UNIVERSIDADE DE LISBOA

FACULDADE DE MEDICINA VETERINÁRIA

Retrospective study of blood lead poisoning in Bald Eagles (*Haliaeetus leucocephalus)* in Virginia, United States of America, from 2017 to 2021

SOFIA MASCARENHAS NETO DE MELO SOARES

ORIENTADOR:
Doutor Virgílio da Silva Almeida

TUTORA:
Dra. Karra Pierce

2023

UNIVERSIDADE DE LISBOA

FACULDADE DE MEDICINA VETERINÁRIA

Retrospective study of blood lead poisoning in Bald Eagles (*Haliaeetus leucocephalus*) in Virginia, United States of America, from 2017 to 2021

SOFIA MASCARENHAS NETO DE MELO SOARES

DISSERTAÇÃO DE MESTRADO EM MEDICINA VETERINÁRIA

JÚRI
PRESIDENTE:
Doutora Maria Manuela Castilho Monteiro de Oliveira
VOGAIS:
Doutor Virgílio da Silva Almeida
Doutora Anabela de Sousa Santos Silva Moreira

ORIENTADOR:
Doutor Virgílio da Silva Almeida

TUTORA:
Dra. Karra Pierce

2023

DECLARAÇÃO RELATIVA ÀS CONDIÇÕES DE REPRODUÇÃO DA DISSERTAÇÃO

Nome: Sofia Mascarenhas Neto de Melo Soares

Título da Tese ou Dissertação: Retrospective study of blood lead poisoning in Bald Eagles (Haliaeetus leucocephalus) in Virginia, United States of America, from 2017 to 2021

Ano de conclusão (indicar o da data da realização das provas públicas): 2023

Designação do curso de Mestrado ou de Doutoramento: Mestrado Integrados em Medicina Veterinária

Área científica em que melhor se enquadra (assinale uma):

☐ Clínica ☐ Produção Animal e Segurança Alimentar

☐ Morfologia e Função x☐ Sanidade Animal

Declaro sobre compromisso de honra que a tese ou dissertação agora entregue corresponde à que foi aprovada pelo júri constituído pela Faculdade de Medicina Veterinária da ULISBOA.

Declaro que concedo à Faculdade de Medicina Veterinária e aos seus agentes uma licença não-exclusiva para arquivar e tornar acessível, nomeadamente através do seu repositório institucional, nas condições abaixo indicadas, a minha tese ou dissertação, no todo ou em parte, em suporte digital.

Declaro que autorizo a Faculdade de Medicina Veterinária a arquivar mais de uma cópia da tese ou dissertação e a, sem alterar o seu conteúdo, converter o documento entregue, para qualquer formato de ficheiro, meio ou suporte, para efeitos de preservação e acesso.

Retenho todos os direitos de autor relativos à tese ou dissertação, e o direito de a usar em trabalhos futuros (como artigos ou livros).

Concordo que a minha tese ou dissertação seja colocada no repositório da Faculdade de Medicina Veterinária com o seguinte estatuto (assinale um):

1. x☐ Disponibilização imediata do conjunto do trabalho para acesso mundial;
2. ☐ Disponibilização do conjunto do trabalho para acesso exclusivo na Faculdade de Medicina Veterinária durante o período de ☐ 6 meses, ☐ 12 meses, sendo que após o tempo assinalado autorizo o acesso mundial*;

* Indique o motivo do embargo (OBRIGATÓRIO)

Nos exemplares das dissertações de mestrado ou teses de doutoramento entregues para a prestação de provas na Universidade e dos quais é obrigatoriamente enviado um exemplar para depósito na Biblioteca da Faculdade de Medicina Veterinária da Universidade de Lisboa deve constar uma das seguintes declarações (incluir apenas uma das três):

1. É AUTORIZADA A REPRODUÇÃO INTEGRAL DESTA TESE/TRABALHO APENAS PARA EFEITOS DE INVESTIGAÇÃO, MEDIANTE DECLARAÇÃO ESCRITA DO INTERESSADO, QUE A TAL SE COMPROMETE.
2. É AUTORIZADA A REPRODUÇÃO PARCIAL DESTA TESE/TRABALHO (indicar, caso tal seja necessário, nº máximo de páginas, ilustrações, gráficos, etc.) APENAS PARA EFEITOS DE INVESTIGAÇÃO, MEDIANTE DECLARAÇÃO ESCRITA DO INTERESSADO, QUE A TAL SE COMPROMETE.
3. DE ACORDO COM A LEGISLAÇÃO EM VIGOR, (indicar, caso tal seja necessário, nº máximo de páginas, ilustrações, gráficos, etc.) NÃO É PERMITIDA A REPRODUÇÃO DE QUALQUER PARTE DESTA TESE/TRABALHO.

Faculdade de Medicina Veterinária da Universidade de Lisboa, 3 de julho de 2023

Assinatura: Sofia Soares

“Wildlife is the barometer of our planet. If it is in danger, we are all in danger.”

David Attenborough

Acknowledgments

First and foremost, my most heartfelt acknowledgment to my parents Ana and Pedro, sister Leonor and grandparents José, Lisete, Patrocínia and Rui. They are my support system, who have always encouraged my dreams, provided me with all the tools needed and helped me through all the obstacles along the way. Everything I have become today I owe them.

To my supervisor Professor Virgílio Almeida, I would like to express my sincere gratitude for his unwavering support and motivation, especially for providing guidance and feedback. I am also extremely thankful for my co-supervisor, Dra. Karra Pierce of the Wildlife Center of Virginia (WCV) not only for all the knowledge transmitted, but also for the hospitality and kindness.

I had the pleasure of working with the WCV staff, students and volunteers, who received me with open arms and shared with me their passion for wildlife. To Dra. Olivia, I wish to thank for giving me more confidence in my work; to Jess, thank you for teaching me the way through the laboratory; to my fellow vet externs – Andrew, Ana and Clarrissa, I am thankful to have shared this experience with such hard-working and enthusiastic people; to all my house roommates, thank you so much for making me feel at home!

In addition, I would like to highlight my experience in CRAS HV UTAD. I owe a debt of gratitude to the team, Dr. Roberto, Dra. Filipa, Dr. Luís and Dr. Diogo, for inspiring me to pursue knowledge and showing me a standard of excellence in wildlife rehabilitation. Moreover, I appreciate all the valuable comments given to this dissertation. My time at CRAS HV UTAD will always have a special place in my heart.

I am thankful to have met such wonderful and passionate people, during all my other externships. In particular, Inês and Catarina, I am lucky to have met you and I appreciate our friendship very much. To Jorge I am thankful for all the support and shared love for nature.

I could not have undertaken this academic journey without my friends, Inês, Joana, Mafalda and Mariana. Thank you for all the help, support and laughs, but more importantly for making my university experience better and unforgettable.

To all my childhood friends, words cannot express my gratitude for all the good memories, lessons and for growing up with me. A special appreciation for Daniela, Débora, Joana B., Joana L., Madalena, Margarida N., Margarida T., Nádia and Rita.

Lastly, I am extremely grateful to everyone who contributed in any way to my veterinarian knowledge and experience during all these academic years.

Thank you!

ESTUDO RETROSPECTIVO DE INTOXICAÇÃO POR CHUMBO NO SANGUE EM ÁGUIAS-DE-CABEÇA-BRANCA (*Haliaeetus leucocephalus*) EM VIRGINIA, ESTADOS UNIDOS DA AMÉRICA, DE 2017 A 2021

Resumo

Devido à posição trófica na cadeira alimentar e comportamento oportunista de alimentação, a Águia-de-cabeça-branca (*Haliaeetus leucocephalus)* tem predisposição a bioacumular contaminantes presentes no ambientes e fontes de alimento. Logo, monitorizar esta espécie permite obter informações relevantes acerca do ecossistema em que habita e também de outros animais selvagens e seres humanos.

Apesar de que medidas terem sido implementas para reduzir a exposição ao chumbo, a intoxicação por chumbo ainda é uma causa atual de mortalidade e morbilidade de animais selvagens. Em 2980 cadáveres de Águia-de-cabeça-branca submetidos a diagnóstico entre 1975 e 2013, a principal causa de morte foi intoxicação (25.6%), com a intoxicação por chumbo correspondendo a 63.5% desta taxa de mortalidade. Mais estudos podem fornecer informações importantes sobre este problema e identificar possíveis fontes e vias de exposição ao chumbo.

Com o objetivo de avaliar a incidência de intoxicação por chumbo no sangue e avaliar possíveis associações entre as concentrações de chumbo no sangue e fatores intrínsecos da admissão, um estudo retrospetivo foi realizado. Este estudo incluiu 191 Águia-de-cabeça-branca admitidas no Centro de Reabilitação de Animais Selvagens de Virginia entre 2017 e 2021 e avaliou os parâmetros: estágio de vida, peso, condição corporal, mês e ano de admissão, circunstância e município de resgate.

Com o objetivo de avaliar as associações entre dados da admissão e concentração de chumbo no sangue, o teste qui-quadrado de *Pearson* foi aplicado e nos casos com frequências esperadas <5, o teste exato de Fisher foi realizado. Para quantificar estas associações, foram obtidas as respetivas *Odds Ratios*. Alem disso, a estimativa da tendência de casos durante o período de estudo foi realizada com a regressão linear.

Em conclusão, a intoxicação por chumbo continua a ser uma ameaça relevante com prevalência significativa na população de Águia-de-cabeça-branca de Virgínia. Este estudo destaca a necessidade de mais medidas de mitigação e esforços de conservação para diminuir a exposição e disponibilidade do chumbo na vida selvagem e ambiente. Adicionalmente, o conceito de *One Health* é realçado, uma vez que a contaminação ambiental por chumbo está intimamente relacionada com atividades antropogénicas que causam efeitos adversos na saúde da vida selvagem e humana.

Palavras-chave: *Haliaeetus leucocephalus*, chumbo, intoxicação, Virgínia, Estados Unidos da América

RETROSPECTIVE STUDY OF BLOOD LEAD POISONING IN BALD EAGLES (*Haliaeetus leucocephalus*) IN VIRGINIA, UNITED STATES OF AMERICA, FROM 2017 TO 2021

Abstract

Due to its trophic position on the food chain and opportunistic feeding behavior, the Bald Eagle (*Haliaeetus leucocephalus)* is prone to bioaccumulate contaminants present in the environment and food sources. Hence, monitoring this species can provide valuable information about the ecosystems it inhabits, as well as other wildlife and humans.

Although measures have been taken to reduce lead exposure, lead poisoning is still a current cause of mortality and morbidity to wildlife. In 2,980 Bald Eagles carcasses submitted for diagnosis between 1975 and 2013, the main cause of death was poisoning (25.6%), with lead intoxication accounting for 63.5% of this mortality rate. Further research can provide important insight into this issue and help identify the sources and pathways of lead exposure.

With the aim assessing the incidence of blood lead poisoning and evaluating possible associations between blood lead concentrations and intrinsic factors on admission, a retrospective study was carried out. This study included 191 Bald Eagles admitted to the Wildlife Center of Virginia between 2017 and 2021 and evaluated the parameters: life stage, weight, body condition score, the month and year of admission, circumstance and county of rescue. In order to evaluate the associations between information at intake and blood lead concentrations, a Person's chi-square test was applied, while in cases with expected frequencies <5, Fisher's exact test was performed. To quantify and evaluate these associations, the respective Odd Ratios were obtained. Moreover, the estimation of the caseload trend during the study period was performed by the linear regression model.

The study showed that life stage*,* the month of admission and the county of rescue had a significant association with the outcome of blood lead concentrations ($p < 0.05$). Additionally, in agreement with previous studies, adults and admissions in January showed to have higher odds of having blood lead poisoning concentrations, whereas admission in June had lower odds of this outcome.

In conclusion, lead poisoning remains a relevant threat with significant prevalence in the Bald Eagle population of Virginia. This study highlights the need for more mitigation measures and conservation efforts to decrease lead exposure and its availability to wildlife and the environment. Moreover, it is emphasized the One Health concept, as environmental contamination with lead is closely related to anthropogenic activities causing adverse effects on both wildlife and human health.

Keywords: *Haliaeetus leucocephalus*, lead, poisoning, Virginia, United States of America

ESTUDO RETROSPECTIVO DE INTOXICAÇÃO POR CHUMBO NO SANGUE EM ÁGUIAS-DE-CABEÇA-BRANCA (*Haliaeetus leucocephalus*) EM VIRGINIA, ESTADOS UNIDOS DA AMÉRICA, DE 2017 A 2021

Resumo alargado

O chumbo é um metal pesado que ocorre naturalmente e é altamente tóxico. Este metal pode atuar como um veneno inespecífico, causando efeitos nocivos nos organismos. Através de mecanismo de ação como a competição por recetores de cálcio, o espectro de efeitos fisiológicos do chumbo é amplo. Devido à grande utilização deste metal em atividades antropogénicas, este encontra-se presente no nosso ambiente. A exposição ao chumbo em baixas concentrações pode resultar diversos efeitos subletais na vida selvagem, enquanto concentrações mais elevadas podem ser fatais. Apesar das medidas implementadas para reduzir esta exposição ao chumbo, a intoxicação por chumbo continua a ser uma causa de morbilidade e mortalidade na fauna selvagem.

A Águia-de-cabeça-branca é uma espécie emblemática e exclusiva do América do Norte. Esta espécie tem um comportamento alimentar oportunista em que a sua dieta varia com sazonalidade e região, sendo a disponibilidade de alimento um fator crucial. Desta forma, estas águias podem consumir presas vivas como mortas, desde peixe a carcaças de mamíferos. No passado as Águias-de-cabeça-branca foram gravemente afetadas por contaminantes ambientais, nomeadamente a utilização do inseticida DDT, nos anos 60. O que resultou que nos Estados Unidos da América foi registado um declínio no número dos casais nidificantes, modificando o seu estado de conservação para ameaçadas. Logo, as Águias-de-cabeça-branca são consideradas valiosos bioindicadores para monitorizar concentrações de contaminantes no ecossistema e as consequências da sua exposição na vida selvagem, devido à sua posição trófica na rede alimentar, hábitos alimentares e longevidade.

Diversos autores revelam os efeitos prejudiciais da exposição ao chumbo na população das Águias-de-cabeça-branca. Adicionalmente, vários estudos evidenciam a associação entre a atividade de caça que utiliza munição de chumbo com os casos de intoxicação por chumbo registados. Apesar de esforços para reduzir o uso deste metal pesado, a intoxicação por chumbo continua a ser uma ameaça para esta população e, também outras espécies selvagens.

Desta forma, surge a necessidade de mais estudos para fornecerem informações relevantes acerca desta ameaça, com o objetivo de identificar as fontes e vias de exposição de chumbo para compreender melhor o impacto da exposição ao chumbo. Além disso, a investigação deste tema pode desempenhar um papel fundamental no desenvolvimento de estratégias mais eficazes para prevenir ou mitigar este problema para a vida selvagem e saúde humana.

Esta dissertação apresenta um estudo retrospetivo com base nos registos das concentrações de sangue de chumbo nas Águias-de-cabeça-branca no momento da admissão no *Wildlife Center of Virgínia*. Todos os casos de Águias-de-cabeça-branca admitidas de 1 de janeiro a 31 de dezembro de 2021 foram exportados do sistema informativo *WILD-One* e foram excluídos todos os indivíduos em que não foram medidos o níveis de concentração de chumbo, sendo que a amostra populacional consistiu em 191 indivíduos.

Este estudo tem como objetivo avaliar possíveis associações entra as concentrações de chumbo no sangue e fatores intrínsecos do indivíduo no momento de admissão, tais como estágio de vida, mês e ano de admissão, município de resgate, circunstância de resgate, peso corporal e condição corporal das Águias-de-cabeça-branca admitidas no Wildlife Center of Virginia entre 2017 e 2021.

As concentrações de chumbo no sangue foram testadas na *LeadCare®II Blood Lead Analyser* em amostras recolhidas durante a admissão das Águias-de-cabeça-branca. Posteriormente, concentrações de chumbo no sangue ≥0.2 ppm foram consideradas como níveis elevados de chumbo, ou seja, intoxicação por chumno. Enquanto concentrações <0.2 ppm foram classificados como níveis de chumbo de referência. A decisão de atribuir estes níveis de chumbo no sangue a estes valores de concentrações foram baseadas em estudos anterior, de forma a facilitar à interpretação e discussão de resultados.

Para a analisar a associação estatística entre os parâmetros em estudo e a as concentrações de chumbo no sangue foi realizado um teste qui-quadrado de *Pearson* e em células com contagens inferiores a 5, o teste exato de *Fisher* foi aplicado. De forma a avaliar e quantificar a associação estatística foi calculado o *odds ratio.* Para finalizar, para estimar a tendência do número de casos durante o período em estudo foi realizada um modelo de regressão linear. Para todos os testes estatísticos foi aplicado um intervalo de confiança de 95%, ou seja, os resultados apenas foram considerados estatisticamente significativos quando $p < 0.05$.

A amostra populacional investigada consistiu maioritariamente de indivíduos adultos, sendo o grupo dos outros estágios de vida menos representados. Relativamente a peso e condição corporal, a classe de peso mais representadas foi a de 3kg a 4 kg e a de condição porral de 2 a 3.O ano de 2017 foi o ano com mais Águias-de-cabeça-branca admitidas com mais casos de elevadas concentrações de chumbo no sangue. Em relação a mês de admissão, enquanto junho foi o mês com mais indivíduos admitidos, janeiro apresentou mais casos de intoxicação por chumbo. A distribuição de casos pelos municípios de resgate foi bastante ampla com 63 municípios representados. A circunstância de resgate mais exibida foi a indeterminada. Por fim, este estudo revelou que 28% dos indivíduos tinham concentrações consideradas como elevadas e 75% dos indivíduos tinham concentrações de chumbo detetáveis no sangue.

A analise estatística demonstrou que apenas estágio de vida, mês de admissão e município de resgate tinham associações estatisticamente significativas com as concentrações de chumbo no sangue elevadas. Posteriormente, os resultados indicam que adultos e admissões em janeiro tinham maiores probabilidades de ter elevadas concentrações de chumbo no sangue. Ao contrário de admissões em Junho que revelaram ter menos probabilidades de ter este nível de contrações de chumbo no sangue. A regressão linear mostrou que o grupo total e de referência tinham significância estatística e revelavam uma tendência negativa dos casos.

A maioria dos resultados deste estudo estavam de acordo com estudos anteriores realizados, mais especificamente os resultados da análise estatística que indicam que casos de intoxicação por chumbo são mais prováveis em adultos e em admissões em janeiro. Embora nenhum destes parâmetros em estudo possam ser utilizados isoladamente como evidência para a fonte de chumbo, quando consideradas em conjunto, é muito provável que munições de caça tenham sido uma importante fonte de exposição ao chumbo.

Concluindo, a intoxicação por chumbo continua a ser uma ameaça relevante para a vida selvagem, com grande relevância na população das Águias-de-cabeça-branca em Virgínia. Embora seja uma condição complicada de controlar, compreender os fatores envolvidos é de extrema importância desenvolver estratégias mais eficazes para a sua mitigação.

Numa perspetiva de *One Health,* este estudo destacou a interconexão entre a saúde da vida selvagem e humana, uma vez que as fontes de chumbo estão intimamente ligadas a atividade da caça e outras ações antropogénicas. Utilizando as Águias-de-cabeça-branca como sentinelas da contaminação ambiental, é possível identificar os riscos potencias para os humanos que consomem animais selvagens caçados, uma vez que a munição de chumbo pode ser uma fonte desta contaminação. Desta forma através de estudos de espécies da fauna selvagem é possível monitorizar a saúde ambiental, da vida selvagem e humana.

Apesar de vários esforços efetuados, como campanhas educacionais e a promoção de alternativas a munições de chumbo, este estudo realça a necessidade da implementação de medidas de mitigação à exposição de chumbo mais eficientes.

Por último é importante realçar a importância de uma base de dados corretamente gerida por um centro de reabilitação de animais selvagens que tem a capacidade de providenciar dados importantes para melhor compreensão das ameaças da vida selvagens e respetivas ações conservacionistas. Adicionalmente, é possível detetar doenças emergentes e melhorar o futuro dos procedimentos de resgate, reabilitação e libertação.

Palavras-chave: *Haliaeetus leucocephalus*, chumbo, intoxicação, Virgínia, Estados Unidos da Améria

Introductory Note

The present work was carried out in the Virginia Wildlife Center in the United States of America as part of the author's training period during the 6th year of the Integrated Master's in Veterinary Medicine. The preliminary results of this study have already resulted in:

- An open communication (Annex I) and won 2nd place in the scientific poster competition (Annex II) in Fauna's XI International Conference, 17th to 19th of March, 2023, Lisbon, Portugal.

TABLE OF CONTENTS

List of figures

List of graphics

List of tables

List of abbreviations, initials and acronyms

μg – Microgram

μL – Microliter

μm- Micrometer

ALAD – Delta aminolevulinic acid dehydratase

ASV – Anodic Stripping Voltammetry

CERAS – Centro de Estudos e Recuperação de Animais Selvagens (Center for the Study and Recovery of Wild Animals)

CERVAS - Centro de Ecologia, Recuperação e Vigilância de Animais Selvagens (Center for Ecology, Recovery and Surveillance of Wild Animals)

CRAS HVUTAD - Centro de Recuperação de Animais Selvagens do Hospital Veterinário da Universidade de Trás-os-Montes e Alto Douro (Wild Animal Recovery Center of the Veterinary Hospital of the University of Trás-os-Montes and Alto Douro)

DDT- Dichlorodiphenyltrichloroethane

DDE - Dichlorodiphenyldichloroethylene

dL - Deciliter

GREFA – Grupo de Rehabilitación de la Fauna Autócna y su Hábitat (Group for the Rehabilitation of Native Fauna and its Habitat)

Kg – Kilogram

MIMV - Masters in Veterinary Medicine

mV - Millivolt

OR - Odds ratio

Pb – Lead

PCBs – Polychlorinated biphenyl

PPIX – Protoporphyrin IX

ppm – Part per million

WCV – The Wildlife Center of Virginia

U.S. – United States of America

1. Activities developed during the curricular traineeship

The final mandatory externship of the Integrated Masters in Veterinary Medicine (MIMV) took place in Waynesboro, Virginia, United States of America, in the Wildlife Center of Virginia.

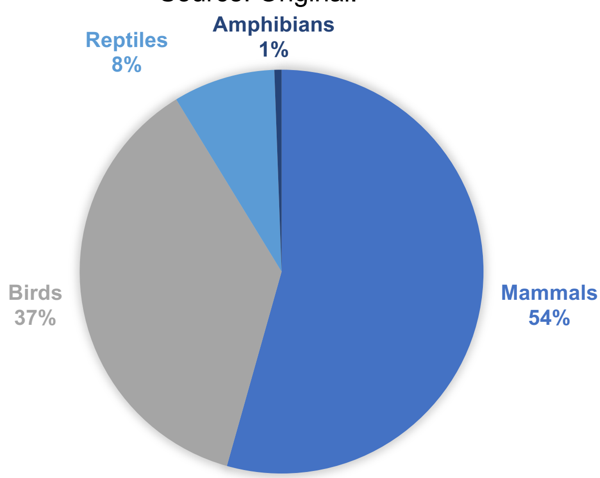
The Wildlife Center of Virginia is a non-profit teaching and referral hospital for native wildlife, formed in 1982. The center's missions are to provide quality health care to native wildlife, conduct health studies, disseminate information to the public and train a corps of wildlife practitioners. The indoor facilities are composed of a veterinary clinic, radiology room, diagnostics laboratory, surgery room, pathology isolation facility, infant care unit, patients in-care rooms, as well as offices for administration and outreach staff. In addition, outdoor facilities have enclosures for non-releasable educational animals. For recovering patients, there are flight pens, a bear enclosure, turtle pens, an aviary, deer fawn pens and a mammal's enclosure.

