

JOURNAL OF WILDLIFE REHABILITATION

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INTERNATIONAL WILDLIFE REHABILITATION COUNCIL

THE *Journal of Wildlife Rehabilitation* is designed to provide useful information to wildlife rehabilitators and others involved in the care and treatment of native wild species with the ultimate purpose of returning them to the wild. The journal is published by the International Wildlife Rehabilitation Council (IWRC), which invites your comments on this issue. Through this publication, rehabilitation courses offered online and on-site in numerous locations, and its outreach to those in the profession, the IWRC works to disseminate information and improve the quality of the care provided to wildlife.



ABOVE:
Flamingos (*Phoenicopterus roseusi*) in Mozambique. IUCN red list category–Least concern.

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ON THE COVER:
Orphaned koala joey *(Phascolarctos cinereus)* in care at Magnetic Island Koala Hospital, Horseshoe Bay, QLD, Australia

PHOTO ©MAGNETIC ISLAND KOALA HOSPITAL. USED WITH PERMISSION.

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INTERNATIONAL WILDLIFE REHABILITATION COUNCIL

Providing evidence-based education and resources on wildlife rehabilitation to move the field of wildlife rehabilitation forward; to promote wildlife conservation and welfare; and to mitigate human-wildlife conflicts worldwide, through better understanding of wild animal ecology, behavior, and welfare.

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EDITORIAL

Helen Van Doninck Hopefuls Across the Globe

The 2024 applicant pool for the Helene Van Doninck Scholarship was exceptionally strong. There were ten applicants from eight countries: Bangladesh, Canada, Chile, Columbia, Ecuador, Latvia, South Africa, and the United States. The individuals spanned the range from youth relatively recently involved in wildlife rehabilitation to established professionals with over ten years in the field. Reading the applicants and support letters, I saw a common theme amongst the applicants, they all embodied Helene's energy for wildlife care and community outreach.

Dr. Helene Marie Van Doninck was passionate in her education of the public, especially regarding the dangers of lead ammunition. Her sense of humor, depth of knowledge, and understanding of people gained her entry into circles that could be otherwise unwelcoming to a veterinarian and avid wildlife rehabilitator who was proposing change. Helene's veterinary and scientific knowledge regarding lead toxicity and the effects on wildlife (especially eagles), persuaded people to make lifestyle changes. Her friendly, non-threatening demeanor when presenting the information, gained their trust as willing partners to protect wildlife and human beings.

The wildlife rehabilitation community came together to remember Helene by creating and contributing to a fund which supports public education. The purpose of this scholarship fund is to advance public education and advocacy for wildlife rehabilitation by supporting wildlife rehabilitators looking to attend conferences, trainings, or other events to teach or learn about public education for animal welfare and conservation.

This year's applicants work at the human wildlife interface. Most combine wildlife rehabilitation practice and attention to animal welfare with an in-depth understanding of One Health and the passion to work with the public on these critical and interconnected realms. I saw again and again within the short space allowed on the application that these individuals demonstrate the balance of medical and technical skill with a deep appreciation for humanity and the passion to connect humans with the broader world. Our chosen recipients and many other applicants show Helene's strength of character and her determination and focus to positively alter the status quo through education and example, striving to make a positive difference for wildlife in our world. In short, they make me hopeful.

IWRC is pleased to manage this fund on behalf of the larger community. The Helene Marie Van Doninck scholarship is funded through community contributions and the awards granted each year may vary depending upon donor support. You can support the fund by visiting https://theiwrc.org/helene-scholarship/ or calling IWRC at 866-871-1869.

—Kai Williams
IWRC Executive Director

Diana Huang

We mourn the loss of Diana Huang, who served as IWRC Membership Chair since 2015. A dedicated veterinarian, educator, and wildlife rehabilitator, Diana's profound impact on our community is deeply felt. Her enthusiasm for teaching about wildlife and promoting peaceful coexistence shone brightly, especially in her work with the Ohio Wildlife Center and her own non-profit, For All Species Education. Diana was renowned for her expertise in neonatal care, handling everything from squirrels to bats with unparalleled skill and compassion. She was always ready to lend her knowledge and assistance, regardless of the hour, embodying a dedication that inspired all who knew her. Beyond her professional and volunteer roles, Diana was a fervent supporter of the performing arts alongside her husband, George, reflecting her vibrant spirit and passion for life. Diana's legacy is that of kindness, expertise, and unwavering commitment to wildlife rehabilitation.

