PREVALENCE OF ANTICOAGULANT RODENTICIDES IN FERRUGINOUS HAWK NESTLINGS AND EVALUATION OF A NOVEL METHOD TO RAPIDLY ASSESS EXPOSURE

by

Ariana Joyce Dickson

A thesis

submitted in partial fulfillment

of the requirements for the degree of

Master of Science in Raptor Biology

Boise State University

August 2020

BOISE STATE UNIVERSITY GRADUATE COLLEGE

DEFENSE COMMITTEE AND FINAL READING APPROVALS

of the thesis submitted by

Ariana Joyce Dickson

Thesis Title: Prevalence of Anticoagulant Rodenticides in Ferruginous Hawk Nestlings and Evaluation of a Novel Method to Rapidly Assess Exposure

Date of Final Oral Examination: 08 May 2020

The following individuals read and discussed the thesis submitted by student Ariana Joyce Dickson, and they evaluated her presentation and response to questions during the final oral examination. They found that the student passed the final oral examination.

James R. Belthoff, Ph.D.	Co-chair, Supervisory Committee
Todd E. Katzner, Ph.D.	Co-chair, Supervisory Committee
Kristen A. Mitchell, Ph.D.	Member, Supervisory Committee

The final reading approval of the thesis was granted by James R. Belthoff, Ph.D. and Todd E. Katzner, Ph.D., co-chairs of the Supervisory Committee. The thesis was approved by the Graduate College.

ACKNOWLEDGMENTS

This research could not have been possible without all of the amazing support that I received from incredible people and organizations during this process. First and foremost, I wish to express my deepest gratitude towards my two advisors, Dr. Jim Belthoff and Dr. Todd Katzner, for their unwavering guidance and advice throughout these past three years at Boise State University. They both inspired, challenged, and helped me to achieve lofty goals in not just my research but also in my personal and professional development. I cannot imagine a better duo to serve as mentors and help me succeed in graduate school. I also want to express appreciation for my committee members: Drs. Kristen Mitchell and Brian Smith. Dr. Mitchell provided me with instrumental background toxicology knowledge that served as the backbone for my research and contributed novel perspectives with respect to my research questions and pushed me to be more interdisciplinary and incorporate translational science. I am deeply indebted to Dr. Smith for being the catalyst for this research, his continuous support, providing me with the opportunity to work on such a transformative project, and for being an ex-officio member of my thesis committee. I would like to extend my sincere thanks to my funding sources: The United States Fish and Wildlife Service and the Raptor Research Center at Boise State University.

I am extremely thankful for the field assistance that I received; without such support I would not have been able to collect samples from three different states. I cannot begin to express my thanks to Matt Stuber who took me under his wing, provided me

with the practical skills to succeed in raptor research, and was always ready to help at the drop of a hat in any way possible. Thank you also to Steve Alsup, Talia Jolley, Jamie Yurick, Joe Weldon, and the Birds of Prey National Conservation Area Partnership, for your friendship, your dedication towards ferruginous hawk conservation, and for crucial field assistance. I am also extremely grateful to Patricia Ortiz for helping me secure and amend numerous permits, spending countless hours organizing, archiving and shipping samples, and overall being a pillar of support during my research. I also extend many thanks to Zach Wallace and the Wyoming Natural Diversity Database, for helping me locate and access ferruginous hawk nests, and for supporting and guiding me in the field. I also extend heartfelt thanks to Mike Lockhart, who opened up his home to my field technicians and provided an immense amount of field support and knowledge. This research would not have been possible without the help of my field assistants: Leticia Camacho, Georgia Isted, and Bailey Walker. Thank you for the many hard and long hours you contributed to this project, especially when it came to carrying ladders and heavy batteries in the desert heat, and your continued friendship. To Georgia, I am incredibly thankful that you came across the pond to work in the Katzner lab for a year; your positivity and passion for raptor conservation inspired me, and I'll always cherish the adventures we shared. I would also like to extend my deepest gratitude to my lab mate and friend, Pat Dumandan. Not only did Pat play a vital role in collecting samples in the field, but she made the hard times enjoyable, especially by supplying the best puns, and aiding me in numerous ways within graduate school. I also extend my thanks to Aran Meyer, Mike Sherman, Miranda Middleton, Cooper Mark, the City of Fort Collins and Colorado Parks and Wildlife for providing field assistance and advice. Thank you to

Gregory Kaltenecker and the Intermountain Bird Observatory for assistance in collecting samples from migratory birds of prey.

Special thanks go to Dr. Barnett Rattner for his insightful suggestions and extensive knowledge of clotting time assays and anticoagulant rodenticides; to Dr. Marjory Brooks and the Comparative Coagulation Section of the Animal Health Diagnostic Center at Cornell University for their advice and analyses; Dr. Cat Barr; Dr. Peter Jowett; the Louisiana State University Animal Disease Diagnostic Laboratory; the Texas A&M Veterinary Medical Diagnostic Laboratory; and the Veterinary Diagnostic Laboratory at Michigan State University for assistance in sample analyses.

I wish to acknowledge the immense amount of support and love that I received from my family. They provided me with unwavering encouragement, patience and guidance throughout these past three years. To my parents, I thank you for always supplying me with the courage and foundation to pursue my dreams. I would also like to thank my boyfriend, Ty Traver, for his patience, assistance with coding, and his inexplicable ability to cheer me up. I'm also incredibly thankful for the friendships that I developed during this time with my fellow graduate students. They provided me with a strong sense of community here in Boise and subsequent support system. I am extremely grateful to all of the aforementioned people and organizations throughout this project, and I hope that I will be given the opportunity to pay it forward.

ABSTRACT

Anticoagulant rodenticides (ARs) are compounds commonly used to control rodent pests by inhibiting an enzyme critical for synthesis of clotting factors in their blood. Secondary and tertiary poisoning of non-target species frequently occur, especially of predators that consume rodents, including many species of raptors. Although raptor exposure to ARs has been documented on at least three continents, patterns, pathways and the sub-lethal effects of exposure are not well studied. This has created a substantial need to monitor the effects of ARs in free-living populations. I evaluated the prevalence of secondary, non-target exposure to anticoagulant rodenticides experienced by a predatory raptor, and I tested the performance and suitability of technology originally developed for human patients taking oral anticoagulant drugs as a novel way to rapidly assay for AR exposure in free-living raptors.

To assess the risk of ARs to birds of prey in the western United States, I surveyed ferruginous hawk nestlings (*Buteo regalis*) in Idaho, Wyoming and Colorado. These hawks inhabit shrub steppes, grasslands, and deserts, many of which are modified by agriculture, wind power, and oil and gas development. Rodenticides are often deployed in or near developed areas to reduce numbers of burrowing mammals. Targeted species include ground squirrels (*Urocitellus* spp.) and prairie dogs (*Cynomys* spp.), which often compose a large proportion of ferruginous hawk diet. I evaluated the prevalence and concentrations of eight different ARs from 173 blood samples from ferruginous hawk nestlings at 60 nest sites in 2018 and 2019. I also collected 117 citrated plasma samples

and analyzed them for biomarkers of AR exposure and indicators of sample quality: prothrombin time (PT), thrombin time (TCT), and fibrinogen concentration. To elucidate possible exposure pathways, I collected and analyzed 54 liver samples from hawk prey and four livers from opportunistically collected dead hawks. There were no ARs detected in any hawk blood or livers, but brodifacoum was present in one rodent liver at a minute concentration (0.003 ppm). Prothrombin time (PT) of 117 hawk nestlings averaged 29.8 ± 4.8 (SD) seconds (range: 21.3 – 41.2 sec). Sex was a strong predictor of PT, with female nestlings exhibiting longer PT. These findings aid in understanding the risk of AR exposure of ferruginous hawk nestlings in Idaho, Wyoming and Colorado and contribute important baseline information on PT of wild birds.

I also evaluated the potential of a point-of-care device, the Coag-Sense® PT/INR Monitoring System manufactured by CoaguSense Inc. (Fremont, California), to rapidly detect AR exposure in living birds of prey. The Coag-Sense® device delivered repeatable (i.e., precise) PT measurements on avian blood samples collected from four species of migrating raptors (Intraclass Correlation Coefficient > 0.9). However, PT measurements reported by the Coag-Sense system from 81 ferruginous hawk (*Buteo regalis*) nestlings were not correlated ($r = -0.017$) to those measured by standardized laboratory techniques (i.e., the accuracy of the Coag-Sense® was low). The Coag-Sense® device therefore did not accurately measure PT in this species of bird and is unlikely to do so in other birds of prey, perhaps because it uses mammalian rather than avian thromboplastin as an activator of clotting. However, this device has potential use on non-human mammals.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AR	Anticoagulant rodenticide
FGAR	First generation anticoagulant rodenticide
SGAR	Second generation anticoagulant rodenticide
EPA	United State Environmental Protection Agency
PT	Prothrombin time
LD_{50}	Median lethal dose
INR	International normalized ratio
TCT	Thrombin clotting time
$t_{1/2}$	Half-life
HPLC	High-performance liquid chromatography
LC/MS/MS	Liquid chromatography with tandem mass spectrometry
CV	Coefficient of variation
LOD	Limit of detection
VIF	Variance inflation factor
AIC	Akaike information criterion
POC	Point-of-care
ISI	International Sensitivity Index

THESIS INTRODUCTION

Rodents have associated with humans since as early as the Neolithic era (van den Brink et al., 2018). They are seen as pests because of their effects on food and agricultural crops, their ability to act as reservoirs of disease, and their potential to damage human structures (van den Brink et al., 2018). Chemical compounds to reduce rodent populations (rodenticides) have been used for more than a century, and they remain in heavy use (Buckle and Smith, 2015; van den Brink et al., 2018). Anticoagulant rodenticides (ARs), or compounds that prevent blood from clotting, are the most commonly selected method to reduce commensal pests (van den Brink et al., 2018).

After the discovery of the hemorrhagic properties of dicoumarol in the 1940s, numerous synthetic analogs were developed and registered for use (Hadler and Buckle, 1992; Duxbury and Poller, 2001). In 1952, the drug warfarin was the first of the analogs to be registered as a rodenticide, and it quickly became the main compound used for rodent control (Duxbury and Poller, 2001). ARs work in the liver by competitively inhibiting an enzyme that is essential to the clotting factor synthesis cycle (Hadler and Buckle, 1992; Duxbury and Poller, 2001). This cycle starts with vitamin K, which is used to produce blood clotting factors II, VII, IX, and X through gamma-carboxylation. Afterwards, vitamin K is left in the inactive form, 2,3-epoxide. The epoxide reductase enzyme then reactivates 2,3-epoxide into the active form, allowing clotting synthesis to continue (Hadler and Buckle, 1992). When ARs are present, epoxide reductase enzyme activity is inhibited, hindering the recycling of vitamin K, thus critically reducing clotting

factor levels until hemorrhaging ensues (Hadler and Buckle, 1992). Because of this mode of action, there is a time delay between when animals ingest the AR and when toxicity occurs. In the case of accidental poisoning, the effects of AR can be reversed via treatment with supplemental vitamin K (Hadler and Buckle, 1992). Moreover, bait-shy rodents do not experience distressing symptoms after initially ingesting the toxicant, ensuring that they feed repeatedly on the bait, and eventually allowing for a lethal dose to be consumed (Hadler and Buckle, 1992). Additionally, poisoning with ARs was once seen as relatively humane (Rowsell et al., 1979), though that perception has changed in recent years (Littin et al., 2014).

There are two major groups of ARs that are delineated by their chemical structure: hydroxycoumarins and indandiones (Valchev et al., 2008). *Hydroxycoumarins* have a 4-hydroxycoumarin ring with a side chain group attached to the 3-position, whereas *indandiones* have a 1,3 indanedione structure with a side chain group attached at the 2-position (Murphy and Lugo, 2015). Hydroxycoumarin compounds are more commonly employed and include coumachlor, coumafuryl, coumatetralyl, warfarin, brodifacoum, bromadiolone, difencoum, difethialone and flocoumafen (Valchev et al., 2008). Indandione compounds include chlorophacinone, diphacinone, pindone and valone (Valchev et al., 2008). These rodenticides are further classified as either "first generation anticoagulant rodenticides" (FGARs), which are older and require multiple doses to be lethal, or "second-generation anticoagulant rodenticides" (SGARs), which are newer and only need a single-dose or "single-feed" to be effective (Valchev et al., 2008; van den Brink et al., 2018). FGARS include warfarin, pindone, coumafuryl, coumachlor, isovaleryl indanedione, chlorophacinone, and diphacinone and represent the initial ARs

developed. Because of heavy use of ARs beginning in the 1950's, rodents built up a resistance to the original FGAR analogs, so effectiveness waned; thus, new SGAR derivatives were developed (Buckle and Smith, 2015). SGARs are all hydroxycoumarin compounds and include bromadiolone, difenacoum, brodifacoum, flocoumafen, and difethialone (Valchev et al., 2008; van den Brink et al. 2018). In comparison to FGARs, SGAR compounds have higher overall potency, a greater affinity for liver binding sites, and a longer half-life in animal tissues (Eason et al., 2010; Laakso et al., 2010). Worldwide, SGARs are now the most widely used chemicals for rodent control (Buckle and Smith, 2015).

Such heavy and widespread use of ARs, especially SGARs, has led to heightened concerns about hazards to non-target wildlife. In fact, this use has frequently led to the unintentional exposures of non-target animals, especially raptors, belonging to numerous taxa and in widely distributed geographic locales, including New Zealand, Malaysia, Africa, Denmark, Norway, France, Spain, the United Kingdom, and North America (Salim et al., 2014; Nakayama et al., 2019; Serieys et al., 2019). Most commonly, non-target wildlife is exposed by directly consuming bait containing anticoagulant rodenticides; this is called primary exposure (Valchev et al., 2008). Another route is the ingestion of contaminated prey (dead or alive), called secondary exposure, which is likely how raptors encounter these toxicants (Valchev et al., 2008; Horak et al., 2018). Because there is a time delay from when the target pest species consumes a lethal dose to when that individual finally perishes, the rodent may continue feeding and accumulating ARs, ultimately reaching a concentration many times greater than what is needed to cause mortality (Stansley et al., 2014). Even after death, SGARs are particularly persistent in

tissues, resulting in the accumulation of toxicants. This increases the potential for secondary exposure to non-target predatory wildlife (Stansley et al., 2014). Additionally, ARs alter rodent behavior, rendering them more susceptible to predation (Cox and Smith, 1992). Less frequent routes of exposure include direct skin contact (Spiller et al., 2003) and through drinking water contaminated with the toxicants (Valchev et al. 2008; Gómez-Canela et al., 2014; Kotthoff et al., 2019).

For non-target avian species that have been tested, the median lethal dose (LD_{50}) varies by compound and species but has been found to range from 0.26 - 3,158 mg/kg after exposure (Nakayama et al., 2019). Compared to rodents and some other species of birds, raptors appear to be more sensitive and susceptible to ARs, which may help explain the higher frequency of reported AR poisonings for this group. For instance, one such AR, diphacinone, is 20 - 30 times more acutely toxic to American kestrels (*Falco sparverius*) than to mallards (*Anas platyrhynchos*; Rattner et al., 2011). Possible reasons behind the increased susceptibly of raptors include decreased rates of AR metabolism by both cytochrome P450 and vitamin K 2,3-epoxide reductase inhibition, longer elimination times, and the additional binding of ARs to proteins (Horak et al., 2018; Nakayama et al., 2020).

Less understood are the effects of non-lethal doses of ARs to non-target wildlife at both a population and individual level. Some previously discovered sub-lethal effects include lower occupancy rates, decreased breeding performance, decreased feeding activity, increased sensitivity to further exposure, hemorrhage following injury, induced neuropathology, and immunosuppression (Mosterd and Thijssen, 1991; Eason et al., 1999; Salim et al., 2014; Serieys et al., 2015; Kalinin et al., 2017).

The U.S. Environmental Protection Agency (EPA) concluded that SGARs pose a substantial risk to non-target wildlife and issued a risk mitigation decision for ten different rodenticides (US EPA, 2008). This decision halted the sale of brodifacoum, difenacoum and difethialone in retail outlets beginning in June 2011. Currently, SGARs are no longer registered for use in products geared towards residential consumers and are only available to licensed pest controllers (US EPA, 2008). To apply SGARs, the EPA has a requirement for bait to be bought in bulk at a minimum of 16 pounds (7.3 kg) by professional users, minimizing the general public's access to these toxicants. In addition, the EPA has issued site restrictions for the application of SGARs, including using bait stations when applying for outdoor, above-ground use to help limit non-target wildlife exposure (EPA, 2017).

Even with these restrictions, SGARs are still heavily used by pest management professionals. In Massachusetts, 97% (n = 35) of companies that provide rodent control services reported using chemical rodenticides, with the SGAR bromadiolone being most commonly used (57%, n = 30) in 2015 (Memmott et al., 2017). FGARs, such as chlorophacinone and diphacinone, are still commercially available to the general public in ready-to-use bait stations that are labeled for use indoors or outdoors within 50 feet (15.2 m) of buildings (EPA, 2017).

Rodents are also pests in agricultural settings where they cause an estimated $50 billion annually in crop damage, so rodenticides are commonly used to limit losses (Eason et al., 2010). Even though data on sales in the AR market are largely considered confidential, there is some indication of how prevalent and widespread ARs are used around agriculture. For example, there were more than 450 tons of AR product sold for

agricultural purposes in California in 2007 alone (Rattner et el., 2014). Two FGARs, Rozol® (chlorophacinone) and Kaput-D® (diphacinone), are registered in 10 states to control black-tailed prairie dog (*Cynomys ludivicianus*) populations (Vyas et al., 2017). In Colorado, approximately 216 kg of Rozol® was applied to 4,080 black-tailed prairie dog colonies in 2010 (Vyas et al., 2013). To mitigate the potential effects that these poisoned prairie dogs (*Cynomys* spp.) have on non-target predators, the EPA mandates that the applicators must return to the site multiple times to collect and dispose of carcasses (EPA, 2017). The degree to which applicators adhere to this regulation is unclear, and there is evidence that this rule is sometimes ignored (Vyas et al., 2013; Memmott et al., 2017). Currently, there is still a high dependence on ARs for rodent control, variation in levels of awareness of the risk that ARs pose to non-target wildlife, and inconsistences in AR regulation adherence (Memmott et al., 2017).

Thus, given the widespread and heavy use of rodenticides in residential, agricultural, and rangeland systems, there is a huge need to monitor their potential effects on non-target wildlife populations. My thesis consists of two chapters focused on (1) the prevalence of non-target AR exposure of a predatory bird of prey, and (2) an investigation of a rapid assessment tool for AR exposure of free-living raptors.

Chapter 1 summarizes field surveys I conducted in the western United States to characterize non-target anticoagulant rodenticide exposure of free-living raptors. My overarching goal was to describe the interplay among raptor behavior, rodent type and behavior, land cover, and anthropogenic factors in non-target exposure to ARs. I used free-living ferruginous hawks (*Buteo regalis*) breeding in Idaho, Wyoming, and Colorado as a case study of a species potentially at risk of secondary AR poisoning. Ferruginous

hawks are rodent specialists that feed primarily on ground squirrels (*Urocitellus* spp.) and prairie dogs (*Cynomys* spp.), both of which are common pest targets for ARs (Giovanni et al. 2007; Ng et al., 2017). In fact, ferruginous hawks preferentially forage at AR-treated black-tailed prairie dog colonies (Vyas et al., 2017), indicating that this hawk species is at risk for secondary exposure. I used three diagnostic metrics to assess the risk of AR exposure to ferruginous hawk nestlings: whole blood assays, assays of opportunistically collected livers from hawks and hawk prey, and clotting-time assays (prothrombin time, PT).

In my second chapter, I evaluated the ability of a point-of-care (POC) device originally developed for human use to rapidly assess AR exposure within birds of prey. There is a clear need to develop methods for rapid assessment of AR exposure in birds of prey because of the potential impacts of ARs on non-target wildlife. Furthermore, an easily deployed AR assay will aid in understanding the prevalence and sub-lethal effects of AR exposure in raptor populations (Hindmarch et al., 2019). Human patients on oral anticoagulant therapy commonly take warfarin, a FGAR, and they must be closely monitored (Wells et al., 2007). To improve safety and decrease cost to patients, multiple POC devices have been developed to measure PT, a metric used to adjust drug dosage (Wells et al., 2007). PT is also a useful biomarker of AR exposure in birds of prey (Murray and Tseng, 2008; Rattner et al., 2010; Hindmarch et al., 2019). Specifically, I tested the precision and accuracy of PT estimates from the Coag-Sense® PT/INR Monitoring System manufactured by CoaguSense Inc. (Fremont, California), one such POC device, using avian blood samples.

My studies were approved by the Boise State University Animal Care and Use Committee (protocols #006-AC18-003 and #AC17-015), and all of the appropriate biosafety protocols (protocol #IBC18-006) and State and Federal permits were obtained to conduct this research (Colorado Parks and Wildlife scientific collection license #19trb2091a, state of Idaho wildlife collection /banding/ possession permits #110728 and #990121, Wyoming Chapter 33 permit #729, US Geological Survey Federal Bird Banding Permits #23715 and #22929).

References

Buckle, A.P., and Smith, R.H. (Eds.), 2015. Rodent pests and their control, 2nd edition. CAB, Wallingford, Oxfordshire; Boston, MA, USA.

Cox, P., and Smith, R.H., 1992. Rodenticide ecotoxicology: Pre-lethal effects of anticoagulants on rat behavior. Proceedings of the Fifteenth Vertebrate Pest Conference. 15,165–170.

Duxbury, and B.M., Poller, L., 2001. State-of-the-art review: The oral anticoagulant saga: past, present, and future. Clinical Applied Thrombosis/Hemostasis. 7, 269–275.

Eason, C.T., Milne, L., Potts, M., Morriss, G., Wright, G.R.G., Sutherland, O.R.W., 1999. Secondary and tertiary poisoning risks associated with brodifacoum. New Zealand Journal of Ecology. 23, 7.

Eason, C.T., Fagerstone, K.A., Eisemann, J.D., Humphrys, S., O'Hare, J.R., Lapidge, S.J., 2010. A review of existing and potential New World and Australasian vertebrate pesticides with a rationale for linking use patterns to registration requirements. International Journal of Pest Management. 56, 109–125.

EPA. 2017. Restrictions on rodenticide products. Environmental Protection Agency. URL https://www.epa.gov/rodenticides/restrictions-rodenticide-products. (accessed Dec 3 2017).

Giovanni, M.D., Boal, C.W., Whitlaw, H.A., 2007. Prey use and provisioning rates of breeding ferruginous and Swainson's hawks on the southern Great Plains, USA. The Wilson Journal of Ornithology. 119, 558–569.

Gómez-Canela, C., Barata, C., Lacorte, S., 2014. Occurrence, elimination, and risk of anticoagulant rodenticides and drugs during wastewater treatment. Environmental Science and Pollution Research. 21, 7194–7203.

Hadler, M.R., and Buckle, A.P., 1992. Forty-five years of anticoagulant rodenticides - past, present and future trends. Proceedings of the Fifteenth Vertebrate Pest Conference. 15, 149–155.

Hindmarch, S., Rattner, B.A., Elliott, J.E., 2019. Use of blood clotting assays to assess potential anticoagulant rodenticide exposure and effects in free-ranging birds of prey. Science of the Total Environment. 657, 1205–1216.