The veterinary team was composed of Dra. Karra Pierce, the director of Veterinary Services, two veterinary interns, Dra. Olivia Schiermeyer and Dra. Marit Bakken, as well as two licensed Veterinary Technicians, Jess Ransier and Rachel Wolffe.

I was a part of the veterinary externship program, which is designed to offer students practical experience under the mentorship of qualified wildlife veterinarians. The externship had a duration of 10 weeks with 2 days of rest per week, starting on the 4th of July until the 11th of September with a total of 650 working hours accomplished. During this period, the Wildlife Center of Virginia admitted 1041 animals of which 566 were mammals, 384 birds, 85 reptiles and 6 amphibians.

Graph 1 – Animals admitted (%) by the taxonomical class during the externship.

Source: Original.



The days started with the treatment of in-care patients, during which I was able to assist in administering oral and injectable medication, fluid therapy, laser therapy, physical therapy and wound care. On most days, we would have around 50 or more in-care animals to administrate proper treatment. When this task was completed and new animals arrived, all these patients would be assessed and their fate decided.

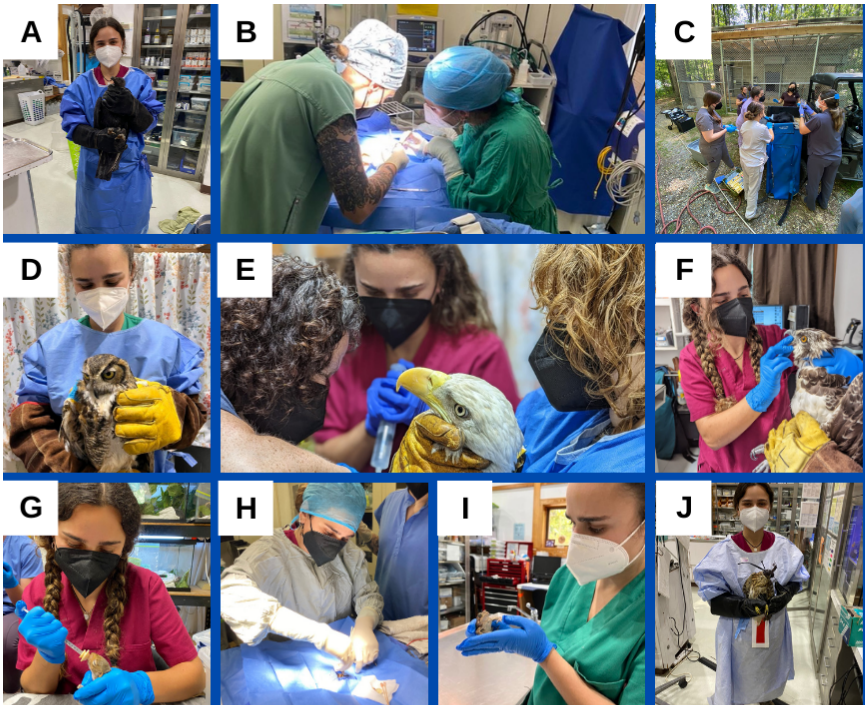
As an extern student, I was responsible for the first approach to the new intakes, in which the protocols varied according to their species. In raptor's species, the protocol of entry was composed of a complete ophthalmic exam, followed by a physical exam. If the patient was stable, a blood collection was made and the animal was sedated with isoflurane for a dorsoventral, laterolateral and ventrodorsal radiographs. A lead blood test and an emergency panel were performed with the previously collected blood, which gave us information about lead blood concentration, hematocrit and total protein, respectively. If the patient stayed under our care, a blood count, as well as biochemistries, were scheduled. Additionally, an oral swab was collected in species belonging to the family *Columbidae* and *Accipitridae* to test for trichomoniasis. As for members of the *Chelonidae* family, apart from a physical exam, dorsoventral, laterolateral and craniocaudal radiographs were performed and a blood count, as well as biochemistries, were scheduled. Animals of the *Passeriformes* order were given a quick physical examination, due to their stressful nature, and if possible, a fecal sample was taken for a coprological exam. All the rabies reservoir species and waterfowl species more susceptible to the avian influenza virus were handled the minimum possible and their physical exam was always performed with the personal protective equipment. As a veterinarian extern, I then proposed a medical approach (if necessary), further exams, adequate medications with the correct dose calculated and a prognosis for the patient. After making all these decisions, the externs meet with the veterinary intern to discuss the treatment plan for the patient.

Moreover, I was able to assist in surgeries, under the role of a surgeon's assistant, such as an amputation of a hind limb in an Eastern Box Turtle *(Terrapene carolina carolina)*, lancing of several aural abscesses in Eastern Box Turtles and an eye evisceration in a Great Horned Owl *(Bubo virginianus)* (Figure 1). As an anesthesiologist, I was present in numerous other surgeries, including an eye evisceration, the lance of aural abscesses, limb amputations and wound debriding procedures. Under the guidance of the veterinarian technicians in the laboratory, I had the opportunity to perform blood counts, total protein, hematocrit and blood lead concentrations and observe fecal smears, in the microscope.

At the end of the day, veterinary externs have the task of maintaining updated medical records on the online platform Wild-One. This assignment entails uploading all the annotations made about physical exams, medications, and diagnostics performed, as well as the treatment plan and prognosis, concerning the daily new entries in the center. In addition, I also had the opportunity to participate in educational and wildlife awareness events for the public, like the release of an adult Bald Eagle (Figure 1).

The Wildlife Center of Virginia (WCV) is a world reference for wildlife rehabilitation centers, that emphasizes deeply in education and training with the help of a multidisciplinary team of great experience. As such, the externship provided me the opportunity to contact the different areas that compose the center and that contribute to the aim of conserving the native fauna. Furthermore, it provided me not only with extensive knowledge in rehabilitation medicine, but also critical clinical and ecological thinking.

Figure 1- Activities developed in the veterinary externship program at the WCV.
Source: Original.



Legend: A) Admission of a Black Vulture (*Coragypus atratus*); B) Eye evisceration surgery on a Great Horned Owl (*Bubo virginianus*); C) Chemical immobilization of an American Black Bear (*Ursus americanus*); D) Admission of a Great Horned Owl (*Bubo virginianus*); E) Performing fluid therapy subcutaneously on a Bald Eagle (*Haliaeetus leucocephalus*); F) Admission of an Osprey (*Pandion haliaetus*); G) Orphan Eastern Gray Squirrel (*Sciurus carolinensis*) H) Surgery of a leg amputation on an Eastern Box Turtle (*Terrapene carolina carolina*); I) Admission of an American Toad (*Anaxyrus americanus*); J) Admission of Cooper's Hawk (*Accipiter cooperii*).

Previously to this experience, I made several complementary externships in other referral wildlife rehabilitation centers from August 30th, 2021, to June 26th, 2022, taking place in Grupo de Rehabilitación de la Fauna Autócna y su Hábitat in Spain (GREFA), The Vale Wildlife Hospital and Rehabilitation Centre in the United Kingdom and in Portugal, in Centro de Ecologia, Recuperação e Vigilância de Animais Selvagens (CERVAS), Centro de Estudos e Recuperação de Animais Selvagens (CERAS) and Centro de Recuperação de Animais Selvagens do Hospital Veterinário da Universidade de Trás-os-Montes e Alto Douro (CRAS HV-UTAD). All the skills I acquired in these voluntary externships provided me with the practical experience and competences to attend the externship program at the WCV.

2. Introduction

Lead (Pb) is a naturally occurring heavy metal that is highly toxic. It can act as a nonspecific poison, causing deleterious effects on living organisms (Bey and Meador 2011). Lead can be released via numerous routes to the environment and due to anthropogenic activities, it is now ubiquitous (Eisler 2000). Exposure to Pb in low concentrations may result in a wide range of sublethal effects in wildlife, as higher concentrations can lead to death (Demayo et al. 1982; Hunt 2012; Russell and Franson 2014). Although measures have been taken to reduce lead exposure, lead poisoning remains a threat to wildlife populations (Avery 2009; Redig 2009).

Bald Eagles are considered to be valuable biosentinels in North America for monitoring ecosystem contaminants concentrations and the consequences of their exposure on wildlife, due to their trophic position in the food web and their longevity (Cesh et al. 2008). As a result, Bald Eagles have been the subject of many studies focused on the effects of contaminants, after suffering dramatic population declines linked to dichlorodiphenyltrichloroethane (DDT) use in the United States of America, during 1950s through 1970s. Despite the restriction on DDT and the recovery of eagles' population, these birds continue to succumb to environmental contaminants, including lead (Russell and Franson 2014). For example, in 2,980 Bald Eagles carcasses submitted for diagnosis to the National Wildlife Health Center between 1975 and 2013, the main cause of death was poisoning (25.6%), with lead poisoning accounting for 63.5% of this mortality rate (Russell and Franson 2014).

Despite several studies conducted on lead exposure in eagles across North America and efforts to reduce the use of this heavy metal, lead poisoning remains a current cause of morbidity and mortality (Wayland et al. 2004). Further research can provide important insight into this threat, by identifying the sources and pathways of lead exposure, helping to understand the impact on the Bald Eagle's population. In addition, it could play a critical role in identifying the extent of lead exposure in the environment. Thereby enabling the development of more effective strategies to prevent or mitigate this issue for both wildlife and human health.

In order to contribute to further knowledge on this issue, a retrospective study was carried out investigating 191 Bald Eagles admitted at the Wildlife Center of Virginia from 2017 to 2021.

3. Literature review

3.1. The Bald eagle

3.1.1. Taxonomy and morphologic features

The Bald Eagle is classified as *Haliaeetus Leucocephalus* (Linneaus, 1766), within the *Accipitriformes* Order and *Accipitridae* Family. Two subspecies have been recognized, namely the southern subspecies, later renamed *Haliaeetus leucocephalus leucocephalus*, after the mention of the northern bald eagle, *Haliaeetus leucocephalus washingtoniensis* (Audobon, 1827). These populations are thought to vary in size, weight, and distribution, being the southern individuals smaller and their habitat ranging from the Southern United States to Northwestern Mexico (Buehler 2022). However geographic ranges have never been drawn and it has been suggested that the variation described may simply be not accurate, leading to not being recognized by ornithologists (Palmer et al. 1998).

H. leucocephalus is one of the largest birds of prey in North America with a wingspan that ranges between 168 to 255 cm and a total body length varying between 71-96 cm (Palmer et al. 1998). Being a raptors' species, reverse sexual dimorphism is present (Newton 1979), with females having an average weight of 5.35 kg tending to be larger compared to males that weigh an average of 4.13 kg (Dunning 2008). This species' physical features change throughout different age classes, yet there is no reliable aging determination due to its individual variability (Mccollough' 1989; Wheeler and Economidy 2018). In an immature bird, the plumage goes through a sequence, starting with a dark stage followed by phases with variable amounts of white (Buehler 2022). Progressively the blackish gray bill and cere slowly transition to an orange-yellow color, likewise, the iris changes from a dark brown to a yellow, the adult form (Mccollough' 1989). The adult plumage is commonly attained at 4 years of age or older and is distinctively colored with a pure white tail and head contrasting with the dark brown body (Wheeler and Economidy 2018) (Figure 2).

Figure 2 - Bald Eagles' morphological features, from individuals admitted at the WCV.
Source: Original.

Legend: A juvenile (A) and an adult (B) Bald Eagle admitted at the WCV.

3.1.2. Reproductive cycle and lifespan

After reaching sexual maturity between 4 and 5 years of age, individuals can compose a breeding pair (Hoyo et al. 2010). The Bald Eagle is a monogamous species, except in cases of the death of the breeding partner, that attempts to reproduce in one nest per year with a clutch size of usually 1 to 3 eggs (Stalmaster 1987).

Depending on latitude, adults start nest-building activities one to three months before egg-laying (Buehler 2022). In Alaska, north of the United States of America, adults build nests in April (F. C. Robards and King 1966), contrasting with the south, in Florida, nest-build can start as early as September (Broley 1947). Important nest habitat characteristics are generally large trees capable of holding the large nest (Andrew and Mosher 1982; Wood et al. 1989) and closeness to water (Andrew and Mosher 1982). Bald eagle nests have been found in areas with human activities as close as 100 meters, showing a certain level of tolerance (Andrew and Mosher 1982; Wood et al. 1989). Nevertheless, human interference causes numerous detrimental effects, such as decreased productivity, thus decreased nestling survival (Anthony and Isaacs 1989), displacement from preferred habitats (Schirato and Parson 2006), alteration of behaviors such as frequency of feeding and brooding nestlings (Steidl and Anthony 2000).

The incubation time is 35 days, both sexes incubate, hunt and feed the offspring that depart the nest at 8-14 weeks (Buehler 2022). The variability of nest departure is due to the growth rate, which is influenced by sex and hatching order (Bortolotti 1986). Fledglings rely on their parents for food up to 6 weeks after leaving the nest (Kussman 1977). Young eagles learn how to hunt on their own, following their parents (Buehler 2022 Oct 7) and progressively become more independent, leaving their natal area at 17 to 24 weeks (Gerrard et al. 1974; Kussman 1977). At this time, juveniles initiate a 4 to 5 years nomadic period, with movements influenced by heritable tendencies, availability of food sources and weather (Buehler 2022).

The longevity of wild Bald Eagles can reach 30 years or more (Winder and Watkins 2020). According to other raptors' survival patterns, it is believed they experience high mortality in the first year of life, followed by a survival increase (Buehler 2022).

3.1.3. Feeding behaviors

The Bald Eagle is an opportunistic forager, meaning its diet depends on food availability that varies with season and region, with the capacity to consume its prey alive, fresh or as carrion (Grubb 1995; Watson 2002). The diet ranges from fish, birds, mammals, reptiles, amphibians and crustaceans (Buehler 2022), but when available, fish seems to be the preferable choice (Wright 1953). Although eagles can show preferences, prey availability seems to be the most determinant factor of their diet (Knight and Knight 1983).

During the winter, there is a relative lack of food and a bigger energy requirement, for thermal reasons (Stalmaster and Gessaman 1984). Most of the wintering eagles are found near water where they feed on fish and waterfowl, often taking the dead, crippled, or otherwise vulnerable animals (Griffin et al. 1982). Mammalian carrion is also an important alternate source of food during this time, especially during deer harvest months, when the same carcasses can be consumed for several days (Neumann 2009; Warner et al. 2014).

3.1.4. Habitat Requirements

The *Haliaeetus leucocephalus* typically inhabits mature forests and wetlands nearby bodies of water, such as lakes, reservoirs, rivers, coastal and estuaries areas, offering foraging opportunities (Garrett et al. 1993; Buehler 2022).

The forest area is usually composed of old-growth and mature trees with patches and openings, resulting in an unobstructed view of prey enhancing the chances of its detection (Stalmaster 1976; Garrett et al. 1993). A wide range of trees are used to perch, roost and nest, physical characteristics seem to be more important than tree species (Garrett et al. 1993). Trees are usually tall with large diameters and high limbs, preferred perch locations may have the following attributes: low disturbance, proximity to potential food sources, short distance to nest, a favorable panorama of surroundings and easily accessible to land and departure (Stalmaster 1987; Caton et al. 1992; Garrett et al. 1993).

During the breeding season, nest selection sites vary geographically, likewise with the presence of other breeding pairs and habitat suitability (Garrett et al. 1993), but the more influential factor is food availability (Swenson et al. 1986). Bald eagles can choose nest sites in decadent or more commonly used, healthy trees (Swenson et al. 1986) with a suitable structure to hold the large nest (Livingston et al. 1990).

Areas with human disturbance are less inhabited (Caton et al. 1992), undeveloped habitats are more favorable for reproduction success (Anthony and Isaacs 1989; Gende et al. 1998). Nonetheless, active nests were found in urban development regions, suggesting that *H. leucocephalus* has some tolerance to human disturbance (Wood et al. 1989).

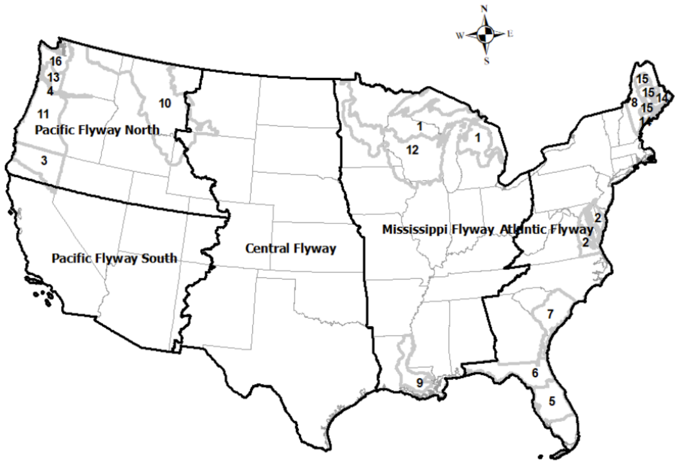
3.1.5. Geographic distribution and population status in the United States of America

Concerning population distribution, Bald Eagles are an endemic North American species, being a native avian species to Canada, the United States and portions of northern Mexico (Buehler 2022) (Figure 3).

According to the United States Fish and Wildlife Service's report, the most updated population estimation is 316,700 individuals, including 71,467 occupied nesting territories in the lower 48 states, excluding Alaska and the southwestern U.S. (U. S. Fish and Wildlife

Service 2020). Due to the limitations of data and evidence from Alaska, it is hard to assess with accuracy the population size (Millsap et al. 2016).

Figure 3 - Bald Eagle management units and survey strata.
Management units are defined by dark lines and are labeled on the figure.
Source: U.S. Fish and Wildlife Service Final Report: Bald Eagle Population Size: 2020 Update.



Legend: Survey strata are 1) Boreal Transition; 2) Chesapeake Bay; 3) California Highlands, 4) Columbia River; 5) Central Florida; 6) Northern Florida; 7) Southeastern Lowlands; 8) Maine Lowlands; 9) Mississippi Valley; 10 Northern Rockies; 11) Oregon; 12) Prairie Transition; 13) Southern Cascades; 14) Upper Atlantic Coast; 15) Main Highlands; 16) Western Cascades.

Nevertheless, this is the state with the biggest breeding population of Bald Eagles in the United States, with an estimation of 15,000 occupied nests (Gould et al. 2009) (Table 1).

Table 1 - Estimates of occupied nests from the integrated aerial survey and eBird relative abundance data by bald eagle management unit 2018-2019.
Source: U.S. Fish and Wildlife Service Final Report: Bald Eagle Population Size: 2020 Update (adapted).

Region	Mean	SD	Median (20^{th} Quantile)
Atlantic Flyway	19,685	142	19,074(15,691)
Central Flyway	7,167	71	6,867(5,197)
Mississippi Flyway	36,973	219	36,038(30,967)
Pacific Flyway North	9,674	52	9,488 (8,239)
Total	73,499	275	71,467 (60,094)

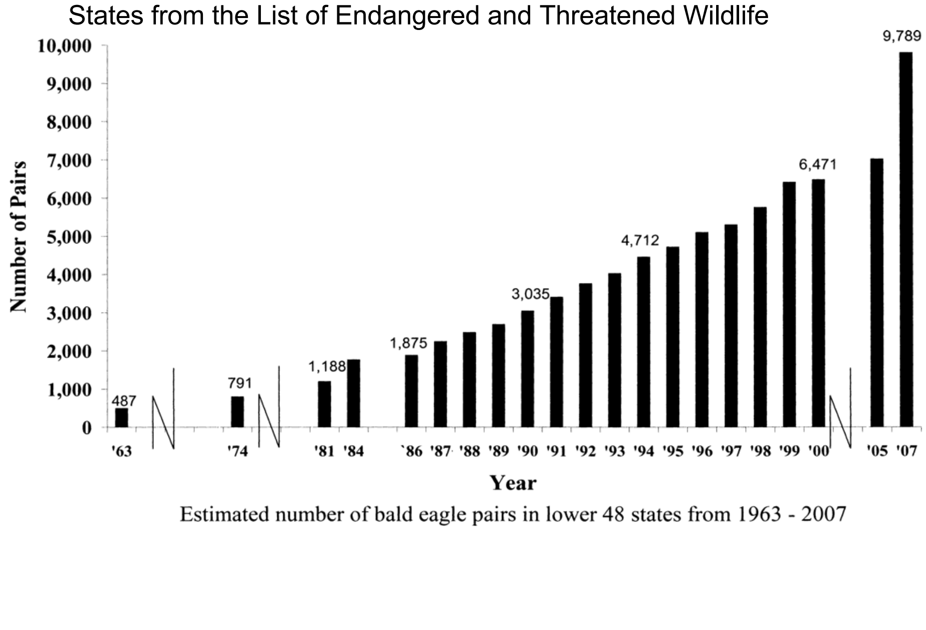
3.1.6. Conservational and legal status in the United States of America

Although in 1782 the United States of America recognized the Bald Eagle as the national symbol, this emblematic specie suffered drastic population variations due to human persecution, habitat destruction and pesticide contamination, especially by DDT (Fredrick C. Robards and King 1966; Buehler 2022).

Starting in the 19th century the population suffered a major decline and by the 20th century, this species was threatened with extinction thus in response, the United States created the Bald and Golden Eagle Protection Act of 1940 (16 U.S.C 668–668d). By 1963, with only 417 nesting pairs of bald eagles known in the lower 48 states (Sprunt and Ligas 1964), the specie was in danger of extinction. Leading to the southern subspecies being listed as Endangered under the Endangered Species Preservation Act of 1966 (Public Law 89-669). Later in 1978, the entire population in the contiguous United States was listed under the Endangered Species Act of 1973 (Public Law 93-205).

Due to the recovery efforts and protection actions, in 1995, they were down listed to a less critical category of Threatened (60 FR 36000). Later based on the population increase to 9,789 breeding pairs, in 2007 the Bald Eagle was delisted from the List of Endangered and Threatened Wildlife (72 FR 37345).(Graph 1). Nonetheless, it is to be noted, this species continues to be protected under the Migratory Bird Treaty Act since 1972 (16 U.S.C. 703-712) and the Bald and Golden Eagle Protection Act of 1940 (16 U.S.C 668–668d). In addition, since 2016, the Bald Eagle is classified as Least Concern by the International Union for Conservation of Nature and Natural Resources (Birdlife International 2016).

Graph 1 - Estimated number of Bald Eagles pairs in lower 48 states from 1963 to 2007.
Source: Endangered and Threatened Wildlife and Plants: Removing the Bald Eagle in the Lower 48 States from the List of Endangered and Threatened Wildlife



3.1.7. Threats and Conservation Efforts

3.1.7.1. Human Disturbance and Habitat Destruction or Modification

Urban development and related human activity are increasing, along with the rise of population, posing a great threat to the environment and wildlife associated, including the Bald Eagle. The most negatively impactful activities are the direct cutting of trees, recreational use of the shoreline and the polluting of waterways (60 FR 36000). Therefore, leading to habitat loss, most significantly the loss of shoreline nesting, perching, and roosting sites, as well as access to aquatic foraging areas (Buehler et al. 1991).

Human disturbance and alterations of habitat, such as tree cutting, can influence nest distribution, since Bald Eagles tend to prefer nest sites in undeveloped areas (Fraser et al. 1985; Anthony and Isaacs 1989; Buehler et al. 1991; Gende et al. 1998) containing suitable perch trees for foraging and other activities (Chandler et al. 1995). Furthermore, nest productivity is also affected, hence higher nesting success in places where there is less human intrusion (Anthony and Isaacs 1989; Gende et al. 1998).

Even though, this specie's nesting habitat is preferably closer to water, the human presence and settlements are enough to make them nest farther from shorelines (Fraser et al. 1985; Anthony and Isaacs 1989; Watts and Whalen 1997), with the possibility of keeping them away from available food sources and it could impair productivity (Schirato and Parson 2006). Likewise, Bald Eagles may also respond negatively to structures themselves or habitat modifications, because, even during seasons with low human activity, they avoided developed areas (Buehler et al. 1991). In the long term, the Bald Eagle's capacity to cope with human activity and the ability to manage appropriate breeding habitats will determine the size and stability of breeding populations (Watts et al. 2008).

