: ‘W’ (Weathered); ‘U’ (Unweathered); and ‘C’ (Control). We divided diet as ‘P’ (Pellet) or ‘S’ (Seed). We denoted Allium supplementation with ‘A’ (Allium) or ‘N’ (No Allium).
Fig. 4.2. Median values for overall weight trend (g) from the Lead×Diet dosing trial. The numbers 0, 1, and 5 on the X-axis represent the number of lead shot given each bird. The diet type is distinguished by ‘PELLET’ (commercial pellet food) and ‘SEED’ (Mixed Seeds).
CHAPTER 5
CONCLUSIONS

Two out of the three most recent diet studies with chukars (*Alectoris chukar*) have encountered individuals with ingested lead pellets (Walter and Reese 2003, Larsen et al. 2007), even though diet research with chukars from pre-1980 failed to report lead-pellet ingestion (Zembel 1977, Knight et al. 1979). I observed chukars with ingested lead pellets both during and outside the hunting season, and from multiple years within the duration of my study. The variability in the frequency of lead ingestion I observed is likely due to fluctuations in available diet items, reliance on free water, and other patterns of habitat use. Because recent findings document lead ingestion by chukars, whereas previous research did not, I consider that these results support a general accumulation of lead shot in soils within chukar habitat. Nevertheless, it is possible that more antiquated research merely omitted such information.

By the late 1960s, 10 western states harbored sufficiently large chukar populations to allow a hunting season (Christensen 1970). After a few years following their establishment, the popularity of hunting chukars within the western U.S. began to quickly increase. Hunting and recreational target shooting continue to deposit lead in wildland soils at a much faster rate than this lead can migrate away from the soil surface or dissolve. As long as these shooting practices persist, densities of lead pellets in wildland soils will continue to increase and the problem of lead ingestion and poisoning in chukars and sympatric species will likely worsen.

Of the 466 wild-harvested chukars from my sample, 43 (9.2%) contained an ingested lead pellet. This frequency of lead-pellet ingestion is the second highest that I
have observed for wild populations of upland game-birds. I only encountered a single ingested lead pellet in all individuals with ingested lead. Additionally, other authors have documented only 1 or 2 ingested lead pellets in chukars (Walter and Reese 2003, Kreager et al. 2008). The overall frequency of lead ingestion by chukars for both of these studies was slightly lower than that of my research—5.7% in gizzards and 7.1% in crops for Walter and Reese (2003), and 8% overall for pen-raised chukars in Kreager et al. (2008). In Kreager et al. (2008), a much greater frequency of pen-reared ring-necked pheasants (34%) (Phasianus colchicus) ingested much greater range of lead pellets/bird (1-66) vs. chukars in the study. The investigation site for this research with chukars and pheasants had densities of lead in soils that were slightly higher and more consistently greater (Holdner et al. 2004) than those I observed in my study area.

Because I did not find more than one lead pellet in any wild chukars with ingested lead, there is evidence for multiple explanations for the frequency of lead-pellet ingestion in this species, they: 1) quickly void an ingested lead pellet, 2) promptly succumb to the effects of lead poisoning with the ingestion of a single lead pellet, particularly during stressful periods like raptor migration and winter, or 3) ingest lead in a sufficiently infrequent manner making the presence of multiple pellets in their digestive tract uncommon. Combining my experimental and sampling results, I found little evidence to support the first hypothesis, but found that explanation 2 is highly plausible. Other upland birds have been documented with many more ingested lead pellets (Lewis and Legler 1968, Shulz et al. 2002, Kreager et al. 2008), which supports hypothesis 3 for chukars. Nevertheless, explanations 2 and 3 are not mutually exclusive because although ingestion may only occur in ~12% of my sample, the birds that do ingest lead can still
quickly succumb to lead poisoning. Moreover, differences in frequency of lead ingestion can vary greatly among sites within a study area. Multiple authors support this lead-availability hypothesis, citing that although they found extremely high densities of lead in soils or multiple ingested pellets in birds, they observed a low frequency of ingestion overall (Lewis and Legler 1968, Best et al. 1992, Schulz et al. 2002). Additionally, captive chukars that I habituated to develop a search image for food or grit items similar to lead pellets voluntarily ingested significantly more shot than chukars vs. reference birds, particularly when lead shot was available in a higher density. This preponderance of evidence supports a hypothesis of availability (explanation 3), while not discounting that of acute lead toxicosis (explanation 2). Ultimately, whether one or many lead pellets are ingested, my evidence shows that both paths can lead to morbidity and death.