Horak, K.E., Fisher, P.M., Hopkins, B., 2018. Pharmacokinetics of anticoagulant rodenticides in target and non-target organisms, in: van den Brink, N.W., Elliott, J.E., Shore, R.F., Rattner, B.A. (Eds.), Anticoagulant Rodenticides and Wildlife. Springer Science, New York, New York, USA. pp. 87–108.

Kalinin, S., Marangoni, N., Kowal, K., Dey, A., Lis, K., Brodsky, S., van Breemen, R., Hauck, Z., Ripper, R., Rubinstein, I., Weinberg, G., Feinstein, D.L., 2017. The long-lasting rodenticide brodifacoum induces neuropathology in adult male rats. Toxicological Sciences. 159, 224–237.

Kotthoff, M., Rüdel, H., Jürling, H., Severin, K., Hennecke, S., Friesen, A., Koschorreck, J., 2019. First evidence of anticoagulant rodenticides in fish and suspended particulate matter: spatial and temporal distribution in German freshwater aquatic systems. Environmental Science and Pollution Research. 26, 7315–7325.

Laakso, S., Suomalainen, K., Koivisto, S., 2010. Literature review on residues of anticoagulants in non-target animals. TemaNord. 541, 1–47.

Littin, K.E., Fisher, P., Beausoleil, N.J., Sharp, T., 2014. Welfare aspects of vertebrate pest control and culling: ranking control techniques for humaneness. Revue Scientifique et Technique. 33, 281–289.

Memmott, K., Murray, M., Rutberg, A., 2017. Use of anticoagulant rodenticides by pest management professionals in Massachusetts, USA. Ecotoxicology. 26, 90–96.

Mosterd, J.J., and Thijssen, H.H., 1991. The long-term effects of the rodenticide, brodifacoum, on blood coagulation and vitamin K metabolism in rats. British Journal of Pharmacology. 104, 531–535.

Murphy M.J., Lugo A.M, 2015. Superwarfarins. In: Ramesh, C.G, (Ed.). Handbook of Toxicology of Chemical Warfare Agents, 2nd edition. Academic Press, London, Oxford, UK; San Diego, CA, USA; Waltham, MA, USA.

Murray, M., Tseng, F., 2008. Diagnosis and treatment of secondary anticoagulant rodenticide toxicosis in a red-tailed hawk (*Buteo jamaicensis*). Journal of Avian Medicine and Surgery. 22, 41–46.

Nakayama, S.M.M., Morita, A., Ikenaka, Y., Mizukawa, H., Ishizuka, M., 2019. A review: poisoning by anticoagulant rodenticides in non-target animals globally. Journal of Veterinary Medical Science. 81, 298–313.

Nakayama, S.M.M., Morita, A., Ikenaka, Y., Kawai, Y.K., Watanabe, K.P., Ishii, C., Mizukawa, H., Yohannes, Y.B., Saito, K., Watanabe, Y., Ito, M., Ohsawa, N., Ishizuka, M., 2020. Avian interspecific differences in VKOR activity and inhibition: Insights from amino acid sequence and mRNA expression ratio of VKORC1 and VKORC1L1. Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology. 228, 108635.

Ng, J., Giovanni, M. D., Bechard, M. J., Schmutz, J. K., Pyle, P., 2017. Ferruginous Hawk (*Buteo regalis*). In: Rodewald, P.G.,(Ed), The Birds of North America. Cornell Lab of Ornithology, Ithaca, NY, USA. https://birdsna.org/Species-Account/bna/species/ferhaw.

Rattner, B.A., Horak, K.E., Warner, S.E., Johnston, J.J., 2010. Acute toxicity of diphacinone in northern bobwhite: Effects on survival and blood clotting. Ecotoxicology and Environmental Safety. 73, 1159–1164.

Rattner, B.A., Horak, K.E., Warner, S.E., Day, D.D., Meteyer, C.U., Volker, S.F., Eisemann, J.D., Johnston, J.J., 2011. Acute toxicity, histopathology, and coagulopathy in American kestrels (*Falco sparverius*) following administration of the rodenticide diphacinone. Environmental Toxicology and Chemistry. 30, 1213–1222.

Rattner, B.A., Horak, K.E., Lazarus, R.S., Goldade, D.A., Johnston, J.J., 2014. Toxicokinetics and coagulopathy threshold of the rodenticide diphacinone in eastern screech- owls (*Megascops asio*). Environmental Toxicology and Chemistry. 33, 74–81.

Rowsell, H.C., Ritcey, J., Cox, F., 1979. Assessment of humaneness of vertebrate pesticides. In: Proceedings of the Canadian Association for Laboratory Animal Science. The Canadian Association for Laboratory Animal Science, Calgary, Canada. pp 236-249.

Salim, H., Noor, H.M., Omar, D., Hamid, N.H., Abidin, M.R.Z., Kasim, A., Salim, H., Md Rawi, C.S., Ahmad, A.H., 2014. Sub-lethal effects of the anticoagulant rodenticides bromadiolone and chlorophacinone on breeding performances of the barn owl (*Tyto alba*) in oil palm plantations. Slovak Raptor Journal. 8, 113–122.

Serieys, L.E.K., Armenta, T.C., Moriarty, J.G., Boydston, E.E., Lyren, L.M., Poppenga, R.H., Crooks, K.R., Wayne, R.K., Riley, S.P.D., 2015. Anticoagulant rodenticides in urban bobcats: exposure, risk factors and potential effects based on a 16-year study. Ecotoxicology. 24, 844–862.

Serieys, L.E.K., Bishop, J., Okes, N., Broadfield, J., Winterton, D.J., Poppenga, R.H., Viljoen, S., Wayne, R.K., O'Riain, M.J., 2019. Widespread anticoagulant poison exposure in predators in a rapidly growing South African city. Science of The Total Environment. 666, 581–590.

Spiller, H.A., Gallenstein, G.L., Murphy, M.J., 2003. Dermal absorption of a liquid diphacinone rodenticide causing coagulopathy. Veterinary and Human Toxicology. 45, 313–314.

Stansley, W., Cummings, M., Vudathala, D., Murphy, L.A., 2014. Anticoagulant rodenticides in red-tailed hawks, *Buteo jamaicensis*, and great horned owls, *Bubo virginianus*, from New Jersey, USA, 2008–2010. Bulletin of Environmental Contamination and Toxicology. 92, 6–9.

US EPA, 2008. Risk mitigation decision for ten rodenticides. USEPA office of prevention, pesticides and toxic substances. http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPP-2006-0955-0764. (accessed 17 Feb 2017)

Valchev, I., Binev, R., Yordanova, V., Nikolov, Y., 2008. Anticoagulant rodenticide intoxication in animals – A review. The Turkish Journal of Veterinary and Animal Sciences. 32, 237–243.

van den Brink, N.W, J. Elliott, R. Shore, B. Rattner, (Eds.). 2018. Anticoagulant Rodenticides and Wildlife. Springer Science, New York, New York, USA.

Vyas, N.B., Hulse, C.S., Meteyer, C.U., Rice, C.P., 2013. Evidence of songbird intoxication from Rozol® application at a black-tailed prairie dog colony. Journal of Fish and Wildlife Management. 4, 97–103.

Vyas, N.B., Kuncir, F., Clinton, C.C., 2017. Influence of poisoned prey on foraging behavior of ferruginous hawks. The American Midland Naturalist. 177, 75–83.

Wells, P.S., Brown, A., Jaffey, J., McGahan, L., Poon, M.-C., Cimon, K., 2017. Safety and effectiveness of point-of-care monitoring devices in patients on oral anticoagulant therapy: a meta-analysis. Open Medicine. 1, 131-146.

CHAPTER ONE: ASSESSING ANTICOAGULANT RODENTICIDE EXPOSURE OF FERRUGINOUS HAWK NESTLINGS

Abstract

Through unintentional secondary poisoning, anticoagulant rodenticides (ARs) threaten raptors - especially species that primarily consume rodents. Although exposure to ARs in free-living raptor populations is documented on at least three continents, patterns and pathways of exposure are not well studied, so potential effects of ARs on raptor populations remain difficult to quantify and mitigate. I evaluated the risk of AR exposure of ferruginous hawks (*Buteo regalis*) in Idaho, Wyoming and Colorado in the western United States. These hawks inhabit shrub steppes, grasslands, and deserts, many of which are modified by agriculture, wind power, and oil and gas development. Rodenticides are often deployed in developed areas to reduce numbers of burrowing mammals. Targeted pest species include ground squirrels (*Urocitellus* spp.) and prairie dogs (*Cynomys* spp.), which compose a large proportion of ferruginous hawk diet. Despite the potential for exposure, no AR residues were detected in the 173 blood samples that I collected from ferruginous hawk nestlings at 60 nest sites over two consecutive breeding seasons (2017 – 2018). To elucidate possible exposure pathways, I also opportunistically collected and analyzed 54 liver samples from prey items and four from recently deceased, opportunistically collected ferruginous hawks. Brodifacoum was detected in one prey liver sample (1.9%) from a ground squirrel, but at a minute concentration (0.003 ppm). I also collected 117 citrated plasma samples from nestling

hawks and analyzed them for prothrombin time (PT) which is a biomarker for AR exposure. Prothrombin time averaged 29.8 ± 4.8 (SD) seconds (range: 21.3 – 41.2 sec). PT values were normally distributed, indicating no evidence of AR exposure. Sex was a strong predictor of PT, with female nestlings exhibiting longer PT than males. These findings aid in understanding the risk of AR exposure to ferruginous hawk nestlings in Idaho, Wyoming and Colorado, and contribute important baseline information on PT.

Introduction

Anticoagulant rodenticides (ARs) have been in use globally since the 1950's to control rodent populations (Hadler and Buckle, 1992). ARs work by inactivating vitamin K epoxide reductase in the liver, which disrupts clotting factor synthesis and subsequently the clotting cascade (Rattner et al., 2014). The vitamin K-dependent clotting mechanism is highly conserved in vertebrates, enabling ARs to have toxic effects on non-target species (Doolittle and Feng, 1987; van den brink et al., 2018). In fact, recent studies document exposure of a high percentage of non-target animals of a variety of predator and prey taxa, including those that prey on or scavenge rodents (Nakayama et al., 2019).

Secondary exposure to ARs typically occurs through the ingestion of contaminated prey tissues, leading to the bioaccumulation and biomagnification of ARs within non-target predatory wildlife (Giraudoux et al., 2006). Adding to the risk of secondary exposure to predators, rodents can live 1-3 weeks after ingesting ARs (EPA, 2017). After initial exposure to ARs, rodents experience lethargy, loss of attentiveness, disruption of their internal biorhythm, hemorrhaging, and altered predator responses. The combination of these symptoms makes them easier targets for predatory wildlife (Cox

and Smith, 1992; EPA, 2017). In particular, it appears that species of predators that specialize on rodents, or those that are opportunistic scavengers, are at the highest risk of secondary AR exposure (van den Brink et al., 2018).

The prevalence of AR residues in birds of prey is most frequently documented in the course of a post-mortem evaluation. ARs accumulate and persist within the liver, often resulting in detectable and high concentrations (Vadenbroucke et al., 2008). However, less is known about the ante-mortem effects of AR exposure experienced by individuals in free-living populations. Liver assays are limited in their ability to provide information on potentially subtle sub-lethal effects, or on the timing and frequency of AR exposure events, and they are not applicable for free-living animals (Hindmarch et al., 2019). In contrast, more readily available tissues, such as blood, can be assayed for AR residues, although this method typically only provides information about recent exposure (Martínez-Padilla et al., 2017; Abernathy et al., 2018).

In addition to direct assays for AR compounds in blood plasma, clotting time assays, such as PT, can be effective biomarkers for AR exposure in live animals (Murray and Tseng, 2008; Rattner et al., 2010; Hindmarch et al., 2019). PT measures the functional activity of the common coagulation pathway by calculating the time in seconds that it takes for a fibrin clot to form (Morrisey et al., 2003; Campbell, 2015). PT is measured by adding tissue thromboplastin and calcium to the plasma to activate the clotting cascade, which converts prothrombin to thrombin and subsequently converts fibrinogen to a fibrin clot (Fig 1.1; Morrisey et al., 2003; Rattner et al., 2010). ARs inhibit the vitamin K-dependent synthesis of a series of clotting factors (II [prothrombin], VII, IX and X), resulting in an increased PT (Rattner et al., 2010). PTs greater than 125%

of normal estimates are usually considered to be indicative of AR exposure (Shlosberg and Booth, 2006).

Baseline estimates of PT are not well established for many species (Hindmarch et al., 2019). For those species in which PT has been measured, the estimates can vary widely by study, population and laboratory (Table 1.1). For example, two recent studies estimated mean PT for barn owls (*Tyto alba*) as 8.7 sec and 22.6 sec (Webster et al., 2015; Hindmarch et al., 2019). PT estimates in mammals can vary because of inherent laboratory variation, but also because of vitamin K supply, animal age and sex, time of blood collection, and elevation (Bertolucci et al., 2005; Patot et al., 2006; Lemini et al., 2007; Sakata et al., 2007). However, such influences are less well known for avian populations.

In North America and Europe, Buteo hawks appear to have a relatively high risk of AR exposure (López-Perea and Mateo, 2018). For instance, AR prevalence in red-tailed hawks (*Buteo jamaicensis*), rough-legged buzzards (*Buteo lagopus*), and common buzzards (*Buteo buteo*) was 76%, 84%, and 94%, respectively (Stone et al., 1999; Stone et al., 2003; Murray, 2011; Christensen et al., 2012). Ferruginous hawks (B. *regalis*) in North America are potentially at similar risk for AR exposure but have not been well studied. Ferruginous hawks inhabit open landscapes including shrub steppes, grasslands, canyonlands, and deserts (Bechard and Schmutz, 1995). These open landscapes are important for natural gas development, wind power generation, rangelands, and crop production, and in each case rodent populations are often managed through the application of ARs. Higher levels of AR exposure occur in non-target species that inhabit rangelands, specifically in areas with a high density of cattle (Shore et al., 2006; Geduhn

et al., 2015; López-Perea et al., 2019). Adding to the risk of encountering ARs, ferruginous hawks feed primarily on ground squirrels (*Urocitellus* spp.) and prairie dogs (*Cynomys* spp.), both of which are common targets of ARs (Bechard and Schmutz, 1995; Vyas et al., 2017). Moreover, ferruginous hawks preferentially forage at black-tailed prairie dog (*Cynomys ludovicianus*) colonies treated with ARs, which increases the likelihood of secondary exposure (Vyas et al., 2017).

The objectives of my research were as follows: 1) to assess AR exposure of ferruginous hawks in relation to land cover type and distance to anthropogenic influences, and 2) to elucidate possible exposure pathways by assessing ARs in hawk prey species. To address my first objective, I sampled and assayed whole blood samples for AR residues from ferruginous hawk nestlings across a large geographic area that included three states in the western United States. I also collected citrated plasma samples and assessed clotting time as a biomarker for AR exposure and examined how this metric varied in nestlings in free-living populations. Lastly, I obtained and assayed livers from a small number of dead ferruginous hawks that were opportunistically collected in collaboration with other researchers and wildlife rehabilitators in my study areas to understand if a discrepancy is present when assaying different tissue types (livers vs. blood). To address my second research objective, I collected livers from common species of ferruginous hawk prey within my study areas and assayed them for AR residues to identify prey bases that may increase the risk of secondary exposure to ferruginous hawks.

Methods

Although I also collected samples from adult ferruginous hawks and their prey, I primarily focused my survey of AR exposure on nestlings for a number of reasons. First, exposure to contaminants during the reproduction and nestling stage can be the most consequential (Herring et al., 2017). For instance, exposure to ARs during the nestling stage could lead to decreased bone density and poor healing, ultimately manifesting in low body condition (Knopper et al., 2007). Secondly, nestlings have large energy needs during growth, which can result in a high level of rodent consumption (Keeley and Bechard, 2017). This increases the probability that nestlings could encounter contaminated prey. Next, nestlings are only in the nest for a short period of time, generally 38-50 days (Howard, 1975; Ng et al., 2017). The plasma half-life ($t_{1/2}$) of ARs is substantially shorter than in liver tissue, resulting in a short window of detection (Martínez-Padilla et al., 2017; Abernathy et al., 2018). The plasma $t_{1/2}$ depends on individual compound and species, but for mice it ranges from 20.4 – 91.7 d (Nakayama et al., 2019). The amount of time that the nestlings are in the nest is likely to overlap with the plasma $t_{1/2}$ of many AR compounds, providing an accurate reflection of AR exposure during this life-stage and reducing the chance of false negatives. Finally, nestlings are logistically easier to capture than adults.

Study Area

I monitored ferruginous hawk nestlings in southwestern Idaho, southern and central Wyoming, and in a small portion of northeastern Colorado (Fig. 1.2) between May and July in both 2018 and 2019. I sampled hawks from nests on artificial nest platforms, in trees, on the ground, and on other human structures. The nests were located

on both public and private lands in a variety of landcover types, and they varied in their distances to agriculture, energy development, rangelands, and anthropogenic infrastructure. Hawks nesting in Wyoming and Colorado had access to prairie dogs (*Cynomys* spp.) as a primary prey species, whereas in Idaho where prairie dogs do not inhabit, hawks fed primarily on ground squirrels (*Urocitellus* spp.).

Whole Blood Sample Collection and AR Analysis

In both 2018 and 2019, I hand captured ferruginous hawk nestlings after accessing nests with ladders or climbing gear, temporarily removed them from the nest, and restrained them in canisters for blood collection and measurements. I banded each nestling with a U.S. Geological Survey metal leg band and assigned age and sex (when possible) based on morphology (Moritsch, 1985; Gossett, 1993). To ensure that samples were <1% of body mass, only nestlings >0.2 kg were sampled. I used a 25-ga butterfly needle to collect 2 ml of whole blood from the brachial vein, transferred the blood into a 3-ml tube with EDTA (k3), inverted the tube several times for mixing, and kept the blood on ice in a cooler in the field until transfer into a refrigerator for longer term storage at 4°C.

When nestlings were too small for 2 ml to be collected, such as earlier in the breeding season when they were younger, I collected 0.5 ml from each nestling in a single nest and pooled the blood to gain an understanding of the nest-level exposure to ARs. The nestlings that contributed to these pooled samples were later resampled in the same year when they were older and larger, and 2 ml of blood was collected at this time. I also opportunistically collected whole blood from adult ferruginous hawks that other

researchers in my study areas captured using a modified dho ghaza with a mechanical owl lure (Bloom et al., 2007; Jensen et al., 2019).

All whole blood samples were analyzed for the presence of bromadiolone, brodifacoum, difethialone, difenacoum, chlorophacinone, diphacinone, coumatetralyl, pival and warfarin. Analysis was by high-performance liquid chromatography (HPLC) with fluorescence UV detection and mass spectrometry and/or liquid chromatography with tandem mass spectrometry (LC/MS/MS) and was conducted at the Louisiana Animal Disease Diagnostic Laboratory and the Texas A&M Veterinary Medical Diagnostic Laboratory, which are both fully accredited by the American Association of Veterinary Laboratory Diagnostics.

Prothrombin Time, Thrombin Clotting Time, and Fibrinogen Concentration

In 2019, I also collected citrated plasma samples as described by Hindmarch et al. (2019) for analysis of PT, thrombin clotting time (TCT), and fibrinogen concentration. TCT measures the time it takes for fibrinogen to be converted into fibrin after thrombin is added to the plasma. TCT is then used to calculate fibrinogen concentration (Fig. 1.1; Rattner et al., 2010). Fibrinogen concentration is insensitive to vitamin K clotting factor deficiencies, meaning that it is not affected by AR exposure. As such, it is a measure of sample quality because its concentration in blood can be reduced by improper sample collection and handling (Rattner et al., 2010; Hindmarch et al., 2019). Lower fibrinogen concentration also can prolong prothrombin time separate from an AR exposure event and therefore help differentiate a false positive (Hindmarch et al., 2019). Typically, any sample that has a fibrinogen concentration >50 mg/dL is able to support clot formation (Hindmarch et al., 2019).

To collect blood for these assays, I used a 1-ml syringe previously loaded with 0.1 ml of 3.2% sodium citrate to draw 0.9 ml of whole blood from the brachial vein in the wing. Immediately after collection, the blood was transferred into a 1.5 ml microcentrifuge tube, gently inverted several times and kept on ice until all of the nestlings in a nest had been processed. Samples were then centrifuged in the field at 2,000 x *g* for 10 min. The resultant plasma (usually 400-600 µl) was immediately transferred into 1.2 ml cryovials and promptly stored frozen inside a cryoshipper containing liquid nitrogen vapors. Cryovials were kept in the cryoshipper until transfer into a -80°C freezer. Blood samples collected for PT analysis using sodium citrate were collected before whole blood samples for AR analysis were drawn. The citrated plasma was shipped on dry ice to the Comparative Coagulation Laboratory in the Animal Health Diagnostic Center at Cornell University (Ithaca, NY) for analysis of PT, TCT and fibrinogen concentration following the previously described protocol by Rattner et al. (2010, Appendix 1).

Prey Liver and Ferruginous Hawk Tissue Collection and Analysis

I opportunistically collected liver samples from rodents at 29 sites throughout my study areas (Fig. 1.2). Samples were obtained in two ways: 1) through access to 2 g of liver tissue from Piute ground squirrels collected during another ongoing research project, and 2) by collecting livers from all types of prey items found in ferruginous hawk nests at the time of blood sampling (the remainder of the carcasses was replaced in hawk nests). Livers that I dissected from prey items found in hawk nests were immediately frozen and stored inside a cryoshipper containing liquid nitrogen vapors in the field and kept there until transferred into a -80°C freezer. I also collected livers from three ferruginous hawks

found dead in the field and a fourth that was euthanized by a local wildlife rehabilitator in Idaho (21 March – 24 May 2019). All hawk and prey liver samples were shipped on dry ice to either the Texas A&M Veterinary Medical Diagnostic Laboratory (College Station, TX) or the Veterinary Diagnostic Laboratory at Michigan State University (Lansing, MI) where they were assayed for ARs as above.

Spatial Analysis

Ferruginous hawks have home ranges that average approximately 7.0 km^2 in size, ranging from 3.4 – 21.7 km^2 (Olendorff, 1993). Therefore, using Arc GIS (ESRI, 2015; ArcMap 10.4.1), I defined a 2-km radius circular buffer (12.6 km^2 area) around each ferruginous hawk nest site reflecting a large percentage of the presumptive foraging areas and possible overall home range of adults tending the nest. I then used LANDFIRE (2014) to estimate the existing vegetation types within buffers around each nest site in seven categories: water, tree, sparse, herbaceous, developed, shrub, and agriculture (Table A2.1). I divided the number of cells of each cover type by the total number of cells in the 2-km buffer and multiplied by 100 to obtain percent cover by type. Ground elevation (m) was calculated for each nest site using a Digital Elevation Model (DEM; U.S. Geological Survey, 2017).