Several conservational efforts have been applied to minimize these consequences, including guidelines to diminish human disturbance in critical locations, protection measures for nesting and winter roosts sites, reforestation and public education (60 FR 36000).

3.1.7.2. Shooting and trapping

Throughout history, Bald eagles have been persecuted by humans for several reasons. Native Americans used Eagle parts and feathers for religious and cultural purposes, as European settlers perceived them as a threat to livestock (Buehler 2022). Even later in Alaska, a bounty on Eagles was placed to protect salmon fishing, from 1917 until 1952, 128,273 eagles were killed and presented (Fredrick C. Robards and King 1966).

Since 1970, it is believed that human persecution decreased (Buehler 2022) with the help of public education and protection and preservation acts. Additionally, as a measure to cater to the religious needs of Native Americans, a legal and regulated collection of salvaged parts from deceased eagles was established, named the National Eagle Repository in

Commerce City (72 FR 37345). Nonetheless, it is still a cause of mortality, being that from 1975-2013, out of 2,980 Bald Eagles submitted to the National Wildlife Health Center in Madison, Wisconsin, 12.2% of Bald Eagles died of shooting or trapping (Russell and Franson 2014).

3.1.7.3. Pesticides and other Contaminants/Toxics

Due to their position at the top of the food chain, Bald Eagles are prone to bioaccumulate all sorts of contaminants present in the environment and food sources. There is wide agreement that pesticides played a role in the abrupt decrease of Bald Eagle populations, at the mid-point of the 20th century (Hickey and Anderson 1968; Grier 1982; Wiemeyer et al. 1984; Elliot and Harris 2001). Although population numbers made an impressive recovery, organophosphorus and carbamate pesticides, heavy metals, along with other substances are still a current threat to this specie's survival and reproduction (Buehler 2022).

In the 1940s, DDT was developed as an insecticide to combat insect-borne human diseases, and rapidly, it was widely used in agriculture and as a household insecticide (Beyer and Meador 2011). At the same time, the chemical compounds, polychlorinated biphenyl (PCBs) were also being broadly used for industrial and commercial purposes. Effects of these compounds were linked to Bald Eagles' reproductive failure, especially DDT, due to its metabolite dichlorodiphenyldichloroethylene (DDE) (Reichel et al. 1984; Nisbet 1989; Anthony et al. 1993). Through eggshell thinning, leading to egg breakage, and consequently, embryo deaths, DDT and its metabolites lower the reproductive success of avian species, including the Bald Eagle (Newton 1979; Wiemeyer et al. 1984; United Nations Environment Programme. 1989). After a review of the effects on humans, wildlife and the environment, DDT was banned in 1972 (37 FR 13369) and PCBs' commercial usage was later restricted, in 1979 (44 FR 31514 1979). Years after, the bans were followed by reports of increased reproduction of Bald Eagles and decreased DDE contamination, proving their effectiveness and necessity (Grier 1982).

Lead poisoning has been well-documented in wild birds, particularly in waterfowl caused by spent ammunition ingestion (Bellrose 1959). Previously, lead poisoning in Bald Eagles has been linked to contaminated food sources, such as preying on waterfowl contaminated with lead (Pattee and Henner 1983). Although measures have been taken such as the ban on lead ammunition for waterfowl hunting (U. S. Fish and Wildlife Service 1986), the cases of lead poisoning in the Bald Eagle have not ceased (Russell and Franson 2014), suggesting other lead contamination sources. Reports have indicated lead exposure by ammunition fragments in deer offal and carcasses (Hunt et al. 2006; Bedrosian et al. 2012; Cruz-Martinez et al. 2012). Currently, lead poisoning is still a current cause of morbidity and mortality in Bald Eagles (Haig et al. 2014; Russell and Franson 2014; Buehler 2022). In addition, although with lower incidence, contamination by several other substances has also been reported as a cause of death in Bald Eagles, namely cyclodiene, dieldrin, thallium, carbamate, carbofuran and strychnine (Reichel et al. 1984; Nisbet 1989; Russell and Franson 2014).

3.1.7.4. Collisions with Stationary/Moving Structure or Objects

As the Bald Eagle's habitat fuses with human-occupied areas, the relevance of mortality by collision rises (Buehler 2022). One common cause is car trauma, in part due to its opportunistic feeding behaviors and with the increase of scavenging opportunities on the side of the road, this species become more susceptible (Russell and Franson 2014). Electrocution is also a concern, Alaska has documented 241 confirmed deaths by collisions with power lines, from 1978 until 2002 (Harness 2008). Another threat is aircraft collisions, which have consequences not only for wildlife, but also for the aircraft itself and its occupants. From reports of the Federal Aviation Administration, the U.S. Air Force and the U.S. Navy, there has been an increase of 2200% in Bald Eagle collisions (Washburn et al. 2015). More recently, along with the growing interest of wind energy industry, Bald Eagles have become at risk of wind turbine collisions (Nasman et al. 2021).

3.2. Lead

Lead is a toxic heavy metal with several valuable properties, used since ancient civilizations (Hoffman et al. 2003; Riva et al. 2012; Grant 2020). During the Industrial Revolution, there was a rise in the production of lead-containing objects (Hoffman et al. 2003; Franson and Pain 2011; Riva et al. 2012). Later lead was even present in paint and gasoline, but restrictions were placed in many countries due to major health consequences (Riva et al. 2012). Nevertheless, nowadays lead can still be found in weights, batteries, and ammunition, among other products, as well as in anthropogenic activities such as mining, smelting and recycling operations (Ramesh 2012).

Due to the widespread usage of this metal and being one of the most hazardous and cumulative environmental pollutants (Patra et al. 2011), lead is ubiquitously in the environment (Eisler 2000; Goyer and Clarkson 2001). Consequently, biological systems have been increasingly exposed to this metal, whose effects are constantly documented as detrimental (Hoffman et al. 2003). Hence, according to the World Health Organization (2022), no blood lead concentration is known to be safe.

Lead poisoning often causes neurological, hematological, and gastrointestinal dysfunction, although it varies with species, duration of exposure and amount of lead absorbed (Bey and Meador 2011; Ramesh 2012). In addition, due to its physiological effects, namely the capability of competing with calcium binding sites and interrupting calcium-mediated functions, lead can have consequences in all body systems (Goyer and Clarkson 2001).

3.2.1 Lead Poisoning in Wild Birds

3.2.1.1. Sources of Lead

Avian wildlife can be exposed to lead through various sources and routes, due to its extensive availability in the environment. For example, there are cases of lead poisoning in waterbirds due to discarded leaded fishing gear in lakes or other waterways from recreational fishing, resulting in other animals that compose the diet of avian species also being contaminated (Scheuhammer 1996; Slabe et al. 2019). Lead exposure in birds has also been documented from lead-based paint from buildings, mine waste, and metal objects (Sileo and Fefer 1987; Finkelstein et al. 2003; Hansen et al. 2011; Golden et al. 2016). However, there is a consensus in the reviewed literature that the most reported source of lead exposure is ammunition, with oral ingestion being the typical route (Jordan and Bellrose 1951; Bellrose 1959; Scheuhammer and Norris 1996;Scheuhammer and Templeton 1998; Church et al. 2006; Redig and Arent 2008; Helander et al. 2009; Franson and Pain 2011; Bedrosian et al. 2012; Cruz-Martinez et al. 2012; Ramesh 2012; Rideout et al. 2012; Behmke et al. 2015; Golden et al. 2016; Katzner et al. 2018).

The ingestion of ammunition can occur when birds, such as waterfowl, ingest lead shotgun pellets in areas where they are hunted over, presumably as grit or food particles (Bellrose 1959; Redig and Arent 2008). In birds of prey or other scavenging species, ingestion is through unretrievied carcasses that have been shot, prey with already ingested lead, or from lead bullet fragments embedded in the offal or other tissues (Griffin et al. 1982; Pattee and Henner 1983; Redig and Arent 2008; Franson and Pain 2011).

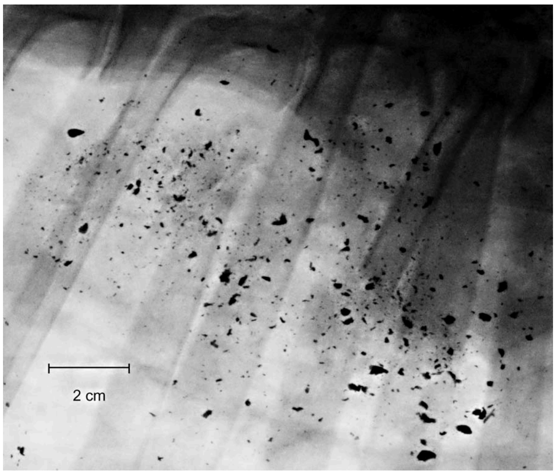
There are several arguments to support ammunition as the main source of lead exposure: greater frequency of lead exposure during or following hunting seasons, direct observation of ammunition in birds and isotopic signatures compatibility from the lead drawn and the manufacture of ammunition (Sheuhammer and Templeton 1998; Cruz-Martinez et al. 2012; Russell and Franson 2014).

A temporal association can be drawn, due to seasonality having a relevant role in lead exposure, especially in bird species that opportunistically forage for food sources such as big game carcasses and offal piles (Cruz-Martinez et al. 2012). For instance, the frequency of lead exposure events and mortality rise during or immediately after game hunting seasons, which was observed in scavenging species after the ban of lead shots for waterfowl hunting in the United States (Bedrosian et al. 2012; Cruz-Martinez et al. 2012; Russell and Franson 2014). The cases that can interfere with this argument are shootings outside of the legal hunting periods or exposure to small game ammunition in other seasons (Golden et al. 2016).

Lead fragments or shots have been discovered during necropsies, radiographs, or directly observed birds feeding on lead-contaminated carcasses (Russell and Franson 2014). Nevertheless, individuals may also be exposed to lead ammunition despite no detection or recovery of lead fragments, this metal could have been already absorbed and expelled, due to the ability to form cast, the majority of birds presenting with clinical signs of illness do not have radiographically detectable lead in the stomach (Redig and Arent 2008). Moreover, in studies with captive scavenging birds dosed with lead shots, its retention varied among individuals of the same species and under similar treatment regimens (Pattee et al. 1981; Carpenter et al. 2003; Golden et al. 2016).

It is possible to measure the lead isotope ratios in human, environmental or wildlife samples and compare them to potential sources of lead (e.g., paint, ammunition, mine tailings). When it is analyzed with other forms of evidence like direct observations, behavioral ecology, or recovery of ingested items, it can help to elucidate how biological systems are exposed to this metal (Sheuhammer and Templeton 1998; Behmke et al. 2015; Golden et al. 2016). For example, a study about the California condors found lead isotope values in free-flying individuals with relatively high blood lead concentrations to be consistent with lead from ammunition (Church et al. 2006). Another relevant factor that exacerbates the risk of lead poisoning is the capability of ammunition to fragment itself (Golden et al. 2016). The hunting firearms currently used have projectiles of various sizes and shapes (Thomas and Guitart 2013). In the case of fragile metals, such as lead, the tendency to break into small pieces increases and lead is dispersed within the carcass (Hunt et al. 2006) (Figure 4). Therefore, the fragmentation of ammunition leads to being more easily ingested and difficult to avoid when consuming contaminated tissues, consequently it becomes more available to multiple individuals (Hunt et al. 2006; Warner et al. 2014).

Figure 4 - Lateral-view radiograph of the mid-thorax of an adult female white-tailed deer killed by a standard copper-jacketed, lead-core, soft-point hunting bullet.
Source: Bullet Fragments in Deer Remains: Implications for lead exposure in avian scavengers (adapted).



3.2.1.2. Exposure Potential and Sensitivity to Lead

Based on the exposure potential and sensitivity, combined with demographic factors, an individual's vulnerability to a toxicant may be evaluated (Golden et al. 2016) A bird's sensitivity and potential to be exposed to lead depend on multiple factors, including its diet, physiology and anatomical characteristics (Franson and Pain 2011; Golden et al. 2016).

Diet can play a very important role, with the capacity of influencing lead's absorption and deposition in tissues (Franson and Pain 2011; Golden et al. 2016). Toxicity can vary with nutritional deficiencies, with low calcium and protein diets increasing the gastrointestinal lead absorption and the total body burden (Eisler 2000; Koranda et al. 1979; Scheuhammer 1996). Specifically, with calcium, lead competes for absorption due to similar chemical properties (Quaterman 1986).

Birds foraging and feeding habits affect exposure to lead. For example, individuals that scavenge on carcasses or offal piles are more likely to be exposed to spent ammunition in the tissues of hunter-killed game (Franson and Pain 2011). Another contributing factor is group feeding behavior, when one individual comes across a contaminated carcass others observe it and subsequently consume the contaminated food source as well (Franson and Pain 2011; Buehler 2022 Oct 7).

Factors such as gender, life stage, species, longevity and even, individual susceptibility influence lead's absorption, tolerance and population impact (Hoffman et al. 1981; Pattee et al. 1981; Scheuhammer 1987; Carpenter et al. 2003; Pain et al. 2009; Krone 2018). It has been documented a greater rate of lead accumulation in reproductively active female birds than in males, it is thought to be associated with the rise of intestinal absorption of calcium during eggshell formation (Scheuhammer 1987). Another period of higher calcium uptake is during bone development in juveniles, resulting in more lead absorbed into the bones (Pain et al. 2009). In studies with experimental dosing, birds revealed individual variability in sensitivity to this element, probably linked to length and number of shot retention, the quantity of lead eroded and individual susceptibility (Hoffman et al. 1981; Pattee et al. 1981). Concerning tolerance between species, authors state that when compared to others, species such as vultures, exhibit a substantially higher tolerance to lead shots (Carpenter et al. 2003). In addition, lead exposure during early life stages in species with long longevity and late maturity, namely eagles and vultures, can result in lead intoxication chronically or sub-chronically (Meretsky et al. 2000). Consequently, it has a more significant impact on these species' populations, due to individuals suffering from the lead poisoning effects without having the chance to reproduce offspring (Meretsky et al. 2000; Krone 2018).

The anatomical characteristics of the avian ventriculus differ with species and can influence the retention and thus to some extent the absorption of metallic lead objects. The gizzards of carnivores, scavengers, and fish-eating birds are adapted for a relatively soft diet, being less muscular than the ones of birds with hard diets, such as grain, vegetation, and insects (Denbow 2000). This effective grinding in this species can enable the erosion of ingested metallic lead, leading to being more available for absorption and subsequent distribution throughout tissues (Jordan and Bellrose 1951). In contrast, raptors, among other species that have a thin-walled stomach and are skilled with forming and regurgitating pellets, or casts, consisting of undigested bones, hair, and feathers of prey (Duke 1986).

Bird's distinct physiology can play a role on lead's breakdown and absorption, leading to a greater vulnerability to lead poisoning (Golden et al. 2016). The stomach's pH in diurnal raptors is around 1, being much lower when compared with psittacines and granivorous birds and even owls, whose stomach pH ranges from 2 to 4 (Duke 1986). Raptors with acidic stomach fluids facilitate the erosion and subsequently absorption in the gastrointestinal tract, which in cases of lead ingestion enables lead solubilization and absorption (Duke 1997; Fisher et al. 2006). Moreover, birds possess a unique trait in gastrointestinal mobility that increases the residence time of ingested materials in the digestive system (Russell and Franson 2014) An adaption hypothesized to allow for greater digestion of nutrients without lengthening the gastrointestinal tract and affecting the ability to fly due to added weight, the contents of the upper ileum and duodenum with the help of periodic reverse peristalsis are moved back into the stomach (Duke 1997).

3.2.1.3. Physiological Effects

The erythropoietic system is one of the most affected, as lead enters the bloodstream, the first measurable consequence is typically the inhibition of heme-biosynthetic enzymes, notably ferrochelatase and delta-aminolevulinic acid dehydratase (ALAD) (Hoffman et al. 1981; Lumeij 1985; Franson and Pain 2011). ALAD is an enzyme responsible for hemoglobin synthesis, which lead can inhibit for several weeks or months, being a very sensitive indicator of lead exposure in birds (Finley et al. 1976; Redig et al. 1991; Carpenter et al. 2003; Pattee et al. 2006). In cases of high exposure, ALAD depression results in anemia characterized by lowered hemoglobin and hematocrit, yet birds can exhibit a certain tolerance and not show any clinical signs (Franson et al. 1983; Pain and Rattner 1988; Franson and Pain 2011). This enzyme is also inhibited in the liver and brain of both birds and mammals, thus possibly having critical effects on these organs' function (Hammond 1973; Buthet et al. 1976; Dieter and Finley 1979). In addition, ferrochelatase is another useful tool to detect lead toxicity in birds, an enzyme that combines ferrous iron and protoporphyrin IX (PPIX) to form heme, causing the accumulation of PPIX in the erythrocytes (Roscoe et al. 1979).

The competition for binding sites with calcium can disrupt this element's action as a regulator of cell function, causing a lot of effects, including neuromuscular (Bressler and Goldstein 1991; Peraza et al. 1998). Moreover, high-dose exposure to lead disrupts the blood-brain barrier, enabling the entrance of molecules into the brain of immature animals, such as albumin that are normally excluded (Clasen et al. 1974; Goldstein 1974; Goldstein et al. 1977). The same effect occurs on ions and water follows, causing edema and an increase in intracranial pressure, consequently a decrease in cerebral perfusion (Ramesh 2012).

3.2.1.4. Time Distribution and Thresholds of Toxicosis

First, lead is absorbed through the gastrointestinal tract and once it reaches the bloodstream, it is distributed throughout the body, including growing feathers (Franson and Pain 2011). Generally, the highest concentrations are found in bone, liver, and kidney, with intermediate concentrations in the brain and blood, and low concentrations in muscle (Custer et al. 1984; Garcia-Fernandez et al. 1995; Bey and Meador 2011).

The liver and kidney are two organs with an active role in the excretion of xenobiotics and it is where high concentrations of lead can be found (Franson and Pain 2011; de Francisco et al. 2016; Krone 2018). It is not clear, which soft tissues have the greater lead accumulation ability, since some studies show higher lead concentrations in the liver than in the kidney, while others reveal the opposite (Bassi et al. 2021). In contrast, the brain has lower lead concentrations due to the blood-brain barrier (Bassi et al. 2021). To contrast, the bone acts like a long-term reservoir, with the hydroxyapatite crystals having a high affinity for lead, hence, a gradual decline in concentrations in soft tissues (Redig and Arent 2008; Franson and Pain 2011).

The diagnosis of lead poisoning in live birds is usually made through blood analysis, while liver and kidney lead levels are used to make a *post-mortem* diagnosis (Redig and Arent 2008). The determination of lead concentrations in blood is a useful and nonlethal way to study lead exposure in birds and live individuals (Franson and Pain 2011) (Table 2). However, lead assessment in blood samples is obtained from a compartment in constant change, with dynamic exchange kinetics, where only a very small amount of the total body burden is being measured (Redig and Arent 2008). In contrast, concentrations in bone are more suited to measure chronicle lead exposure, although the interpretation can be challenging since there is a continuous accumulation and slow release (Franson and Pain 2011). An alternative method is measuring lead in feathers, which reflects exposure at the time of feather growth, a more limited time frame (Golden et al. 2016).

Thresholds of lead toxicosis are difficult to determine, due to species' variability and lack of studies. Additionally, a proper interpretation of tissue lead concentrations in an individual bird is made in combination with several other factors, including species, overall health, type of lead exposure frequency, among others (Franson and Pain 2011).

Table 2 - Suggested Interpretation of Tissue Lead Concentration in Three Orders of Birds.
Source: Franson and Pain 2011 (adapted).

Order	Blood (ppm)	Liver (mg/kg ww)	Kidney (mg/kg ww)
Anseriformes			
Subclinical Poisoning	0.2 - 0.5	2 - 6	2 - 6
Clinical Poisoning	0.50 - 1	6 - 10	6 - 15
Severe Clinical Poisoning	> 1	> 10	> 15
Falconiformes			
Subclinical Poisoning	0.2 - 0.5	2 - 6	2 - 4
Clinical Poisoning	0.5 - 1	6 – 10	4 - 6
Severe Clinical Poisoning	> 1	> 10	> 6
Columbiformes			
Subclinical Poisoning	0.2 - 2	2 - 6	2 - 15
Clinical Poisoning	2- 3	6 -15	15 - 30
Severe Clinical Poisoning	> 3	> 15	> 30

3.2.1.5. Clinical signs and Prognosis

The organism's response to lead comprises a wide spectrum of responses, from subtle physiological effects to severe pathological changes, with the magnitude, duration and previous history of lead exposure playing an important role (Golden et al. 2016).

Acute exposure to high levels of lead is generally typified by a fairly good body condition and can result in sudden death without any previous signs (Franson and Pain 2011; Pain et al. 2019). While clinical signs are often associated with chronic lead cases, with individuals presenting anorexia, low fat reserves, muscle wasting and debilitation (Golden et al. 2016; Pain et al. 2019). Clinical signs may include lethargy, anemia, green diarrhea with staining of the vent, wing droop, ataxia, loss of balance and coordination (Redig and Arent 2008; Franson and Pain 2011; Pain et al. 2019). Ultimately, lead exposure causes alterations in growth, locomotion, balance, body and organ mass, thermoregulation, depth perception, scavenging and feeding ability, with affected functions of the neurological, cardiological, gastrointestinal, reproductive and immunological systems (Cory-Slechta et al. 1980; Lumeij 1985; Grasman and Scanlon 1995; Gochfeld 2000; Lee et al. 2001; Tobias Krause et al. 2006) (Figure 5).

As far as subclinical incidents, individuals can present different body condition scores and typically have other admission causes (Redig and Arent 2008). It is thought that sublethal exposures can affect health directly, as well as make individuals more susceptible to other threats, namely predation, collisions with objects, hunting and other illnesses (Hunt 2012). Nevertheless, the correlation between causes of death and lead exposure is difficult to accurately establish, thus, the population impact in certain bird species is likely being underestimated (Hunt 2012).

Concerning prognosis is hard to determine, because it must be consider other factors such as species and individual variability, the overall health of the bird and possible interactions with other illnesses (Franson and Pain 2011). In an experimental study of lead-shot poisoning in Bald Eagles, while some individuals succumb within 2-3 weeks, others survived for more than 15 weeks (Pattee et al. 1981). While in Turkey Vultures experimentally lead poisoned, the sample population all survived for more than 20 weeks (Carpenter et. al. 2003). Thus, lead mortality varies within species and dosage method.

Figure 5 - Bald Eagles admitted at the WCV with lead poisoning, showing clinical signs.
Source: The Wildlife Center of Virginia.

A) An adult with general listlessness and overall weakness.

(B) An adult with neurological impairment.

3.2.1.6. Regulations and Measures Implemented in the United States of America

Many approaches have been taken to reduce lead's availability to birds, both regulatory and voluntary actions, varying with each state, reflecting the diverse stances on this issue (Haig et al. 2014).

In terms of legislation, lead shot was completely banned for the hunting of waterfowl in 1991, due to overwhelming evidence of its detrimental effect on the waterfowl and avian scavengers population (Jordan and Bellrose 1951; Bellrose 1959; Pattee and Henner 1983). The effectiveness of the 1991 lead banned was evaluated by numerous studies, with one revealing a decline of 64% in mortality of mallards from lead shot exposure, equating to 1.4 million of 90 individuals along the Mississippi Flyway in 1997 (Anderson et al. 2000b). Although these studies revealed a reduction, the lead exposure in waterfowl and birds that prey upon these species was not eliminated (Stevenson et al. 2005; Avery 2009). In addition to the lead remaining from waterfowl hunting, lead continues to enter the environment through other types of hunting and shooting activities in the United States, such as small game and deer hunting, the most popular type of hunting currently (Golden et al. 2016).