As IWRC's membership chair for 9 years, Diana had the best interest of wildlife rehabilitators at heart. Diana was one of the masterminds behind starting the Coffee & Tea series in March of 2020, she was instrumental in setting up the 2023 Underrepresented Member Initiative, and she managed the creation of the first Disaster Preparedness workbook. Diana put in countless hours working on the Journal of Wildlife Rehabilitation paper database, creating and maintaining our international wildlife rehabilitation center database, and most recently she was overhauling our public wildlife support pages, bringing them into the current decade, work we plan to complete in the coming year. This list just brushes the service Diana Huang performed for IWRC members and for all rehabilitators she could support.

https://www.dignitymemorial.com/obituaries/worthington-oh/diana-huang-11928349



Diana Huang

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Randie Sue Segal

Just like the seasons are changing around me, my dear friend Randie has now stepped over the threshold to her new season, leaving family and friends to mourn, remember, and celebrate her. I imagine her liberated energy surrounding all of us.

Although our friendship only dates back 12 years, it felt like it had always been, and we could pick up at any time – I can't say I felt 'time' between us. We met because of an IWRC collaboration at the Irish Wildlife Rehabilitation Conference – an experience that cemented my commitment to wildlife rehabilitation. That serendipitous encounter with Randie changed my life as she welcomed me within her circle with open arms.

Randie was an integral part of the IWRC – She was determined, inspiring, driven, resourceful, curious and resilient. She advocated for the advancement of wildlife rehabilitation and would've spent countless hours doing so. Her legacy to this field will have had far reaching ripples.

As I reflect on some of the qualities I saw in her, I will remember her most for her welcoming smile, her wisdom, her humor, her sense of wonder, her deep love for all creatures and her spirit of gratitude.

Although this tribute may be brief, I will carry Randie's memory with me forever and leave you with some of her wise words to me: "Life may not be perfect, but there are perfect moments."

Sláinte, my friend!
—Diane Séguin



Randie Sue Segal

As global meetings on climate and biodiversity get underway, coalition of organizations calls for protection of one of Africa's last bastions of marine biodiversity and support of local communities

One of the world's hottest hotspots for marine biodiversity, Mozambique's Inhambane Seascape, is under assault by two massively destructive industries. Conservation organizations are concerned that offshore seismic testing for gas mining and heavy-sands mining of pristine coastal dunes and Miombo forests would be catastrophic for these critical ecosystems—including the heart of the seascape, the idyllic Bazaruto archipelago—if allowed to move forward.

As local communities and organizations fear repercussions for speaking out against these industries, Re:wild, Amphibian Survival Alliance, ALL RISE, BirdLife International, Centro Terra Viva, Marine Megafauna Foundation (MMF), Mission Blue, Natural Justice, ParCo Community Partners, and Our Children's Earth Foundation are calling on the global community to speak up and urge the government of Mozambique to protect this irreplaceable seascape and to invest in Inhambane's local communities.

This call to action comes just ahead of the 2024 United Nations Biodiversity Conference of the Parties (COP16) and the 2024 United Nations Climate Change Conference (COP29), and as Mozambique counts votes from its Oct. 9 election. Read more here.



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Dugong in the Bazaruto Archipelago.

Catastrophic 73% decline in the average size of global wildlife populations in just 50 years reveals a 'system in peril'

WASHINGTON, DC *(October 9, 2024)*—There has been a catastrophic 73% decline in the average size of monitored wildlife populations* in just 50 years (1970-2020), according to World Wildlife Fund's (WWF) Living Planet Report 2024. The report warns that parts of our planet are approaching dangerous tipping points driven by the combination of nature loss and climate change which pose grave threats to humanity.

The Living Planet Index, provided by the Zoological Society of London (ZSL), tracks almost 35,000 vertebrate populations of 5,495 species from 1970-2020. The steepest decline is in freshwater populations (85%), followed by terrestrial (69%) and then marine (56%).

Habitat loss and degradation and overharvesting, driven primarily by our global food system are the dominant threats to wildlife populations around the world, followed by invasive species, disease and climate change.