Statistical Analyses

All statistical analyses were performed using R software version 3.5.1 (R Core Team, 2018). Because ARs were not detected in birds, I was unable to model the risk of AR exposure as a function of landcover correlates and prey base. Normality of the clotting time data was assessed using the Shapiro-Wilk test. To model the variation of PT as a function of ecological factors, I created competing linear-mixed effects models using

the package “lme4” (Bates et al., 2015). In these models, mass, sex, age, percent shrub cover, percent herbaceous cover, elevation, and time of blood collection were predictor variables with PT as the response variable. I used nest site as a random effect to account for the dependency among observations as multiple nestlings were frequently sampled at each nest (Fig. A2.1). Multicollinearity among predictor variables was assessed by computing variance inflation factors (VIF) using the package “car” (Fox and Weisberg, 2019). Variables that had a variance inflation factor greater than five were removed from the final model set. Final fixed covariates (Table 1.2) included sex, age in days, the elevation of the nest site, the time of day when each blood sample was collected, and percent shrub cover around the nest site. Nestlings in which a putative sex could not be assigned were removed from analysis. Because the continuous predictor variables were at different scales of measurement, I standardized them prior to analysis using the ‘scale’ function in base R, which subtracts the mean and divides by the standard deviation of the data set.

I created a global model that included all five final fixed effects and a random effect for nest site. Next I employed the model selection function “dredge” in the package “MuMIn” (Bartoń, 2019) which generated 32 models with every combination of the five fixed effect terms from the global model (Table 1.3 and Fig. 1.3). I compared the models using Akaike’s information criterion (AIC; Akaike, 1973; Burnham and Anderson, 2002). No model had a majority of weights, indicating high model selection uncertainty (Symonds and Moussalli, 2010). To accommodate for this uncertainty, I then used a full model averaging approach, using the function “model.avg” in the package “MuMIn” (Bartoń, 2019) to estimate PT parameters. Model assumptions were validated by plotting

the residuals against fitted values. Means are given with standard deviations except where indicated.

Results

Whole Blood Sample Collection and AR Analysis

I collected and assayed whole blood samples (Table A2.2) from 60 ferruginous hawk nests in 2018 and 2019 (Table 1.4). Nests were located on artificial structures (n = 52) and on natural substrates (n = 8). At the time of blood collection, estimated age of nestlings was 31 ± 6.3 d (range: 19 – 45 d, n = 171), and they weighed 1.17 ± 0.25 kg (range: 0.71 – 1.72 kg, n = 171). Bromadiolone, brodifacoum, difethialone, difenacoum, chlorophacinone, diphacinone, coumatetralyl, pival and warfarin were not detected in any of the whole blood samples. The limit of detection (LOD) for assays ranged from 0.008 – 0.083 ppm.

Prothrombin Time, Thrombin Clotting Time, and Fibrinogen Concentration

I collected citrated plasma samples from 127 ferruginous hawk nestlings in 2019 from Idaho (n = 57), Wyoming (n = 66) and Colorado (n = 4). I subsequently excluded 10 samples from analysis because they were either hemolyzed or had clot fragments present (Table A2.3). The remaining 117 samples came from 44 nest sites (n = 18, 23, and 3 from ID, WY and CO, respectively). At time of blood collection, these nestlings were 32 ± 6.4 d old (range: 17 – 42 d), weighed 1.2 ± 0.26 kg (range: 0.8 – 1.7 kg), and included 52 putative females, 40 putative males, and 25 individuals for which sex could not be assigned. A subset of 101 samples had sufficient remaining plasma for calculation of fibrinogen concentration. Prothrombin times were normally distributed (Shapiro-Wilk: w = 0.97, p = 0.06), and the coefficient of variation (CV) for PT was 16%. PT was 29.8 ±

4.8 sec (range: 21.3 – 41.2 sec), TCT was 7.1 ± 1.5 sec (range: 1.0 – 17.6 sec) and fibrinogen concentration was 117.0 ± 21.8 mg/dL (range: 67.0 – 178.0 mg/dL).

The model results suggested that PT was higher in females than in males and that sex class was the best predictor for PT (Table 1.5). This model predicted an average PT of 31.66 sec (95% CI 30.33 – 33.00) for female nestlings versus 28.39 sec for males (95% CI = 25.48 – 31.31). There was no association between PT and any of the other predictor variables as their confidence intervals for parameters overlapped zero (Table 1.5).

Prey Liver and Ferruginous Hawk Tissue Collection and Analysis

I analyzed 54 prey livers collected from a total of 29 sites (n = 18, n = 10, n = 1 from ID, WY and CO, respectively; Table 1.6), 13 of which were hawk nests. No ARs were detected prey livers collected directly from ferruginous hawk nests or in the majority of livers collected outside of hawk nests. However, brodifacoum was detected in one sample (1.9%) from a Piute ground squirrel collected in Idaho, but at very low concentration (0.003 ppm, LOD = 0.002 ppm). There were no AR residues detected in the four ferruginous hawk livers analyzed (LOD varied by compound; range: 0.005 – 0.020 ppm).

Discussion

Though I was able to survey a broad geographic area and evaluate a number of different markers for exposure, I detected no evidence of AR exposure in ferruginous hawk nestlings in my study areas. Only one liver sample from a prey species contained ARs, and this was at a minute concentration. No ARs were detected in prey collected directly from hawk nests and all other livers from potential species tested. In evaluating

PT of ferruginous hawks, the low CV (16%) and normally distributed data also support the conclusion that AR exposure was absent. Despite no apparent AR exposure in nestlings during the breeding season, my study provided important baseline information about PT in ferruginous hawks and factors associated with PT variability.

Sex was a strong predictor of PT of nestling hawks, such that females had longer clotting times than males. This trend was not seen in the PT measurements of raptors admitted to a rehabilitation center (Hindmarch et al., 2019), but it parallels patterns in humans and rodents (Lemini et al., 2007; Sakata et al., 2007). This raises the question of whether wild female raptors may disproportionately be affected by AR exposure. That is, if the baseline PT is higher for females as compared to males, then an encounter with an AR that increases PT may push the PT of females into a lethal range with a smaller dose. To the best of my knowledge, this concept has not been explored through research but could result in sex-biased mortality from AR exposure.

My study provided a snapshot of exposure profiles within a specific age structure and season for ferruginous hawks. The substantial differences between my findings, where there was little indication of AR exposure, and those from previous research, where AR exposure in the *Buteo* genus ranges from 44% to 95% (López-Perea and Mateo, 2018), highlights how much remains unknown about AR prevalence, use and effects. For instance, despite an indication of low exposure during the nestling phase, it is possible that AR use varies seasonally, putting ferruginous hawks at risk of encountering these toxicants along their migration route or in their wintering grounds. In fact, Rozol®, a FGAR, is registered in 10 states in the U.S. to control black-tailed prairie dogs from 1 October to 15 March (Vyas et al., 2017). In Europe, the number of AR-poisoned

predators increases in late fall and early spring in conjunction with seasonal application of ARs (Berny et al., 1997; Berny and Gaillet, 2008). In Northern Ireland, most applications occur in the fall and winter (Tosh et al., 2011). This highlights the importance of additional studies to continue to assess the risk of ARs to ferruginous hawks including outside of the breeding season.

Prothrombin Time as a Biomarker for AR Exposure

The PT estimates recorded in ferruginous hawk nestlings in my study were longer than most reported for other avian species (Table 1.1; Fig. 1.4). There was adequate fibrinogen concentration present in the samples, indicating that sample quality was good, so the longer PT values I observed were not caused by improper collection or handling. Outside of sample quality, there are several possible explanations for this observed pattern.

First, variability in PT among species is likely explained in part by inherent differences in coagulation enzymes. For instance, PT in chickens (*Gallus gallus domesticus*) is shorter, ranging from 8.4-13.9 seconds (Stopforth, 1970; Doerr et al., 1975; Morrisey et al., 2003) than brown kiwis (*Apteryx australis mantelli*), which averaged 24.3 sec (Morrisey et al., 2003). Ostriches (*Struthio camelus*) have even longer PT, averaging 73.0 sec, which is suspected to be the result of reduced levels of clotting factors II, V, and VIII in the plasma as compared to other species (Frost et al. 1999). Therefore, the longer PT estimates in ferruginous hawk nestlings compared to previously reported PT values in raptors (Fig. 1.4) could be related to inter-species differences. However, significant intra-species variation could also reflect differences in laboratory methodology and thromboplastin reagents, which could obscure inter-species differences.

For example, the PT of ostriches varies substantially when the type of thromboplastin used for the assay was changed. When a chicken-based reagent is employed for ostriches, the mean PT is 90.1 sec in contrast to using a homologous-based reagent, which results in a mean PT of 73.0 sec (Frost et al., 1999). Moreover, when analyzed separately, two studies reported that the mean PT of barn owls differed by about 10 seconds (Webster et al., 2015; Hindmarch et al., 2019). To better understand inter-species variation in coagulation, further standardization of clotting time assays across laboratories is likely needed.

The age of individuals may also contribute to variability in reported PT. Most previous studies have analyzed PT of adult birds, whereas my study focused on nestlings. This difference in life stage (nestlings vs. adults) may partially explain the higher values that I observed in ferruginous hawks compared to other raptor species. This possibility is supported by studies showing that the coagulation system of healthy human infants differs from that of adults, with the number of vitamin K-dependent factors ranging between 25% to 70% of adult values, contributing to prolonged prothrombin times in infants (Andrew et al., 1988). Whether a similar phenomenon occurs in avian species is unknown because of a general dearth of studies.

Next, exposure to ARs can increase prothrombin time (Rattner et al., 2010), although it does not appear to be the underlying factor for the lengthy prothrombin times observed in ferruginous hawk nestlings. Large disparities in PT within a population can indicate AR exposure, which is usually reflected in a higher CV (Webster et al., 2015). For instance, in a controlled AR dosing experiment using Japanese quail (*Coturnix japonica*), the CV of PT for each experiment ranged from 25-115% depending on the

dose, versus a CV of 10.5% in control quail that did not receive ARs (Webster et al., 2015). The CV of PT in a free-ranging, unexposed barn owl population was 14% (Webster et al., 2015). The CV of PT that I found (16%) in ferruginous hawk nestlings is similar in magnitude to the previously mentioned control group of quail and in the unexposed population of owls, which I interpret as arising from inter-individual differences and not AR exposure events.

Another method for assessing if AR exposure has occurred in individuals is based on identifying PT values are greater than 125% of baseline values (Shlosberg and Booth, 2006). However, baseline PT values are not well established for raptor species, including ferruginous hawks, which are rarely kept in captivity. To account for this missing information, Hindmarch et al. (2019) used a more conservative metric (150% of baseline values) to make conclusions about AR exposure in birds of prey. If the median PT (29.1 sec) of the ferruginous hawk nestlings that I measured is assumed to represent a baseline for this species and age structure, then none of the PT values from the current study exceed 150% of that baseline value (43.7 sec), indicating no AR exposure (Fig. 1.4). Rather than using species-specific thresholds for determining AR exposure, Hindmarch et. al (2019) used the median PT estimate (12.4 sec) established from captive American kestrels (*Falco sparverius)* to assess AR poisoning in a number of raptor species admitted to a wildlife rehabilitation center (Fig. 1.4). If I were to apply a similar threshold, then nestlings with a PT >31.0 seconds would be assumed to have been exposed to ARs, and 40 (34%) ferruginous hawk nestlings in my study would meet this criterion. However, the lack of AR residues detected in any liver sample and the small CV of PT in hawk nestlings both suggest no exposure. Thus, simply using a PT baseline value for American

kestrels likely is not appropriate for ferruginous hawks. This highlights the importance of further research evaluating clotting time in various species and the benefits of using a combination of diagnostic methods to examine AR exposure in free-living populations.

Conclusions

My study is among the first to evaluate the risk of AR exposure of free-living ferruginous hawk nestlings during the breeding season in the western United States. Based on the results from the multiple approaches that I used (e.g., assaying blood for AR residues, examining liver, and measuring aspects of clotting time), it appears that ferruginous hawk nestlings were not encountering ARs. However, AR use on the landscape, and therefore AR exposure in ferruginous hawks, could vary temporally, so surveillance is still necessary, especially because of ongoing population declines in this species and its heightened conservation status. My findings also allow managers and researchers to refocus some of their resources on evaluating the risk of AR exposure of ferruginous hawks outside of the breeding season. In addition, the longer prothrombin times I recorded in ferruginous hawk nestlings, a species where information on clotting time parameters was lacking, indicates substantial inter-species variation when compared to previously studied species of raptors.

Assessing the risk of AR exposure of live birds of prey presents challenges, especially given the short half-lives of AR compounds in the blood of animals. Thus, false negatives are possible when assessing AR exposure in wild birds based solely on blood samples. As such, using a combination of diagnostic metrics may be needed to fully assess exposure in free-living populations. In addition to direct assays, approaches can include measuring clotting parameters, like PT, and assaying other biological

materials, such as feces, pellets and eggs (Eadsforth et al., 1991; Giorgi and Mengozzi, 2010; Sage et al., 2010). Honing methodology to assess AR exposure in free-living populations will provide a better understanding of exposure rates to non-target wildlife and potential sub-lethal effects of the toxicants.

Tables

Table 1.1 Reference values for prothrombin time (PT) using avian thromboplastin in avian species.

Common Name	Species	n	Status	Mean PT (SE) sec	Reference
Umbrella cockatoo	*Cacatua alba*	14	Captive	13.6 (1.2)	Morrisey et al. 2003
Hispaniola Amazon parrot	*Amazona ventrali*	6	Captive	10.1 (0.8)	Morrisey et al. 2003
Brown kiwi	*Apteryx australis mantelli*	4	Captive	24.3 (0.2)	Bailey et al. 2005
Ostrich	*Struthio camelus*	30	Captive	73.0 (2.5)	Frost et al. 1999
Japanese quail	*Coturnix japonica*	≥12	Captive	10.0 (0.3)	Belleville et al. 1982
Japanese quail	*Coturnix japonica*	21	Captive	12.5[1]	Webster et al. 2015
Leghorn chicken	*Gallus gallus domesticus*	12	Captive	8.4 (0.1)	Doerr et al. 1975
Leghorn chicken	*Gallus gallus domesticus*	6	Captive	8.7 (2.0)	Morrisey et al. 2003
Leghorn chicken (3 weeks old)	*Gallus gallus domesticus*	1200	Captive	9.4 (0.1)	Doerr et al. 1975
Chicken	*Gallus gallus domesticus*	100	Captive	11.4	Bigland and Triantaphyllopoulos 1961
Chicken (cockerels)	*Gallus gallus domesticus*	8	Captive	12.0	Stopforth 1970
Chicken (capons)	*Gallus gallus domesticus*	15	Captive	13.4	Stopforth 1970

Common Name	Species	n	Status	Mean PT (SE) sec	Reference
Chicken (pullets)	*Gallus gallus domesticus*	15	Captive	13.9	Stopforth 1970
Barred owl	*Strix varia*	13	Free-ranging, admitted to rehab	13.2 (2.9)	Hindmarch et al. 2019
Great horned owl	*Bubo virginianus*	7	Free-ranging, admitted to rehab	9.3 (0.5)	Hindmarch et al. 2019
Eastern screech-owl	*Megascops asio*	40	Captive	9.3 (0.3)	Rattner et al. 2014
Barn owl (pre-fledging)	*Tyto alba*	11	Free-ranging	8.7 (0.5)	Hindmarch et al. 2019
Barn owl	*Tyto alba*	9	Free-ranging, admitted to rehab	12.1 (2.2)	Hindmarch et al. 2019
Barn owl	*Tyto alba*	61	Free-ranging	22.1[1]	Webster et al. 2015
Barn owl	*Tyto alba*	2	Captive	22.6[1]	Webster et al. 2015
Bald eagle	*Haliaeetus leucocephalus*	8	Free-ranging, admitted to rehab	14.3 (1.5)	Hindmarch et al. 2019
Cooper's hawk	*Accipiter cooperii*	6	Free-ranging, admitted to rehab	8.6 (1.1)	Hindmarch et al. 2019
American kestrel	*Falco sparverius*	49	Captive	12.4 (0.3)	Rattner et al. 2015
Red-tailed hawk	*Buteo jamaicensis*	7	Free-ranging, admitted to rehab	22.5 (9.3)	Hindmarch et al. 2019
Ferruginous hawk (pre-fledging)	*Buteo regalis*	117	Free-ranging	29.8 (0.4)	This study

[1]Median values are presented rather than means.

Table 1.2 **Final fixed covariates used in models to describe the variation in clotting time, as measured by prothrombin time (PT), in free-living ferruginous hawk nestlings in Idaho, Colorado and Wyoming in 2019.**

Covariate	Source	Description	Potential link to PT	Example(s)
Age	Field data	Age in days, based on feather development (Moritsch, 1985)	More developed nestlings may have lower PT as their liver becomes more developed and efficient	Age affects prothrombin activity in humans (Andrew et al., 1988; Sakata et al., 2007)
Time	Field data	The time (in min after midnight) that blood samples were collected	PT will be higher in nestlings sampled in the morning	Diurnal variation in PT occurs in humans and rodents, with higher values earlier in the morning (Bleske et al., 1995; Bertolucci et al., 2005)
Sex	Field data	Putative sex of each nestling based on morphological measurements (Gossett, 1993)	Females are more likely to have elevated PT than males	Female rats, mice and humans have higher PT (Lemini et al., 2007; Sakata et al., 2007)
Elevation	USGS DEM (2014)	The elevation (m) at each nest site	Nestlings in low elevations will have higher PT	Humans traveling to higher altitudes exhibit decreased PT (Patot et al., 2006)
Shrub Cover	LANDFIRE (2014)	An index for vitamin K supply on the landscape. Combined cover of shrub species within a 2-km radius of each nest site (%)	In areas with disturbance, i.e. herbaceous vegetation, there will be more plants that contain vitamin K	Plants in North America contain vitamin K (Phillips et al., 2013). Vitamin K intake can affect PT in humans (Ho et al., 2002)

Table 1.3 **Competing models for exploring prothrombin time (PT) as a function of Age, Elevation, Sex, Shrub Cover, and Time of Day in ferruginous hawk nestlings in Idaho, Wyoming and Colorado in 2019. All models included an intercept and random effect for nest site. The number of parameters (K), log-likelihood (LL), Akaike's Information Criterion corrected for small sample sizes (AIC_c), ΔAIC_c, and Akaike weight (w_i) are provided for each model. Models were later averaged.**

Model Structure	K	LL	AICc	ΔAICc	w_i
Age + Elevation + Sex	6	-255.01	523.01	0.00	0.24
Age + Elevation + Sex + Shrub Cover	7	-254.51	524.35	1.34	0.12
Age + Sex + Shrub Cover	6	-255.70	524.39	1.38	0.12
Elevation + Sex	5	-256.99	524.67	1.66	0.10
Age + Elevation + Sex + Time	7	-254.84	525.02	2.01	0.09
Sex + Shrub	5	-257.41	525.51	2.50	0.07
Elevation + Sex + Time	6	-256.67	526.34	3.33	0.05
Elevation + Sex + Shrub Cover	6	-256.78	526.54	3.53	0.04
Age + Sex + Time + Shrub Cover	8	-254.47	526.67	3.66	0.04
Age + Elevation + Sex + Time + Shrub Cover (Global)	7	-255.70	526.73	3.72	0.04
Sex +Time + Shrub Cover	6	-257.30	527.59	4.58	0.02
Sex	4	-259.60	527.65	4.65	0.02
Elevation + Sex + Time + Shrub Cover	7	-256.58	528.48	5.48	0.02
Sex + Time	5	-259.10	528.90	5.89	0.01
Age + Sex	5	-259.23	529.16	6.15	0.01
Age + Sex + Time	6	-258.80	530.58	7.57	0.01
Age + Elevation	5	-262.73	536.15	13.14	0.00
Age + Elevation + Shrub Cover	6	-262.35	537.68	14.67	0.00
Age + Shrub	5	-263.78	538.25	15.24	0.00
Elevation	4	-264.95	538.37	15.36	0.00
Age + Elevation + Time	6	-262.70	538.40	15.39	0.00
Shrub Cover	4	-265.61	539.68	16.67	0.00
Age + Elevation + Time + Shrub Cover	7	-262.34	540.02	17.01	0.00
Elevation + Shrub Cover	5	-264.83	540.35	17.35	0.00
Elevation + Time	5	-264.86	540.41	17.40	0.00
Age + Time + Shrub	6	-263.74	540.47	17.46	0.00
Intercept Only (Null)	3	-267.61	541.49	18.48	0.00
Time + Shrub	5	-265.60	541.91	18.90	0.00
Elevation + Time + Shrub Cover	6	-264.78	542.55	19.54	0.00
Age	4	-267.14	542.74	19.73	0.00
Time	4	-267.40	543.26	20.25	0.00
Age + Time	5	-266.98	544.65	21.64	0.00

Table 1.4 Number of whole blood samples collected and analyzed for AR residues from ferruginous hawk nests in 2018 – 2019 in three western states in the USA. The parentheses around nest sites indicate the number of nests that were sampled in both 2018 and 2019. Pooled samples contained blood collected from >1 nestling in a single nest to reach the 2-ml minimum required for the AR assay.

State	Nest Sites	Nestlings	Adults	Pooled[1]
Idaho	22 (2)	75	2	11
Wyoming	37 (3)	95	-	-
Colorado	1	1	-	-
Total	60	171	2	11

[1]A total of 36 nestlings contributed to the pooled samples. On average, 3 ± 1.2 nestlings from each nest site were part of a pooled sample, and they were 14.3 ± 3.6 d old (range: 7– 20 d) when sampled.

Table 1.5 **Model averaged regression parameters, SE, and 95% CI for PT in nestling ferruginous hawks in Colorado, Wyoming and Idaho, USA, 2019, derived using a linear-mixed model approach with nest site as a random effect.**

Covariate	Parameter Estimate	SE	95% Confidence Interval
Intercept	31.66	0.67	30.33 – 33.00
Age	0.80	0.75	-0.68 – 2.78
Elevation	-1.11	0.97	-3.01 – 0.79
Sex	-3.27	0.79	-4.85 – -1.69
Shrub Cover	-0.55	0.83	-2.19 – 1.09
Time	-0.08	0.33	-0.74 – 0.58

Table 1.6 Number of livers collected in 2018 and 2019, by species and state, that were screened for AR residues.

Common Name	Species	Idaho	Wyoming	Colorado
Northern pocket gopher	*Thomomys talpoides*	-	2	-
Piute ground squirrel	*Urocitellus mollis*	36[1]	-	-
Thirteen-lined ground squirrel	*Ictidomys tridecemlineatus*	-	2	-
White-tailed prairie dog	*Cynomys leucurus*	-	10	-
Rodent, unidentified		-	3	-
Western meadowlark	*Sturnella neglecta*	-	-	1
Ferruginous hawk	*Buteo regalis*	4	-	-
Total:		40	17	1

[1]Brodifacoum was detected at a low concentration (0.003 ppm, LOD = 0.002 ppm) in one Piute ground squirrel liver collected in Idaho on 4 April 2018. No AR residues were detected in the remaining samples that were tested.