As the 1991 nationwide lead ban proved to not be sufficient to mitigate the several consequences caused by lead exposure on wildlife, states decided to implement more strict state measures. Several states and wetland management agencies do not allow lead ammunition in aquatic environments, regardless of the target species (Haig et al. 2014). Furthermore, about 35 states have established areas in which waterfowl lead shot regulations have expanded to include non-waterfowl species, being more commonly focused on upland game-bird hunting and vary with state, target species, and even with land ownership within a particular state (Thomas 2009). In a more severe case, the California Condor is a critically endangered species with lead poisoning being one of the leading causes of this population decline (Meretsky et al. 2000; Church et al. 2006; Rideout et al. 2012). As a mitigation measure, California state in 2013, became the first state to sign a bill into law prohibiting the use of all lead ammunition when taking any wildlife with a firearm (Assembly Bill 711). In the year following the implementation of this ban, blood lead concentrations significantly declined even in other species, namely Turkey Vultures and Golden Eagles (Kelly et al. 2011).

In addition, numerous state fish-and-game agencies no longer use lead in internal agency operations, such as for some of their control programs for nuisance wildlife species, the U.S. Department of Agriculture Animal and Plant Health Inspection Service Wildlife Services has adopted non-lead ammunition (Haig et al. 2014).

At a smaller scale, important measures have been implemented with the aim of reducing the access to spent lead ammunition, like the redistribution of shot through sediment cultivation, raising water levels in wetlands and providing uncontaminated food to endangered species (Thomas 2009). Other relevant actions are the exchanges and giveaways, acting as a communication means to educate hunters and other users on the hazards of using lead and exposing the benefits of switching to nontoxic alternatives (Haig et al. 2014). Additionally, fishing gear is also another source of lead in the environment and the requirements for the usage of lead vary significantly by state, with more restrictions in areas with substantial groups of waterfowls, such as loons and swans (Haig et al. 2014).

4. Aims of the study

The aim of this study was to assess possible associations between blood lead concentrations and intrinsic host factors on arrival, such as life stage, month and year of admission, county of rescue, circumstances of rescue, body weight and body condition of Bald Eagles admitted to the Wildlife Center of Virginia between 2017 and 2021. To achieve this aim, this study had the following objectives:

1. To characterize the Bald Eagle population admitted at the WCV and tested for blood lead concentration during the 5-year period;
2. To investigate the prevalence and geographical distribution of blood lead poisoning in Bald Eagles rescued;
3. To assess spatial and temporal variation in blood lead concentration in the Bald Eagle population of Virginia;
4. To determine if a correlation exists between admission information and blood lead concentration in Bald Eagles;
5. To evaluate the tendency of blood lead poisoning cases and overall admissions of Bald Eagles;
6. To provide insight to this topic and provide recommendations for conservation and management efforts.

5. Materials and methods

5.1. Type of Study

The type of study conducted was a retrospective study, based on the records of blood lead concentrations of Bald Eagles admitted at the WCV between 2017 and 2021.

5.2. The database and studied parameters

All cases referring to Bald Eagles admissions from January 1st, 2017 to December 31st, 2021 at the WCV were exported from the information system *WILD-One* to the Microsoft® Office 365 Excel.

During the investigated period, 241 Bald Eagles were admitted at the WCV, of which upon arrival: 25 were deceased and 216 were alive. Nevertheless, 25 individuals were euthanized after a physical examination, meaning these animals did not have blood lead levels measured. Based on the criteria of exclusion, namely the measurement of blood lead concentration, the final sample size was 191 Bad Eagles. Each animal admitted has a case number identification and records associated with several parameters, the ones analyzed in this study are listed below.

Most of the Bald Eagle's data did not include sex, as it was classified as undermined in many cases. Since the process of sexing is considerably challenging, as it requires several measurements to be taken into consideration (Garcelon et al. 1985), the WCV team considers the time of sexing not a priority. Therefore, sex data analysis was not performed in this study.

5.2.1. Life stage

The Bald eagle's life stage was recorded in four possible classifications: hatchling, fledgling, juvenile or adult. This classification was based on plumage characteristics assessed on admission.

Hatchlings were generally admitted with no feathers and fledglings had juvenile feathers. The difference between juveniles and adults is the plumage. Juvenile individuals have dark-colored plumage with variable amounts of white and adult Bald Eagles have distinctively white and tail plumage, with a dark brown body (Buehler 2022) (Figure 6).

Figure 6 - Life stages of Bald Eagles admitted at the WCV.
Source: The Wildlife Center of Virginia.

(A) A hatchling, (B) a fledgling, (C), a juvenile and (D) an adult Bald Eagle.

5.2.2. Body weight

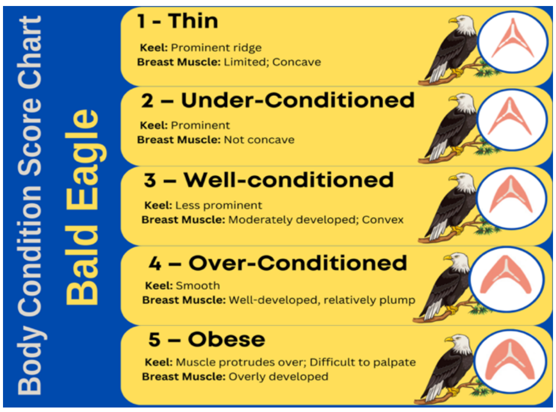
During the physical exam, the patient was always weighted at intake. The measuring unit used for the body weight was the kilogram (kg).

To facilitate the organization and analysis of data six body weight groups were considered: <1 kg, 1kg to <2 kg, 2kg to < 3 kg, 3kg to <4 kg, 4kg to < 5kg and ≥ 5 kg.

5.2.3. Body condition

Body condition was always assessed. The general body condition of most avian species may be evaluated by measuring the amount of subcutaneous adipose tissue, through the palpation of the pectoral muscles (Scott 2021). Based on a 1 to 5 scale, a numeric score is associated with the amount of muscle and fat coverage over the keel (Figure 7).

Figure 7 – Body condition score chart in Bald Eagles.
Source: original.



At the WCV, intermediate scores are used when there is not a consensus on the most accurate body condition score. Therefore, there are 9 possible body condition scores considered and adopted in this study: 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5 and 5. To ease the interpretation of results the body condition scores were divided into 4 categories: ≥1 to <2, ≥2 to <3, ≥3 to <4 and ≥4 to ≤5.

5.2.4. Date of admission

When the animal was admitted at WCV the month and year of admission were recorded.

5.2.5. County of rescue

The WCV always records information about the location of the rescue, including the County of Virginia where the animal was found. This study considered all 95 counties and 38 independent cities, which are recognized county-equivalents.

5.2.6. Circumstances of admission

At the WCV, contact with the rescuers is made through the front desk staff, who documents all the information regarding the animal admitted. The circumstances of admission refer to the reason why the animal is being brought to care. Based on the history told by the person who found the animal and combined with the knowledge of WCV employees, the circumstances of intake are determined. In this study, the circumstances leading to a Bald Eagle's admission to the WCV are divided into 12 categories:

1) Animal interaction – consists of contact with a domestic animal (such as dog or cat) or non-domestic, which led directly or indirectly to the animal's admission. This last subcategory can be further divided into: "different species" or the "same species", as fights between conspecifics can occur due to territorial reasons.

2) Collision – concerns impact with moving objects (such as cars, trucks, motorcycles or other vehicles), as well as a collision with stationary objects (such as walls and doors).

3) Electrocution – contact with an energized power line, resulting in a flow of electricity through the animal's body leading to a need to be taken into care.

4) Entrapment - confinement and restrict movement causing the animal to be entrapped and unable to move freely. This may be due to capturing devices (such as traps and fishing gear) or from others whose primary function is different from capturing animals (such as fences, sporting gear, and landscaping netting).

5) Environment – it contemplates environmental conditions such as extreme weather that directly or indirectly affected the animal and caused its admission. For example, cases of severe weather conditions that disoriented the animal.

6) Inappropriate human possession – this category includes any case where wild animals are removed from their natural habitat and are in human possession, in which the intent can be to be rescued or to be kept as a pet, for unauthorized or untrained rehabilitation.

7) Nest and Habitat disturbance – refers to the disturbance or destruction of a nest or habitat leading to the animal's injury or displacement.

8) Orphan – includes displaced healthy or injured young animals that are dependent on their progenitors for survival but there is a considerable probability that parents have not returned to care for the young or have rejected the youngsters.

9) Petrochemicals / Grease – includes petrochemicals (such as oil, grease, paint, or other products) that may affect the animal found and contribute to its capture.

10) Projectile – includes any propelled object that affects the animal (such as a gunshot, shotgun, rifle, handgun, air gun and BB gun).

11) Referral – consists of the transfers of animals from one rehabilitation facility to another for further rehabilitation, which can be divided between a facility of origin having the adequate permit or not.

12) Undetermined – this category encompasses all unknown events and indeterminate causes. In the context of this study, most cases refer to when rescuers find Bald Eagles that are grounded, and not able to fly or move for a long period, but the cause is not certain.

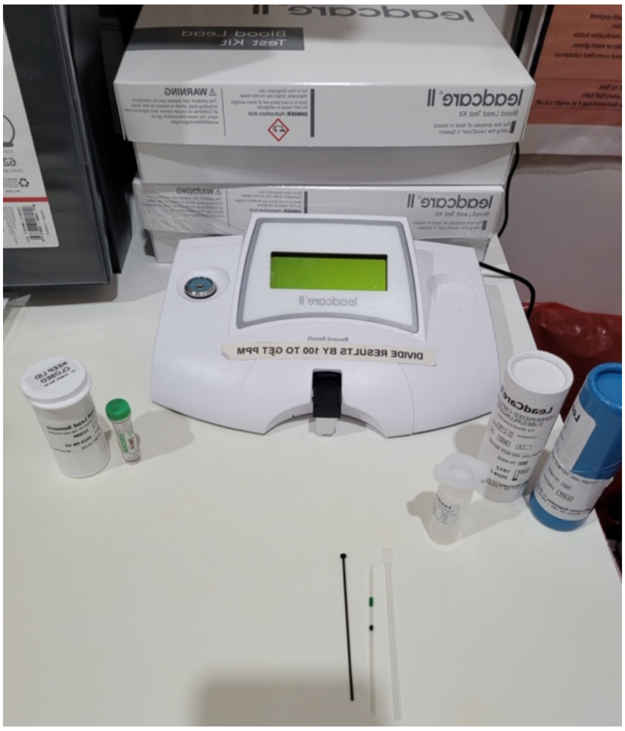
5.2.7. Blood Lead Concentration Analysis

The WCV's intake protocol for raptors includes the collection of a blood sample for the analysis of blood lead concentration. If possible, blood samples are collected from the basilic vein; other options include the jugular vein and tibiotarsal vein (Doneley 2016) (Figure 8). Upon collection, the blood sample is transferred to a heparin tube and awaits the blood lead quantification.

Figure 8 - Blood collection in the basilic vein of a Bald Eagle.
Source: The Wildlife Center of Virginia

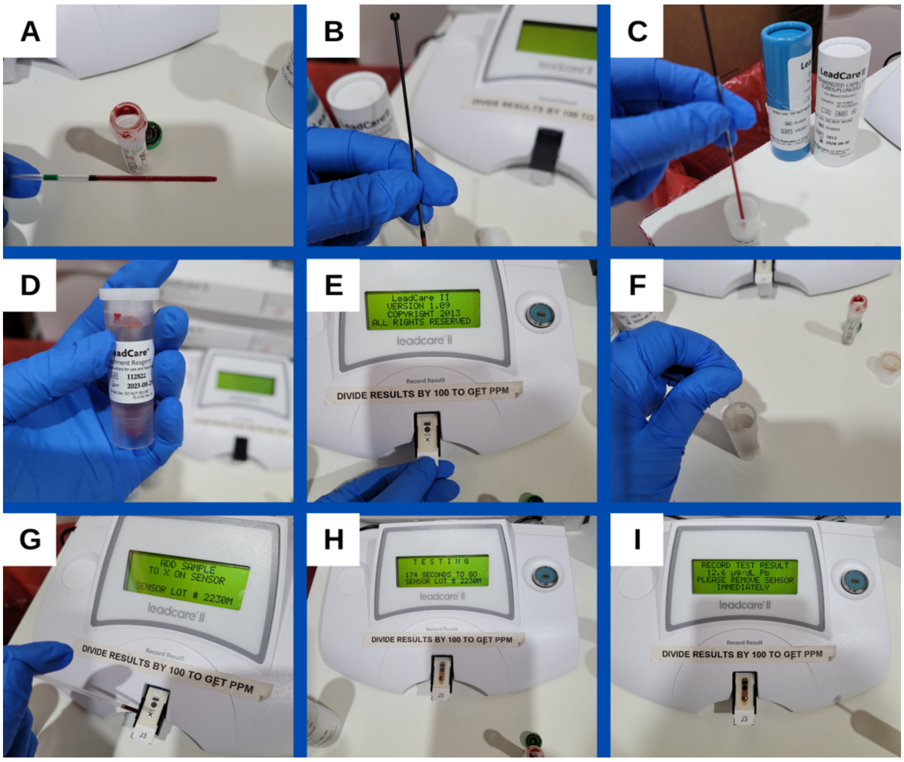
The amount of lead in the blood samples was determined by the LeadCare® II Blood Lead Analyzer, using the technique of Anodic Stripping Voltammetry (ASV) (Figure 9).

Figure 9 - The LeadCare® II Blood Analyser.
Source: original.



The ASV relies on two electrochemical reactions: the reduction process that results in the ionized lead form receiving two electrons and converting to its metal form, due to a high negative potential. The other reaction is the stripping cycle, which occurs when the electrode potential is shifted from a negative to a more positive value, reaching a potential at which lead oxidizes/reduces. Leading to the neutral lead giving up its two electrons and returning to its original ionized state. The electron transfer that occurs creates an electrical current that is directly proportional to the concentration of lead in the sample. The LeadCare® II Blood Lead Analyzer kit contains a Treatment Reagent tube with 250 μL of the diluted hydrochloric acid solution, in which the whole blood is added. After the blood is mixed with this reagent it is placed on the system's sensor, composed of gold particles. Using controlled potential electrolysis at -530 mV, after 140 seconds a square-wave waveform is superimposed on the gold particle electrode. This results in the deposited lead being stripped off the gold particles. The area of the resulting lead peak is converted to a temperature-corrected blood lead concentration by comparison to an internal calibration curve stored in the electronics module (Figure 10).

Figure 10 - Steps to perform a blood lead analysis of a blood sample on the LeadCare® II Blood Lead Analyzer.
Source: original



Legend: A) From the blood sample, the capillary tube is filled until the black line, holding it with a green band on top; B and C) The full content of the capillary tube is dispensed to the place the treatment reagent tube, with the help of a plunger; D) The treatment agent tube is inverted 8 to 10 times to mix the sample completely, until it turns brown; E) The sensor is inserted completely into the analyzer; F) With the transfer dropper, a small of the sample is drawn; G) The dropper tip is placed on the X on the sensor and drop is put.; H) The analyzer displayed a message to wait 3 minutes (180 seconds); I) The test results are provided.

The result is given in micrograms (μg) of lead per deciliter (dL) of whole blood. However, the WCV team converts it to part per million (ppm) to ease the comparison between other reference facilities and laboratories. The reportable range of the test is 3.3 to 65 μg/dL (0.033 ppm to 0.65ppm) meaning the window displays *Low* when the result is less than 3.3 μg/dL (0.033 ppm) and it displays *High*, indicating a blood lead test outcome greater than 65 μg/dL (0.65 ppm).

In this study, blood lead concentrations ≥0.2 ppm were considered as having elevated lead levels, while concentrations <0.2 ppm were classified as having background levels of lead. The decision to assign blood lead levels with elevated or background was based on lead exposure categories reported by Franson and Pain (2011), in which blood lead concentrations

≥ 0.2 ppm are considered to result in sub-clinical lead poisoning. Moreover, Cruz-Martinez et al (2021) also utilized this categorization of lead concentrations, which simplifies the description and analysis of data, thus, facilitating the comparison and discussion of results.

5.3. Software for data recording and analysis

5.3.1. Microsoft Excel

Using *Microsoft® Office 365 Excel version 220544* was used for an exploratory data analysis. This provided a comprehensive overview of the information and enabled the identification and exclusion of any conflicting data and the creation of tables and graphs. The absolute and relative frequencies of the qualitative variables were determined, as well as the maximum and minimum values of the quantitative variables. Thus, it was possible to determine the most appropriate statistical test for each group of data.

5.3.2. R software

To further characterize and interpret the data obtained, *The R Project for Statistical Computing, version 4.2.3.* for *macOS 11* was used.

To assess statistical associations between life stage, body weight, body condition scores, county of rescue, year, month of admission and blood lead concentrations, the Pearson's chi-squared test was used and in cases of the expected cell counts less than 5, the Fisher's Exact Test was applied. In order to evaluate and quantify the strength of these associations, Odds ratio (OR) was estimated.

For all statistical tests applied, it was used a 95% confidence interval, meaning the results were only considered statistically significative when $p < 0.05$

To estimate the caseload trend during the study period a linear regression model was used. The linear regression can be used to identify any overall patterns or trends in the number of cases over time by determining whether the number of cases would increase or decrease over time.

6. Results

6.1. Sample description

This study investigated 191 Bald Eagles. All eagles that arrived deceased or were euthanized at admission without having blood lead concentration measured (n = 50) were excluded.

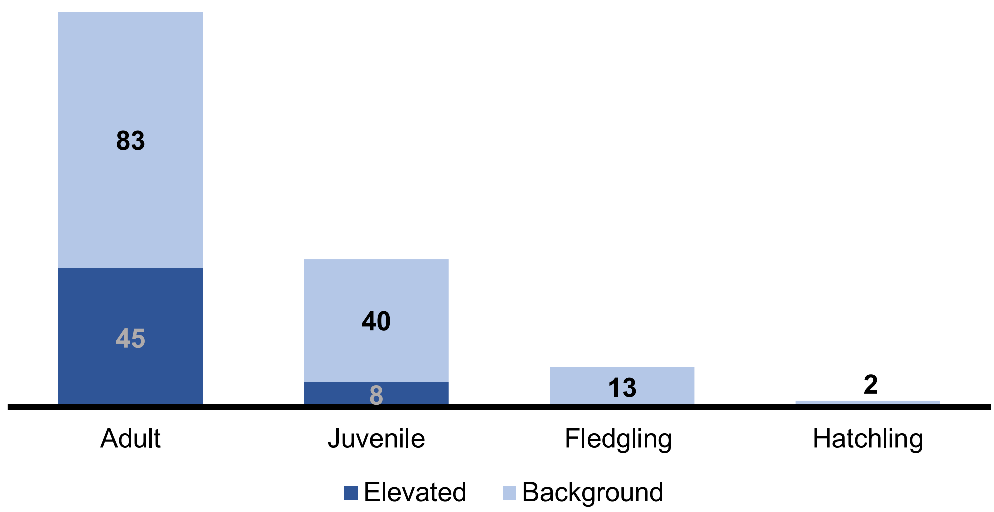
Eagles were distributed into two groups: the elevated group, referring to blood lead concentrations ≥ 0.2 ppm and the background group considering cases with blood lead concentrations < 0.2 ppm.

6.1.1. Life stage

The population sample of this study consisted of 128 (67.0%) adults, 48 juveniles (25.1%), 13 fledglings (6.9%) and 2 hatchlings (1.0%).

Regarding blood lead concentrations, the elevated group consisted of 45 adults (84.9%) and 8 juveniles (15.1%), meanwhile, fledglings and hatchlings did not have any cases of lead concentrations ≥ 0.2 ppm. The background group is comprised of 83 adults (60.1%), 40 juveniles (29.0%), 13 fledglings (9.4%) and 2 hatchlings (1.4%).

Graph 2 - Absolute frequencies of life stages on the Bald Eagles investigated.



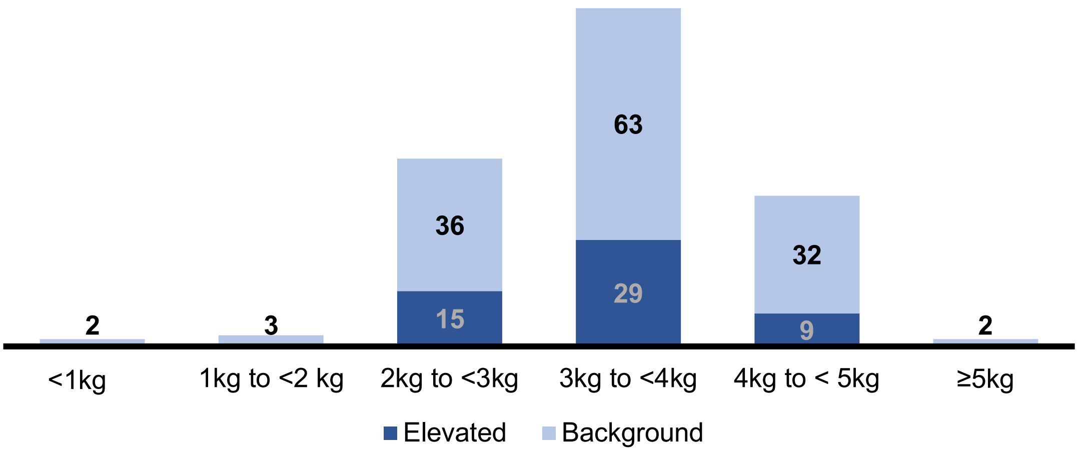
6.1.2. Weight

Body weights varied between 0.4 kg and 5.31 kg, with an average weight of 3.25 kg and a median of 3.0 kg. As mentioned in section 5.2.2., body weights were divided into 6 groups: <1kg, 1kg to <2kg, 2kg to <3kg, 3kg to <4kg, 4kg to <5kg and ≥ 5kg. The distribution was the following: 2 eagles with <1kg (1.4%), 4 eagles with 1 to <2 kg (2.2%), 51 eagles with

2kg to <3kg (26.1%), 92 eagles with 3kg to <4 kg (45.7%), 40 eagles with 4kg to <5kg (23.2%) and 2 eagles with ≥5 kg (1.4%).

Based on lead levels at intake, the elevated group revealed the following distribution: in 3kg to <4 kg with 29 cases (54.7%), in 2kg to <3 kg with 15 cases (28.3%) and 4kg to <5kg with 9 cases (17.0%). In addition, the body weights of the elevated group ranged from 2.05kg to 4.91kg, with an average weight of 3.40kg. The background group showed an average weight of 3.36kg, ranging between 0.4kg to 5.31kg.

Graph 3 - Absolute frequencies of weights on the Bald Eagles investigated.