Significant declines in wildlife populations negatively impact the health and resilience of our environment and push nature closer to disastrous tipping points– critical thresholds resulting in substantial and potentially irreversible change. Regional tipping points, such as the decimation of North American pine forests, the destruction of the Amazon rainforest, and the mass die-off of coral reefs, have the potential to create shockwaves far beyond the immediate region, impacting food security, livelihoods, and economies.** Read the rest here.

Research expedition reveals greater protection needed for the critically endangered North Atlantic right whale

WASHINGTON, DC *(15 October 2024)*—The latest report reveals new insights into North Atlantic right whale behavior, distribution and habitat use that send a stark message about the urgent need for more effective and expanded protection measures.

The report, R/V Song of the Whale: 2023 North Atlantic right whale surveys, was released today by IFAW drawing on data from its commissioned research project aboard the Song of the Whale, a purpose-built whale research vessel.

"Right whales face numerous hazards as they migrate from calving grounds off the US Southeast to feeding grounds in the US Northeast and Canada," said Kathleen Collins, Senior Marine Campaign Manager at IFAW. Read more. ◆

Physiological stress response of orphaned koala joeys to visitors: A pilot study

Harsh Pramila Pahuja,[1*] Izzy Bee[2], Ali Bee,[2] and Edward Jitik Narayan[1]



PHOTO © ANDRAS DEAK. DREAMSTIME.COM.

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ABSTRACT
Koala (*Phascolarctos cinereus*) joey rescues are increasing over the past decade, and rehabilitation of a joey requires extensive care, and close proximity and handling by humans. These novel environments are likely to present a suite of biotic and abiotic stressors during rehabilitation. In this study, we aimed to monitor the physiological response of orphaned koala joeys to potential stressors. A total of 92 faecal samples were analysed for faecal cortisol metabolites (FCMs) using a polyclonal R4866 cortisol enzyme-immunoassay which has been previously validated in koalas. The iterative baseline method was used to establish FCM profiles of all individuals, and to identify significant peaks in FCM concentrations. Visitor events were identified and confirmed as an acute stressor based on the FCM profiles of the koala joeys. All koala joeys were found to have a significant rise in FCM concentrations after each visitor encounter. These results provide the first record of acute stress response of koala joeys to visitors. We suggest that visitor encounters be kept to a minimum and perhaps avoided altogether, especially for young rehabilitating joeys.

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Introduction

The koala (*Phascolarctos cinereus*) is an iconic marsupial endemic to Australia.[1] Despite being an Australian icon, in February 2022, the conservation status of koalas was upgraded to "endangered" due to their starkly declining population.[2] Retrospective studies assessing the trends of koala admissions to wildlife hospitals highlight that the clinical outcome for the majority of these koalas is that they either naturally succumb to their injuries and/or diseases, or they need to be humanely euthanized due to their critical condition.[3–6] Increased mortality of adult female koalas is likely to cause

more rescues of young orphans because during the early stages of life, joeys are sustained by their mothers. A study by Taylor-Brown et al.[6] indicated that koala joeys formed the second largest group (after possums) that were rescued as orphans. Furthermore, as highlighted in Pahuja and Narayan,[7] koala joey rescues increased slightly from 2014–2021 in Adelaide, South Australia.

Koala joeys are born in an altricial state, which is followed by an approximate 6-month period of pouch occupancy.[8] During this extensive dependent period, maternal milk, eucalyptus and "pap" constitute the diet of joeys.[9,10] When orphaned, of course, this process needs to be replicated by humans and requires extensive care, close proximity, and handling. Although crucial for long-term survival, being raised by a human in captivity is likely to serve as a "stressor" for the koala joey because of the novel environment.

A stressor is commonly defined as any noxious stimuli that could potentially cause an imbalance in the animal's homeostatic system.[11] When confronted with a stressor, the body elicits an array of responses, one of which is the neuroendocrine activation of the hypothalamic-pituitary-adrenal (HPA) axis.[12] This results in the secretion of glucocorticoids (GCs) which have a range of effects (e.g., energy mobilisation for fight-or-flight response) that allow the animal to achieve homeostasis (i.e., cope with the stressor).[11] As a result, GCs are often referred to as the stress hormones,[13] and short-term elevation of GCs are indicative of acute stress.[14] However, prolonged GC elevation can have a deleterious impact on an animal's health, given the diverse biological effects of GCs on various body systems, and is thus indicative of chronic stress.[14,15] GCs (or their metabolites) can be measured in a range of sample types, such as blood, feces, and fur. However, given the ease of collection and minimal handling associated with collecting scat samples, monitoring fecal glucocorticoid metabolites (FGMs) is the most commonly employed method in studies on Australian marsupials.[16]