Figures

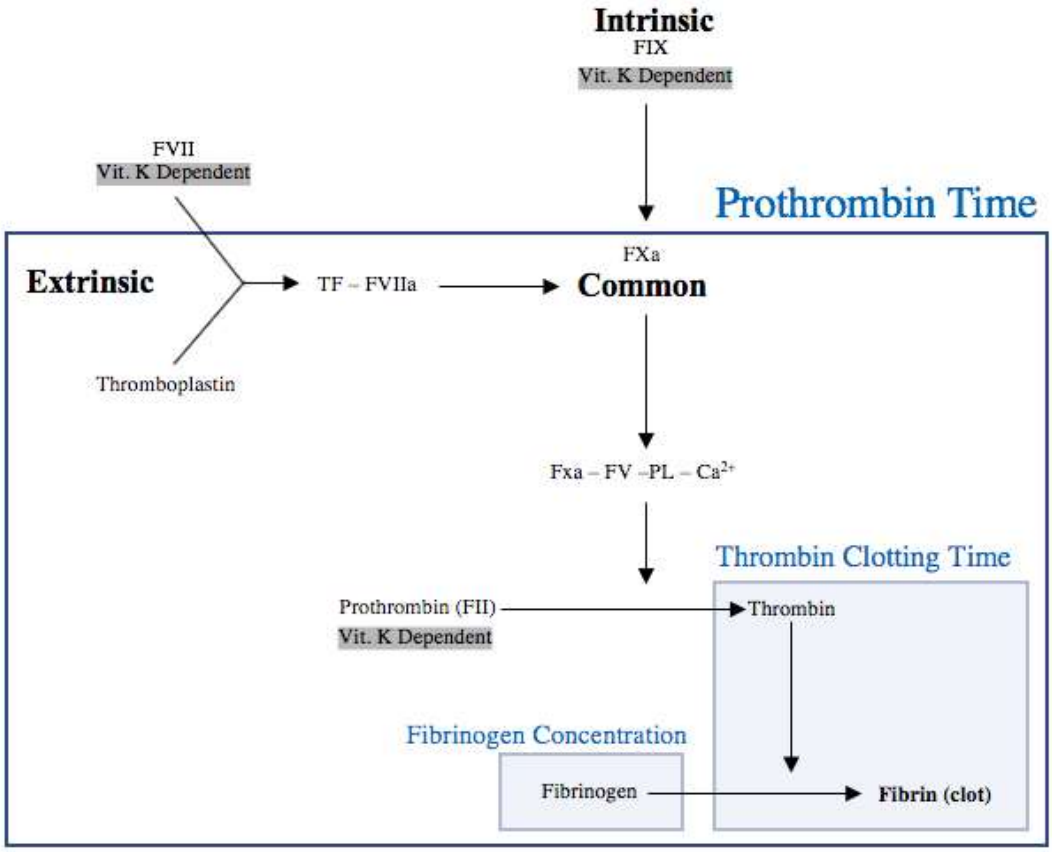

Figure 1.1 Simplified schematic of the coagulation pathway in birds (adapted from Rattner et al. 2010). The specific components of the pathway measured with prothrombin time, thrombin clotting time and fibrinogen concentration are indicated in the blue boxes.

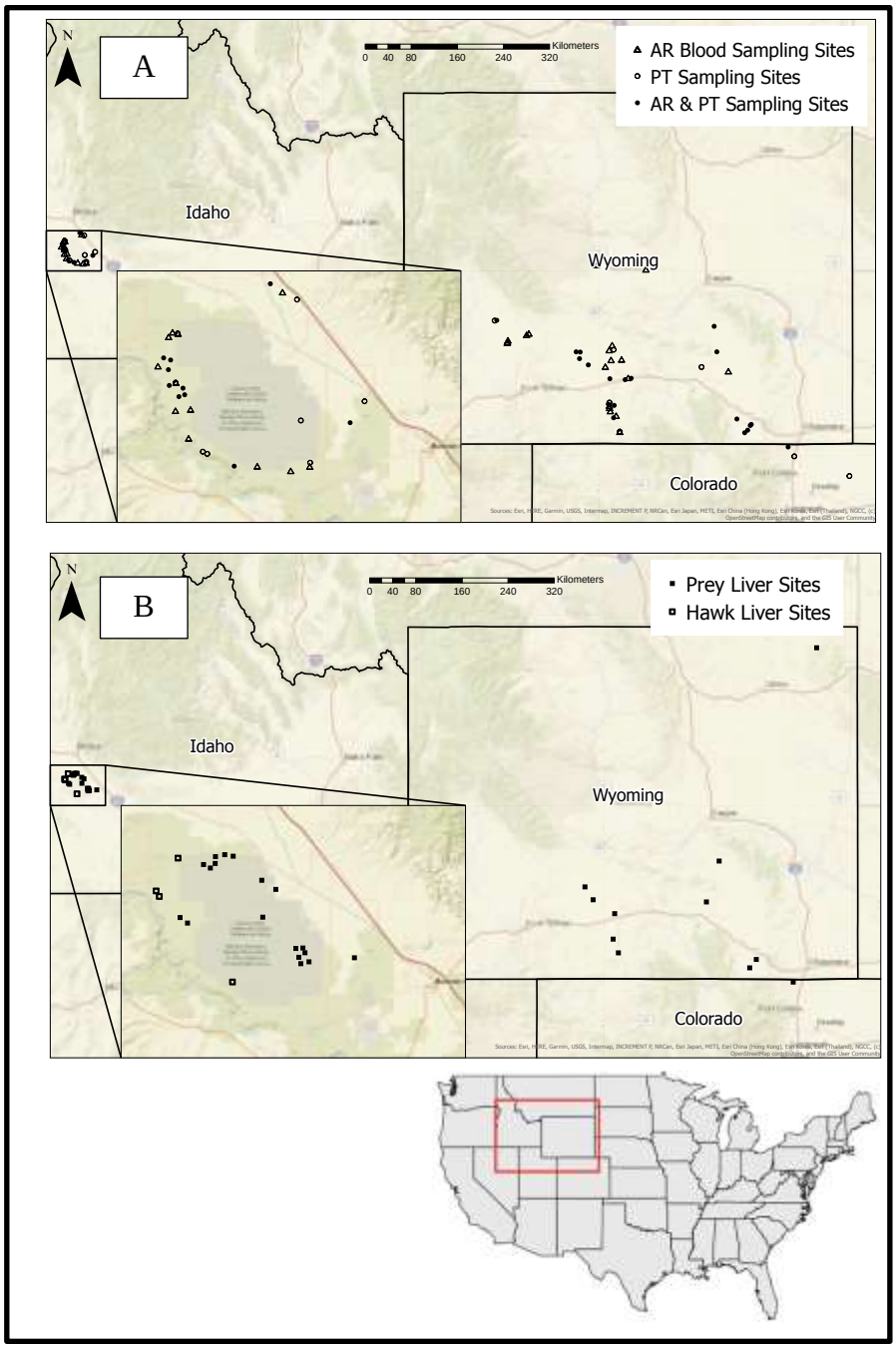

Figure 1.2 Locations of (A) ferruginous hawk anticoagulant rodenticide (AR) blood collection sites in 2018 – 2019 (open triangle), prothrombin time (PT) collection sites in 2019 (open circle), and sites where PT and AR samples where both collected (closed circle), and (B) prey liver (closed square) and ferruginous hawk liver (open square) collection sites in 2018 and 2019 in Colorado, Idaho and Wyoming, USA.

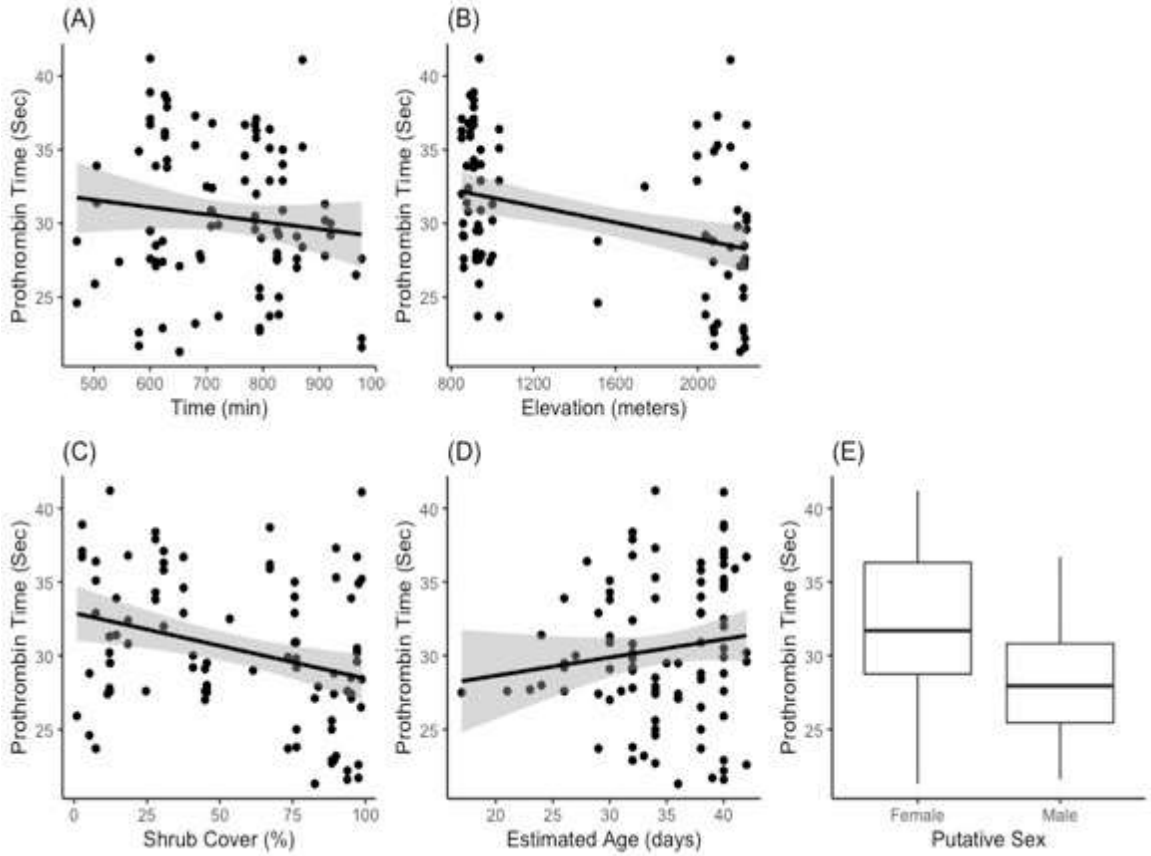

Figure 1.3 Prothrombin time (PT) of nestling ferruginous hawks in Idaho, Wyoming and Colorado, USA, 2019 as a function of (A) blood collection time (in minutes after midnight), (B) elevation, (C) shrub cover, (D) nestling age, and (E) nestling sex. Continuous variables are depicted with 95% confidence intervals (shaded gray). Categorical covariate relationships are depicted with a box plot where the horizontal line is the median, the box represents the interquartile range, and the whiskers represent the minimum and maximum.

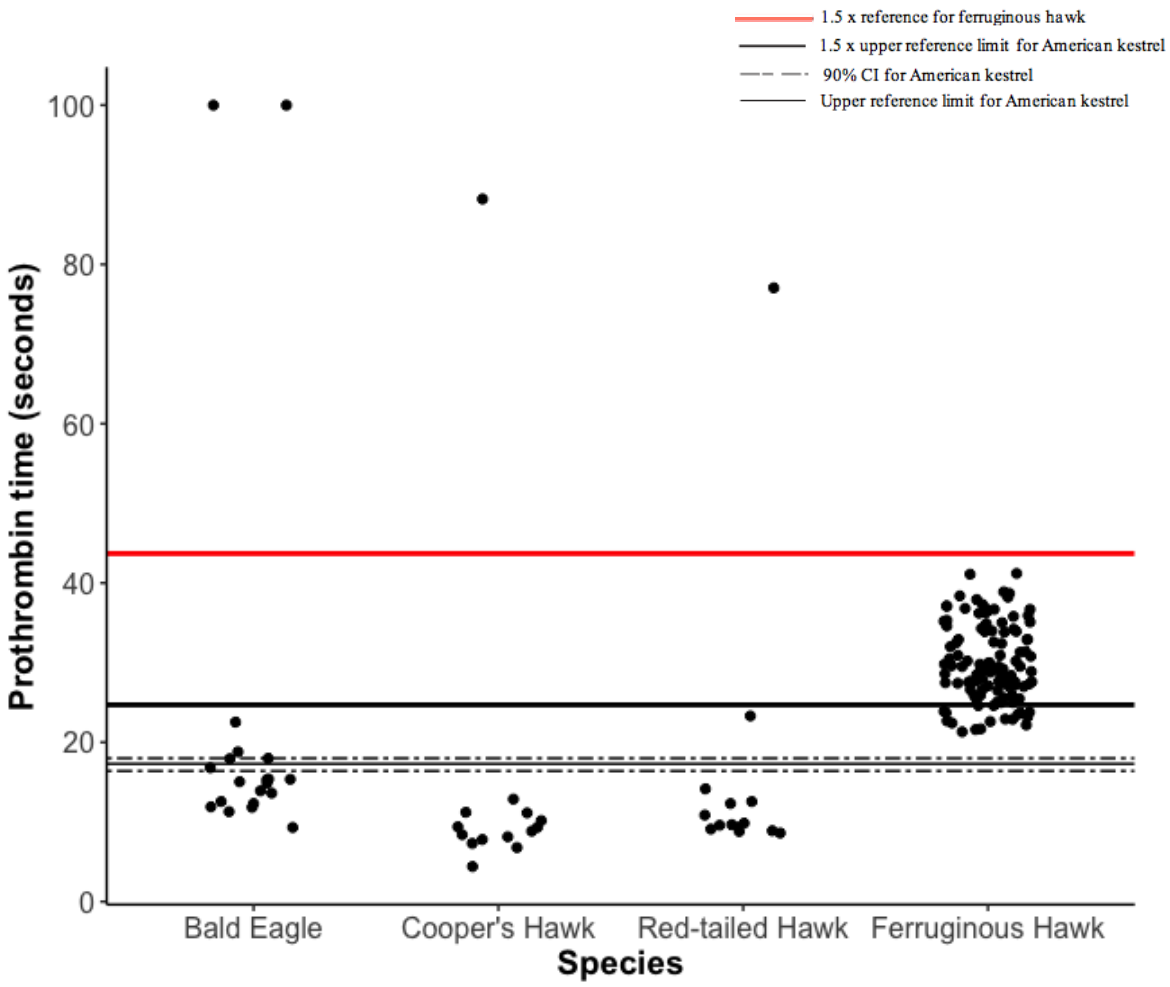

Figure 1.4 Comparison of prothrombin times collected from ferruginous hawk nestlings in this study to bald eagles (*Haliaeetus leucocephalus*), Cooper's hawks (*Accipiter cooperi*), and red-tailed hawks (*Buteo jamaicensis*) admitted to a rehabilitation clinic (from Hindmarch et al. 2019). Values are compared to the upper reference limit (17.3 sec), the 90% confidence interval, and 1.5 X upper reference limit (24.7 sec) from captive adult American kestrels (*Falco sparverius*) and the 1.5 X reference limit (43.7 sec) from ferruginous hawk nestlings.

References

Abernathy, E.V., Hull, J.M., Fish, A.M., Briggs, C.W., 2018. Secondary anticoagulant rodenticide exposure in migrating juvenile red-tailed hawks (*Buteo jamaicensis*) in relationship to body condition. Journal of Raptor Research. 52, 225–230.

Akaike, H., 1973. Maximum likelihood identification of gaussian autoregressive moving average models. Biometrika. 60, 255–265.

Andrew, M., Paes, B., Milner, R., Johnston, M., Mitchell, L., Tollefsen, D., Castle, V., Powers, P., 1988. Development of the human coagulation system in the healthy premature infant. Blood. 72, 1651–1657.

Bailey, C., Fisher, P., Eason, C.T., 2005. Assessing anticoagulant resistance in rats and coagulation effects in birds using small-volume blood samples. Science for Conservation. 249, 1–22.

Bartoń, K., 2019. MuMIn: Multi-Model Inference. R package version 1.43.15. https://CRAN.R-project.org/package=MuMIn\.

Bates, D., Maechler, M., Bolker, B.,Walker, S., 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software. 67, 1–48.

Bechard, M.J., Schmutz, J.K., 1995. Ferruginous hawk (*Buteo regalis*). In: Rodewald, P.G., (Ed). The Birds of North America (P. G. Rodewald, Ed.). Ithaca: Cornell Lab of Ornithology; https://birdsna.org/Species-Account/bna/species/ferhaw.

Belleville, J., Cornillon, B., Paul, J., Baguet, J., Clendinnen, G., Eloy, R., 1982. Haemostasis, blood coagulation and fibrinolysis in the japanese quail. Comparative Biochemistry and Physiology. 71, 219–230.

Berny, P.J., Buronfosse, T., Buronfosse, F., Lamarque, F., Lorgue, G., 1997. Field evidence of secondary poisoning of foxes (*Vulpes vulpes*) and buzzards (*Buteo buteo*) by bromadiolone, a 4-year survey. Chemosphere. 35, 1817–1829.

Berny, P., Gaillet, J-R., 2008. Acute poisoning of red kites (*Milvus milvus*) in France: data from the sagir network. Journal of Wildlife Diseases. 44, 417–426.

Bertolucci, C., Pinotti, M., Colognesi, I., Foà, A., Bernardi, F., Portaluppi, F., 2005. Circadian rhythms in mouse blood coagulation. Journal of Biological Rhythms. 20, 219–224.

Bigland, C.H., Triantaphyllopoulos, D.C., 1961. Chicken prothrombin, thrombin, and fibrinogen. American Journal of Physiology. 200, 1013–1017.

Bleske, B.E., Welage, L.S., Warren, E.W., Brown M.B., Shea M.J., 1995. Variations in prothrombin time and international normalized ratio over 24 hours in warfarin-treated patients. Pharmacotherapy.15, 709–712.

Bloom, P. H., W. S. Clark, Kidd, J. W., 2007. Capture techniques. In: Bird, D.M., Bildstein, K.L., (Eds). Raptor Research and Management Techniques. Hancock House Publishers Ltd, Surrey, BC, Canada; Blaine, Washington, USA. pp. 193–219.

Burnham, K.P., Anderson, D.R., 2002. Model selection and multimodel inference, 2nd edition. Springer, New York, New York, USA.

Campbell, T.W., 2015. Exotic Animal Hematology and Cytology, 4th Edition. John Wiley & Sons, Ames, Iowa, USA.

Christensen, T.K., Lassen, P., Elmeros, M., 2012. High exposure rates of anticoagulant rodenticides in predatory bird species in intensively managed landscapes in Denmark. Archives of Environmental Contamaination and Toxicology. 63, 437–444.

Cox, P., Smith, R.H., 1992. Rodenticide ecotoxicology: Pre-lethal effects of anticoagulants on rat behavior. Proceedings of the Fifteenth Vertebrate Pest Conference. 15,165–170.

Doerr, J.A., Wyatt, R.D., Hamilton, P.B., 1975. Investigation and standardization of prothrombin times in chickens. Poultry Science. 54, 969–980.

Doolittle, R.F., Feng, D.F., 1987. Reconstructing the evolution of vertebrate blood coagulation from a consideration of the amino acid sequences of clotting proteins. Cold Spring Harbor Symposia on Quantitative Biology. 52, 869–874.

Eadsforth, C.V., Dutton, A.J., Harrison, E.G., Vaughan, J.A., 1991. A barn owl feeding study with [^{14} c]flocoumafen-dosed mice: Validation of a non-invasive method of monitoring exposure of barn owls to anticoagulant rodenticides in their prey. Pesticide Science. 32, 105–119.

EPA. 2017. Restrictions on rodenticide products. Environmental Protection Agency. https://www.epa.gov/rodenticides/restrictions-rodenticide-products. (accessed Dec 3 2017)

ESRI (Environmental Systems Resource Institute). 2015. ArcMap 10.4.1. ESRI, Redlands, California.

Fox, J., and Weisberg, S., 2019. An R Companion to Applied Regression, 3rd edition. Sage, Thousand Oaks, CA, USA. https://socialsciences.mcmaster.ca/jfox/Books/Companion/.

Frost, C.L., Naudé, R.J., Oelofsen, W., Jacobson, B., 1999. Comparative blood coagulation studies in the ostrich. Immunopharmacology. 45, 75–81.

Geduhn, A., Jacob, J., Schenke, D., Keller, B., Kleinschmidt, S., Esther, A., 2015. Relation between intensity of biocide practice and residues of anticoagulant rodenticides in red foxes (*Vulpes vulpes*). PloS One.10, e0139191.

Giorgi, M., and Mengozzi, G., 2010. An HPLC method for the determination of bromadiolone plasma kinetics and its residues in hen eggs. Journal of Chromatographic Science. 48, 714–720.

Giraudoux, P., Tremollières, C., Barbier, B., Defaut, R., Rieffel, D., Bernard, N., Lucot, É., Berny, P., 2006. Persistence of bromadiolone anticoagulant rodenticide in *Arvicola terrestris* populations after field control. Environmental Research. 102, 291–298.

Gossett, D.N., 1993. Studies of ferruginous hawk biology: I. Recoveries of banded ferruginous hawks from presumed eastern and western subpopulations. II. Morphological and genetic differences of presumed subpopulations of ferruginous hawks. III. Sex determination of nestling ferruginous hawks. M.S. Thesis, Boise State University, Boise, Idaho.

Hadler, M.R., and Buckle, A.P., 1992. Forty-five years of anticoagulant rodenticides - past, present and future trends. Proceedings of the Fifteenth Vertebrate Pest Conference. 15, 149–155.

Herring, G., Eagles-Smith, C.A., Buck, J., 2017. Characterizing golden eagle risk to lead and anticoagulant rodenticide exposure: A review. Journal of Raptor Research. 51, 273–292.

Hindmarch, S., Rattner, B.A., Elliott, J.E., 2019. Use of blood clotting assays to assess potential anticoagulant rodenticide exposure and effects in free-ranging birds of prey. Science of The Total Environment. 657, 1205–1216.

Ho, C.H., Lin, M.W., You, J.Y., Chen, C.C., Yu, T.J., 2002. Variations of prothrombin time and international normalized ratio in patients treated with warfarin. Thrombosis Research. 107, 277–280.

Howard, R.P., 1975. Breeding ecology of the ferruginous hawk in northern Utah and southern Idaho. M.S. Thesis, Utah State University, Logan.

Jensen, M.K., Hamburg, S.D., Rota, C.T., Brinker, D.F., Coles, D.L., Manske, M.A., Slabe, V.A., Stuber, M.J., Welsh, A.B., Katzner, T.E., 2019. An improved mechanical owl for efficient capture of nesting raptors. Journal of Raptor Research. 53, 14–25.

Keeley, W.H., Bechard, M.J., 2017. Nesting behavior, provisioning rates, and parental roles of ferruginous hawks in New Mexico. Journal of Raptor Research. 51, 397–408.

Knopper, L.D., Mineau, P., Walker, L.A., Shore, R.F., 2007. Bone density and breaking strength in UK raptors exposed to second generation anticoagulant rodenticides. Bulletin of Environmental Contamination and Toxicology. 78, 249–251.

LANDFIRE, 2014. Existing Vegetation Type Layer, LANDFIRE 1.4.0, U.S. Department of the Interior, Geological Survey. http://landfire.cr.usgs.gov/viewer/ (accessed 8 Febuary 2020).

Lemini, C., Jaimez, R., Franco, Y., 2007. Gender and inter-species influence on coagulation tests of rats and mice. Thrombosis Research. 120, 415–419.