The presence of ingested lead in chukars was common June-January, but increased liver lead during June-October was dissimilar from that of November-January. I encountered 19 instances of ingested lead for June-October (n=221) and 20 during November-January (n=193). Contrastingly, I observed 14 events of increased liver lead for June-October (n=97), but failed to find a single occurrence during November-January (n=24). Therefore, chukars apparently succumb to lead poisoning more frequently during periods of high stress such as raptor migration and winter. Nevertheless, although most chukar mortalities resulting from lead toxicosis probably occur during November-March, sub-lethal and lethal effects of lead poisoning are possible year round. Investigators should pay close attention to seasonal aspects of lead ingestion and its effects when estimating the consequences of lead-pellet ingestion in wildlife.
Food and grit items that are commonly ingested by wild chukars can be similar in size, shape, and color to lead pellets in many of the popular shot sizes. Specific reasons for an increased frequency of lead-pellet ingestion by chukars include: 1) the arid climate, rocky topography (Walter and Reese 2003), and alkaline soils of chukar habitat, which reduce the rate of pellet settlement (Schranck and Dollahon 1975) and dissolution (Stansley et al. 1992), 2) similarities in appearance between lead pellets and grit or food sources (Gionfriddo and Best 1999, Schulz et al. 2002, Mateo 2009), 3) density of lead pellets in soils, especially concerning concentration areas of lead deposition such as water sources (Best et al. 1992), roads, and sites used for target shooting, 4) hunting pressure, and 5) their propensity to probe soils intensely for food (Gionfriddo and Best 1999). As I mentioned previously, in captive chukars the frequency of lead-pellet ingestion definitely increases with pellet density (availability), provided that the individuals in question possess a search image for food or grit items similar to lead pellets. Consequently, the aforementioned mentioned reasons for lead ingestion likely combine to determine consumption frequencies.

Chukars are also prone to the consequences of lead-pellet ingestion. Factors exacerbating the consequences of lead ingestion by chukars include: 1) presence of a well-developed muscular grinding gizzard with a low pH (Pain 1996, Pain et al. 2009), 2) consumption of pebbles to aide in food digestion, which accelerates lead dissolution (Pain 1996, Pain et al. 2009), 3) stressful periods of predation (Pain 1996), such as peak migration of raptors, 4) extreme daily and seasonal weather events and temperature changes (Pain 1996), and 5) disease (Pain 1996). Chukars should be considered at high
risk for lead-pellet ingestion and subsequent poisoning because they fulfill each of these factors that influence lead ingestion and absorption.

I determined that mean values for the concentration of lead in liver tissue appear to be greatest at ≤1.5km for water sources and ≤1.0km for roads. Although most estimates of the average size for seasonal home range of chukars are <1km² (Lindbloom 1998, Walter 2002), movements of much greater distances have been documented (Lindbloom 1998, Walter 2002, Larsen et al. 2010). Great variability in size for seasonal home range inter-annually has been observed as well (Larsen et al. 2010). Assuming that water sources and roads do have an effect on the risk of increased lead exposure for chukars, these effects are pronounced up to linear distances that are greater than most estimates for the average size of seasonal home range in chukars. This discrepancy suggests: 1) some chukars have greater movements to water than the average estimates for size of seasonal home range, 2) the size of seasonal home ranges are larger than current estimates suggest, or 3) water and roads have no significant effect on lead ingestion. Spatially intensive research should be conducted with an emphasis on correctly estimating the size of seasonal home range for chukars to improve assumptions for the effects of spatial factors on the frequency of lead ingestion.

I estimated a density of 1,712,134 lead pellets/Ha for soils at a systematically-chosen area used for target shooting within occupied mourning dove (Zenaida macroura) and chukar habitat. My estimates for randomly-selected springs within occupied mourning dove and chukar habitat were as high as 58,600 lead pellets/ha. The highest estimates for man-made water catchments (guzzlers) and reference points were only 2,445 lead pellets/ha. Guzzlers appear to be a valid management tool to mitigate the
frequency and effects of lead ingestion on wildlife, while simultaneously providing a source of free water to many wildlife species. I confirmed that settlement of lead pellets in rocky, alkaline soils within semi-arid habitats is relatively slow. Researchers sampling soil in these types of habitat will probably find all existent lead pellets in approximately the top 1 cm of soil.