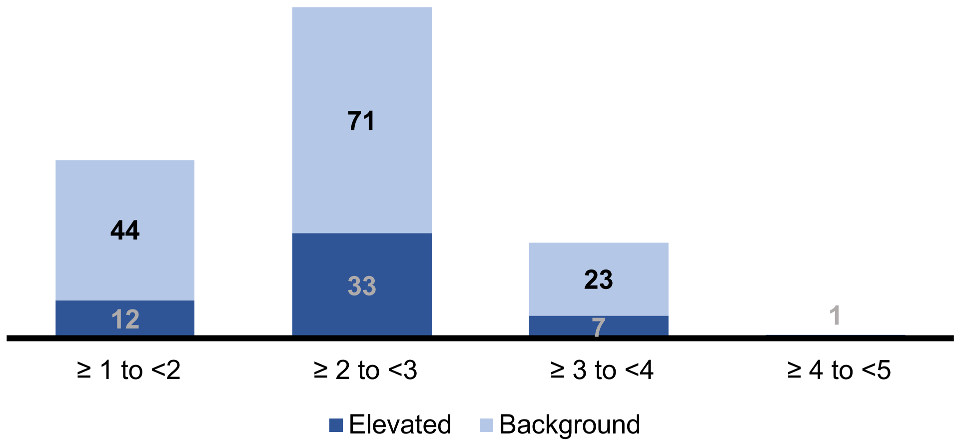


6.1.3. Body condition

The average body condition score at intake was 2.12 and a median of 2, with scores ranging from scores of 4 to 1. As previously stated in section 5.2.3., to facilitate the analysis of data, body conditions scores were divided into 4 categories: ≥1 to <2, ≥2 to <3, ≥3 to <4 and ≥4 to <5. The category with the most prevalence in the population sample was ≥2 to <3 with 104 eagles (54.5%), followed by ≥1 to <2 with 56 eagles (29.3%), then ≥3 to <4 with 30 eagles (15.7%) and lastly ≥4 to <5 with 1 eagle (0.5%).

The category of ≥2 to <3 was also the most prevalent in the elevated group with 33 eagles (63.2%), followed by category ≥1 to <2 with 12 eagles (22.6%), category ≥3 to <4 with 7 eagles (13.2%) and lastly, category ≥4 to <5 with only one eagle (1.9%) This group had an average body condition of 2.26, scores ranging between 1 to 4, whereas the background group had an average body condition of 2.06 ranging from 1 to 3.5.

Graph 4 - Absolute frequencies of body conditions on the Bald Eagles investigated.

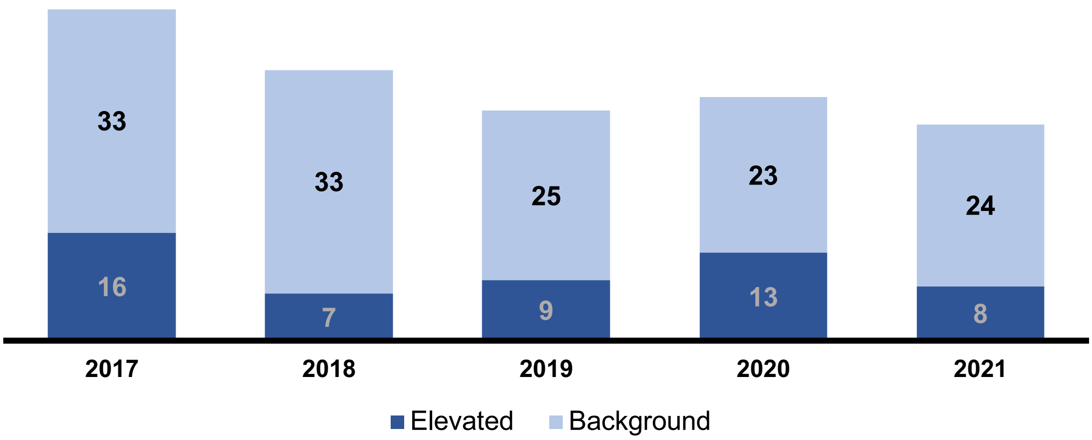


6.1.4. Date of Admission

As far as the number of Bald Eagles admitted each year, during the 5 years of study, 2017 had the highest number of cases, with 49 (25.7%). Following a descending order of 40 cases in 2018 (20.9%), 36 cases in 2020 (18.8%), 34 cases in 2019 (17.8%) and 32 cases in 2021 (16.8%).

Referring to lead analysis, 2017 was also the year with more cases with concentrations ≥ 0.2 ppm, accounting for 16 cases (30.2%). While 2020 had 13 cases (24.5%), 2019 had 9 cases (17.0%), 2021 had 8 cases (15.1%) and 2018 had 7 cases (13.2%).

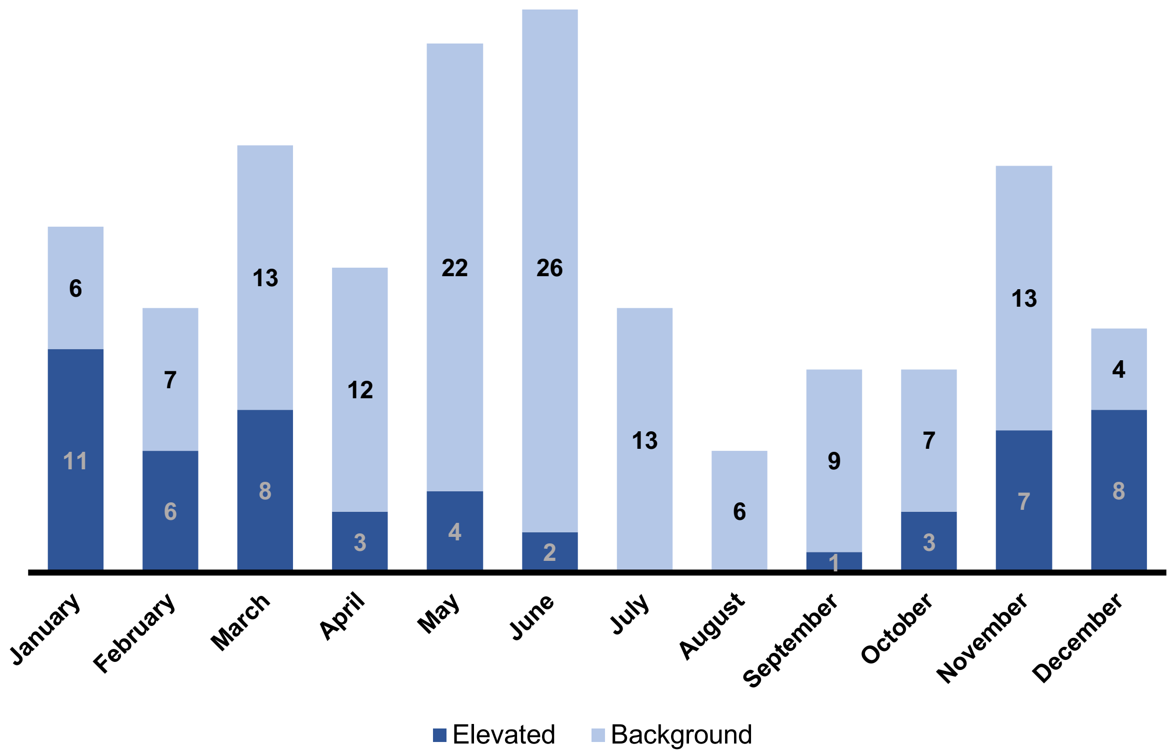
Graph 5 - Absolute frequencies of the number of Bald Eagles investigated by year of admission.



When analyzing the month of admission, relative frequencies were the following: 17 January (8.9%), 13 February (6.8%), 21 March (11.0%), 15 April (7.9%), 26 May (13.6%), 28 June (14.7%), 13 July (6.8%), 6 August (3.1%), 10 September (5.2%), 10 October (5.2%), 20 November (10.5%) and 12 December (6.3%). The month of June had the most cases of Bald Eagles, while August was the month that had the least.

Whereas January was the month with more eagles in the elevated group, with 11 individuals (20.8%). Followed by December and March with 8 individuals (15.1%), then November with 7 individuals (13.2%), February with 6 individuals (11.3%), May with 4 individuals (7.5%), October and April with 3 individuals (5.7%), June with 2 individuals (3.8%) and September with only one individual (1.9%).

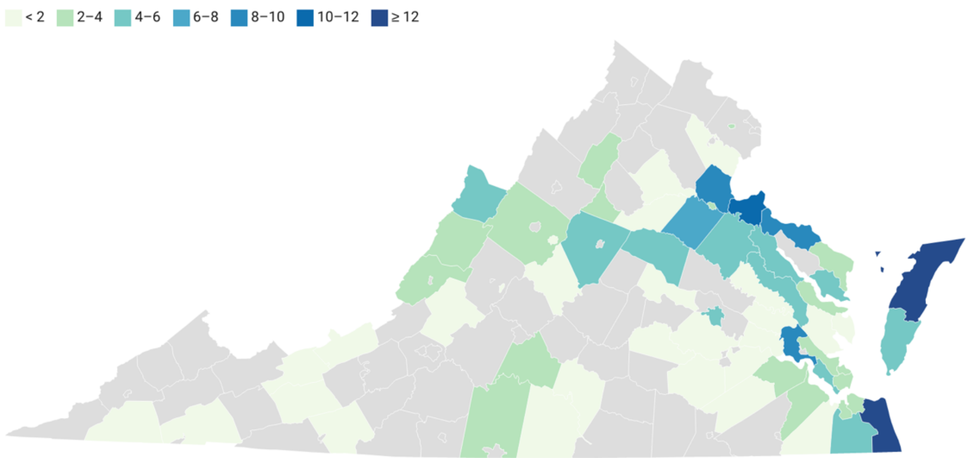
Graph 6 - Absolute frequencies of the number of Bald Eagles investigated by month of admission.



6.1.5. County of rescue

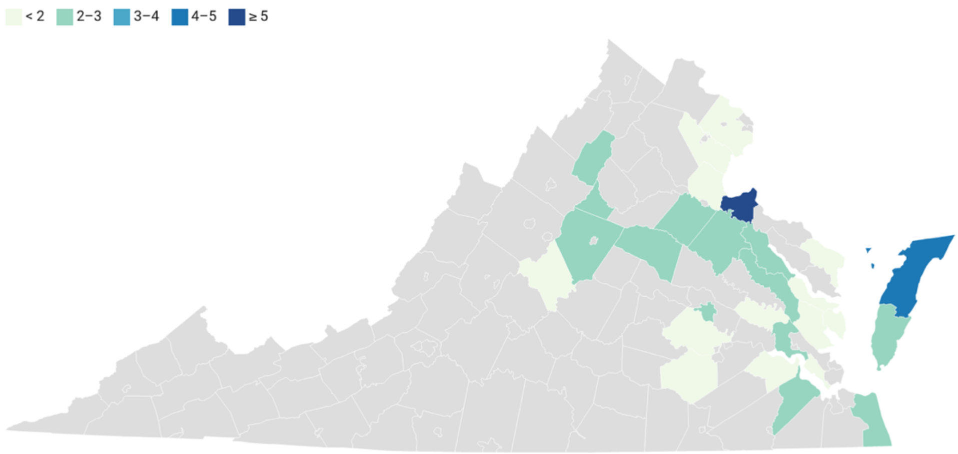
The counties of rescue were comprised of 63 counties and independent cities, with the maximum of cases per county being 13 and 27 counties having just one case. Relative frequencies and absolute frequencies are represented in Annex III.

Graph 7 - Distribution on the map of Virginia of absolute frequencies by county and the independent city of the rescue of the Bald Eagles included in this study.
Source: Created with Datawrapper



The distribution of the elevated group was by 29 counties, ranging from 6 cases in The King George County to only 1 case in 13 counties (Annex III).

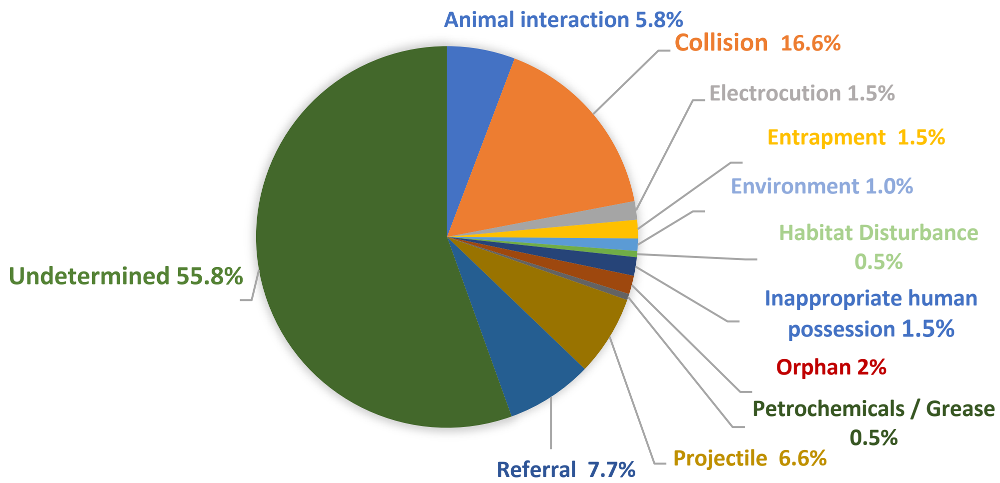
Graph 8 - Distribution on the map of Virginia of absolute frequencies of cases of "Elevated" blood lead concentrations by county and the independent city of the rescue of the Bald Eagles investigated.
Source: Created with Datawrapper



6.1.6. Circumstances of rescue

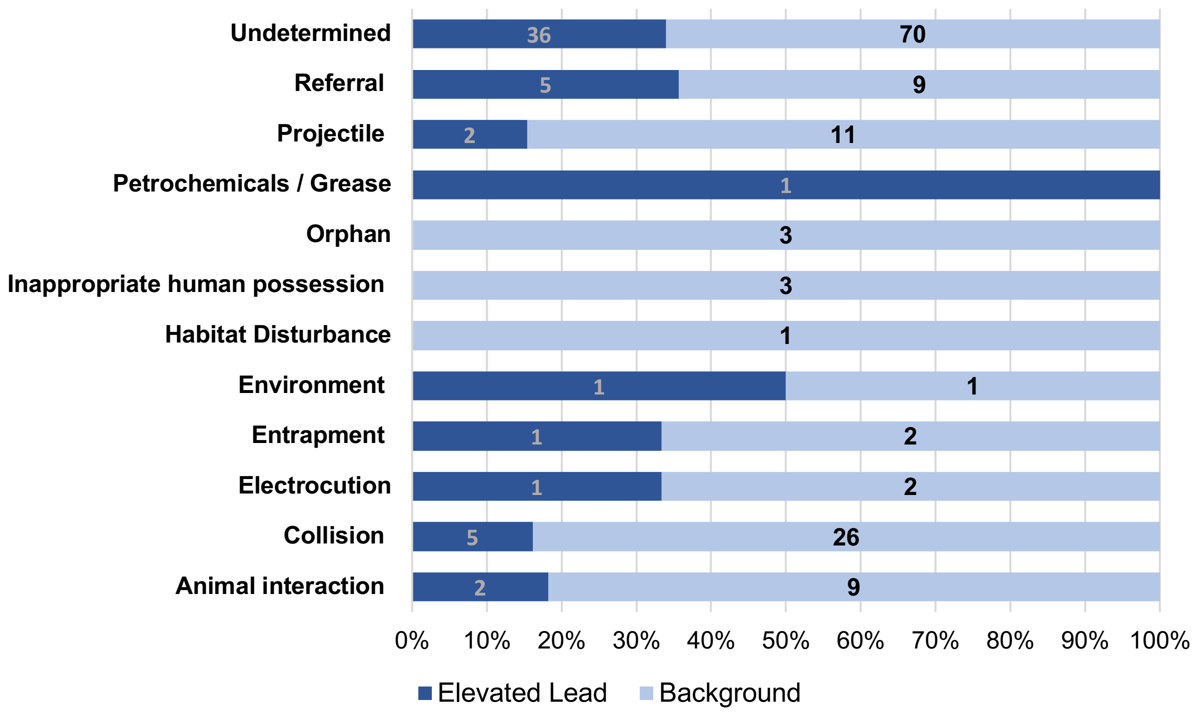
The most prevalent circumstance of rescue was "undetermined", with 106 Bald Eagles having an undermined reason for admission (55.8%). As far of the rest categories, the distributions were the following: 11 cases were "animal interaction" (5.8%), 31 cases were "collision" (16.6%), 3 cases were "electrocution" (1.5%), 3 cases were "entrapment" (1.5%), 2 cases were "environment" (1.0%), 1 case was "habitat disturbance" (0.5%), 2 cases were "inappropriate human possession" (1.0%), 3 cases were "orphan" (1.5%), 1 case was "petrochemicals/grease" (0.5%), 13 cases were "projectile" (6.6%) and 15 cases were a referral (7.7%) (Graph 9).

Graph 9 – Absolute frequencies of circumstances of rescue of the Bald Eagles investigated.



While in the elevated group the circumstance of rescue with most cases was also undetermined with 36 cases (66.7%), followed by referral and collision, with 6 and 5 cases respectively (9.3%), the animal interaction and projectile with 2 cases (3.7%) and lastly electrocution, entrapment, environment and petrochemicals/grease with only 1 case (1.9%).

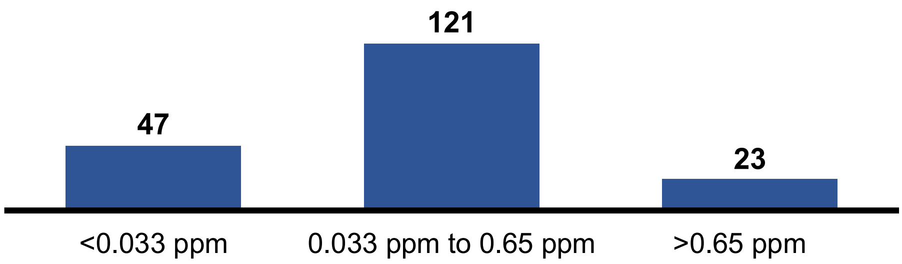
Graph 10 - Absolute frequencies circumstances of rescue of the Bald Eagles investigated.



6.1.7. Blood lead concentration

Concerning blood lead concentrations at admission, if considering the limited of detections of the LeadCare® II Blood Lead Analyzer, the results were: 47 cases with <0.033 ppm (24.6%), 121 cases with 0.033ppm to 0.65ppm (63.4%) and 23 cases with >0.65ppm (12.0%). Meaning, 75.4% of the Bald Eagles from this study had detectable blood lead concentration (>0.033 ppm).

Graph 11 - Absolute frequencies of blood lead concentrations at intake of the Bald Eagles investigated, by the limits of detection of the LeadCare® II Blood Lead Analyzer.



Based on the classification of Franson and Pain (2011) for blood lead poisoning concentrations: elevated (≥0.2 ppm) and background (<0.2 ppm). The elevated group included 53 Bald Eagles (28%), while the background group included more Bald Eagles with 138 (72%).

6.2. Statistical analysis

The association between the variables of life stage, body condition, body weight, month and year of admission circumstances and county of rescue presented on intake with the outcome of blood lead concentrations categorized as elevated *versus* background, were tested through Chi-squared test, Fisher's exact test and Odds Ratio (see Annex IV and Annex V).

First, the parameters when analyzed in groups, life stage (p=0.0027), month of admission (p=8.33e-06) and county of rescue (p=7.621e-07) were the only with a p<0.05, revealing an association statistically significant with the outcome of blood lead concentrations. While parameters such as body weight (p=0.735), body condition (p=0.196), year of admission (p=0.394) and circumstances of rescue (p=0.363) showed an association with not statistical significance.

Secondly, parameters with an association statistically significant were analyzed individually. The results indicated that adults (OR=3.7; IC 95% [1.57;9.8]; p=0,001) and admissions in January (OR=5.7; IC 95% [1.8;19.9]; p=0,001) had higher chances (OR<1) of having elevated blood lead concentrations. Despite February (OR=2.3; IC 95% [0.6;8.7];

p=0.19), March (OR=1.7; IC 95% [0.57;4.78]; p=0,30), October (OR=1.12; IC 95% [0.18;5.15]; p=1), November (OR=1.46; IC 95% [0.46;4.23; p=0,43) and December (OR=1.32; IC 95% [0.27;5.21]; p=0,74) also presenting higher odds of exhibiting the outcome of elevated (OR<1), these variables had a *p*>0.05. Moreover, these months also had a confidence interval for the odds ratio including the value of 1, which means that it is not possible to conclude the existence of a statistically significant association.

While admissions admission in June (OR=0.17; IC 95% [0.02;0.72]; p=0,006) had lower odds (OR<1), this indicates that there is a negative association between the two variables. Other variables such as juvenile (OR=0.43; IC 95% [0.16;1.04; p=0,06), April (OR=0.63; IC 95% [0.11;2.47]; p=0,74), May (OR=0.43; IC 95% [0.10;1.36]; p=0,16) and September (OR=0.27 ; IC 95% [0.006;2.08]; p=0,28) also had lower odds (OR<1), but the *p*>0.05.

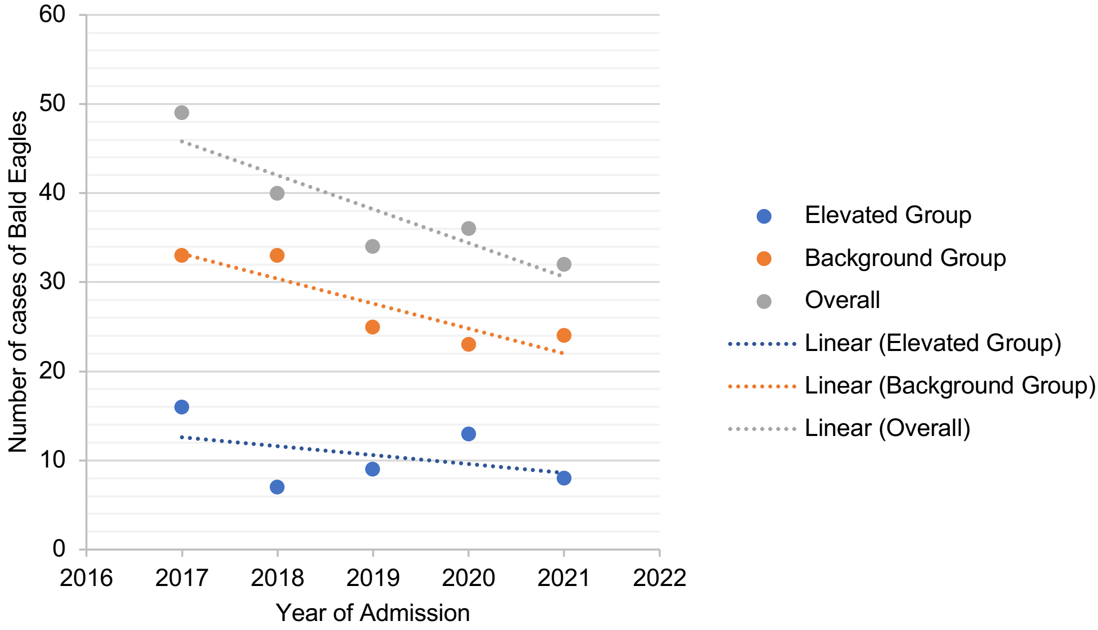
Some variables indicated no association with the outcome studies (OR=0) and not to be statistically significant. These are the case of fledgling (OR=0; IC 95% [0;0.81]; p=0,16), hatchling (OR=0 ; IC 95% [0;13.91]; p=0,16), July (OR=0; IC 95% [0;0.8]; p=0,16) and August (OR=0; IC 95% [0; 2.19]; p=0,16).

Although the county of rescue showed to be statistically significant (*p* = 7.621e-07), the odds ratio was not performed. The distribution of county of rescue data resulted in insufficient data, with cell frequencies very low (Annex III). In this case, it would not be appropriate to draw any conclusion from the analysis.

6.2.1. Number of cases tendency from 2017 to 2021

A linear regression examination was performed to investigate further the number of cases per year in the three groups (elevated, background and overall) throughout the five years of study. The elevated group did not show to be statistically significant *(B=2029.6, t=-0.80, R^2=-0.42, F (1,3)=0.64; p>0.05)*, whereas the background and overall group revealed to be, *(B=5680.8, t=-3.36, R^2= -0.89, F (1,3)=11.30; p<0.05) and (B =7710.4, t =-3.45, R^2 =-0.89, F (1,3)=11.90; p<0.05)* respectively.

Graph 12 – Linear regression of cases of Bald Eagles by year of admission in the elevated group, background group and overall group.