López-Perea J.J., Mateo R., 2018. Secondary Exposure to Anticoagulant Rodenticides and Effects on Predators. In: van den Brink, N., Elliott, J., Shore, R., Rattner, B., (Eds) Anticoagulant Rodenticides and Wildlife. Emerging Topics in Ecotoxicology (Principles, Approaches and Perspectives). Springer Science. New York, New York, USA.

López-Perea, J.J., Camarero, P.R., Sánchez-Barbudo, I.S., Mateo, R., 2019. Urbanization and cattle density are determinants in the exposure to anticoagulant rodenticides of non-target wildlife. Environmental Pollution. 244, 801–808.

Martínez-Padilla, J., López-Idiáquez, D., López-Perea, J.J., Mateo, R., Paz, A., Viñuela, J., 2017. A negative association between bromadiolone exposure and nestling body condition in common kestrels: management implications for vole outbreaks. Pest Management Science. 73, 364–370.

Moritsch, M., 1985. Photographic guide for aging nestling ferruginous hawks. Bureau of Land Management, Boise, Idaho, USA.

Morrisey, J.K., Paul-Murphy, J., Fialkowski, J.P., Hart, A., Darien, B.J., 2003. Estimation of prothrombin times of Hispaniolan Amazon parrots (*Amazona ventralis*) and umbrella cockatoos (*Cacatua alba*). Journal of Avian Medicine and Surgery. 17, 72–77.

Murray, M., Tseng, F., 2008. Diagnosis and treatment of secondary anticoagulant rodenticide toxicosis in a red-tailed hawk (*Buteo jamaicensis*). Journal of Avian Medicine and Surgery. 22, 41–46.

Murray, M., 2011. Anticoagulant rodenticide exposure and toxicosis in four species of birds of prey presented to a wildlife clinic in Massachusetts, 2006–2010. Journal of Zoo and Wildlife Medicine. 42, 88–97.

Nakayama, S.M.M., Morita, A., Ikenaka, Y., Mizukawa, H., Ishizuka, M., 2019. A review: poisoning by anticoagulant rodenticides in non-target animals globally. Journal of Veterinary Medical Science. 81, 298–313.

Ng, J., Giovanni, M. D., Bechard, M. J., Schmutz, J. K., Pyle, P., 2017. Ferruginous hawk (*Buteo regalis*). In: Rodewald, P.G.,(Ed). The Birds of North America. Cornell

Lab of Ornithology, Ithaca, New York, USA. https://birdsna.org/Species-Account/bna/species/ferhaw.

Olendorff, R.R., 1993. Status, biology, and management of ferruginous hawks: a review. Raptor Research and Technical Assistance Center, Bureau of Land Management, Boise. 84pp.

Patot, M.C.T.V., Hill, A.E., Dingmann, C., Gaul, L., Fralick, K., Christians, U., Honigman, B., Salman, M.D., 2006. Risk of impaired coagulation in warfarin patients ascending to altitude (>2400 m). High Altitude Medicine & Biology. 7, 39–46.

Phillips, K.M., Pehrsson, P.R., Agnew, W.W., Scheett, A.J., Follett, J.R., Lukaski, H.C., Patterson, K.Y., 2014. Nutrient composition of selected traditional United States Northern Plains Native American plant foods. Journal of Food Composition and Analysis. 34, 136–152.

R Core Team, 2018. R: A language and environment for statistical computing. R Foundation for Statistical Computing,Vienna, Austria. https://www.R-project.org/.

Rattner, B.A., Horak, K.E., Warner, S.E., Johnston, J.J., 2010. Acute toxicity of diphacinone in northern bobwhite: Effects on survival and blood clotting. Ecotoxicology and Environmental Safety. 73, 1159–1164.

Rattner, B.A., Horak, K.E., Lazarus, R.S., Goldade, D.A., Johnston, J.J., 2014. Toxicokinetics and coagulopathy threshold of the rodenticide diphacinone in eastern screech- owls (*Megascops asio*). Environmental Toxicology and Chemistry. 33, 74–81.

Rattner, B., Horak, K.E., Lazarus, R., Schultz, S., Knowles, S., Abbo, B., Volker, S., 2015. Toxicity reference values for chlorophacinone and their application for assessing anticoagulant rodenticide risk to raptors. Ecotoxicology. 24, 720–734.

Sage, M., Fourel, I., Cœurdassier, M., Barrat, J., Berny, P., Giraudoux, P., 2010. Determination of bromadiolone residues in fox faeces by LC/ESI-MS in

relationship with toxicological data and clinical signs after repeated exposure. Environmental Research. 110, 664–674.

Sakata, T., Okamoto, A., Morita, T., Kokubo, Y., Sato, K., Okayama, A., Tomoike, H., Miyata, T., 2007. Age- and gender-related differences of plasma prothrombin activity levels. Thrombosis and Haemostasis. 97, 1052–1053.

Shlosberg, A., Booth, L., 2006. Veterinary and Clinical Treatment of Vertebrate Pesticide Poisoning – A Technical Review. Lincoln, NZ. 101 pp.

Shore, R.F., Malcolm, H.M., Mclennan, D., Turk, A., Walker, L.A., Wienburg, C.L., Burn, A.J., 2006. Did foot-and-mouth disease-control operations affect rodenticide exposure in raptors? The Journal of Wildlife Management. 70, 588–593.

Stone, W.B., Okoniewski, J.C., Stedelin, J.R., 1999. Poisoning of wildlife with anticoagulant rodenticides in New York. Journal of Wildlife Diseases. 35, 187–193.

Stone, W.B., Okoniewski, J.C., Stedelin, J.R., 2003. Anticoagulant rodenticides and raptors: recent findings from New York, 1998-2001. Bulletin of Environmental Contamination and Toxicology. 70, 34–40.

Stopforth, A., 1970. A study of coagulation mechanism in domestic chickens. Journal of Comparative Pathology. 80, 525–533.

Symonds, M.R.E., Moussalli, A., 2011. A brief guide to model selection, multimodel inference and model averaging in behavioural ecology using Akaike's information criterion. Behavioral Ecology and Sociobiology. 65, 13–21.

Tosh, D.G., Shore, R.F., Jess, S., Withers, A., Bearhop, S., Ian Montgomery, W., McDonald, R.A., 2011. User behavior, best practice and the risks of non-target exposure associated with anticoagulant rodenticide use. Journal of Environmental Management. 92, 1503–1508.

U.S. Geological Survey, 2017. One meter Digital Elevation Models (DEMs) – USGS National Map 3DEP Downloadable Data Collection: U.S. Geological Survey.

van den Brink, N.W, J. Elliott, R. Shore, B. Rattner, (Eds.). 2018. Anticoagulant rodenticides and wildlife. Springer Science, New York, New York, USA.

Vandenbroucke, V., Bousquet-Melou, A., De BACKER, P., Croubels, S., 2008. Pharmacokinetics of eight anticoagulant rodenticides in mice after single oral administration. Journal of Veterinary Pharmacology and Therapeutics. 31, 437–445.

Vyas, N.B., Kuncir, F., Clinton, C.C., 2017. Influence of poisoned prey on foraging behavior of ferruginous hawks. The American Midland Naturalist. 177, 75–83.

Webster, K., Harr, K., Bennett, D., Williams, T., Cheng, K., Maisonneuve, F., Elliott, J., 2015. Assessment of toxicity and coagulopathy of brodifacoum in Japanese quail and testing in wild owls. Ecotoxicology. 24, 1087–1101.

APPENDIX 1

Prothrombin Time, Thrombin Clotting Time, and Fibrinogen Concentration Assays

The following protocol was conducted by the Comparative Coagulation Laboratory in the Animal Health Diagnostic Center at Cornell University (Ithaca, NY). PT assays required prior preparation of avian thromboplastin. Chicken brain tissue was obtained from a pathology service (Cornell University) from sentinel birds submitted for necropsy for health surveillance purposes. Avian brain thromboplastin was prepared from the harvested brain tissue following a previously described protocol (Rattner et al., 2010). In brief, brain tissues were homogenized in 10 volumes of cold acetone, the homogenate centrifuged at 1,500 x *g* for 10 min, and the supernatant discarded. The pellet was re-suspended in 5 ml of acetone, homogenized, centrifuged, acetone decanted, and this procedure was repeated. The pellet was then again re-suspended in acetone and vacuum filtered using a Buchner funnel with a glass microfiber filter. The material retained on the filter was dried overnight in a vacuum desiccator, removed from the microfiber filter, ground with a mortar and pestle, transferred into cryotubes in 40 mg aliquots, and then stored frozen at –80°C until use in PT assays.

The clotting time tests (PT, thrombin time) were performed using a semi-automated coagulation instrument (Start 4, Diagnostica Stago, Parsippany, NJ) with mechanical endpoint detection. The test samples were thawed at 37°C for 10 min and then held on wet ice until diluted in an equal volume of imidazole buffered saline (pH 7.4) just before assay. The plasma dilutions were assayed in duplicate, and mean clotting times were used in calculations. To assay PT, frozen aliquots (40 mg) of avian thromboplastin were allowed to equilibrate at room temperature for 15 min before the addition of 2.0 ml of 25 mM CaCl2, followed by incubation at 42°C and pulse vortexing for 15 min. The suspension was then centrifuged at 1,000 x g for 20 min and the

recovered supernatant combined with equal volume 25 mM $CaCl_2$. The PT assays were performed by incubating 100 µl dilute plasma at 37°C for 60 sec. Coagulation was then initiated by the addition of 200 µl of a warm (37°C) calcium and thromboplastin solution. The thrombin time was determined by incubating 100 µl of dilute plasma at 37°C for 60 sec, and then adding 100 µl of room temperature bovine thrombin reagent (50 U/mL thrombin, Triniclot fibrinogen, Trinclot, Wicklow, Ireland). Fibrinogen was measured in an automated coagulation instrument (STACompact, Diagnostica Stago) via the Clauss method using the manufacturer's human thrombin reagent and a human fibrinogen standard (Fibrinogen, Diagnostica Stago). Both PT and TCT assays were done in duplicate. The samples for fibrinogen were assayed as a single replicate to minimize the amount of plasma used.

References

Rattner, B.A., Horak, K.E., Warner, S.E., Johnston, J.J., 2010. Acute toxicity of diphacinone in Northern bobwhite: Effects on survival and blood clotting. Ecotoxicology and Environmental Safety. 73, 1159–1164.

APPENDIX 2

Chapter 1 Tables and Figures

Table A2.1 **The categories used to define land cover type within a 2-km radius circular buffer around each ferruginous hawk nest. Existing vegetation type data was derived from LANDFIRE (2014).**

Veg Category	Description
Water	Open water, snow, and ice
Sparse	Barren, no dominant vegetation type, bedrock, scree, and talus
Herb	Introduced annual and perennial grassland and forbland, riparian woodlands, wet meadow, inland marches and prairies, heathland, tidal march, tidal flat, prairies, modified-managed prairie grassland, peatlands, tallgrass prairies, glades and barrens
Shrub	Deciduous shrubland, introduced upland vegetation shrub, woody wetland vegetation, introduced riparian vegetation, chaparral, shrub-dominated aspen forest, desert scrub, big and low sagebrush shrubland and steppe, salt desert scrub, mountain mahogany woodland and shrub, dwarf shrubland, willow shrubland
Tree	Upland deciduous, evergreen and mixed forest, riparian woodland, riparian forests, Douglas fir, lodgepole pine, ponderosa pine, birch, juniper woodlands, pinyon-juniper, aspen
Agriculture	Pasture, hay, cultivated crops, irrigated agriculture, vineyard, row crop, wheat, and aquaculture
Developed	Roads, buildings, and developed open space

Table A2.2 **Information about the individual ferruginous hawks (n = 171 nestlings, n = 2 adults) from which whole blood samples were collected and analyzed for AR residues from 2018 – 2019 in Idaho (ID), Wyoming (WY) and Colorado (CO), USA. No AR residues were detected in any of the blood samples.**

Individual ID	Nest ID	Date	State	Sex[1]	Life Stage	Age (d)	Mass (g)
Big Baja 181	Big Baja 181	5/15/18	ID	F	Adult	-	1863
1207-81372	Slater's Flat	5/25/18	ID	F	Nestling	34	1427
1207-81373	Slater's Flat	5/25/18	ID	F	Nestling	33	1389
1207-81374	Cow Pasture	5/25/18	ID	F	Nestling	30	1292
1207-81375	Cow Pasture	5/25/18	ID	F	Nestling	30	1269
1207-81376	Cow Pasture	5/25/18	ID	F	Nestling	30	1241
1207-81377	Surprise	5/25/18	ID	F	Nestling	35	1370
1207-81378	Surprise	5/25/18	ID	F	Nestling	38	1479
1957-31839	Slater's Flat	5/25/18	ID	M	Nestling	34	1031
1957-31840	Slater's Flat	5/25/18	ID	M	Nestling	34	1002
1957-31841	Cow Pasture	5/25/18	ID	M	Nestling	30	917
1957-31842	Cow Pasture	5/25/18	ID	M	Nestling	30	794
1957-31843	Surprise	5/25/18	ID	M	Nestling	38	1068
1957-31844	Surprise	5/25/18	ID	M	Nestling	38	980
1207-82006	Dead Pigeon B	5/29/18	ID	M	Nestling	30	1001
1957-41016	Dead Pigeon B	5/29/18	ID	M	Nestling	32	1038
1957-41017	Dead Pigeon B	5/29/18	ID	M	Nestling	32	1034
1957-41018	Dead Pigeon B	5/29/18	ID	M	Nestling	32	1098
1967-06492	Moore's Road Central	6/01/18	ID	F	Nestling	36	1455
1967-06493	Guzzler	6/01/18	ID	F	Nestling	29	1381
1967-06494	Guzzler	6/01/18	ID	F	Nestling	29	1507
1967-06495	Guzzler	6/01/18	ID	F	Nestling	28	1338
1957-41019	Moore's Road Central	6/01/18	ID	M	Nestling	36	1007
1957-41020	Moore's Road Central	6/01/18	ID	M	Nestling	36	1008
1967-06496	Cabin	6/04/18	ID	F	Nestling	30	1374
1967-06497	Cabin	6/04/18	ID	F	Nestling	30	1339
1967-06798	Cabin	6/04/18	ID	F	Nestling	28	1383
18WY7701	Buff ID 77	6/08/18	WY	U	Nestling	18	706
1207-81379	Shadescale Exclosure	6/08/18	ID	F	Nestling	34	1295
1207-81380	Shadescale Exclosure	6/08/18	ID	F	Nestling	32	1269
1207-81381	Shadescale Exclosure	6/08/18	ID	F	Nestling	36	1423
1207-81382	Big Baja 77-81	6/08/18	ID	F	Nestling	45	1625
1207-81383	Moore's Road West	6/08/18	ID	F	Nestling	36	1349

Individual ID	Nest ID	Date	State	Sex[1]	Life Stage	Age (d)	Mass (g)
1207-81384	Moore's Road West	6/08/18	ID	F	Nestling	32	1333
1207-81385	Moore's Road West	6/08/18	ID	F	Nestling	36	1511
1207-81386	Moore's Road West	6/08/18	ID	F	Nestling	34	1395
1207-81387	Coyote Exclosure B	6/08/18	ID	F	Nestling	28	1136
1207-81388	Coyote Exclosure B	6/08/18	ID	F	Nestling	28	1290
1207-81389	Coyote Exclosure B	6/08/18	ID	F	Nestling	32	1301
1957-31845	Shadescale Exclosure	6/08/18	ID	M	Nestling	36	946
1957-31846	Tank Road	6/08/18	ID	M	Nestling	36	1007
1957-31847	Tank Road	6/08/18	ID	M	Nestling	35	942
1957-31848	Tank Road	6/08/18	ID	M	Nestling	33	947
1957-31849	Tank Road	6/08/18	ID	M	Nestling	36	946
1957-38150	Moore's Road West	6/08/18	ID	M	Nestling	32	1044
1957-41039	Coyote Exclosure B	6/08/18	ID	M	Nestling	30	834
1957-41040	Coyote Exclosure B	6/08/18	ID	M	Nestling	30	800
18WYFH1B01	FH-1B	6/10/18	WY	U	Nestling	21	806
18WYFH1B02	FH-1B	6/10/18	WY	U	Nestling	17	985
18WYFH1B03	FH-1B	6/10/18	WY	U	Nestling	23	903
18WY0702	Buff ID 7	6/10/18	WY	F	Nestling	26	1199
1967-06499	FH-5	6/10/18	WY	F	Nestling	32	1542
1967-06500	FH-5	6/10/18	WY	F	Nestling	28	1533
18WY0703	Buff ID 7	6/10/18	WY	M	Nestling	24	732
1957-41021	FH-5	6/10/18	WY	M	Nestling	32	1139
1957-41022	FH-5	6/10/18	WY	M	Nestling	30	1167
1207-82007	FH-6	6/11/18	WY	F	Nestling	31	1356
1207-82008	FH-6	6/11/18	WY	F	Nestling	29	1429
18WY0402	Buff ID 4	6/11/18	WY	F	Nestling	24	1329
1967-03146	Tank Road	6/11/18	ID	F	Adult	-	1999
18WYFH701	FH-7	6/11/18	WY	M	Nestling	20	856
18WYFH702	FH-7	6/11/18	WY	M	Nestling	19	851
1957-41023	Buff ID 4	6/11/18	WY	M	Nestling	30	1096
1957-41024	Buff ID 4	6/11/18	WY	M	Nestling	28	1091
1957-41025	FH-6	6/11/18	WY	M	Nestling	31	1032
18WYFH3802	FH-8	6/12/18	WY	F	Nestling	17	995
18WYFH3803	FH-8	6/12/18	WY	F	Nestling	19	1053
18WYFH3801	FH-8	6/12/18	WY	M	Nestling	21	851
18WYFH2104	FH-21	6/13/18	WY	U	Nestling	24	885
1088-09141	Bryan's Run	6/13/18	ID	F	Nestling	44	1621
1207-81390	Moore's Road East	6/13/18	ID	F	Nestling	39	1494

Individual ID	Nest ID	Date	State	Sex[1]	Life Stage	Age (d)	Mass (g)
1207-81391	Moore's Road East	6/13/18	ID	F	Nestling	36	1372
1207-81393	Section 16 Farm	6/13/18	ID	F	Nestling	30	1330
1207-81394	Henderson Draw	6/13/18	ID	F	Nestling	26	950
1207-81395	Bryan's Run	6/13/18	ID	F	Nestling	44	1660
1207-82009	Interstate 1	6/13/18	WY	F	Nestling	31	1432
1207-82010	Interstate 1	6/13/18	WY	F	Nestling	28	1419
1207-82011	FH-21	6/13/18	WY	F	Nestling	26	1295
18WYINT301	Interstate 3	6/13/18	WY	F	Nestling	23	863
18WYINT302	Interstate 3	6/13/18	WY	F	Nestling	24	914
1207-81392	Section 16 Farm West	6/13/18	ID	M	Nestling	35	1323
18WYINT303	Interstate 3	6/13/18	WY	M	Nestling	26	894
1957-41026	Interstate 1	6/13/18	WY	M	Nestling	29	1039
1957-41027	FH-21	6/13/18	WY	M	Nestling	26	991
1957-41028	FH-21	6/13/18	WY	M	Nestling	26	937
1957-41041	Big Baja 230	6/13/18	ID	M	Nestling	35	816
1957-41042	Moore's Road East	6/13/18	ID	M	Nestling	35	839
1957-41043	Moore's Road East	6/13/18	ID	M	Nestling	36	988
1957-41044	Section 16 Farm West	6/13/18	ID	M	Nestling	34	1274
1957-41045	Section 16 Farm West	6/13/18	ID	M	Nestling	33	971
1957-41046	Section 16 Farm West	6/13/18	ID	M	Nestling	36	885
1957-41047	Section 16 Farm	6/13/18	ID	M	Nestling	32	839
1957-41048	Section 16 Farm	6/13/18	ID	M	Nestling	32	866
1957-41049	Section 16 Farm	6/13/18	ID	M	Nestling	31	799
1957-41050	Henderson Draw	6/13/18	ID	M	Nestling	26	803
1957-41051	Henderson Draw	6/13/18	ID	M	Nestling	25	822
1957-41052	Bryan's Run	6/13/18	ID	M	Nestling	44	1023
1957-41053	Bryan's Run	6/13/18	ID	M	Nestling	44	991
1957-41054	Bryan's Run	6/13/18	ID	M	Nestling	44	1055
18WYWCC01	Wild Cow Camp	6/14/18	WY	U	Nestling	17	934
1207-81396	Coyote South	6/14/18	ID	F	Nestling	34	1414
1207-81397	Coyote Butte B	6/14/18	ID	F	Nestling	32	1471
1207-81398	Coyote Butte A	6/14/18	ID	F	Nestling	42	1650
1207-82012	FH-23	6/14/18	WY	F	Nestling	30	1360
1207-82013	FH-23	6/14/18	WY	F	Nestling	31	1440
1957-41029	FH-23	6/14/18	WY	M	Nestling	32	1018
1957-41030	FH-23	6/14/18	WY	M	Nestling	31	1022
1957-41055	Coyote South	6/14/18	ID	M	Nestling	35	927
1957-41056	Coyote South	6/14/18	ID	M	Nestling	32	839

Individual ID	Nest ID	Date	State	Sex[1]	Life Stage	Age (d)	Mass (g)
1957-41057	Coyote South	6/14/18	ID	M	Nestling	34	970
1207-82014	FH-29	6/15/18	WY	F	Nestling	28	1364
1207-82015	FH-29	6/15/18	WY	F	Nestling	30	1452
18WYFH2901	FH-29	6/15/18	WY	F	Nestling	25	1234
18WYFH3003	FH-30	6/15/18	WY	F	Nestling	26	1430
18WYRED01	Red Desert	6/15/18	WY	F	Nestling	24	1269
18WYFH3002	FH-30	6/15/18	WY	M	Nestling	20	890
18WYRED02	Red Desert	6/15/18	WY	M	Nestling	25	911
18WYRED03	Red Desert	6/15/18	WY	M	Nestling	22	925
18WYRED04	Red Desert	6/15/18	WY	M	Nestling	27	943
1957-41031	FH-30	6/15/18	WY	M	Nestling	28	1029
1957-41032	FH-29	6/15/18	WY	M	Nestling	29	1113
18WY5601	Buff ID 56	6/16/18	WY	F	Nestling	26	1219
18WY6502	Buff ID 65	6/16/18	WY	F	Nestling	20	956
18WY6503	Buff ID 65	6/16/18	WY	F	Nestling	21	955
18WY6702	Buff ID 67	6/16/18	WY	F	Nestling	20	1214
18WY6501	Buff ID 65	6/16/18	WY	M	Nestling	19	765
18WY6701	Buff ID 67	6/16/18	WY	M	Nestling	24	844
18WY6703	Buff ID 67	6/16/18	WY	M	Nestling	24	955
18WYCL02	Chain Lakes	6/16/18	WY	M	Nestling	26	962
1957-41033	Chain Lakes	6/16/18	WY	M	Nestling	31	1034
1207-82016	Pathfinder	6/19/18	WY	F	Nestling	34	1620
1957-41034	Pathfinder	6/19/18	WY	M	Nestling	34	1107
1957-41035	Pathfinder	6/19/18	WY	M	Nestling	30	1053
1207-82017	Jenga Rock	6/21/18	WY	F	Nestling	42	1545
1207-82018	Jenga Rock	6/21/18	WY	F	Nestling	41	1558
1957-41036	Jenga Rock	6/21/18	WY	M	Nestling	40	1066
1207-82019	Windmill 1	6/25/18	WY	F	Nestling	42	1721
1207-82020	Buff ID 2	6/25/18	WY	F	Nestling	29	1574
18WYWIN02	Windmill 2	6/25/18	WY	F	Nestling	28	1295
1957-31851	Windmill 1	6/25/18	WY	M	Nestling	40	1112
1957-31852	Buff ID 2	6/25/18	WY	M	Nestling	30	1074
1957-31853	Buff ID 2	6/25/18	WY	M	Nestling	31	1085
1957-31854	Buff ID 2	6/25/18	WY	M	Nestling	31	1021
1957-41037	Windmill 2	6/25/18	WY	M	Nestling	28	976
1957-41038	Windmill 1	6/25/18	WY	M	Nestling	42	1130
1207-82021	Sandstone City	6/26/18	WY	F	Nestling	31	1476
1207-82022	Sandstone City	6/26/18	WY	F	Nestling	29	1426

Individual ID	Nest ID	Date	State	Sex[1]	Life Stage	Age (d)	Mass (g)
1207-82023	Buff ID 44	6/26/18	WY	F	Nestling	30	1410
1957-31855	Sandstone City	6/26/18	WY	M	Nestling	30	1135
1957-31856	Buff ID 44	6/26/18	WY	M	Nestling	32	1068
1207-82024	Waltman	6/27/18	WY	F	Nestling	41	1552
1957-31857	Waltman	6/27/18	WY	M	Nestling	40	1121
1957-31858	Waltman	6/27/18	WY	M	Nestling	40	1028
1957-31859	Nebo	6/27/18	WY	M	Nestling	31	1088
1207-82025	Interstate 2	6/28/18	WY	F	Nestling	28	1372
1207-82026	Mines 4	6/28/18	WY	F	Nestling	42	1528
1207-82027	Mines 2	6/28/18	WY	F	Nestling	40	1489
1207-82028	Mines 2	6/28/18	WY	F	Nestling	40	1395
1207-82029	Buff ID 77	6/28/18	WY	F	Nestling	32	1549
1207-82030	Buff ID 78	6/28/18	WY	F	Nestling	30	1354
1957-31860	Interstate 2	6/28/18	WY	M	Nestling	28	1005
1957-31861	Buff ID 76	6/28/18	WY	M	Nestling	32	952
1207-82031	Pinedale 2	6/29/18	WY	F	Nestling	42	1644
2207-01921	Cow Pasture	5/31/19	ID	F	Nestling	34	1497
2207-01925	Surprise	6/02/19	ID	F	Nestling	40	1631
2207-01944	Dead Pigeon B	6/11/19	ID	F	Nestling	40	1471
19WYFH2701	FH-27	6/16/19	WY	U	Nestling	26	1240
19COSSN01	SSN01	6/26/19	CO	U	Nestling	21	817
2207-01959	Windmill 2	6/27/19	WY	F	Nestling	36	1466
2187-04462	Jenga Rock	6/27/19	WY	M	Nestling	40	1126
2207-01964	Pathfinder	6/29/19	WY	F	Nestling	30	1196
2187-04465	Pacific	6/29/19	WY	M	Nestling	39	1090
2207-01972	Pinedale 2	7/02/19	WY	F	Nestling	40	1586

[1]Presumptive sex (M = Male, F = Female) based on morphological measurements at time of capture. U = unknown sex.