Cortisol is the principal GC produced by koalas[17] and is increasingly acknowledged as a practical endocrine indicator of stress.[16,18] Whilst there have been a number of studies investigating the physiological stress response of marsupials,[14,15,19–21] including koalas,[22–24] there is a paucity of literature on the capacity of koalas to respond to the stressors of captivity. To improve the rehabilitation outcomes and ensure the welfare of koalas in captivity, it is imperative to understand the potential stressors of captivity, and how these animals respond to it.

In the present study, we monitored the fecal cortisol metabolites (FCMs) of three orphaned koala joeys undergoing rehabilitation over a period of one month before release. During this period, we also monitored potential stressors faced by the joeys during rehabilitation. Of particular interest was to examine whether koala joeys have a measurable physiological stress response to these stressors. We hypothesized that acute stressors are likely to cause a physiological change in the joeys which will be reflected and capture as elevated FCMs. The primary aim of this study was to expand the current knowledge on koala stress biology by monitoring FCMs within the context of potential stressors. To our knowledge, this is the first study to report on the physiological stress response of koala joeys to potential stressors.

Material and Methods

Animal Ethics

All procedures in this research were performed in accordance with the relevant guidelines and regulations set by The University of Queensland Animal Ethics Committee: Native or Exotic Wildlife and Marine Animals (NEWMA) (approval number 2022/AE000035).

Study Site and Animals

All koala joeys were housed at the Magnetic Island Koala Hospital, 3 Corica Crescent, Horseshoe Bay, QLD, Australia 4819 (GPS coordinates: 19°7'26.06"S, 146°51'34.06"E). A total of 3 orphaned koala joeys [1 male (M1) and 2 females (F1 and F2)] were studied based on whichever was currently in care during the period of data collection (2 April 2022 through 2 May 2022). All koala joeys were housed individually in a 6 m × 6 m × 3.5 m (L × W × H) enclosure donated by the Glencore Australia, Copper Refineries Pty. Ltd. The enclosure was designed with a cement slab floor, insulated roof, snake proof mesh, and trim deck metal, and furnished with logs, branches, ropes, and polyvinyl pipes of varying dimensions. The joeys were provided with ad libitum water and feed. The feed consisted of a mix of various species of *Eucalyptus* leaves freshly cut and sourced from a local *Eucalyptus* plantation. The *Eucalyptus* leaves were changed daily and mainly consisted of (but were not limited to) lemon scented gum (*Corymbia citriodora*), river red gum (*E. camaldulensis*), swamp mahogany (*E. robusta*), red mahogany (*E. resinifera*), mountain mahogany (*E. notabilis*), and tallow (*E. microcorys*) The age for all koala joeys during data collection was estimated by the veterinarian to be between 12–15 months based on tooth wear and body weight. No joeys had any history of disease, and all remained clinically healthy throughout the study period as reported by the veterinarian.

Data collection (Faecal sample collection and recording stressors)

In order to evaluate daily changes in FCM concentrations, fresh feces were collected routinely from all three koala joeys daily, over a one-month study period. One faecal sample (comprising on average of 3–5 fecal pellets) per day per animal was collected during routine cleaning of the enclosure (0900h to 1100 h), with minimal disturbance to the animal. Samples were collected by hand, using a new pair of gloves, and immediately transferred into a resealable polyethylene ZipLock© bag and labeled with the animal's name, date, and the time of sample collection. All feces were removed from each enclosure at the end of each day so that we could ensure fresh fecal sample collection on the following day. The samples were then stored at –20°C until they were sent to the

laboratory on ice via overnight freight. Upon delivery, they were immediately refrozen at –20°C until processing.

The staff at the Magnetic Island Koala Hospital also observed and recorded any potential stressors. A range of stressors have been identified for marsupials in captivity,[14,16,23] including (but not limited to) maintenance activities in and around the enclosure, moving animals to a different enclosure, visitor encounters, loud noises, extreme environmental events, and handling for routine health checks. A non-exhaustive list of potential stressors was used as a reference to observe and record potential stressors that could be affecting the koala joeys.