7. Discussion

The sample population mainly consisted of adult individuals (67%) and was also the predominant life stage (85%) in the elevated blood lead concentration group, which is consistent with previous studies (Craig et al. 1990; Hunt et al. 2006; Cruz-Martinez et al. 2012; Warner et al. 2014). Conversely, juveniles accounted for 25% of the sample population and only 15% of the elevated group. The statistical analysis also revealed life stage to be statistically significant (p = 0.0027), with adult Bald Eagles having greater odds (OR = 3.7; $1.5<OR<9.8$) of exhibiting blood lead poisoning.

Cruz-Martinez et al. (2012) investigated 1277 bald eagles admitted to the Raptor Center of the University of Minnesota from 1996 to 2006. Adult individuals were the most represented group in the sample and in the group with elevated levels. This result may be associated to the aggressive behavior of adults feeding in contaminated scavenging sites during winter, leading them to have first choice at consuming the carcasses and likely choosing tissues at bullet wound sites (Saito 2009; Buehler 2022 Oct 7). Another explanation for this finding is that Bald Eagles are exposed to lead throughout their lifespan, resulting in lead being stored in internal tissues and bone (Barbosa et al. 2005; Franson and Pain 2011). Thus, adults are more prone to accumulate a higher total lead body burden (Slabe et al. 2019). Moreover, blood lead concentrations indicate current exposure or mobilization of lead previously stored (Franson and Pain 2011).

The representation of hatchlings and fledglings in the population was relatively low, accounting for only 1% and 7%, respectively, and there were no reported cases of blood lead poisoning among these life stages. Yet, there is a lack of studies of lead's impact on Bald Eagle nestlings in comparison to adults. Furthermore, in studies reporting lead concentrations in live nestlings it is applied the same threshold of lead concentrations used for adults, which might not be accurate (Anthony et al. 1993b; Bruggeman et al. 2018; Slabe et al. 2019). Studies have shown that younger birds are more sensitive to the effects of lead (Burger et al. 1994; Gochfeld 2000). Despite these limitations, Bruggerman et al (2018) performed a blood analysis in 188 nestlings revealing 43% of blood lead concentrations below the limit of detection, while the remaining 57% of concentrations ranged from 0.002 ppm to 0.264 ppm, with only one individual having a blood lead levels ≥ 0.02ppm. This study also showed a correlation in lead concentrations between siblings from the same nest, indicating they receive similar exposures. Another study showed similar blood lead concentrations between Bald Eagles and Ospreys nestlings, associated with a common food source, the fish, which was sampled from the area studied and had lead concentrations above the limit of detection (Slabe et al. 2019).

These findings could potentially be attributed to the fact that nestlings are born in spring, after the fall hunting season of ungulates and upland, and fish usually comprises most of their diet (Bruggeman et al. 2018; Buehler 2022 Oct 7). Therefore, they are less likely to consume carcasses contaminated with lead ammunition, a common source of lead poisoning (Bruggeman et al. 2018). Unlike adults, nestlings need to have recent exposure to lead to have blood lead concentrations, which can come from sources such as fishing tackle, spent ammunition and prey with lead in their tissue (Scheuhammer and Norris 1996; Fisher et al. 2006). Additionally, there is evidence that young birds, in their growing phase, absorb and deposit lead into the bone at higher rates, resulting in lower concentrations in blood and soft tissues (Pain et al. 1995).

Referring to weights at intake, the 3kg to <4 kg was the weight category more present in the sample (48%) and elevated group (55%). This result is not consistent with the fact that adults were the life stage predominant in both groups and weights averages vary between 4kg to 5 kg, depending on gender (Dunning 2008). Moreover, the second weight category more frequent in the groups in question was 2kg to <3kg and then followed by 4kg to <5kg. Nevertheless, it is worth noting that animals admitted to wildlife rehabilitation facilities are often in poor health or debilitated and have low body condition scores, which may add bias to the weight values. In addition, lead poisoning can affect body mass, by decreasing fat reserves and muscle (Golden et al. 2016; Pain et al. 2019). However, in the present study, the variable weight was not associated with the outcome of elevated or background blood lead concentration levels (p=0.7351).

The weight distribution bias could explain the fact that the average weight of the elevated group was 3.40kg, being more than the average weight of the background group, 3.36kg. The background group had a wider range of values, including life stages with lower body masses, compared to the elevated group which was comprised mainly of adults. These findings oppose the results of Franson and Russell (2014), which showed the group with lead-poisoned Bald Eagles was considerably lighter than the non-lead-poisoned eagles.

Body condition is closely related to the variable of weight and was not statistically significant (p= 0.196). The same was observed in the study of Warner et al. (2014), where lead concentrations and body conditions were not significantly statistically correlated.

The elevated group showed an average body condition of 2.26, with scores ranging between 1 to 4. These results were notably higher and more varied than the background group, which presented an average of 2.06 and a range of 1 to 3.5. Since non-adults comprise 40% of the background group, it is relevant to consider that young birds often have often lower body condition scores compared to adults due to their flight muscles are not fully developed (Scott 2021). This may lead to lower body condition scores, especially since the admission records are made by externs at the WCV, which are mainly students who may not be so experienced.

In a study of Bald Eagles experimentally dosed with lead, emaciation was the major gross lesion reported (Pattee et al. 1981). In acute exposure to lead, animals typically exhibit good body condition, compared to chronic cases of lead, often associated with poor body condition (Franson and Pain 2011). This might justify the Bald Eagles in this study with body condition scores of ≥3 to <4 and ≥4 to <5, which might suggest lead poisoning cases of acute nature, rather than chronic. According to Franson and Russell (2014) results, more than half of the Bald Eagles with lead poisoning were characterized by emaciation or poor body condition. This was not observed in the presented results of the elevated group, only 23% were categorized with scores of thin (≥1 to <2) and 62% were under-conditioned (≥2 to <3).

The year of admission also not revealed statistical significance (*p*= 0.3943). However, a linear regression test was applied to investigate the number of cases per year in the elevated, background and overall groups throughout the period of study. The elevated group was not statistically significant, while the background and overall group were and showed a significant decrease in the number of cases. This tendency is contrary to the total of cases the WCV has admitted in this 5-year time, which progressively increased (2017: 2,768; 2018: 3,162; 2019: 3,345; 2020: 3,727; 2021: 3,804). The decrease in the number of Bald Eagles admitted may be a sign of improvement in social awareness, better conservation efforts and a decrease in this species' threats. Other factor that might influenced these results are changes in the migration patterns of Bald Eagles or in the number of nesting pairs in Virginia. Although, according to the Wildlife Report, the number of nesting pairs in the Atlantic Flyway has increased compared to the 2016 report (U. S. Fish and Wildlife Service 2020). It also must be considered that this study only included animals that were admitted alive and stayed in care, since the animals that were euthanized after the physical exam did not have the lead concentrations measured. Hence, there could be some biases, since not all Bald Eagles admitted at the WCV were included in this study.

As far as the number of Bald Eagles in the elevated group, the year 2020 was the second year with highest cases and no conclusion could be assessed regard the linear regression. Research studies investigating lead levels tendency in Bald eagles produced varied results, with some indicating an increase while others showing the opposite (Cruz-Martinez et al. 2012b; Bruggeman et al. 2018). Nevertheless, it is important to note that each study has unique characteristics, such as sample size, geographical locations and exclusion criteria that make direct comparisons difficult. Therefore, it is important to consider a range of studies and to evaluate each one individually, taking into account its specific design and limitations.

The month of admission revealed to be statistically significant (*p*= 8.33e-06). The statistical analysis showed that admissions in January have greater odds of exhibiting blood lead poisoning (OR = 5.7; 1.8<OR<19.9). While admissions in February, March, October, November and December also have higher odds, but were not statistically significant $p>0.05$. These temporal results are in agreement with previous studies, which have associated this lead poisoning seasonality with hunting season (Neumann 2009 Jan; Redig 2009; Strom et al. 2009; Cruz-Martinez et al. 2012; Slabe et al. 2022).

In Wisconsin, a study showed an increased prevalence of lead poisoning in Bald Eagles during October and January which overlaps with hunting season suggesting lead ammunition as primary source of exposure (Strom et al. 2009). Whereas in the Neumann (2009) study, cases of lead poisoning increased through the winter, more specifically December and January, during the peak of the deer harvest season. Another spike of cases in March was observed, which was not detected in this study. A possible hypothesis for this finding is migration movements during spring, involving eagles traveling across the country passing through Wisconsin. Therefore, deer carcass scavenging opportunities could have been expanded. While in May there were no documented lead poisoning cases and cases were only seen in October. According to Neumann (2009), the timing of poisoning cases is not linked to upland game hunting, since it happens mainly during October and pheasant and rabbit carcasses are less available due to weather conditions, unlike deer carcasses.

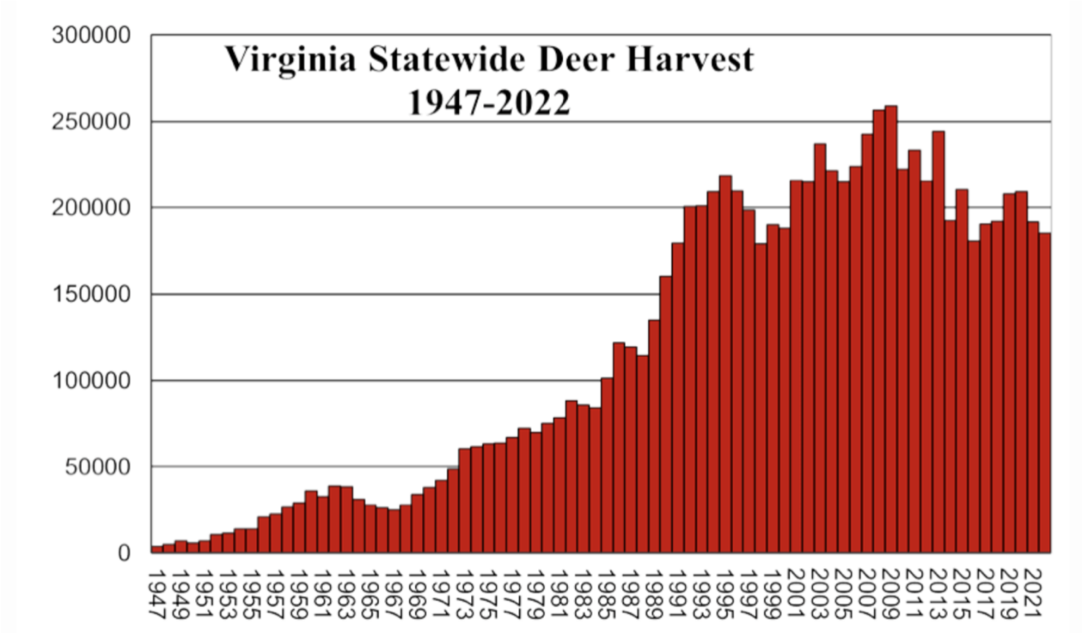
Cruz-Martinez et al. (2012) identified a seasonal pattern of elevated levels of lead in eagles starting in October and December and January having the most cases. In addition, the study also stated that the proportion of cases, declined from February to May. Comparing to this study, the results were the same, except December that had the same number of cases such as March and the decline was until June. Although June was the month with more Bald Eagles admitted overall, it was the month with lower odds of having blood lead poisoning (OR = 0.17; $0.01<OR<0.7$). In addition, admission in April, May and September also have lower odds, but showed no statistical significance $p>0.05$.

Slabe et al. (2019) conducted a study on piscivorous raptors, Ospreys and Bald Eagles, in the Chesapeake Bay region of Maryland and Virginia. They found lead in fish, a shared food source. As a result, during breeding season when the diet of these two species consists of mainly fish, the blood lead concentrations were similar. However, during the non-breeding season, Bald Eagles showed higher concentrations, which must be due to diet variations, such as scavenging habits during the winter, unlike Osprey (Bedrosian et al. 2012b). Through seasonality and based on the differences between lead concentrations and feeding habits of both species, it supports the idea that spent ammunition can be a lead source.

In the state of Virginia, white-tailed deer hunting is the most popular game species, representing 90% of hunting licenses purchased. This species' population is estimated to be between 850000 to 1000000 deer in Virginia, with 190582 deer harvest in 2021 (Graph 12).

The hunting season for deer in Virginia varies depending on the county and hunting method used. In general, the use of firearms for hunting deer starts in October until March.

Graph 13 - Virginia deer harvest from 1947 to 2022.
Source: Virginia Department of Wildlife Resources.



When a deer is hunted, it is common to undress the carcass and leave the offal pile in the field. This contaminated viscera is ingested by scavenging species, including Bald Eagles (Bedrosian et al. 2012; Warner et al. 2014). In a study that collected and radiographed offal piles from deer hunted with shotguns or a muzzle load firearm, 36% of the offal piles contained lead fragments (Warner et al. 2014). Another study investigating eviscerated deer killed with lead bullets, 74% of the carcasses had more than 100 visible fragments. Moreover, 90% of offal piles contained lead fragments (Hunt et al. 2006).

Based on the high numbers of deer harvest in Virginia, feeding habits of Bald Eagles and seasonality in lead exposure, spent ammunition seems to be closely linked to lead poisoning in this species.

There are still lead poisoning cases occurring outside of the hunting season that may be attributed to other sources of lead in the environment. Bald Eagles are known to prey upon waterfowl, particularly when waterfowl populations are abundant (Buehler 2022 Oct 7). Despite the lead ban on waterfowl hunting, legacy lead shot is still present in the environment. Therefore, waterfowl can unintentionally ingest and become exposed to lead (Bellrose 1959; Redig and Arent 2008). Additionally, Bald eagles as a piscivorous species can be also exposed to lead-contaminated fish and consequently, lead-based fishing equipment (Scheuhammer et al. 2003; Slabe et al. 2019). While other potential sources of lead, such as industrial waste and mining exist in the environment, it is unlikely that Bald Eagles would be frequently exposure to these sources (Warner et al. 2014). Moreover, the literature does not provide any documented examples of such exposure occurring.

Referring to the county of rescue, although the variable showed to be statistically significant (p=7.621e-07), further analysis were not performed because most of the counties of rescue in the elevated group only accounted for one case, thus it would be impossible to draw conclusions.

It is important to highlight that the location of the rescue of the Bald Eagle is not indicative of the place where the animal was exposed to lead. Hence, it becomes difficult to make reliable inferences about the source and extend of the lead contamination base upon solely this variable.

Previous studies included place of rescue as a parameter but categorized it in different ways. In the study of Cruz-Martinez et al. (2012) the recovery location was categorized as recovered from either the rifle or shotgun zone, based on the legal firearm deer hunting zone prescribed by the departments of resources in each year. In some cases, the location was categorized as unknown, when the information was not available. The analysis showed that eagles recovered from the rifle zones had a higher risk for elevated lead levels as compared to those that were recovered from shotguns. Redig (2009) investigated the correlation between the recovery location and deer hunting zones. His results were that more cases of Bald Eagles lead poisoned occurred in deer hunting rifle zones.

While the circumstance surrounding an animal's rescue can provide valuable insight into the condition upon admission to a wildlife rehabilitation center, such assessments are inherently subjective and rely on the competences and knowledge of the rescuer. In this study, this variable was not statistically significant with blood lead concentrations (p=0.3631).

The circumstance of rescue with more cases of high blood lead concentrations was undetermined, as it was also the most common cause of admission. This finding is most likely associated with the wide range of effects that lead can cause on an animal's physiology and behavior (Golden et al. 2016). These effects may include impaired locomotion, coordination, growth, body and organ mass, thermoregulation, vision, foraging and feeding, as well as dysfunction in the neurological, cardiological, gastrointestinal, reproductive and immunological systems (Cory-Slechta et al. 1980; Lumeij 1985; Grasman and Scanlon 1995; Gochfeld 2000; Lee et al. 2001; Tobias Krause et al. 2006). As a result of these complex and varied effects, the animal might be unable to fly or move properly, making it easier to catch and admit to a rehabilitation center. However, the underlying reason for its impaired mobility may be multifactorial and difficult to ascertain.

Despite the decrease in human persecution of Bald Eagles, 12.2% of the study's population of Neumann (2009) had projectile injuries, in comparison with the 6% found in this research. These injuries could be the result of various human activities, such as hunting, shooting or accidental discharge of firearms. Interestingly, only two of these cases had high lead blood concentrations, which is not entirely surprising. Lead fragments from ammunition can be absorbed more quickly into an animal's bloodstream if in contact with an acidic environment, such as the digestive system (Golden et al. 2016). In contrast with fragments in other tissues, such as muscle, in which lead will be absorbed over time. While projectile injuries can cause physical damage to the animal's tissue and organs, they do not always result in lead exposure.

As far as subclinical incidents, individuals can present different body condition scores and typically have other admission causes (Redig and Arent 2008). Sublethal exposures can affect health directly, as well as make individuals more susceptible to other threats, namely predation, collisions with objects, hunting and others illnesses (Hunt 2012). Nevertheless, the correlation between causes of death and lead exposure is difficult to accurately establish, thus, the population impact in certain bird species is likely being underestimated (Hunt 2012).

Bald Eagles admitted due to inappropriate human possession, habitat disturbance and orphan did not have any documented cases of blood lead poisoning. This result was expected, since these are admission motives mostly associated with younger animals and elevated blood lead concentrations were more frequent in adult individuals in this study. As for referrals, when an animal is transferred from another wildlife rehabilitation center, the true circumstance of rescue is not recorded, which could lead to some bias.

This study found that 28% of the admitted Bald Eagles had blood lead concentrations of 0.2 ppm or higher, a result similar to the study conducted by Cruz-Martinez et al. (2012), which reported that 26% of the individuals with elevated lead concentrations, ≥0.2 ppm. However, it is relevant to emphasize that there is no consensus on blood lead concentration thresholds for specific species, as most concentrations values are established for birds as a whole or by order, such as for Falconiformes (Franson and Pain 2011). For instance, experimental lead poisoning studies in Turkey Vultures and Bald Eagles shown different levels of sensitivity to lead burden (Pattee et al. 1981; Carpenter et al. 2003). These findings suggest the need to establish species-specific thresholds for lead exposure. As a result, Ecke et al. (2017) proposed 0.025 ppm for Golden Eagles as threshold for blood lead concentration, while Cade (2007) study proposed 0.1 ppm for the California Condor.

The prevalence of detectable blood lead concentrations was 75% in the sample population. Although the concentrations were not considered poisoning, studies show the several effects that sub-lethal exposure of lead may have. In fact, sub-lethal concentrations of lead may induce morphological, physiological and behavioral effects (Hoffman et al. 1981; O'Halloran et al. 1989; Gochfeld 2000; Kelly and Kelly 2005; Hunt 2012; Ecke et al. 2017). Additionally, studies proposed that Bald Eagles with sub-lethal concentration of lead become more susceptible to other causes of morbidity and mortality and could hinder the population growth of this species (Kiladmer and Redig 1997; Neumann 2009 Jan).

7.1. Limitations of this Study

This chapter discusses the study's limitations, namely factors to consider when interpreting the results. Identifying and acknowledging these limitations is crucial to understand the results and make informed conclusions. By being transparent about potential sources of error, the validity of results can be better evaluated and help the design of future studies.

Beginning with the usage of a single blood sample to determine lead poisoning. According to Fry (2009), sporadic monitoring of lead concentrations in the blood of live birds is inadequate, as it may fail to capture peak values or entire exposure events due to lead's short half-life in blood. A single sample provides only information on concentrations at the time of collection and does not indicate whether the levels are increasing, decreasing, or stable, or whether birds that were previously exposed still have detectable lead in their blood (Haig et al. 2014). Furthermore, blood lead concentrations from blood can occur from either current exposure or mobilization of lead previously stored in internal tissues (Barbosa et al. 2005). Lead is stored in bone from past exposure events and can be mobilized and released to blood and tissue regularly, thus causing increased blood lead concentrations during eggshell production or periods of physiological stress (Franson and Pain 2011, Langner et al 2015). These limitations most likely led to underestimating the true pervasiveness of lead within the Bald Eagles investigated in this study. Nonetheless, collecting blood successively leads to stress and could ultimately worsen the prognosis of the animal. In addition, our sample population included only live birds and measuring lead concentrations in live animals is usually done through blood analysis (Redig and Arent 2008).

Another limitation of this study was the usage of the LeadCare® II Blood Lead Analyzer to measure blood lead concentration. The analyzer range of detection is from 0.033ppm to 0.65ppm. One way to address the limitation of a sample being out of the detection range would be to dilute the sample and through estimates determine the actual concentration. Although, this is not an accurate method and could lead to more errors. Another option would be to send the blood sample to another facility to be measured the exact concentration. As a result, a more comprehensive understanding of the scope of blood lead concentrations could be achieved. Although this is a common practice in samples >0.65 ppm at the WCV, it is not always applied. This happens due to the cost involved and not being a prioritized action, since the information would not change the treatment for the patient at the WCV. Additionally, it must be considered that the precision and accuracy of measurements within the detection range of 0.033ppm to 0.65ppm may be influenced by factors such as machine calibration, which could introduce measurement error.

Lastly, this study's results may have been impacted by the uneven distribution of animals across life stages, which could introduced errors and affected the accuracy of some conclusions. Moreover, the inability to determine the gender of the individuals reduced the amount of knowledge produced about lead exposure in the Bald Eagles population.

8. Conclusions

Lead poisoning remains a relevant threat to wildlife with high prevalence in the Bald Eagle population of Virginia. While it is a complex condition to control, understanding the factors involved is very important to develop more effective strategies to mitigate it.

This study focused on lead concentrations in the blood of alive rehabilitation patients, providing a valuable overview of the lead concentrations in Bald Eagles. Despite the limitations mentioned, it provided a useful insight into the extent of lead exposure in Bald Eagles, as lead levels were measured in all in-care patients. This information is particularly beneficial for wildlife centers that do not have access to in-house lead measurement, as lead poisoning is a common occurrence among birds and can never be entirely ruled out as a possible diagnosis due to widespread availability.

This study concluded that adult Bald Eagles and admissions in January have higher odds of having elevated blood lead concentrations, whereas admission in June had, lower odds of showing blood lead poisoning. These findings are in agreement with previous published studies.

Similar surveys can provide critical information on short- and long-term of wildlife species and help identifying sources of toxicants to which they are exposed. None of the seven variables analyzed can be used as standalone evidence as to the source of the lead, however, considered as a whole, it is highly likely that spent ammunition was an important source of lead exposure.

From a One Health perspective, this study highlighted the interconnection between wildlife and human health, as lead sources are closely linked with hunting activities and other anthropogenic actions. By using Bald Eagles as sentinels of environmental contamination, this study sheds light on the potential risks to people who consume game animals, since lead ammunition can be a source of lead exposure. This way, through research on sentinel wildlife species, it is possible to monitor environmental, wildlife and human health.

To reduce lead exposure, alternative metals are currently available to replace lead in ammunition and pose less environmental threats. In addition, there is a growing movement towards banning the use of lead in ammunition and tackle for hunting and fishing activities, as well as increasing awareness of the consequences of lead. Several efforts have already been implemented, such as education campaigns and the removal of lead from environment through cleanup efforts. This study reinforces the need for the implementation of these mitigation measures.

Furthermore, lead poisoning could seriously affect wildlife protected species populations, particularly the case of Bald Eagles, as an apex predator, with long longevity and late maturity.

Finally, can never be overemphasized the great value that a well-managed database from a wildlife rehabilitation center can provide, as a key-source of data to generate information for a better understanding of wildlife threats, respective conservation actions, targeted disease surveillance and future improvement of rescue, rehabilitation and release procedures.

9. References

37 FR 13369. 1972. Consolidated DDT Hearings. United States of America: Federal Register, Rules and Regulations.

44 FR 31514. 1979. PCBs; Manufacturing, Processing, Distribution in Commerce and Use Bans. United States of America: Federal Register, Rules and Regulations.