Table A2.3 **Information about the individual ferruginous hawks (n = 117) from which citrated plasma samples were collected in 2019 and analyzed for prothrombin time (PT), thrombin clotting time (TCT) in Wyoming (WY) and Colorado (CO), USA. Only a subset (n = 101) had sufficient plasma volume to measure fibrinogen concentration (Fib. Conc.).**

								Clotting Time Assays		
Nestling ID	Nest ID	Date	Time (min)[1]	State	Sex[2]	Age (days)	Mass (g)	PT (sec)	TCT (sec)	Fib Conc. (mg/dL)
2187-04421	Cow Pasture	5/31/19	600	ID	M	36	985	29.5	6.7	120
2207-01921	Cow Pasture	5/31/19	600	ID	F	34	1497	41.2	7.4	107
2207-01922	Cow Pasture	5/31/19	600	ID	F	35	1492	29.5	8.3	122
2207-01923	Surprise	6/2/19	600	ID	F	40	1488	37.1	7.0	145
2207-01924	Surprise	6/2/19	600	ID	F	40	1609	36.7	7.5	-
2207-01925	Surprise	6/2/19	600	ID	F	40	1631	38.9	7.1	126
2207-01926	Cabin	6/3/19	600	ID	F	40	1415	37.1	8.0	127
2207-01927	Cabin	6/3/19	788	ID	F	38	1429	36.3	7.9	-
2207-01928	Cabin	6/3/19	788	ID	F	40	1432	32.0	7.3	-
2207-01929	Cabin	6/3/19	788	ID	F	38	1318	35.8	9.1	-
2187-04426	Moore's Road East	6/6/19	788	ID	M	34	879	27.9	9.0	76
2187-04429	Section 16 Farm	6/6/19	688	ID	M	38	1017	34.0	6.1	119
2187-04430	Section 16 Farm	6/6/19	835	ID	M	38	1063	30.9	6.1	114
2207-01930	Section 16 Farm	6/6/19	835	ID	F	38	1506	32.9	7.0	115
2207-01931	Section 16 Farm	6/6/19	835	ID	F	38	1481	35.0	6.7	107
2187-04434	Split Rim	6/7/19	835	ID	M	30	1017	27.0	6.7	67
2187-04435	Split Rim	6/7/19	690	ID	M	26	1025	27.6	7.1	98
2207-01932	Section 16 Farm W	6/7/19	860	ID	F	31	1668	27.6	7.8	93

Nestling ID	Nest ID	Date	Time (min)[1]	State	Sex[2]	Age (days)	Mass (g)	PT (sec)	TCT (sec)	Fib Conc. (mg/dL)
2207-01934	Split Rim	6/7/19	860	ID	F	30	1441	29.1	6.6	103
2187-04436	Range 17	6/8/19	860	ID	M	40	1066	25.9	6.3	114
2187-04438	Moore Road Central	6/8/19	721	ID	M	38	1062	23.7	17.6	-
2207-01935	Moore Road Central	6/8/19	502	ID	F	40	1606	29.9	6.6	106
2207-01936	Spoon Downstream	6/8/19	920	ID	F	27	1194	30.0	7.2	106
2207-01937	Spoon Downstream	6/8/19	721	ID	F	26	1162	29.2	6.7	118
2187-04440	Cinder Butte Road	6/10/19	920	ID	M	29	1017	27.4	6.9	124
2187-04441	Shadscale Exclosure	6/10/19	812	ID	M	30	992	33.8	8.7	103
2187-04442	Shadscale Exclosure	6/10/19	812	ID	M	30	921	34.3	9.0	105
2187-04443	Indian Creek	6/10/19	812	ID	M	29	965	23.7	7.5	120
2187-04444	Bryan's Run	6/10/19	910	ID	M	32	1052	27.8	7.9	111
2187-04445	Bryan's Run	6/10/19	910	ID	M	32	1027	30.2	8.9	92
2207-01938	Shadscale Exclosure	6/10/19	910	ID	F	32	1291	38.4	6.6	105
2207-01939	Shadscale Exclosure	6/10/19	812	ID	F	32	1459	37.9	7.8	84
2207-01940	Indian Creek	6/10/19	630	ID	F	30	1349	35.1	6.9	109
2207-01941	Indian Creek	6/10/19	630	ID	F	29	1405	32.9	6.7	97
2207-01942	Indian Creek	6/10/19	630	ID	F	28	1349	36.4	8.0	101
2207-01943	Bryan's Run	6/10/19	630	ID	F	30	1359	31.3	7.2	116
19IDCEB01	Coyote Exclosure	6/11/19	545	ID	M	26	849	33.9	8.4	98
19IDCEB02	Coyote Exclosure	6/11/19	505	ID	F	24	1027	31.4	6.8	101
19IDMRW01	Moore's Road West	6/11/19	505	ID	F	21	1110	27.6	6.0	147
19IDMRW02	Moore's Road West	6/11/19	825	ID	F	26	1167	29.5	6.7	141
19IDMRW03	Moore's Road West	6/11/19	710	ID	F	23	1267	27.7	5.2	163
19IDMRW04	Moore's Road West	6/11/19	710	ID	M	24	932	28.0	6.0	140

Nestling ID	Nest ID	Date	Time (min)[1]	State	Sex[2]	Age (days)	Mass (g)	PT (sec)	TCT (sec)	Fib Conc. (mg/dL)
19IDMRW05	Moore's Road West	6/11/19	710	ID	F	17	831	27.5	6.0	130
2187-04446	Coyote South	6/11/19	825	ID	M	32	1055	32.4	8.1	111
2187-04447	Coyote South	6/11/19	825	ID	M	32	991	30.8	7.7	108
2207-01944	Dead Pigeon B	6/11/19	825	ID	F	40	1471	38.7	7.2	84
2207-01945	Dead Pigeon B	6/11/19	825	ID	F	40	1528	36.2	7.9	78
2207-01946	Dead Pigeon B	6/11/19	626	ID	F	41	1550	35.9	7.2	88
2207-01947	Coyote South	6/11/19	626	ID	F	32	1422	36.8	6.1	114
19WYFH1A01	FH-1A	6/14/19	626	WY	U	22	800	23.9	6.7	108
19WYBI401	Buff ID 4	6/15/19	631	WY	U	28	1199	32.6	5.9	137
19WYBI402	Buff ID 4	6/15/19	768	WY	U	24	1044	34.2	6.0	136
19WYBI403	Buff ID 4	6/15/19	768	WY	U	30	1234	28.5	6.6	130
19WYFH3801	FH-38	6/15/19	768	WY	U	24	843	25.8	8.6	156
19WYFH3802	FH-38	6/15/19	565	WY	U	24	879	23.5	8.9	96
19WYFH701	FH-7	6/15/19	565	WY	U	24	797	27.3	5.1	178
19WYFH702	FH-7	6/15/19	671	WY	U	24	909	25.8	6.5	164
19WYFH2701	FH-27	6/16/19	671	WY	U	26	1240	29.8	5.0	141
19WYFH2702	FH-27	6/16/19	1040	WY	U	24	933	29.9	8.2	117
19WYFH2703	FH-27	6/16/19	1040	WY	U	28	992	24.6	9.2	101
19WYFH2901	FH-29	6/16/19	1040	WY	U	24	1200	25.1	7.3	134
19WYFH2902	FH-29	6/16/19	679	WY	U	30	1511	26.6	7.3	126
19WYFH2903	FH-29	6/16/19	679	WY	U	30	1431	25.9	7.0	120
19WYRED01	Red Desert	6/16/19	797	WY	U	26	1182	27.5	6.6	116
19WYRED02	Red Desert	6/16/19	922	WY	U	26	1206	27.0	6.5	120
19WYRED03	Red Desert	6/16/19	679	WY	U	24	894	25.5	7.5	-

Nestling ID	Nest ID	Date	Time (min)[1]	State	Sex[2]	Age (days)	Mass (g)	PT (sec)	TCT (sec)	Fib Conc. (mg/dL)
2187-04453	FH-30	6/16/19	922	WY	M	32	1042	29.0	8.0	114
19WYBI6801	Buff ID 68	6/17/19	922	WY	U	19	1001	28.9	6.1	135
19WYBI6802	Buff ID 68	6/17/19	815	WY	U	24	1117	29.3	6.1	144
19WYBI6803	Buff ID 68	6/17/19	815	WY	U	24	850	25.5	6.2	159
19WYFH2101	FH-21	6/20/19	815	WY	U	20	981	28.6	5.4	130
19WYFH2102	FH-21	6/20/19	650	WY	U	20	1018	22.4	8.1	-
19WYFH901	FH-9	6/20/19	650	WY	U	26	933	26.6	6.7	86
19WYFH902	FH-9	6/20/19	975	WY	U	24	843	27.6	7.0	153
2187-04454	Interstate 3	6/21/19	975	WY	M	33	794	23.2	8.5	-
2187-04455	Interstate 2	6/21/19	828	WY	M	34	1064	25.0	7.1	134
2207-01954	Interstate 3	6/21/19	828	WY	F	34	1485	37.3	6.3	145
2207-01955	Interstate 3	6/21/19	828	WY	F	34	1354	35.3	6.0	127
2207-01956	Interstate 2	6/21/19	680	WY	F	32	1425	29.2	6.5	109
2207-01957	Interstate 2	6/21/19	680	WY	F	32	1315	23.8	8.3	-
2187-04456	7438A	6/25/19	680	CO	M	38	1006	28.8	6.5	149
2187-04457	7438A	6/25/19	470	CO	M	34	964	24.6	7.0	121
19COSSN01	SSN01	6/26/19	470	CO	U	21	817	38.2	5.3	163
2187-04458	Windmill 1	6/27/19	700	WY	M	34	1020	22.7	7.4	126
2187-04459	Windmill 1	6/27/19	794	WY	M	32	941	22.9	7.0	125
2187-04460	Windmill 1	6/27/19	975	WY	M	34	930	25.6	6.9	126
2187-04461	Jenga Rock	6/27/19	794	WY	M	40	1102	22.2	10.6	-
2187-04462	Jenga Rock	6/27/19	794	WY	M	40	1126	21.6	7.4	122
2207-01958	Windmill 2	6/27/19	975	WY	F	36	1467	27.1	6.1	-
2207-01959	Windmill 2	6/27/19	975	WY	F	36	1466	21.3	6.3	92

Nestling ID	Nest ID	Date	Time (min)[1]	State	Sex[2]	Age (days)	Mass (g)	PT (sec)	TCT (sec)	Fib Conc. (mg/dL)
2207-01960	Windmill 1	6/27/19	652	WY	F	38	1589	25.0	6.1	134
2207-01961	Jenga Rock	6/27/19	652	WY	F	40	1577	27.6	6.7	109
1088-08527	WOTR	6/28/19	794	WY	F	40	1524	22.9	6.0	138
2187-04463	WOTR	6/28/19	622	WY	M	40	989	28.8	8.0	109
2187-04464	WOTR	6/28/19	622	WY	M	36	1021	27.4	7.9	108
1088-08528	Pacific	6/29/19	622	WY	F	42	1549	22.6	4.9	140
2187-04465	Pacific	6/29/19	580	WY	M	39	1090	21.7	6.6	-
2207-01962	Pacific	6/29/19	580	WY	F	40	1599	34.9	5.4	146
2207-01963	Pathfinder	6/29/19	708	WY	F	32	1389	29.8	6.2	114
2207-01964	Pathfinder	6/29/19	580	WY	F	30	1196	30.9	6.2	116
2187-04467	Sandstone City	6/30/19	708	WY	M	34	1179	33.9	6.7	121
2187-04468	Sandstone City	6/30/19	786	WY	M	34	1111	27.4	11.9	83
2187-04469	Sandstone City	6/30/19	786	WY	M	34	1044	28.5	6.7	100
2187-04470	Buff ID 2	6/30/19	786	WY	M	42	1160	29.6	8.4	94
2187-04484	Buff ID 2	6/30/19	786	WY	M	42	1127	36.7	8.0	73
2207-01966	Sandstone City	6/30/19	610	WY	F	36	1665	27.1	5.8	111
2207-01967	Buff ID 2	6/30/19	610	WY	F	40	1555	30.5	6.4	102
2207-01968	Buff ID 2	6/30/19	610	WY	F	42	1690	30.2	6.0	102
2187-04471	SSN02	7/1/19	610	CO	M	40	896	32.5	6.6	115
2207-01969	Buff ID 4	7/1/19	768	WY	F	40	1564	34.6	7.0	94
2207-01970	Buff ID 4	7/1/19	768	WY	F	40	1599	36.7	7.0	82
2207-01971	Buff ID 4	7/1/19	768	WY	F	38	1679	32.9	6.4	110
0788-59254	Pinedale 1	7/2/19	700	WY	F	38	1426	26.5	4.2	-
2187-04474	Pinedale 2	7/2/19	870	WY	M	40	1103	35.2	6.5	-

Nestling ID	Nest ID	Date	Time (min)[1]	State	Sex[2]	Age (days)	Mass (g)	PT (sec)	TCT (sec)	Fib Conc. (mg/dL)
2187-04475	Pinedale 2	7/2/19	870	WY	M	38	1064	28.4	6.4	-
2207-01972	Pinedale 2	7/2/19	870	WY	F	40	1586	41.1	6.0	-
2187-04422	Cow Pasture	5/31/19	965	ID	M	34	958	27.6	6.4	127

[1]Time is recorded as number of minutes after midnight.
[2] Presumptive sex based on morphology at time of capture.

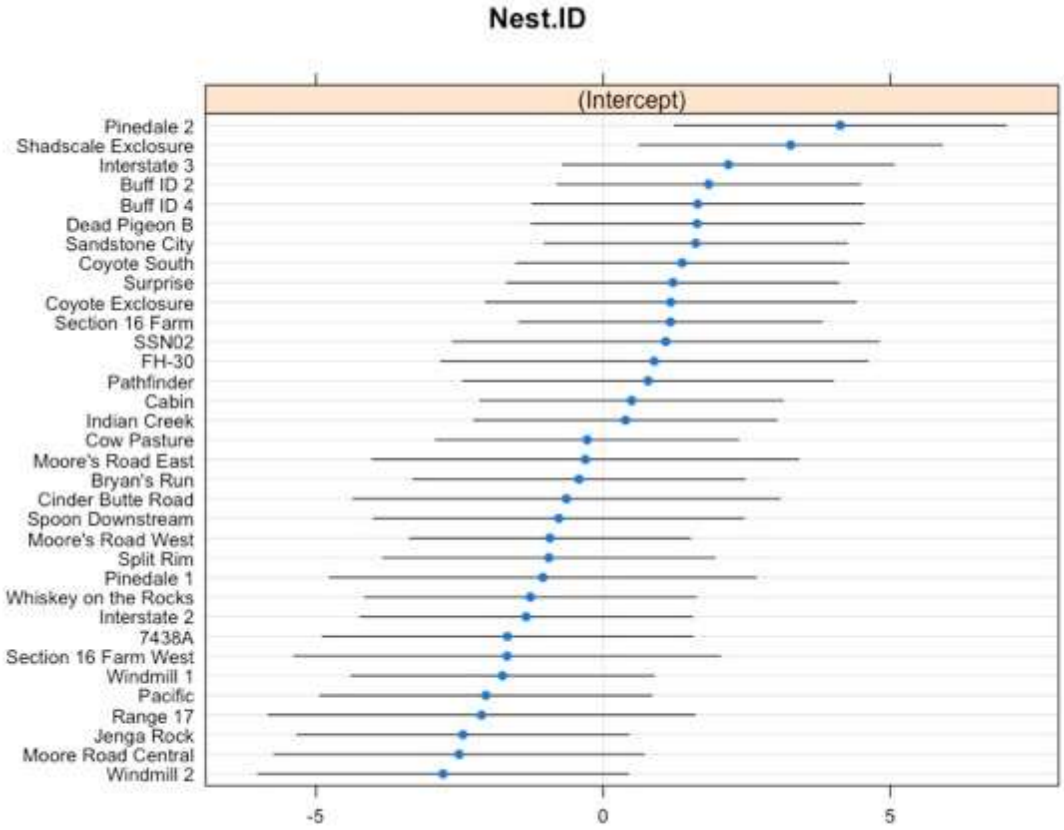

Figure. A2.1 **The random effect (intercept) of nest site on the estimated prothrombin time (PT).**

CHAPTER TWO: EVALUATING A RAPID FIELD ASSESSMENT SYSTEM FOR ANTICOAGULANT RODENTICIDE EXPOSURE OF RAPTORS

Abstract

Anticoagulant rodenticides (ARs) are compounds that are commonly used to control rodent pests by inhibiting an enzyme critical for clotting factor synthesis. Secondary and tertiary poisoning of non-target species frequently occurs, especially of predators. Raptors often hunt rodents, so secondary exposure to ARs is of conservation concern for many species of raptors. Indeed, post-mortem analysis of hepatic tissue from a large number of raptor species from around the world have revealed AR poisoning. Less understood is the prevalence of AR exposure and any possible sub-lethal effects in free-living raptor populations. Multiple point-of-care (POC) devices have been developed to measure PT and decrease costs incurred to human patients on anticoagulant drugs. I evaluated the potential of using one of these POC devices, the Coag-Sense® PT/INR Monitoring System manufactured by CoaguSense Inc. (Fremont, California), as a way to rapidly detect AR exposure in living birds of prey making use of small blood samples. The Coag-Sense® device delivered repeatable PT measurements on avian blood samples collected from four species of migrating raptors (Intraclass Correlation Coefficient >0.9). However, PT measurements reported by the Coag-Sense® system from 81 ferruginous hawk (*Buteo regalis*) nestlings were not correlated to those measured by standardized laboratory techniques ($r = -0.017$). The Coag-Sense® device therefore did not accurately measure PT in this species of bird and is unlikely to do so in other birds of prey. However, this device has potential use on non-human mammals.

Introduction

Anticoagulant rodenticides (ARs) have a long, global history as an effective and successful pesticide. Anticoagulants have been the main choice for rodent pest management because of their effectiveness in controlling pest populations, affordability, and the readily available treatment with vitamin K in the case of accidental poisoning (Hadler and Buckle, 1992; Jacob and Buckle, 2018). In the 1970s, evolution of AR resistance in some rodents to "first generation" compounds propelled the development of "second generation anticoagulant rodenticides" or SGARs (Pelz et al., 2005). These newer compounds have higher potency, a greater affinity to binding sites in the liver, and a longer half-life (Laakso et al., 2010).

ARs work by blocking a liver enzyme that is essential to the clotting factor synthesis cycle, depleting the level of clotting factors over time. The diminishing number of clotting factors subsequently increases clotting time, or prothrombin time (PT), to a point where hemorrhaging ensues (Rattner et al., 2010). This clotting mechanism is conserved among vertebrates, so ARs can have toxic effects on non-target species through secondary poisoning (Doolittle and Feng, 1987). The increased toxicity of SGARs exacerbates the negative effects on non-target wildlife, leading to conservation concern surrounding their use (Nakayama et al., 2019).

Accumulation of ARs is usually measured within the liver of deceased animals using high-performance liquid chromatography (HPLC) with fluorescence UV detection and mass spectrometry and/or liquid chromatography with tandem mass spectrometry (LC/MS/MS; Vandenbroucke et al., 2008; Hindmarch et al., 2019; Nakayama et al., 2019). Unfortunately, analysis of liver is only useful in post-mortem cases and therefore

cannot shed light on levels, timing or frequency of exposure in free-living wildlife, nor can it cast light on sub-lethal effects of AR exposure (Hindmarch et al., 2019). Analyzing AR concentrations in blood from living animals would be preferable, but such AR exposure assessments are challenging because they require large volumes of blood, they are expensive, and because ARs have a short half-life in blood. For example, ARs were detected in 94% of 24 livers but 0% of 10 blood samples in caracal (*Caracal caracal*) cats in South Africa (Serieys et al., 2019).