Most lead pellets ingested by chukars are likely deposited through recreational target shooting and hunting of sympatric mourning doves. Nevertheless, chukars are a popular game species and some lead accumulation in soils of chukar habitat is doubtless because of chukar hunting itself. If lead poisoning adversely affects chukar populations in Utah, localized extinctions could potentially occur. Such extinctions may reduce hunter interest and would subsequently decrease license sales and the resultant management funds that benefit many wildlife species.

Lead caused extended bouts of peripheral neuropathy and lethargy that resulted in severe weight loss in captive chukars. I also documented 7 mortalities and 10 separate morbidities during my dosing trials. As few as one #6 lead pellet can cause morbidity or mortality in captive chukars. Weathered lead was more toxic to chukars and more commonly retained for the trial duration. A mixed seed diet significantly exacerbated the effects of lead ingestion on captive individuals. Wild onion supplementation demonstrated benefits to chukars against lead poisoning.

Of 121 liver samples I had analyzed, 5 (4.1%) contained lead concentrations consistent with those that were capable of inducing mortality in captive chukars dosed with lead and fed a mixed-seed diet. About 2/3 of captive chukars retained lead shot long enough to reach tissue concentrations consistent with clinical poisoning. If captive
chukars are an accurate index of wild individuals, it is probable that between 8 and 12% of the chukar population in Utah succumbs to lead poisoning. This value may be higher, when taking stressful conditions and the potential of under-estimating the frequency of lead-pellet ingestion into consideration. Even using the low end of the spectrum, potential annual losses of chukars in Utah to lead poisoning would be in the thousands. Lead poisoning of chukars in Utah is a probable detriment to the sustainability of certain populations. Some populations likely face little risk from lead poisoning; whereas small, isolated, and heavily hunted populations that ingest Indian ricegrass (*Stipa hymenoides*), use springs, or frequent areas used for target shooting are at high risk of increased lead exposure and its consequences. Hunting pressure likely varies throughout western Utah, and accordingly so does lead-shot consumption by chukars within this area. Nevertheless, lead-pellet ingestion by chukars in my study area is widespread.

Additional research endeavors regarding lead contamination of wildlife species in Utah should focus on the consequences of lead ingestion and poisoning in wild chukars, mourning doves, and any other sympatric species fulfilling the factors that increase lead ingestion and exacerbate its absorption. Additionally, investigating the risks to human and wildlife consumers of animal species harvested or wounded with lead ammunition is warranted. Finally, the influence of the frequency, size, and duration of wild onion dosage on its benefit to lead-dosed chukars would be highly informative.

Anti-lead sentiments should not be misconstrued as anti-hunting campaigns. Hunting is a viable source of recreational and economic opportunity, and a useful tool for wildlife management and conservation, but lead ammunition is toxic and has no known beneficial function in biological organisms. Nontoxic alternatives are capable and widely
available. Preventable poisoning of protected wildlife species is an unnecessary form of mortality (Neumann 2009).

Unfortunately, nontoxic shot is a very controversial issue with two opposing sides: 1) hunters, who believe anti-lead equates with anti-hunting and an infringement on their rights (Anderson 1992, Schulz et al. 2007), and 2) environmentalists and scientists, who consider lead contamination as a preventable detriment to desirable species and ecosystems. The benefits of the nontoxic regulations of 1991 to U.S. waterfowl populations are voiced by the following authors: 1) Anderson et al. (2000) attributed an estimated 64% reduction in mortality from lead poisoning to the switch from lead to nontoxic shot, which consequently spared an estimated 1.4 million ducks in the 1997 fall continental flight, 2) Thomas (2009) stated that the adoption of nontoxic shot for waterfowl has been the most effective tool used by the individual hunter for the conservation of waterfowl in North America, and 3) both Anderson et al. (2000) and Thomas and Guitart (2005) state that the contribution of the switch to nontoxic shot to the survivorship of birds exceeds that of continental habitat manipulations and improvements. Ultimately, the problem of lead ingestion and concomitant toxicosis can only be completely eliminated through the implementation of additional nontoxic regulations for hunting and shooting.

**LITERATURE CITED**


APPENDIX
Justin Bingham
Utah State University
5230 Old Main Hall
Logan, UT 84322-5230

Dear Justin,

You have my permission and encouragement to use in your M.S. Thesis any of the manuscripts or publications we have been working on. You led out in the initiation of research, data analyses and composition of the manuscripts and thus it is most appropriate that this work be included in your thesis.

Sincerely yours,

Randy Larsen, Assistant Professor of Wildlife and Wildlands Conservation, Brigham Young University.