60 F.R. 1995. Endangered and Threatened Wildlife and Plants; Final Rule to Reclassify the Bald Eagle From Endangered to Threatened in All of the Lower 48 States. United States of America: Federal Register Rules and Regulations.

72 FR 37345. 2007. Endangered and Threatened Wildlife and Plants; Removing the Bald Eagle in the Lower 48 States From the List of Endangered and Threatened Wildlife. United States of America: Federal Register, Rules and Regulations.

Anderson WL, Havera SP, Zercher BW. 2000. Ingestion of Lead and Nontoxic Shotgun Pellets by Ducks in the Mississippi Flyway. J Wildl Manage. 64(3):848. doi:10.2307/3802755.

Andrew JM, Mosher JA. 1982. Bald Eagle nest site selection and nesting habitat in Maryland. Journal of Wildlife Management. 46(2):382–390.

Anthony RG, Garrett MG, Schuler CA. 1993a. Environmental Contaminants in Bald Eagles in the Columbia River Estuary. J Wildl Manage. 57(1):10. doi:10.2307/3808994.

Anthony RG, Garrett MG, Schuler CA. 1993b. Environmental Contaminants in Bald Eagles in the Columbia River Estuary. J Wildl Manage. 57(1):10. doi:10.2307/3808994.

Anthony RG, Isaacs FB. 1989. Characteristics of Bald Eagle Nest Sites in Oregon. J Wildl Manage. 53(1):148. doi:10.2307/3801322.

Avery D. 2009. Regulation of Lead-based Ammunition Around the World. The Peregrine Fund. Doi:10.4080/ilsa.2009.0115

Barbosa F, Tanus-Santos JE, Gerlach RF, Parsons PJ. 2005. A Critical Review of Biomarkers Used for Monitoring Human Exposure to Lead: Advantages, Limitations, and Future Needs. Environ Health Perspect. 113(12):1669–1674. doi:10.1289/ehp.7917.

Barrett MW, Karstad LH. 1971. A Fluorescent Erythrocyte Test for Lead Poisoning in Waterfowl. J Wildl Manage. 35(1):109. doi:10.2307/3799878.

Bassi E, Facoetti R, Ferloni M, Pastorino A, Bianchi A, Fedrizzi G, Bertoletti I, Andreotti A. 2021. Lead contamination in tissues of large avian scavengers in south-central Europe. Science of The Total Environment. 778:146130. doi:10.1016/j.scitotenv.2021.146130.

Bedrosian B, Craighead D, Crandall R. 2012a. Lead Exposure in Bald Eagles from Big Game Hunting, the Continental Implications and Successful Mitigation Efforts. PLoS One. 7(12). doi:10.1371/journal.pone.0051978.

Bedrosian B, Craighead D, Crandall R. 2012b. Lead Exposure in Bald Eagles from Big Game Hunting, the Continental Implications and Successful Mitigation Efforts. PLoS One. 7(12). doi:10.1371/journal.pone.0051978.

Behmke S, Fallon J, Duerr AE, Lehner A, Buchweitz J, Katzner T. 2015. Chronic lead exposure is epidemic in obligate scavenger populations in eastern North America. Environ Int. 79:51–55. doi:10.1016/j.envint.2015.03.010.

Bellrose FC. 1959. Lead Poisoning as a Mortality Factor in Waterfowl Populations. Illinois Natural History Survey Bulletin. 27(1–6):235–288. doi:10.21900/j.inhs.v27.172.

Bey WN, Meador JP. 2011. Environmental Contaminants in Biota. Boca Raton: CRC Press.

Beyer WN, Franson JC, Locke LN, Stroud RK, Sileo L. 1998. Retrospective Study of the Diagnostic Criteria in a Lead-Poisoning Survey of Waterfowl. Arch Environ Contam Toxicol. 35(3):506–512. doi:10.1007/s002449900409.

Beyer WN, Meador JP. 2011. Environmental Contaminants in Biota: Interpreting Tissue Concentrations. 2nd ed. Taylor & Francis Group.

Birdlife International. 2016. *Haliaeetus leucocephalus*. The IUCN Red List of Threatened Species 2016.

Bortolotti GaryR. 1986. Influence of Sibling Competition on Nestling Sex Ratios of Sexually Dimorphic Birds. The American Naturalist .:496–507.

Bressler JP, Goldstein GW. 1991. Mechanisms of lead neurotoxicity. Biochem Pharmacol. 41(4):479–484. doi:10.1016/0006-2952(91)90617-E.

Broley CL. 1947. Migration and nesting of Florida Bald Eagles. Wilson Bulletin. 59:3–20.

Bruggeman JE, Route WT, Redig PT, Key RL. 2018. Patterns and trends in lead (Pb) concentrations in bald eagle (Haliaeetus leucocephalus) nestlings from the western Great Lakes region. Ecotoxicology. 27(5):605–618. doi:10.1007/s10646-018-1933-5.

Buehler DA. 2022 Oct 7. Bald Eagle (Haliaeetus leucocephalus). Rodewald PG, Mlodinow SG, editors. Birds of the World. doi:10.2173/bow.baleag.02.

Buehler DA, Mersmann TJ, Fraser JD, Seegar JKD. 1991. Effects of Human Activity on Bald Eagle Distribution on the Northern Chesapeake Bay. J Wildl Manage. 55(2):282. doi:10.2307/3809151.

Burger J, Pokras M, Chafel R, Gochfeld M. 1994. Heavy metal concentrations in feathers of common loons (Gavia immer) in the Northeastern United States and age differences in mercury levels. Environ Monit Assess. 30(1):1–7. doi:10.1007/BF00546196.

Buthet J-P, Roels H, Hubermont G, Lauwerys R. 1976. Effect of lead on some parameters of the heme biosynthetic pathway in rat tissues in vivo. Toxicology. 6(1):21–34. doi:10.1016/0300-483X(76)90004-4.

Cade TJ. 2007. Exposure of California Condors to Lead From Spent Ammunition. Journal of Wildlife Management. 71(7):2125. doi:10.2193/2007-084.

Carpenter JW, Pattee OH, Fritts SH, Rattner BA, Wiemeyer SN, Royle JA, Smith MR. 2003a. Experimental lead poisoning in Turkey Vultures (cathartes aura). J Wildl Dis. 39(1):96–104. doi:10.7589/0090-3558-39.1.96.

Carpenter JW, Pattee OH, Fritts SH, Rattner BA, Wiemeyer SN, Royle JA, Smith MR. 2003b. Experimental Lead Poisoning in Turkey Vultures (*Cathartes aura)* J Wildl Dis. 39(1):96–104. doi:10.7589/0090-3558-39.1.96.

Caton EL, McClelland BR, Patterson DA, Yates RE. 1992. Characteristics of Foraging Perches Used by Breeding Bald Eagles in Montana. Wilson Bull. 104(1):136–142.

Cesh LS, Williams TD, Garcelon DK, Elliott JE. 2008. Patterns and Trends of Chlorinated Hydrocarbons in Nestling Bald Eagle (Haliaeetus leucocephalus) Plasma in British Columbia and Southern California. Arch Environ Contam Toxicol. 55(3):496–502. doi:10.1007/s00244-007-9125-y.

Chandler SK, Fraser JD, Buehler DA, Seegar JKD. 1995. Perch Trees and Shoreline Development as Predictors of Bald Eagle Distribution on Chesapeake Bay. http://www.jstor.org/stable/3808946.

Church ME, Gwiazda R, Risebrough RW, Sorenson K, Chamberlain CP, Farry S, Heinrich W, Rideout BA, Smith DR. 2006. Ammunition is the Principal Source of Lead Accumulated by California Condors Re-Introduced to the Wild. Environ Sci Technol. 40(19):6143–6150. doi:10.1021/es060765s.

Clasen RA, Hartmann JF, Starr AJ, Coogan PS, Pandolfi S, Laing I, Becker R, Hass GM. 1974. Electron Microscopic and Chemical Studies of the Vascular Changes and Edema of Lead Encephalopathy A Comparative Study of the Hminwn and Experimental Disease.

Craig TH, Connelly JW, Craig EH, Parker TL. 1990. Lead Concentrations in Golden and Bald Eagles. Wilson Bull.(102):130–133.

Cruz-Martinez L, Redig PT, Deen J. 2012. Lead from spent ammunition: a source of exposure and poisoning in Bald Eagles. Human–Wildlife Interactions*: Vol*. 6: Iss. 1, Article 11. doi: 10.26077/sdgs-cs63

Custer TW, Franson JC, Pattee OH. 1984. Tissue lead distribution and hematologic effects in American Kestrels (Falco Sparverius L.) fed biologically incorporated lead. J Wildl Dis. 20(1):39–43. doi:10.7589/0090-3558-20.1.39.

Demayo A, Taylor MC, Taylor KW, Hodson P V., Hammond PB. 1982. Toxic effects of lead and lead compounds on human health, aquatic life, wildlife plants, and livestock. C R C Critical Reviews in Environmental Control. 12(4):257–305. doi:10.1080/10643388209381698.

Dieter MP, Finley MT. 1979. δ-aminolevulinic acid dehydratase enzyme activity in blood, brain, and liver of lead-dosed ducks. Environ Res. 19(1):127–135. doi:10.1016/0013-9351(79)90041-0.

Doneley B. 2016. Avian Medicine and Surgery in Practice: Companion and Aviary Birds. 2nd ed. CRC Press.

Duke G. 1986. Alimentary Canal: Secretion and Digestion, Special Digestive Functions, and Absorption. In: Sturkie PD, editor. Avian Physiology. 4th ed. New York: Springer-Verlag. p. 289–302.

Duke GE. 1997. Gastrointestinal physiology and nutrition in wild birds. Proceedings of the Nutrition Society. 56(3):1049–1056. doi:10.1079/PNS19970109.

Dunning JBJr. 2008. Avian Body Masses Second Edition.

Ecke F, Singh NJ, Arnemo JM, Bignert A, Helander B, Berglund ÅMM, Borg H, Bröjer C, Holm K, Lanzone M, et al. 2017. Sublethal Lead Exposure Alters Movement Behavior in Free-Ranging Golden Eagles. Environ Sci Technol. 51(10):5729–5736. doi:10.1021/acs.est.6b06024.

Eisler R. 2000. Handbook of Chemical Risk Assessment. CRC Press.

Elliot J, Harris ML. 2001. An ecotoxicological assessment of chlorinated hydrocarbon effects on bald eagle populations. Reviews in Toxicology. 4(1):1–60.

Finkelstein ME, Gwiazda RH, Smith DR. 2003. Lead Poisoning of Seabirds: Environmental Risks from Leaded Paint at a Decommissioned Military Base. Environ Sci Technol. 37(15):3256–3260. doi:10.1021/es026272e.

Finley MT, Dieter MP, Locke LN. 1976. δ-Aminolevulinic acid dehydratase: Inhibition in ducks dosed with lead shot. Environ Res. 12(2):243–249. doi:10.1016/0013-9351(76)90032-3.

Fisher IJ, Pain DJ, Thomas VG. 2006. A review of lead poisoning from ammunition sources in terrestrial birds. Biol Conserv. 131(3):421–432. doi:10.1016/j.biocon.2006.02.018.

Francisco ON, Feeney D, Armién AG, Wuenschmann A, Redig PT. 2016. Correlation of brain Magnetic Resonance Imaging of spontaneously lead poisoned bald eagles (Haliaeetus leucocephalus) with histological lesions: A pilot study. Res Vet Sci. 105:236–242. doi:10.1016/j.rvsc.2016.02.010.

Franson CJ, Pain DJ. 2011. Lead in Birds. In: Beyer WN, Meador JP, editors. Environmental Contaminants in Biota, Interpreting Tissue Concentrations. 2nd ed. CRC Press. p. 563–594.

Franson JC, Sileo L, Pattee OH, Moore JF. 1983. Effects Of Chronic Dietary Lead In American Kestrels (Falco Sparverius). J Wildl Dis. 19(2):110–113. doi:10.7589/0090-3558-19.2.110.

Fraser JD, Frenzel LD, Mathisen JE. 1985. The Impact of Human Activities on Breeding Bald Eagles in North-Central Minnesota. J Wildl Manage. 49(3):585. doi:10.2307/3801676.

Garcelon DK, Martell MS, Redig PT, Buoen LC. 1985. Morphometric, Karyotypic, and Laparoscopic Techniques for Determining Sex in Bald Eagles. J Wildl Manage. 49(3):595. doi:10.2307/3801678.

Garcia-Fernandez AJ, Sanchez-Garcia JA, Jimenez-Montalban P, Luna A. 1995. Lead and cadmium in wild birds in southeastern spain. Environ Toxicol Chem. 14(12):2049–2058. doi:10.1002/etc.5620141207.

Garrett MG, Watson JW, Anthony RG. 1993. Bald Eagle Home Range and Habitat Use in the Columbia River Estuary. J Wildl Manage. 57(1):19. doi:10.2307/3808995.

Gende SM, Willson MF, Marston BH, Jacobson M, Smith WP. 1998. Bald Eagle nesting density and success in relation to distance from clearcut logging in Southeast Alaska. Biol Conserv. 83(2):121–126. doi:10.1016/S0006-3207(97)00078-5.

Gerrard P, Gerrard JM, Whitfield WA, Maher WJ. 1974. Post-fledging movements of juvenile Bald Eagles. Blue Jay.:218–226.

Gochfeld JBM. 2000. Effects of lead on birds (laridae): a review of laboratory and field studies. Journal of Toxicology and Environmental Health, Part B. 3(2):59–78. doi:10.1080/109374000281096.

Golden NH, Warner SE, Coffey MJ. 2016. A Review and Assessment of Spent Lead Ammunition and Its Exposure and Effects to Scavenging Birds in the United States. p. 123–191.

Goldstein GW. 1974. Pathogenesis of Lead Encephalopathy. Arch Neurol. 31(6):382. doi:10.1001/archneur.1974.00490420048005.

Goldstein GW, Wolinsky JS, Csejtey J. 1977. Isolated brain capillaries: A model for the study of lead encephalopathy. Ann Neurol. 1(3):235–239. doi:10.1002/ana.410010306.

Gould R, Whittington DM, Allen G. 2009. Final Environmental Assessment, Proposal to Permit Take as Provided Under the Bald and Golden Eagle Protection Act. Washington, D.C., U.S.A.

Goyer RA, Clarkson TW. 2001. Toxic Effects of Metals. In: Klaassen CD, editor. Casarett and Doullis Toxicology: The Basic Science of Poisons. 6th ed. New York: McGraw-Hill. p. 861–867.

Grant LD. 2020. Lead and Compounds. In: Environmental Toxicants. Wiley. p. 627–675.

Grier JW. 1982. Ban of DDT and Subsequent Recovery of Reproduction in Bald Eagles. Science (1979). 218(4578):1232–1235. doi:10.1126/science.7146905.

Griffin CR, Baskett TS, Sparrowe RD. 1982. Ecology of Bald Eagles wintering near a waterfowl concentration. Washington.

Grubb TG. 1995. Food Habits of Bald Eagles Breeding in the Arizona Desert. http://www.jstor.org.

Haig SM, D'Elia J, Eagles-Smith C, Fair JM, Gervais J, Herring G, Rivers JW, Schulz JH. 2014. The persistent problem of lead poisoning in birds from ammunition and fishing tackle. Condor. 116(3):408–428. doi:10.1650/CONDOR-14-36.1.

Hammond PB. 1973. The relationship between inhibition of δ-aminolevulinic acid dehydratase by lead and lead mobilization by ethylenediamine tetraacetate (EDTA). Toxicol Appl Pharmacol. 26(3):466–475. doi:10.1016/0041-008X(73)90283-4.

Hansen JA, Audet D, Spears BL, Healy KA, Brazzle RE, Hoffman DJ, Dailey A, Beyer WN. 2011. Lead exposure and poisoning of songbirds using the Coeur d'Alene River Basin, Idaho, USA. Integr Environ Assess Manag. 7(4):587–595. doi:10.1002/ieam.201.

Harness RE. 2008. Bald Eagle (Haliaeetus leucocephalus) Electrocutions and Artificial Food Sources in Alaska. Environment Concerns in Rights-of-Way Management 8th International Symposium.:383–387. doi:10.1016/B978-044453223-7.50046-0.

Helander B, Axelsson J, Borg H, Holm K, Bignert A. 2009. Ingestion of lead from ammunition and lead concentrations in white-tailed sea eagles (Haliaeetus albicilla) in Sweden. Science of The Total Environment. 407(21):5555–5563. doi:10.1016/j.scitotenv.2009.07.027.

Hickey JJ, Anderson DW. 1968. Chlorinated Hydrocarbons and Eggshell Changes in Raptorial and Fish-Eating Birds. Science (1979). 162(3850):271–273. doi:10.1126/science.162.3850.271.

Hoffman DJ, Pattee OH, WIEMEYER SNWSN, MULHERN BMB. 1981. Effects Of Lead Shot Ingestion On Delta-Aminovulinic Acid Dehydratase Activity, Hemoglobin Concentration, And Serum Chemistry in Bald Eagles. J Wildl Dis. 17(3):423–431. doi:10.7589/0090-3558-17.3.423.

Hoffman DJ, Rattner B, Burton GAJ, Cairns JJ. 2003. Handbook of Ecotoxicology. 2nd Edition. Lewis.

Hoyo J del, Elliott A, Sargatal J, Christie DA. 2010. Handbook of the Birds of the World. Barcelona: Lynx Edicions.

Hunt WG. 2012. Implications of Sublethal Lead Exposure In Avian Scavengers. Journal of Raptor Research. 46(4):389–393. doi:10.3356/JRR-11-85.1.

Hunt WG, Burnham W, Parish CN, Burnham KK, Mutch B, Oaks JL. 2006. Bullet Fragments in Deer Remains: Implications for Lead Exposure in Avian Scavengers. Wildl Soc Bull. 34(1):167–170. doi:10.2193/0091-7648(2006)34[167:bfidri]2.0.co;2.

Jordan JS, Bellrose FC. 1951. Lead Poisoning in Wild Waterfowl. Biological notes; 26. Urbana, Ill : Illinois Natural History Survey.

Katzner TE, Stuber MJ, Slabe VA, Anderson JT, Cooper JL, Rhea LL, Millsap BA. 2018. Origins of lead in populations of raptors. Anim Conserv. 21(3):232–240. doi:10.1111/acv.12379.

Kelly A, Kelly S. 2005. Are Mute Swans with elevated blood lead levels more likely to collide with overhead power lines? Waterbirds. 28(3):331–334. doi:10.1675/1524-4695(2005)028[0331:AMSWEB]2.0.CO;2.

Kelly TR, Bloom PH, Torres SG, Hernandez YZ, Poppenga RH, Boyce WM, Johnson CK. 2011. Impact of the California Lead Ammunition Ban on Reducing Lead Exposure in Golden Eagles and Turkey Vultures. PLoS One. 6(4):e17656. doi:10.1371/journal.pone.0017656.

Kiladmer JL, Redig PT. 1997. Sixteen Years of Lead Poisoning in Eagles, 1980-95: An Epizootiologic View. J Raptor Res. 31(4):327–332.

Knight SK, Knight RL. 1983. Aspects of food finding by wintering Bald Eagles.

Krone O. 2018. Lead Poisoning in Birds of Prey. In: Birds of Prey. Cham: Springer International Publishing. p. 251–272.

Kussman J v. 1977. Post-fledging behavior of the northern Bald Eagle, Haliaeetus leucocephalus, in the Chippewa National Forest, Minnesota. [Minneapolis]: University of Minnesota.

Livingston SA, Todd CS, Krohn WB, Owen RB. 1990. Habitat Models for Nesting Bald Eagles in Maine. J Wildl Manage. 54(4):644. doi:10.2307/3809363.

Lumeij JT. 1985. Clinicopathologic aspects of lead poisoning in birds: a review. Veterinary Quarterly. 7(2):133–138. doi:10.1080/01652176.1985.9693969.

Mccollough' MA. 1989. Molting Sequence and Aging of Bald Eagles. The Wilson Bulletin . 101(1):1–158.

Meretsky VJ, Snyder NFR, Beissinger SR, Clendenen DA, Wiley JW. 2000. Demography of the California Condor: Implications for Reestablishment. Conservation Biology 14(4): 957-967.

Millsap BA, Bjerre ER, Otto MC, Zimmerman GS, Zimpfer NL. 2016. Bald and Golden Eagles: Population demographics and estimation of sustainable take in the United States, 2016 update; U.S. Fish and Willdife Service.

Nasman K, Bay K, Mattson T, Leckband J, Becker D. 2021. Predicting Bald Eagle Collision at Wind Energy Facilities. J Wildl Manage. 85(3):520–530. doi:10.1002/jwmg.21994.

Neumann K. 2009 Jan. Bald Eagle Lead Poisoning in Winter. The Peregrine Fund. doi:10.4080/ilsa.2009.0119.

Newton I. 1979. Population ecology of raptors. Buteo Books, Vermillion, SD.

Nisbet ICT. 1989. Organochlorines, Reproductive Impairment and Declines in Bald Eagle Haliaeetus leucocephalus Populations: Mechanisms and Dose-response Relationships. In Raptors in the Modern World.:483–490.

O'Halloran J, Myers AA, Duggan PF. 1989. Some sub-lethal effects of lead on mute swan Cygnus olor. J Zool. 218(4):627–632. doi:10.1111/j.1469-7998.1989.tb05004.x.

Pain DJ, Fuller M, Pokras M, Hunt WG. 2009. A global update of lead poisoning in terrestrial birds from ammunition sources. The Peregrine Fund, Boise, Idaho, USA. Doi:10.4080/ilsa.2009.0108.

Pain DJ, Mateo R, Green RE. 2019. Effects of lead from ammunition on birds and other wildlife: A review and update. Ambio. 48(9):935–953. doi:10.1007/s13280-019-01159-0.

Pain DJ, Rattner BA. 1988. Mortality and hematology associated with the ingestion of one number four lead shot in black ducks,Anas rubripes. Bull Environ Contam Toxicol. 40(2):159–164. doi:10.1007/BF01881033.

Pain DJ, Sears J, Newton I. 1995. Lead concentrations in birds of prey in Britain. Environmental Pollution. 87(2):173–180. doi:10.1016/0269-7491(94)P2604-8.

Palmer RS, Gerrard JS, Stalmaster M v. 1998. Handbook of North American Birds. Palmer RS, editor. New Haven: Yale University Press.

Patra RC, Rautray AK, Swarup D. 2011. Oxidative Stress in Lead and Cadmium Toxicity and Its Amelioration. Vet Med Int. 2011:1–9. doi:10.4061/2011/457327.

Pattee OH, Carpenter JW, Fritts SH, Rattner BA, Wiemeyer SN, Royle JA, Smith MR. 2006b. Lead poisoning in captive Andean Condors (*Vultur Gryphus*). J Wildl Dis. 42(4):772–779. doi:10.7589/0090-3558-42.4.772.

Pattee OH, Henner SK. 1983. Bald eagles and waterfowl: The lead shot connection. Transactions of the North American Wildlife and Natural Resources Conference. 48:230–237.

Pattee OH, Wiemeyer SN, Mulhern BM, Sileo L. 1981. Experimental Lead-Shot Poisoning in Bald Eagles. http://www.jstor.orgURL:http://www.jstor.org/stable/3808728.

Peraza MA, Ayala-Fierro F, Barber DS, Casarez E, Rael LT. 1998. Effects of micronutrients on metal toxicity. Environ Health Perspect. 106(1):203–216. doi:10.1289/ehp.98106s1203.

Public Law 89-669. 1966. Endangered Species Preservation Act of 1966. United States of America.

Public Law 93-205. 1973. Endagered Species Act of 1973. United States of America.

Quaterman J. 1986. Lead. In: Trace Elements in Human and Animal Nutrition. Vol. 2. 5th ed. New York: Elsevier. p. 281–317.