Because of the challenges associated with detecting ARs in blood, some studies have used clotting time assays, such as PT, as a biomarker for AR exposure (Murray and Tseng, 2008; Rattner et al., 2010; Hindmarch et al., 2019). This is because when exposed to ARs, clotting time in birds of prey is prolonged in relative proportion to the amount of AR ingested (Rattner et al., 2010). This use of clotting time as a biomarker creates opportunity to adapt technologies developed in other fields to understand wildlife exposure to ARs. In fact, around the time that ARs were gaining popularity as a method to control rodent pests, the same compounds also became the main preventative and treatment option for blood clots in humans (Pelz et al., 2005). Warfarin was one of the first such drugs heavily used (Fuentes et al., 2018). This drug can cause adverse effects when out of therapeutic range, so patients must be closely monitored by regularly measuring their clotting or prothrombin time (Pirmohamed, 2006; Wells et al., 2007). This need for constant monitoring has fueled the development of point-of-care (POC) devices that allows patients to self-test and adjust their drug dosage accordingly (Pirmohamed, 2006; Wells et al., 2007).

These recently developed POC devices for humans may be a feasible and relatively inexpensive way to detect AR exposure in free-living wildlife under field conditions. Benefits from an easy to use AR rapid assessment system could include the ability to rapidly treat exposed birds in the field, collect data on possible sub-lethal effects, and be able to assay ARs in smaller birds for which the blood volumes needed for laboratory assessment of AR presence preclude their use. Such an approach has worked before. For instance, field scientists and wildlife rehabilitation centers working with sick or injured birds test them for lead exposure using a point-of-care testing system originally designed for humans, e.g., the LeadCare® device (Rodriguez-Ramos et al., 2009).

Free-living raptors (birds of prey) are among the non-target species commonly exposed to ARs (Cox and Smith, 1992; EPA, 2017; van den Brink et al., 2018; Nakayama et al., 2019). Thus, my objective was to evaluate the potential use of a POC device originally developed for humans as a way to rapidly assess AR exposure in free-living birds of prey. I had two research questions: 1) How precise are PT measurements in bird blood from a commercially available POC device originally developed for humans, and 2) how accurate are the PT measurements collected for avian samples from that same POC device.

Methods

Coag-Sense® PT/INR Monitoring System

I used a Coag-Sense® PT/INR Monitoring System manufactured by CoaguSense Inc. (Fremont, California) and commercially available from Wilburn Medical USA (Kernersville, North Carolina) to measure the clotting response of free-living raptors (Fig. 2.1). The Coag-Sense® PT/INR Monitoring System is one of several POC

diagnostic devices that quantifies PT, measured in seconds, and the international normalized ratio (INR), which is unitless. INR is a standardized PT metric used to account for variation inherent in different reagents. INR incorporates an assigned International Sensitivity Index (ISI) metric for each thromboplastin. The ISI indicates how the particular thromboplastin compares to the international reference (Kitchen and Preston, 1999). INR is calculated as:

$$INR = \left(\frac{PT\ (patient)}{PT\ (reference)}\right)^{ISI}$$

INR is then used to adjust drug dosage for humans (Duxbury and Poller, 2001; Harris et al., 2013). According to the manufacturer's user manual, the Coag-Sense® PT/INR Monitoring System has built-in quality control software to undergo self-checks every time the machine is powered on to ensure that the timing function, battery level, mechanical and optical functions can operate within specification. If there are any problems detected during this self-check, an error message will be displayed, and the device will not test the sample. The control software also assures performance accuracy during sample testing, such as controlling a heater to ensure that the temperature of each sample is consistent (Review Memorandum, 2018). Once inside the device, a blood sample is mixed with a recombinant rabbit thromboplastin reagent to activate clotting factors (Harris et al., 2013). The resultant clot blocks an infrared photosensor and the rate at which the sensor is blocked allows the unit to measure speed of clotting (Review Memorandum, 2018). INR is calculated from PT measurements, and the Coag-Sense® displays and stores both the PT and INR measurements for each sample.

Intra-Device Precision

Before any quantitative instruments can be used for research or other clinical applications, their precision, or the extent that their measurements can be reproduced, should be evaluated (Koo and Li, 2016). To evaluate the precision of PT measurements from the Coag-Sense® PT/INR Monitoring System when using avian blood samples, I obtained and used two Coag-Sense® devices and evaluated samples collected from birds during Sept. – Oct. 2018 and 2019. Blood samples were collected from free-living raptors captured during fall migration at the Intermountain Bird Observatory's Lucky Peak research station located near Boise, Idaho, USA (43°36'18" N, 116° 3'36" W). Hawks were trapped by station personnel using lures, mist nets, bow nets and Dho-gazza traps and then restrained, usually for < 30 min, in canisters until processed (Bloom et al., 2007). During processing, morphometric measurements were collected, sex and age were estimated based on morphology and plumage, and each bird was given a uniquely numbered U.S. Geological Survey metal leg band. Subsequently, I pricked the bird's wing vein with a 25-gauge needle and collected the pooled blood with two non-heparinized microcapillary tubes, and then transferred the blood into two Coag-Sense® monitoring devices to measure clotting time and precision. I refer to the duplicate samples from an individual but measured by the two different devices as replicates 1 and 2.

Comparison of Prothrombin Time Estimates

To address my second research question, I evaluated the accuracy of the measurements collected from the Coag-Sense® PT/INR Monitoring System using blood samples from free-living ferruginous hawk (*Buteo regalis*) nestlings. To do this, I

compared paired PT estimates of an individual derived from two different techniques: using the POC device in the field versus analyzing a citrated plasma sample using a standard and previously validated laboratory technique (Rattner et al., 2010). In contrast to the analysis using the POC device, the laboratory analysis used avian thromboplastin, rather than rabbit thromboplastin, to active the clotting cascade. Commercially available thromboplastins based on mammalian tissues are able to initiate the clotting cascade in avian blood samples and have shown potential promise for use in birds (Guddorf et al., 2014).

I collected blood samples from nestling hawks in southwestern Idaho, southern and central Wyoming, and northern Colorado (Fig. 2.2) during May – July 2019. I hand captured nestlings from their nests, restrained them in metal canisters, took morphological measurements (Hull and Bloom, 2001), estimated their age and sex (Moritsch, 1985; Gossett, 1993), and collected blood samples from the brachial vein of each bird (Monks and Forbes, 2007). First, I collected 0.9 ml of blood in a 1-ml syringe preloaded with 0.1 ml of 3.2% sodium citrate (i.e., a citrated sample; Hindmarch et al. 2019). Immediately after the first sample was taken, I collected an additional 10 µl of blood from the same bird in a non-heparinized microcapillary tube and transferred it into the Coag-Sense® device. Then I transferred the citrated blood sample into a 1.5 ml-microcentrifuge tube, gently inverted it several times, and stored it on ice until all of the nestlings at a site had been similarly sampled. I then centrifuged the citrated blood samples in the field at 2,000 x g for 10 min. The resultant plasma (usually 400 – 600 µl) was transferred into 1.2 ml cryovials using micropipettes and promptly frozen and stored inside a cryoshipper containing liquid nitrogen (Hindmarch et al., 2019). Cryovials were

transferred into a -80°C freezer upon returning from the field. The citrated plasma samples were shipped on dry ice to the Comparative Coagulation Laboratory in the Animal Health Diagnostic Center at Cornell University (Ithaca, NY) for analysis of PT according to the protocol by Rattner et al. (2010, Appendix 1).

Statistical Analysis

All statistical analyses were performed using R software version 3.5.1 (R Core Team, 2018). Normality of the data was confirmed with Shapiro-Wilk tests. To assess the precision of the POC device when measuring PT on avian blood samples, I calculated the intraclass correlation coefficient (ICC) for both the INR and PT measurements using a single-rating, absolute-agreement, two-way mixed effects model (Koo and Li, 2016) in the package "irr" (Gamer et al., 2019).

To assess the accuracy of the Coag-Sense® measurements, I used a Pearson's correlation analysis to examine the association between the paired PT measurements generated by the POC device and the standard laboratory technique. I also evaluated the null hypothesis that the mean difference between the paired PT measurements was zero. Because differences were not normally distributed, I employed the non-parametric Wilcoxon signed-rank test to examine the agreement between the two different methods. Finally, I calculated the coefficient of variation and compared the variability of the PT measurements estimated by each type of assay. I only used samples that did not have clot fragments present prior to analysis or had not hemolyzed during PT analysis. Means are given with standard deviations.

Results

Intra-Device Precision

I obtained replicate blood samples from six individuals of four migratory species: northern goshawk (*Accipiter gentilis*; n = 1), Cooper's hawk (*A. cooperi*; n = 2), sharp-shinned hawk (*A. cooperii*; n = 2), and merlin (*Falco columbarius*; n = 1; Table 2.1). The individuals I sampled included birds in their hatching year (HY, n = 3), second year (SY, n = 2), and older (after second year; ASY, n = 1), and individuals of both sexes were obtained (Table 2.1).

There was a high degree of precision in the PT measurements collected by the two different Coag-Sense® devices with respect to the two replicates (Table 2.2; Fig 2.2; ICC > 0.9 indicate excellent repeatability; Koo and Li 2016). Similarly, INR values were also repeatable between the two devices (Table 2.2; ICC > 0.9).

Comparison of Prothrombin Time Estimates

I collected both citrated plasma and Coag-Sense® readings from 89 ferruginous hawk nestlings in 40 different nests. Eight samples either hemolyzed or had clot fragments present, which left 81 sample pairs suitable for analysis. There were 3 ± 1.0 nestlings per nest (range: 1 – 5) of which I sampled 2 ± 1.0 (range 1 – 4). The sampled nestlings were 33 ± 6.3 d of age (n = 81), ranging from 19 – 42 d. Thirty-two (37%) of the ferruginous hawk nestlings analyzed were putative males, 34 (38%) were female, and 15 (17%) were too young to predict sex.

The accuracy of PT measurements, as defined above, collected by the Coag-Sense® device was poor. PT measurements from the Coag-Sense® were, in nearly every instance, higher than those from the laboratory, and the mean difference (21.5 ± 13.8 sec,

n = 81) was significantly greater than zero (Wilcoxon signed-rank test: Z = 7.10, p < 0.001). Measurements collected from the Coag-Sense® device also had a larger range and were more variable overall compared to those from the laboratory (Table 2.3). Finally, there was no correlation between the PT estimates measured using the Coag-Sense® system and those measured in the laboratory (Pearson's correlation analysis: r = -0.017, p = 0.88, n = 81, Fig.2.3).

Discussion

There is a clear need to develop methods for rapid assessment of AR exposure in birds because of an increased conservation concern surrounding the impacts of ARs on non-target wildlife, how much is still unknown about the sub-lethal effects of ARs, and the prevalence of AR exposure in raptor populations (Hindmarch et al., 2019; Serieys et al., 2019). Thus, I evaluated the ability of the Coag-Sense® PT/INR Monitoring System to precisely and accurately measure PT in avian blood samples as a biomarker for AR exposure. Although the device was precise, indicated by the high ICC value (Table 2.2) from replicate PT measurements, it turned out to be inaccurate. Overall, average PT measurements from the Coag-sense® device were substantially higher and more variable than measurements derived using standard laboratory techniques. Furthermore, the Coag-sense® measurements did not correlate with the standard laboratory analyzed values. These results reveal that the use of the Coag-sense® system is unsuitable for rapidly assessing AR exposure in birds of prey.

One potential explanation for these results is that the particular reagent within the device was not able to activate the clotting cascade as it would in human and possibly other mammalian species. The POC device I used relies on commercially available

reagents to activate the clotting cascade (Harris et al., 2013). Because these commercially available reagents are based on rabbit thromboplastin, they may not be suitable for avian blood, although they had shown some promise in previous studies (Guddorf et al., 2014). Avian thromboplastin is not commercially available because of its lack of stability and therefore most commercial laboratories do not have the means to assay avian PT (Doerr, 1975; Webster et al., 2015). While not suitable for avian samples, the Coag-Sense® and other similar POC devices may be a great tool in rapidly assessing AR exposure in mammalian non-target wildlife (Guddorf et al., 2014). Specifically, one POC device, the CoaguChek®-XS, was shown to accurately and precisely measure PT in domestic dogs and horses (Newbould and Norman, 2013; Kelmer et al., 2014; Berlin et al., 2019).

To better assess AR exposure in raptor populations, research paths could include evaluating other POC devices or modifying these POC devices to include a more suitable reagent to assess PT in avian samples. In this study, I only evaluated one type of POC device for avian use. There are currently 18 different POC devices available for human use, and these devices use varying methods to estimate PT (Harris et al., 2013). In terms of modifying the device, changing the commercial reagent from rabbit to bovine thromboplastin may improve PT estimates from avian samples (Guddorf et al., 2014). An easily deployed methodology to assess AR exposure in field conditions would significantly improve our knowledge of the prevalence and effects in free-living raptor populations.

Using PT measurements derived from standard laboratory techniques has been proposed as a way to assess AR exposure (Murray and Tseng, 2008; Rattner et al., 2010; Hindmarch et al., 2019). However, field collection of samples and subsequent analysis

pose challenges. Among the challenges is that collecting citrated plasma samples requires a precise 1:9 sodium citrate to whole blood ratio, and quick access to ultra-low temperatures to prevent sample degradation. Moreover, avian thromboplastin requires substantial effort and expertise to prepare (Guddorf et al., 2014). Six hours after preparation, avian thromboplastin begins to become unstable, leading to reduced activity and artificially prolonging PT estimates (Doerr et al., 1975; Morrisey et al., 2003). Hence, variation between laboratories arises between differences in methodologies and batches of thromboplastin. To reduce variation between laboratories, a standardized methodology needs to be employed or analysis should be conducted at a single laboratory (Hindmarch et al., 2019). To account for differences in thromboplastins, a metric that can be compared globally for wildlife may need to be implemented, such as how INR was established for human patients to standardize differences in reagents used globally. These challenges make measuring PT a difficult way to monitor AR exposure in wildlife on any wide scale basis and underscore the ongoing need for the development of a new way to rapidly assess AR exposure in birds of prey.

Tables

Table 2.1 **The species, age, sex and PT/INR measurements (replicate 1 and 2) to examine the repeatability of Coag-Sense® values in birds of prey captured near Boise, Idaho, USA. Age codes: HY = hatching year, SY = second year, ASY = after second year.**

				INR		PT (sec)	
Year	**Species[1]**	**Age**	**Sex**	**Rep 1**	**Rep 2**	**Rep 1**	**Rep 2**
2018	Sharp-shinned hawk	SY	M	5.8	5.9	75.0	73.0
2018	Northern goshawk	HY	M	3.2	3.2	39.5	39.6
2018	Sharp-shinned hawk	ASY	F	4.1	4.3	52.0	55.4
2018	Cooper's hawk	SY	M	3.0	3.5	37.0	44.2
2019	Cooper's hawk	HY	F	3.5	3.9	38.4	43.1
2019	Merlin	HY	F	2.0	2.1	23.4	25.8

[1]See text for scientific name.

Table 2.2 **Precision assessment for INR and PT using the Coag-Sense® monitoring system on avian blood samples obtained from migrating raptors (n = 6) in the fall of 2018 and 2019 in Idaho. Data are presented as median (interquartile range) for the Coag-Sense® system (CS) Replicates 1 and 2; ICC, intraclass correlation coefficient (95% confidence interval); and P, corresponding p-value for ICC.**

Coagulation Test	CS Replicate 1	CS Replicate 2	ICC (95% CI)	P
INR	3.35 (3.05 - 3.95)	3.70 (3.28 - 4.20)	0.976 (0.64 – 1.00)	0.002
PT	38.90 (37.40 - 48.88)	43.70 (0.48 - 52.60)	0.972 (0.772 – 1.00)	< 0.001

Table 2.3 Summary of prothrombin time (PT) estimates in seconds for ferruginous hawk nestlings (n = 81) from Idaho, Wyoming and Colorado in 2019. Estimates were derived from using the Coag-Sense® monitoring system in the field and from using standard laboratory techniques (see text).

Method	Mean PT	Median PT	Range	Std. Deviation	CV[1]
Laboratory	29.8	29.2	21.3 - 41.2	4.82	16.2 %
Coag-Sense®	49.2	50.0	13.10 - 88.8	15.82	32.2 %

[1]Coefficeient of variation

Figures

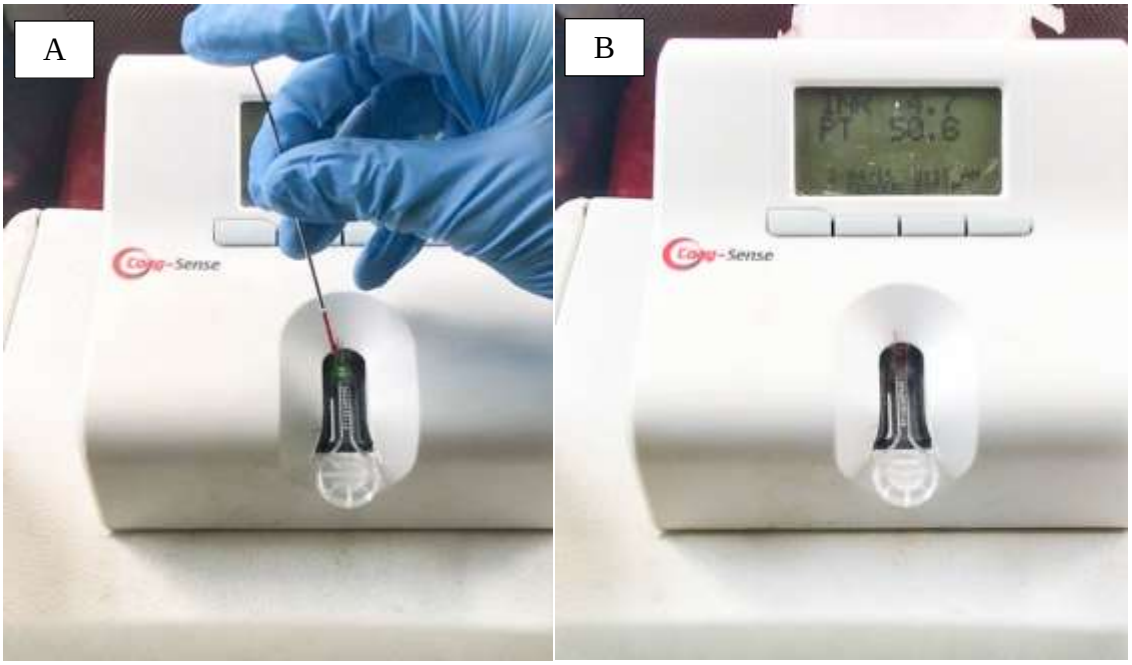

Figure 2.1 The Coag-Sense® PT/INR Monitoring System. Once a strip is inserted correctly and the device calibrates for 30 seconds, a 10 µl blood sample can be placed onto the strip (A). The Coag-Sense® then measures and displays prothrombin time (PT) and international normalized ratio (INR) for a sample (B).

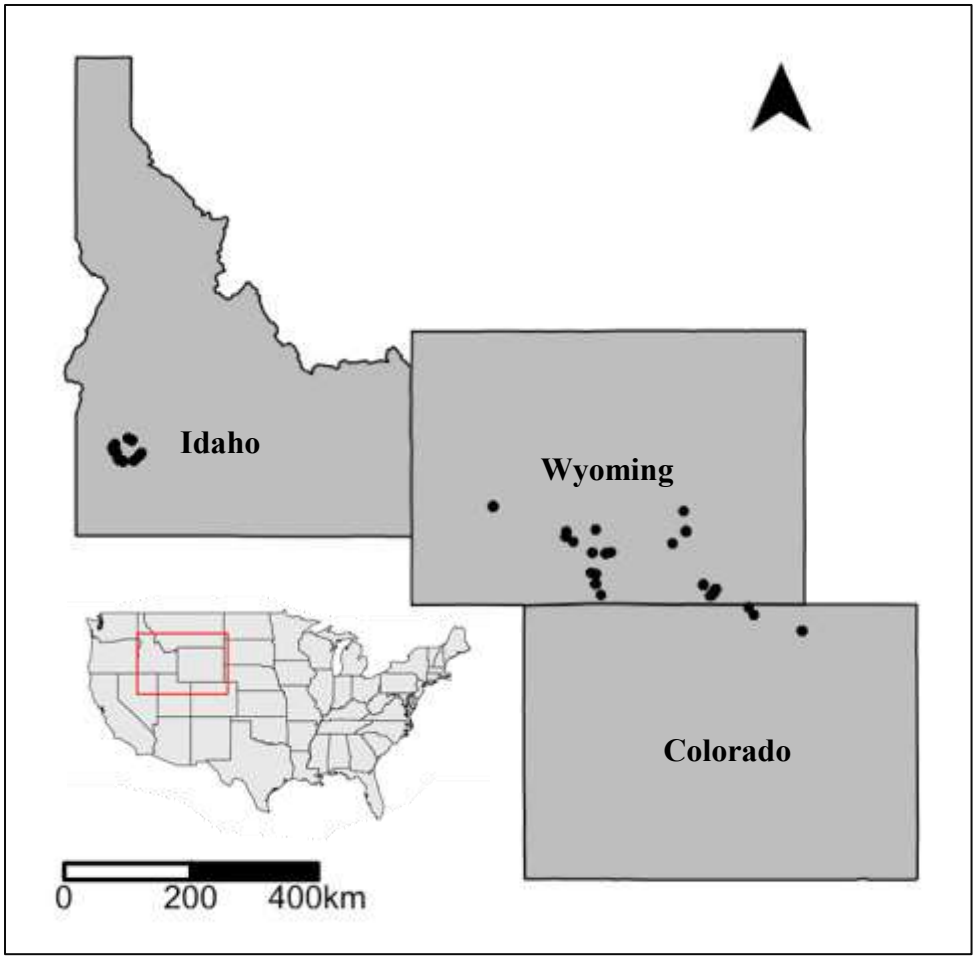

Figure 2.2 Location of ferruginous hawk nests where blood sampling for the measurement of prothrombin time (PT) and international normalized ratio (INR) was conducted in Idaho, Wyoming, and Colorado in the western United States during 2019.