Ramesh CG. 2012. Veterinary Toxicology. 2nd ed. Elsevier.

Redig P. 2009. Potential Sources of Lead Exposure for Bald Eagles: a Retrospective Study. The Peregrine Fund, Boise, Idaho, USA. Doi: 10.4080/ilsa.2009.0208

Redig PT, Arent LR. 2008. Raptor Toxicology. Veterinary Clinics of North America: Exotic Animal Practice. 11(2):261–282. doi:10.1016/j.cvex.2007.12.004.

Redig PT, Lawler EM, Schwartz S, Dunnette JL, Stephenson B, Duke GE. 1991. Effects of chronic exposure to sublethal concentrations of lead acetate on heme synthesis and immune function in red-tailed hawks. Arch Environ Contam Toxicol. 21(1):72–77. doi:10.1007/BF01055559.

Reichel WL, Schmeling SK, Cromartie E, Kaiser TE, Krynitsky AJ, Lamont TG, Mulhern BM, Prouty RM, Stafford CJ, Swineford DM. 1984. Pesticide, PCB, and lead residues and necropsy data for bald eagles from 32 states-1978–81. Environ Monit Assess. 4(4):395–403. doi:10.1007/BF00394177.

Rendon A. 2013. Hunting: nonlead ammunition. California: California Assembly Bill.

Rideout BA, Stalis I, Papendick R, Pessier A, Puschner B, Finkelstein ME, Smith DR, Johnson M, Mace M, Stroud R, et al. 2012. Patterns of mortality in free-ranging California Condors (*Gymnogyps Californianus*). J Wildl Dis. 48(1):95–112. doi:10.7589/0090-3558-48.1.95.

Riva MA, Lafranconi A, D'orso MI, Cesana G. 2012. Lead Poisoning: Historical Aspects of a Paradigmatic "Occupational and Environmental Disease." Saf Health Work. 3(1):11–16. doi:10.5491/SHAW.2012.3.1.11.

Robards F. C., King JG. 1966. Nesting and productivity of Bald Eagles, southeast Alaska–1966. US Bureau Sport Fisheries and Wildlife.

Robards Fredrick C., King JG. 1966. Nesting and Productivity of Bald eagles: Southeast Alaska, 1966. U.S. Department of the Interior B of SF and W, editor. Alaska.

Roscoe DE, Nielsen SW, Lamola AA, Zuckerman D. 1979. A Simple, Quantitative Test For Erythrocytic Protoporphyrin In Lead-Poisoned Ducks. J Wildl Dis. 15(1):127–136. doi:10.7589/0090-3558-15.1.127.

Russell RE, Franson JC. 2014. Causes of mortality in eagles submitted to the National Wildlife Health Center 1975-2013. Wildl Soc Bull. 38(4):697–704. doi:10.1002/wsb.469.

Saito K. 2009. Lead Poisoning of Steller's Sea Eagle (Haliaeetus pelagicus) and White-tailed Eagle (Haliaeetus albicilla) Caused by the Ingestion of Lead Bullets and Slugs, in Hokkaido, Japan. In: Ingestion of Lead from Spent Ammunition: Implications for Wildlife and Humans. The Peregrine Fund. Doi: 10.4080/ilsa.2009.0304

Scheuhammer AM. 1987. The chronic toxicity of aluminium, cadmium, mercury, and lead in birds: A review. Environmental Pollution. 46(4):263–295. doi:10.1016/0269-7491(87)90173-4.

Scheuhammer AM. 1996. Influence of reduced dietary calcium on the accumulation and effects of lead, cadmium, and aluminum in birds. Environmental Pollution. 94(3):337–343. doi:10.1016/S0269-7491(96)00084-X.

Scheuhammer AM, Money SL, Kirk DA, Donaldson G. 2003. Lead fishing sinkers and jigs in Canada: Review of their use patterns and toxic impacts on wildlife. Canadian Wildlife Service, Occasional Paper.(108):3–11. http://www.cws-scf.ec.gc.ca.

Scheuhammer AM, Norris SL. 1996. The ecotoxicology of lead shot and lead fishing weights. Ecotoxicology. 5(5):279–295. doi:10.1007/BF00119051.

Schirato G, Parson W. 2006. Bald Eagle Management in Urbanizing Habitat of Puget Sound, Washington. Nourthwestern Naturalist. 87(2):138–142.

Scott DE. 2021. Raptor Medicine, Surgery, and Rehabilitation. 3rd ed. CABI.

Sheuhammer AM, Templeton DM. 1998. Use Of Stable Isotope Ratios To Distinguish Sources Of Lead Exposure In Wild Birds. Ecotoxicology. 7(1):37–42. doi:10.1023/A:1008855617453.

Sileo L, Fefer SI. 1987. Paint chip poisoning of Laysan albatross at midway atoll. J Wildl Dis. 23(3):432–437. doi:10.7589/0090-3558-23.3.432.

Slabe VA, Anderson JT, Cooper J, Brown B, Ortiz P, Buchweitz J, McRuer D, Katzner T. 2019. Lead in piscivorous raptors during breeding season in the Chesapeake Bay region of Maryland and Virginia, USA. Environ Toxicol Chem. 38(4):862–871. doi:10.1002/etc.4376.

Slabe VA, Anderson JT, Millsap BA, Cooper JL, Harmata AR, Restani M, Crandall RH, Bodenstein B, Bloom PH, Booms T, et al. 2022. Demographic implications of lead poisoning for eagles across North America. [Internet]. [accessed 2022 December 12]. https://birdsoftheworld.org/bow/.

Sprunt A, Ligas FJ. 1964. Excerpts from convention addresses on the 1963 Bald Eagle count. Audubon 66.:45–47.

Stalmaster M V. 1987. The Bald Eagle. New York : Universe Books.

Stalmaster M v., Gessaman JA. 1984. Ecological Energetics and Foraging Behavior of Overwintering Bald Eagles. Ecol Monogr. 54(4):407–428. doi:10.2307/1942594.

Stalmaster MV. 1976. Winter Ecology and Effects of Human Behavior on Bald Eagles in the Nooksack River Valley, Washington. [Washington]: Western Washington State College.

Steidl RJ, Anthony RG. 2000. Experimental effects of human activity on breeding Bald Eagles. Ecological Applications, vol. 10, no. 1, 2000, pp. 258–68. JSTOR, https://doi.org/10.2307/2641000.

Stevenson AL, Scheuhammer AM, Chan HM. 2005. Effects of Nontoxic Shot Regulations on Lead Accumulation in Ducks and American Woodcock in Canada. Arch Environ Contam Toxicol. 48(3):405–413. doi:10.1007/s00244-004-0044-x.

Strom S, Langenberg J, Businga N, Batten J. 2009. Lead Exposure in Wisconsin Birds. In: Ingestion of Lead from Spent Ammunition: Implications for Wildlife and Humans. The Peregrine Fund, Boise, Idaho, USA. Doi: 10.4080/ilsa.2009.0205

Swenson JE, Alt KL, Eng RL. 1986. Ecology of Bald Eagles in the Greater Yellowstone Ecosystem (95): 3-46.

Thomas V. 2009. The Policy and Legislative Dimensions of Nontoxic Shot and Bullet Use in North America. The Peregrine Fund, Boise, Idaho, USA. Doi: 10.4080/ilsa.2009.0311

Thomas VG, Guitart R. 2013. Transition to Non-toxic Gunshot Use in Olympic Shooting: Policy Implications for IOC and UNEP in Resolving an Environmental Problem. Ambio. 42(6):746–754. doi:10.1007/s13280-013-0393-7.

U. S. Fish and Wildlife Service. 1986. Final supplemental environmental impact statement, use of lead shot for hunting migratory birds in the United States. Washington, D.C.

U. S. Fish and Wildlife Service. 2020. U.S. Fish and Wildlife Service Final Report: Bald Eagle Population Size: 2020 Update. Washington, D.C. U.S.A.

United Nations Environment Programme., International Labour Organisation., World Health Organization. 1989. DDT and Its Derivatives- Environmental Aspects. World Health Organization.

United States Code. 1940. Bald and Golden Eagle Protection Act. United States of America: Authenticated U.S. Government Information, GPO.

United States Code. 1972. Migratory Bird Treaty Act. United States of America.

Warner SE, Britton EE, Becker DN, Coffey MJ. 2014a. Bald Eagle Lead Exposure in the Upper Midwest. J Fish Wildl Manag. 5(2):208–216. doi:10.3996/032013-JFWM-029.

Washburn BE, Begier MJ, Wright SE. 2015. Collisions Between Eagles and Aircraft: an Increasing Problem in the Airport Environment. Journal of Raptor Research. 49(2):192–200. doi:10.3356/rapt-49-02-192-200.1.

Watson JW. 2002. Comparative Home Ranges and Food Habits of Bald Eagles Nesting in Four Aquatic Habitats in Western Washington. Northwestern Naturalist. 83(3):101–108.

Watts BD, Therres GD, Byrd MA. 2008. Recovery of the Chesapeake Bay Bald Eagle Nesting Population. Journal of Wildlife Management. 72(1):152–158. doi:10.2193/2005-616.

Watts BD, Whalen DM. 1997. Interactions between eagles and humans in the James River Bald Eagle Concentration Area. Williamsburg, VA.

Wheeler BK, Economidy J. 2018. Birds of Prey of the East. Princeton University Press.

Wiemeyer SN, Lamont TG, Bunck CM, Sindelar CR, Gramlich FJ, Fraser JD, Byrd MA. 1984. Organochlorine Pesticide, Polychlorobiphenyl, and Mercury residues in bald eagle eggs --1969- 79- and Their Relationships to Shell Thinning and Reproduction. Arch Environ Contam Toxicol. 13(5):529–549. doi:10.1007/BF01056332.

Winder VL, Watkins MA. 2020. Thirty Years of Bald Eagle Population Recovery and Nesting Ecology in Kansas, 1989–2018. Journal of Raptor Research. 54(3). doi:10.3356/0892-1016-54.3.255.

Wood PB, Edwards TC, Collopy MW. 1989. Characteristics of Bald Eagle Nesting Habitat in Florida. J Wildl Manage. 53(2):441. doi:10.2307/3801148.

World Health Organization. 2022 Aug 31. Lead Poisoning. [Internet]. [accessed 2022 December 8]. https://www.who.int/news-room/fact-sheets/detail/lead-poisoning-and-health

Wright BS. 1953. The Relation of Bald Eagles to Breeding Ducks in New Brunswick. J Wildl Manage. 17(1):55. doi:10.2307/3796805.

10. Annexes

Annex I – Open communication and poster abstract presented at the Fauna's XI International Conference, 17th to 19th of March, 2023.

Blood lead poisoning in Bald Eagles (Haliaeetus leucocephalus) in Virginia, U.S.A., between 2017 and 2021

Soares S.[1], Pierce K. [2,] Almeida V.[3]
[1]CIISA – Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon, Portugal
[2] The Wildlife Center of Virginia, Virginia, United States of America [3] Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS)

Due to its trophic position in the food chain and opportunistic feeding behavior, the Bald Eagle is prone to bioaccumulate contaminants present in the environment and food sources (Stalmaster 1987; Cesh et al. 2008; Buehler 2022). Lead toxicity is a historical and current cause of morbidity and mortality in Bald Eagles (Pattee and Henner 1983; Wayland et al. 2004). In 2,980 Bald Eagles carcasses submitted for diagnosis to the National Wildlife Health Center between 1975 and 2013, the main cause of death was poisoning (25.6%), with lead intoxication accounting for 63.5% of this mortality rate (Russell and Franson2014). There is strong scientific evidence that the ingestion of spent lead ammunition in contaminated viscera is a major source of lead intoxication in the Bald Eagle (Bedrosian et al. 2012; Cruz-Martinez et al. 2012; Bruggeman et al. 2018).

To contribute to further knowledge on this subject, a retrospective study was carried out on all Bald Eagles admitted to the Wildlife Center of Virginia between 2017 and 2021 (n=191). Data related to life stage, weight, body condition score, blood lead concentration, month of admission and county of rescue were retrieved from the WildOne information system. Lead concentrations were measured by using an electrochemical technique called anodic stripping voltammetry in a LeadCare II Blood Lead Analyzer. This system has the following limits of detection: less than 0.033 ppm is reported as "Low" and higher than 0.065 ppm is reported as "High".

Results revealed 144 Bald Eagles had detectable blood lead (75%). Additionally, averages of weights and body score conditions between groups with detectable lead and non-detectable lead were very similar. The highest incidence of blood lead concentration was detected in the period from November to March. A possible explanation for this temporal finding is an association with the deer harvest season. Lastly, lead cases show a wide geographical distribution. In conclusion, most of the Bald Eagles had detectable blood lead concentrations, being most frequent among adult individuals. As far as spatial outcomes, preliminary results indicate that most of Virginia's Bald Eagle population is affected.

Keywords: Bald Eagle, Lead, Poisoning, Virginia

Annex II - Poster presented at the Fauna's IX International Conference, 17th to 19th of March, 2023.

Blood lead poisoning in Bald Eagles (*Haliaeetus leucocephalus*) in Virginia, U.S.A., between 2017 and 2021

Soares S.[1], Pierce K. [2], Almeida V.[3]

[1]CIISA – Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon, Portugal

[2] The Wildlife Center of Virginia, Virginia, United States of America

[3] Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS)

INTRODUCTION

Due to its trophic position in the food chain and opportunistic feeding behavior, the Bald Eagle is prone to bioaccumulate contaminants present in the environment and food sources.

Lead toxicity is a historical and current cause of morbidity and mortality in Bald Eagles. Since 1975-2013, out of the 2,980 Bald Eagle carcasses submitted to the National Wildlife Health Center, poisoning was identified as the cause of death in 25.6%, of which 63.5% was caused by lead.

The literature review reports the ingestion of spent lead ammunition as a major source of lead poisoning in the Bald Eagle. In particular, through shots not retrieved or lead-contaminated viscera discarded from big game animals.

Figure 1 – Two adults and one juvenile Bald Eagle feeding on a deer carcass.

MATERIALS AND METHODS

- **Retrospective study** of all Bald Eagles admitted to the Wildlife Center of Virginia between 2017 and 2021 (N=191).

- **Variables investigated:** life stage, weight, blood lead concentration, body score condition (1-5), the month of admission, and the county of rescue.

Lead concentrations were measured on blood samples by the LeadCare II system that uses an electrochemical technique called anodic stripping voltammetry. This system has the following limits of detection:

- Less than 0.033 ppm is reported as "**Low**";
- Higher than 0.65 ppm is reported as "**High**".

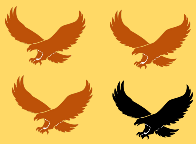
Figure 2 – Adult Bald Eagles admitted at the Wildlife Center of Virginia showing clinical signs of lead poisoning: general listlessness and overall weakness (A) and neurological impairment (B).

Figure 3 – Blood collection from the basilic vein in a Bald Eagle.

RESULTS

Total: 191 Bald Eagles

- 47 individuals with non-detectable blood lead
- 144 individuals with detectable blood lead



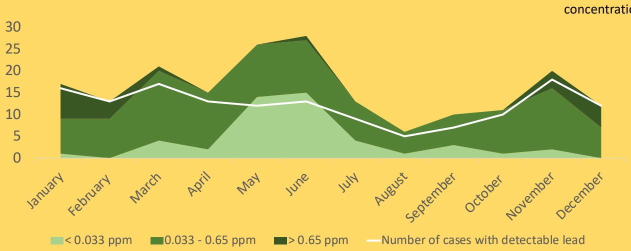
- **3** out of 4 Bald Eagles, had **detectable lead in their blood.**

Life Stage	Detectable lead	Non-detectable lead
Adult (> 5 years)	117 (81.3%)	11 (23.4%)
Juvenile (> 6 months)	23 (15.9%)	25 (53.2%)
Fledgling (2- 6 months)	4 (2.8%)	9 (19.1%)
Hatchling (< 2 months)	0	2 (4.3%)
Total	144 (100%)	47 (100%)

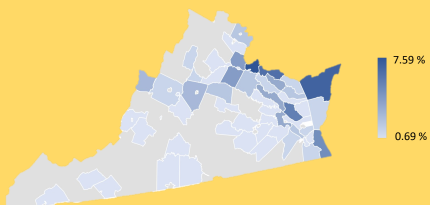
Table 1 – Absolute and relative frequencies of blood lead concentration by life stage.

	Overall				Adults			
	Detectable Lead		Non- Detectable lead		Detectable Lead		Non- Detectable lead	
	Weight	**BCS**	**Weight**	**BCS**	**Weight**	**BCS**	**Weight**	**BCS**
Average	3.3 kg	2.1	3.0 kg	2.1	3.3 kg	2.1	3.0 kg	2.7

Table 2 – Overall and adults' averages of weights and body score condition.



Graph 1 – Monthly absolute frequencies of blood lead concentrations.



Graph 2 – Geographical relative frequencies of blood lead concentration by county of Virginia, U.S.A..

DISCUSSION AND CONCLUSION

- ✓ Most of the Bald Eagles (**75%**) admitted had detectable lead concentrations in their blood.
- ✓ Lead cases were most prevalent among **adult individuals** compared to the other age groups.
- ✓ Weights and body score conditions between groups with detectable lead and non-detectable lead were very similar, suggesting **acute poisoning**.
- ✓ The highest incidence of cases with blood lead concentrations higher than 0.65 ppm was from **November** to **March**. A possible association with the deer harvest season in Virginia starting in **September** until **April** it´s in progress.
- ✓ Lead cases had a **broad geographical distribution**, suggesting that most of Virginia's Bald Eagle population is affected.

This study highlights the high frequency of lead poisoning in Bald Eagles in Virginia and strengthens the need for conservation efforts.

- Bedrosian B, Craighead D, Crandall R. 2012. Lead Exposure in Bald Eagles from Big Game Hunting, the Continental Implications and Successful Mitigation Efforts. PLoS One. 7(12). doi:10.1371/journal.pone.0051978.
- Bruggeman JE, Route WT, Redig PT, Key RL. 2018. Patterns and trends in lead (Pb) concentrations in bald eagle (Haliaeetus leucocephalus) nestlings from the western Great Lakes region. Ecotoxicology. 27(5):605–618. doi:10.1007/s10646-018-1933-5.
- Buehler DA. 2022. Bald Eagle (Haliaeetus leucocephalus). In: Rodewald PG, Mlodinow SG, editors. Birds of the World. Cornell Lab of Ornithology.
- Cesh LS, Williams TD, Garcelon DK, Elliott JE. 2008. Patterns and Trends of Chlorinated Hydrocarbons in Nestling Bald Eagle (Haliaeetus leucocephalus) Plasma in British Columbia and Southern California. Arch Environ Contam Toxicol. 55(3):496–502. doi:10.1007/s00244-007-9125-y.
- Cruz-Martinez L, Redig PT, Deen J. 2012. Lead from spent ammunition: a source of exposure and poisoning in bald eagles. Human-Wildlife Interactions. 6(1):94–104.
- Pattee OH, Henner SK. 1983. Bald eagles and waterfowl: The lead shot connection. Transactions of the North American Wildlife and Natural Resources Conference. 48:230–237.
- Russell RE, Franson JC. 2014. Causes of mortality in eagles submitted to the National Wildlife Health Center 1975-2013. Wildl Soc Bull. 38(4):697–704. doi:10.1002/wsb.469.
- Stalmaster M v. 1987. The Bald Eagle. New York : Universe Books.
- Wayland K, Wilson LK, Elliott JE, Miller MJ, Bollinger T, Langelier K, Keating J, Froese JM. 2004. Mortatlity, Morbility, and Lead Poisoning of Eagles in Western, Canada 1986-98. J Raptor Res. 37(1):8–18.

Annex III – Absolute and relative admission frequencies by location of rescue and blood lead concentrations in Bald Eagles.

County or Independent city of Rescue	Absolut Frequency	Relative Frequency	Elevated Group	Background Group
Accomack County	13	6.8%	4	9
Albemarle County	4	2.1%	2	2
Alleghany County	2	1%	0	2
Amelia County	1	0.5%	0	1
Augusta County	3	1.6%	0	3
Bath County	2	1%	0	2
Bland County	1	0.5%	0	1
Botetourt County	1	0.5%	0	1
Buena Vista (City)	1	0.5%	0	1
Campbell County	2	1%	0	2
Caroline County	4	2.1%	2	2
Carroll County	1	0.5%	0	1
Charles City County	1	0.5%	0	1
Charles County	1	0.5%	0	1
Chesapeake (City)	4	2.1%	0	4
Chesterfield County	1	0.5%	1	0
Culpeper County	1	0.5%	0	1
Dinwiddie County	1	0.5%	1	0
Essex County	4	2.1%	2	2
Fairfax County	3	1.6%	1	2
Fredericksburg (City)	2	1.0%	0	2
Giles County	1	0.5%	0	1
Gloucester County	9	4.7%	4	5
Gloucester Countyro (City)	1	0.5%	1	0
Goochland County	1	0.5%	0	1
Greene County	2	1.6%	2	0
Halifax County	1	0.5%	0	1
Hampton (City)	2	1.6%	0	2
Highland County	4	2.1%	0	4
Isle Of Wight County	2	1.6%	2	0
James City County	8	4.2%	2	6
King And Queen County	5	2.6%	2	3
King George County	11	5.8%	6	5
King William County	1	1%	0	1
Lancaster County	4	2.1%	0	4
Louisa County	4	2.1%	2	2
Mathews County	1	0.5%	1	0
Middlesex County	2	1.6%	1	1
Nelson County	1	0.5%	1	0

New Kent County	1	0.5%	1	0
Newport News (City)	4	2.1%	1	3
Norfolk (City)	3	1.6%	0	3
Northampton County	4	2.1%	1	3
Northumberland County	3	2%	1	2
Orange County	1	0.5%	0	1
Page County	3	1.6%	2	1
Pittsylvania County	2	1.0%	0	2
Poquoson (City)	2	1.0%	0	2
Portsmouth (City)	3	1.6%	0	3
Prince George County	1	0.5%	1	0
Prince William County	1	0.5%	0	1
Richmond County	5	3.1%	2	3
Scott County	1	0.5%	0	1
Spotsylvania County	6	3.1%	2	4
Stafford County	8	4.2%	1	7
Suffolk (City)	1	0.5%	0	1
Surry County	2	1.6%	1	1
Sussex County	1	0.5%	0	1
Virginia Beach (City)	12	6.3%	2	10
Washington County	1	0.5%	0	1
Waynesboro (City)	1	0.5%	0	1
Westmoreland County	9	4.7%	0	9
Wythe County	1	0.5%	0	1
York County	2	1.6%	0	2

Annex IV – Result of Person's chi-square test and Fisher's exact test.

Variable	*p*-value
Life stage	0.0027
Body Weight	0.7351
Body Condition	0.196
Month of Admission	8.338e-06
Year of Admission	0.3943
County of Rescue	7.621e-07
Circumstances of Admission	0.3631

Annex V – Results of the *Odds Ratio*.

Predictor variable	OR	IC 95%	*p-value*
Life Stage			
Adult	3.7	1.5 - 9.8	0.001
Juvenile	0.4	0.2 - 1.04	0.06
Fledgling	0	0 - 0.8	0.02
Hatchling	0	0 - 13	1
Month			
January	5.7	1.8 - 19.9	0.001
February	2.4	0.62 - 8.7	0.19
March	1.70	0.5 - 4.7	1.70
April	0.6	0.1 - 2.4	0.76
May	0.4	0.1 - 4.7	0.17
June	0.17	0.01 - 0.7	0.006
July	0	0 - 0.8	0.02
August	0	0 - 0.7	0.19
September	0.3	0 - 2.1	0.28
October	1.1	0.2 - 5.1	1
November	1.5	0.5 - 4.2	0.43
December	1.3	0.3 - 5.2	0.74

OR (*Odds Ratio*); IC (Confidence interval).