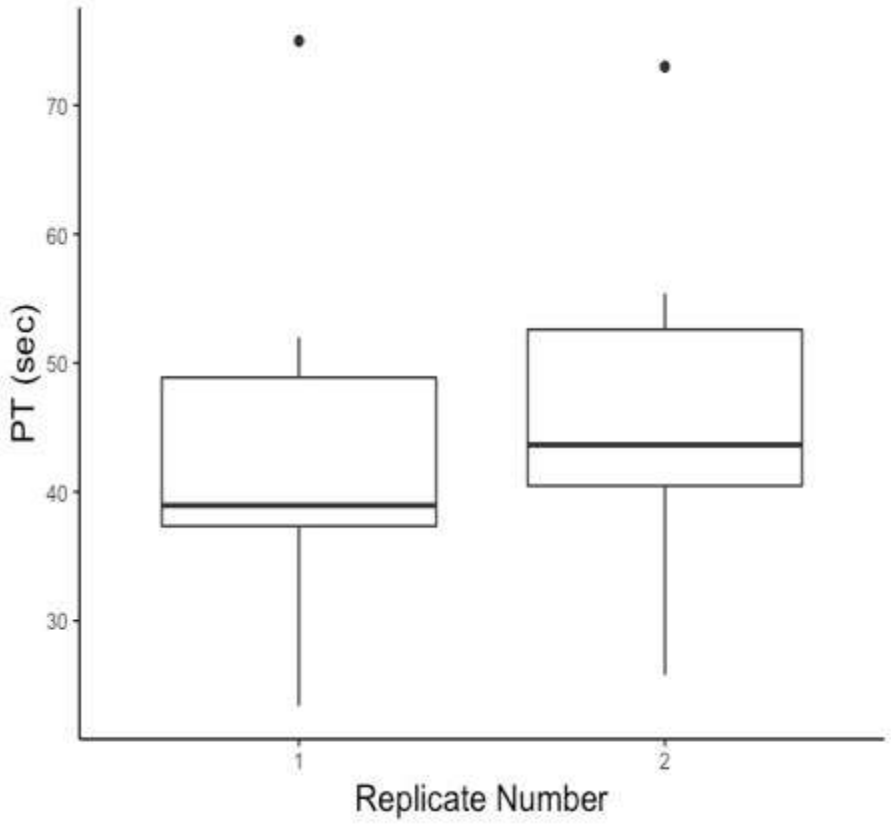

Figure 2.3 Prothrombin time (PT) estimates in migrating birds of prey (n = 6 individuals of 4 species, see Table 2.1) calculated using two different Coag-Sense® devices using samples derived from the same individual. (Replicate 1 and 2). Box plots show median, interquartile range, and the minimum and maximum for each replicate.

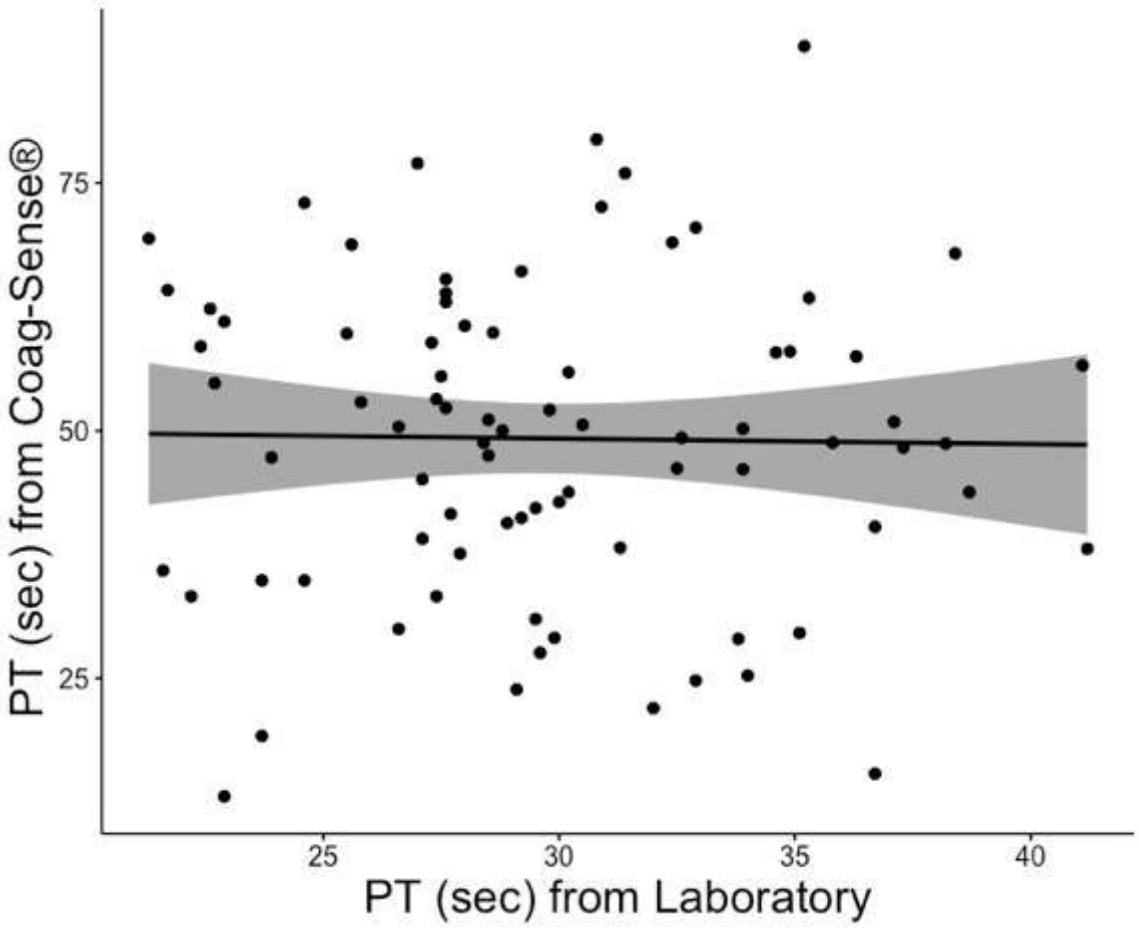

Figure 2.4 Relationship between prothrombin time (PT) estimates in nestling ferruginous hawks obtained from the Coag-Sense® system in the field and those using standard techniques as described by Rattner et al. (2010) in a laboratory (Pearson's correlation analysis: r = -0.017, p = 0.88, n = 81) on samples obtained from the same individual in rapid succession while in the field.

References

Berlin, N., Kelmer, E., Segev, G., Aroch, I., Kelmer, G., 2019. Assessment of the CoaguChek-XS portable prothrombin time point-of-care analyzer for horses. Journal of Veterinary Diagnostic Investigation. 31, 448–452.

Bloom, P. H., W. S. Clark, Kidd, J. W., 2007. Capture techniques. In: Bird, D.M., Bildstein, K.L., (Eds). Raptor Research and Management Techniques. Hancock House Publishers Ltd, Surrey, BC, Canada; Blaine, Washington, USA. pp. 193–219.

Cox, P., Smith, R.H., 1992. Rodenticide ecotoxicology: Pre-lethal effects of anticoagulants on rat behavior. Proceedings of the Fifteenth Vertebrate Pest Conference. 86,165–170.

Doerr, J.A., Wyatt, R.D., Hamilton, P.B., 1975. Investigation and standardization of prothrombin times in chickens. Poultry Science. 54, 969–980.

Doolittle, R.F., Feng, D.F., 1987. Reconstructing the evolution of vertebrate blood coagulation from a consideration of the amino acid sequences of clotting proteins. Cold Spring Harbor Symposia on Quantitative Biology. 52, 869–874.

Duxbury, B.M., Poller, L., 2001. State-of-the-art review: The oral anticoagulant saga: past, present, and future. Clinical Applied Thrombosis/Hemostasis. 7, 269–275.

EPA, 2017. Restrictions on Rodenticide Products. https://www.epa.gov/rodenticides/restrictions-rodenticide-products. (accessed 3 October 2017).

Fuentes, A.V., Pineda, M.D., Venkata, K.C.N., 2018. Comprehension of top 200 prescribed drugs in the us as a resource for pharmacy teaching, training and practice. Pharmacy. 6, 1-10.

Gamer, M., Lemon, J., Singh, P., 2019. irr: Various Coefficients of Interrater Reliability and Agreement. R package version 0.84.1.https://CRAN.R-project.org/package=irr.

Gossett, D.N., 1993. Studies of ferruginous hawk biology: I. Recoveries of banded ferruginous hawks from presumed eastern and western subpopulations. II. Morphological and genetic differences of presumed subpopulations of ferruginous hawks. III. Sex determination of nestling ferruginous hawks. M.S. Thesis, Boise State University, Boise, Idaho.

Guddorf V., Kummerfeld, N., Mischke, R., 2014. Methodical aspects of blood coagulation measurements in birds applying commercial reagents – a pilot study. Berliner und Münchener Tierärztliche Wochenschrift. 127, 322–327.

Hadler, M.R., and Buckle, A.P., 1992. Forty-five years of anticoagulant rodenticides - past, present and future trends. Proceedings of the Fifteenth Vertebrate Pest Conference. 15, 149–155.

Harris, L.F., Castro-López, V., Killard, A.J., 2013. Coagulation monitoring devices: Past, present, and future at the point of care. Trends in Analytical Chemistry. 50, 85–95.

Hindmarch, S., Rattner, B.A., Elliott, J.E., 2019. Use of blood clotting assays to assess potential anticoagulant rodenticide exposure and effects in free-ranging birds of prey. Science of The Total Environment. 657, 1205–1216.

Hull, B., Bloom, P., 2001.The North American banders manual for raptor banding techniques. North American Banding Council, Point Reyes Station, California, USA.

Jacob J., Buckle A, 2018. Use of anticoagulant rodenticides in different applications around the world. In: van den Brink N., Elliott J., Shore R., Rattner B., (Eds) Anticoagulant Rodenticides and Wildlife. Emerging Topics in Ecotoxicology (Principles, Approaches and Perspectives). Springer Science, New York, New York, USA.

Kelmer, E., Segev, G., Codner, C., Bruchim, Y., Klainbart, S., Aroch, I., 2014. Assessment of a portable prothrombin time analyzer (CoaguChek-XS) in dogs. Journal of Veterinary Emergency and Critical Care. 24, 455–460.

Kitchen, S., Preston, F.E., 1999. Standardization of prothrombin time for laboratory control of oral anticoagulant therapy. Seminars in Thrombosis and Hemostasis. 25, 17-25.

Koo, T.K., Li, M.Y., 2016. A guideline of selecting and reporting intraclass correlation coefficients for reliability research. Journal of Chiropractic Medicine. 15, 155–163.

Laakso, S., Suomalainen, K., Koivisto, S., 2010. Literature review on residues of anticoagulants in non-target animals. TemaNord. 541, 1-47.

Monks, D.J., and Forbes, N.A., 2007. Hematological. In: Bildstein, K.L., Bird, D.M., (Eds.), Raptor research and management techniques. Hancock House Publishers Ltd, Surrey, BC, Canada, and Blaine, Washington, USA.

Moritsch, M., 1985. Photographic Guide for Aging Nestling Ferruginous Hawks. Bureau of Land Management, Boise, Idaho, USA.

Morrisey, J.K., Paul-Murphy, J., Fialkowski, J.P., Hart, A., Darien, B.J., 2003. Estimation of prothrombin times of Hispaniolan Amazon parrots (*Amazona ventralis*) and umbrella cockatoos (*Cacatua alba*). Journal of Avian Medicine and Surgery. 17, 72–77.

Murray, M., Tseng, F., 2008. Diagnosis and treatment of secondary anticoagulant rodenticide toxicosis in a red-tailed hawk (*Buteo jamaicensis*). Journal of Avian Medicine and Surgery. 22, 41–46.

Nakayama, S.M.M., Morita, A., Ikenaka, Y., Mizukawa, H., Ishizuka, M., 2019. A review: poisoning by anticoagulant rodenticides in non-target animals globally. Journal of Veterinary Medical Science. 81, 298–313.

Newbould, A., Norman, E., 2013. Comparison of point-of-care analysis using Coaguchek XS and standard laboratory-measured prothrombin time in dogs. New Zealand Veterinary Journal. 61, 18–24.

Pelz, H.J., Rost, S., Hünerberg, M., Fregin, A., Heiberg, A.C., Baert, K., MacNicoll, A.D., Prescott, C.V., Walker, A.-S., Oldenburg, J., Müller, C.R., 2005. The genetic basis of resistance to anticoagulants in rodents. Genetics. 170, 1839–1847.

Pirmohamed, M., 2006. Warfarin: almost 60 years old and still causing problems. British Journal of Clinical Pharmacology. 62, 509–511.

R Core Team, 2018. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Rattner, B.A., Horak, K.E., Johnston, J.J., 2010. Comparative toxicity of diphacinone to northern bobwhite (*Colinus virginianus*) and American kestrels (*Falco sparverius*). USDA National Wildlife Research Center - Staff Publications. 1268.

Review Memorandum, 2018. Substantial Equivalence Determination Decision Summary. http://www.accessdata.fda.gov/cdrh_docs/reviews/K093243.pdf> (accessed 17 October 2019).

Rodriguez-Ramos, J., Gutierrez V., Holfe U., Mateo R., Monslave L., Crespo E., Blanco J.M., 2009. Lead in griffon and cinereous vultures in central Spain: Correlations between clinical signs and blood lead levels. in: Watson, R.T, Fuller, M., Pokras, M., , Hunt W.G., (Eds.), Ingestion of Lead from Spent Ammunition: Implications for Wildlife and Humans, The Peregrine Fund, Boise, Idaho, USA.

Serieys, L.E.K., Bishop, J., Okes, N., Broadfield, J., Winterton, D.J., Poppenga, R.H., Viljoen, S., Wayne, R.K., O'Riain, M.J., 2019. Widespread anticoagulant poison exposure in predators in a rapidly growing South African city. Science of The Total Environment. 666, 581–590.

van den Brink, N.W, J. Elliott, R. Shore, B. Rattner., (Eds.), 2018. Anticoagulant Rodenticides and Wildlife, Springer Science. New York, New York, USA.

Vandenbroucke, V., Bousquet- Melou, A., Backer, P.D., Croubels, S., 2008. Pharmacokinetics of eight anticoagulant rodenticides in mice after single oral administration. Journal of Veterinary Pharmacology and Therapeutics. 31, 437–445.

Webster, K., Harr, K., Bennett, D., Williams, T., Cheng, K., Maisonneuve, F., Elliott, J., 2015. Assessment of toxicity and coagulopathy of brodifacoum in Japanese quail and testing in wild owls. Ecotoxicology. 24, 1087–1101

Wells, P.S., Brown, A., Jaffey, J., McGahan, L., Poon, M.-C., Cimon, K., 2017. Safety and effectiveness of point-of-care monitoring devices in patients on oral anticoagulant therapy: a meta-analysis. Open Medicine. 1, 131-146.

APPENDIX 3

Chapter 2 Table

Table A3.1 **Information about the individual ferruginous hawks (n = 81) from which prothrombin time (PT) was estimated in 2019 using both the Coag-Sense® device and standard laboratory techniques (done in duplicate). Samples were collected in Idaho (ID), Colorado (CO), and Wyoming (WY), USA.**

						Prothrombin Time (sec) Estimation			
Identification	**Sex[1]**	**Age (d)**	**State**	**Nest ID**	**Date**	**Coag-sense®**	**Lab Rep 1**	**Lab Rep 2**	**Lab Mean**
2187-04421	M	36	ID	Cow Pasture	5/31/19	42.2	29.5	29.5	29.5
2187-04422	M	34	ID	Cow Pasture	5/31/19	63.9	27.7	27.5	27.6
2207-01921	F	34	ID	Cow Pasture	5/31/19	38.1	41.2	41.2	41.2
2207-01922	F	35	ID	Cow Pasture	5/31/19	31.0	29.5	29.5	29.5
2207-01926	F	40	ID	Cabin	6/3/19	50.9	37	37.2	37.1
2207-01927	F	38	ID	Cabin	6/3/19	57.5	36.4	36.2	36.3
2207-01928	F	40	ID	Cabin	6/3/19	22.0	32.0	32.0	32.0
2207-01929	F	38	ID	Cabin	6/3/19	48.8	35.9	35.6	35.8
2187-04426	M	34	ID	Moore's Road East	6/6/19	37.6	28.3	27.5	27.9
2187-04429	M	38	ID	Section 16 Farm	6/6/19	25.3	34.1	33.8	34.0
2187-04430	M	38	ID	Section 16 Farm	6/6/19	72.6	31	30.7	30.9
2207-01930	F	38	ID	Section 16 Farm	6/6/19	70.5	32.8	32.9	32.9
2207-01932	F	31	ID	Section 16 Farm W	6/7/19	65.3	27.5	27.6	27.6
2187-04434	M	30	ID	Split Rim	6/7/19	77.0	27.1	26.9	27.0
2187-04435	M	26	ID	Split Rim	6/7/19	52.3	27.7	27.5	27.6
2207-01934	F	30	ID	Split Rim	6/7/19	23.9	29.1	29.0	29.1
2187-04438	M	38	ID	Moore Road Central	6/8/19	19.2	23.8	23.5	23.7
2207-01935	F	40	ID	Moore Road Central	6/8/19	29.1	29.8	29.9	29.9
2207-01936	F	27	ID	Spoon Downstream	6/8/19	42.8	30.0	30.0	30.0
2207-01937	F	26	ID	Spoon Downstream	6/8/19	66.1	29.2	29.2	29.2
2187-04445	M	32	ID	Bryan's Run	6/10/19	55.9	30.3	30.1	30.2

						Prothrombin Time (sec) Estimation			
Identification	**Sex[1]**	**Age (d)**	**State**	**Nest ID**	**Date**	**Coag-sense®**	**Lab Rep 1**	**Lab Rep 2**	**Lab Mean**
2207-01943	F	30	ID	Bryan's Run	6/10/19	38.2	31.5	31.0	31.3
2187-04440	M	29	ID	Cinder Butte Road	6/10/19	53.2	27.8	26.9	27.4
2187-04443	M	29	ID	Indian Creek	6/10/19	34.9	23.9	23.5	23.7
2207-01940	F	30	ID	Indian Creek	6/10/19	29.6	35.2	35.0	35.1
2207-01941	F	29	ID	Indian Creek	6/10/19	24.8	32.8	32.9	32.9
2187-04441	M	30	ID	Shadscale Exclosure	6/10/19	29.0	33.9	33.7	33.8
2207-01938	F	32	ID	Shadscale Exclosure	6/10/19	67.9	38.4	38.3	38.4
19IDCEB01	M	26	ID	Coyote Exclosure	6/11/19	46.1	33.9	33.8	33.9
19IDCEB02	F	24	ID	Coyote Exclosure	6/11/19	76.0	31.1	31.7	31.4
2187-04446	M	32	ID	Coyote South	6/11/19	69.0	32.7	32.1	32.4
2187-04447	M	32	ID	Coyote South	6/11/19	79.4	30.9	30.6	30.8
2207-01944	F	40	ID	Dead Pigeon B	6/11/19	43.8	38.6	38.8	38.7
19IDMRW03	F	23	ID	Moore's Road West	6/11/19	41.6	27.7	27.6	27.7
19IDMRW04	M	24	ID	Moore's Road West	6/11/19	60.6	27.9	28.0	28.0
19WYFH1A01	U	22	WY	FH-1A	6/14/19	47.3	23.7	24.1	23.9
19WYBI401	U	28	WY	Buff ID 4	6/15/19	49.3	32.4	32.7	32.6
19WYBI403	U	30	WY	Buff ID 4	6/15/19	51.1	28.4	28.6	28.5
2207-01969	F	40	WY	Buff ID 4	6/15/19	57.9	34.6	34.5	34.6
2207-01970	F	40	WY	Buff ID 4	6/15/19	40.3	36.7	36.7	36.7
19WYFH3801	U	24	WY	FH-38	6/15/19	52.9	25.9	25.7	25.8
19WYFH701	U	24	WY	FH-7	6/15/19	58.9	26.8	27.7	27.3
19WYFH2703	U	28	WY	FH-27	6/16/19	73.0	24.8	24.3	24.6
19WYFH2902	U	30	WY	FH-29	6/16/19	30.0	26.2	26.9	26.6
19WYRED01	U	26	WY	Red Desert	6/16/19	55.5	27.4	27.5	27.5

						Prothrombin Time (sec) Estimation			
Identification	**Sex[1]**	**Age (d)**	**State**	**Nest ID**	**Date**	**Coag-sense®**	**Lab Rep 1**	**Lab Rep 2**	**Lab Mean**
19WYRED03	U	24	WY	Red Desert	6/16/19	59.8	25.6	25.3	25.5
19WYBI6801	U	19	WY	Buff ID 68	6/17/19	40.7	29	28.8	28.9
19WYFH2101	U	20	WY	FH-21	6/20/19	59.9	28.6	28.5	28.6
19WYFH2102	U	20	WY	FH-21	6/20/19	58.5	22.3	22.5	22.4
19WYFH901	U	26	WY	FH-9	6/20/19	50.4	26.6	26.5	26.6
19WYFH902	U	24	WY	FH-9	6/20/19	63.0	27.6	27.5	27.6
2207-01956	F	32	WY	Interstate 2	6/21/19	41.2	29.2	29.2	29.2
2207-01954	F	34	WY	Interstate 3	6/21/19	48.3	37.3	37.3	37.3
2207-01955	F	34	WY	Interstate 3	6/21/19	63.4	35.4	35.2	35.3
2187-04456	M	38	CO	7438A	6/25/19	50.0	28.9	28.7	28.8
2187-04457	M	34	CO	7438A	6/25/19	34.9	24.7	24.4	24.6
19COSSN01	U	21	CO	SSN01	6/26/19	48.7	38.1	38.3	38.2
2187-04461	M	40	WY	Jenga Rock	6/27/19	33.3	22.5	21.9	22.2
2187-04462	M	40	WY	Jenga Rock	6/27/19	35.9	21.6	21.5	21.6
2187-04458	M	34	WY	Windmill 1	6/27/19	54.8	22.9	22.4	22.7
2187-04459	M	32	WY	Windmill 1	6/27/19	13.1	22.9	22.9	22.9
2187-04460	M	34	WY	Windmill 1	6/27/19	68.8	25.6	25.6	25.6
2207-01958	F	36	WY	Windmill 2	6/27/19	39.1	26.9	27.3	27.1
2207-01959	F	36	WY	Windmill 2	6/27/19	69.4	21.4	21.2	21.3
1088-08527	F	40	WY	WOTR	6/28/19	61.0	23.3	22.5	22.9
1088-08528	F	42	WY	Pacific	6/29/19	62.3	22.6	22.5	22.6
2187-04465	M	39	WY	Pacific	6/29/19	64.2	21.0	22.4	21.7
2207-01962	F	40	WY	Pacific	6/29/19	58.0	34.8	34.9	34.9
2207-01963	F	32	WY	Pathfinder	6/29/19	52.1	29.8	29.7	29.8

						Prothrombin Time (sec) Estimation			
Identification	**Sex[1]**	**Age (d)**	**State**	**Nest ID**	**Date**	**Coag-sense®**	**Lab Rep 1**	**Lab Rep 2**	**Lab Mean**
2187-04470	M	42	WY	Buff ID 2	6/30/19	27.6	29.7	29.4	29.6
2187-04484	M	42	WY	Buff ID 2	6/30/19	15.4	36.7	36.6	36.7
2207-01967	F	40	WY	Buff ID 2	6/30/19	50.6	30.6	30.4	30.5
2207-01968	F	42	WY	Buff ID 2	6/30/19	43.8	30.0	30.3	30.2
2187-04467	M	34	WY	Sandstone City	6/30/19	50.2	32.0	35.7	33.9
2187-04468	M	34	WY	Sandstone City	6/30/19	33.3	27.3	27.4	27.4
2187-04469	M	34	WY	Sandstone City	6/30/19	47.5	28.5	28.4	28.5
2207-01966	F	36	WY	Sandstone City	6/30/19	45.1	27.1	27.1	27.1
2187-04471	M	40	CO	SSN02	7/1/19	46.2	32.4	32.6	32.5
2187-04474	M	40	WY	Pinedale 2	7/2/19	88.8	35.4	35.0	35.2
2187-04475	M	38	WY	Pinedale 2	7/2/19	48.8	28.4	28.4	28.4
2207-01972	F	40	WY	Pinedale 2	7/2/19	56.6	41.2	41.0	41.1

[1]Presumptive sex based on morphology at time of capture