Fecal sample processing and extraction

Fecal sample processing and FCM extraction followed the procedures previously described for koalas (both adults and joeys).[17,22,23] Briefly, frozen fecal samples were weighed and then dried in an oven at 65°C for a 24 hr period, or until completely dried. Once completely dry, the samples were ground into a fine powder using a mortar and pestle. Analytical grade ethanol (10% v/v) was used to clean the mortar and pestle between each individual sample processing to avoid cross-contamination. The ground-up powder was sifted through a fine mesh strainer to remove all coarse particles. A 0.2 ± 0.001 g sample of sifted product was weighed out into a labeled Eppendorf tube and then stored in a –20°C freezer until use.

For hormone extraction, samples were removed from the –20°C freezer and 2 mL of 90% (v/v) analytical grade ethanol solution was added to the Eppendorf tube. Tubes were vortexed at medium-high speed (5 min, 5000 g) on an Eppendorf mini-spin centrifuge for a minimum of 30 sec to thoroughly mix the solution. Tubes were then placed into a water bath (>60°C) for 20 min to allow hormones to dissolve in the solution. While in the bath, tubes were gently manually shaken to ensure feces remained submerged in ethanol and did not spill over the top of the tube. After 30 min, the supernatant was transferred into an Eppendorf tube, closed, and then centrifuged at 3000 g for 5 min to separate any solid fecal residue. If the sample looked turbid or had suspended particles, tubes were centrifuged again for 5 min and then pipetted into a new and clean labeled Eppendorf tube. Following this, for the polyclonal cortisol antibody assay (R4866; Late C. J. Munro, University of California, Davis), 0.6 mL of the aqueous solution was aliquoted into a new and clean labeled Eppendorf tube. The tubes were kept in a laminar flow chamber for 24 hr to allow enough time for the ethanol to completely evaporate. Once ethanol was completely evaporated, 1 mL of assay buffer (39 mM $NaH_2PO_4 \cdot H_2O$, 61 mM $NaHPO_4$, 15 mM NaCl and 0.1% bovine serum albumin, pH 7.0) was added to each tube. The tubes were vortexed at medium-high speed (5 min, 3000 g) in an Eppendorf mini-spin centrifuge for 30 sec, and then centrifuged at 10000 g for 10 min. Following this, 850 µL of supernatant was pipetted into a new and clean labeled Eppendorf tube, ensuring any solid remnants in the solution were avoided.

Enzyme-immunoassay (EIA)

Recent studies have identified tetrahydrocortisol as the predominant metabolite of native cortisol in koala faeces.[25] However, there is currently no EIA available on the market that specifically targets or cross-reacts extensively with this metabolite. The R4866 EIA has previously been validated in koalas using physiological (ACTH challenge), biological, and laboratory validation techniques, and is routinely used for monitoring physiological stress in koalas.[17,23,24,26,27] In addition, this assay has also been validated in the laboratory for monitoring FCMs in koala joeys.[22] Glucocorticoid concentrations for fecal extract using the R4866 assay were determined using a polyclonal anti-cortisol antiserum diluted to 1:15 000, horseradish peroxidase (HRP) conjugated cortisol 1:80 000 and cortisol standards (1.56–400 pg/well). Cross reactivity of the R4866 anti-cortisol antiserum is reported as 100% with cortisol and less than 10% with other steroids (with prednisolone 9.9%, prednisone 6.3%, cortisone 5%, and <1% with corticosterone) tested.[28] Sample extracts were assayed in duplicate on Nunc Maxisorp™ plates (96 wells) (Sigma Aldrich, Sydney, Australia). Plates were coated with 50 µL cortisol antibody diluted to the appropriate concentration in a coating buffer (50 mmol/L bicarbonate buffer, pH 9.6), and left to stand and incubate for a minimum 12 hr in a fridge at 4°C. The plates were washed the next day using an automated plate washer (ELx50, BioTek™, Sursee, Switzerland) with phosphate-buffered saline containing 0.5 mL/L Tween 20 to rinse away any unbound antibody. Stocks of standards, fecal extracts, and horseradish peroxidase labels were diluted to the appropriate concentration in assay buffer. The extraction dilution rates (1:2) were based on the concentrations of pooled samples that resulted in 50% binding in a previous study monitoring FCMs in orphaned koala joeys.[22] For each assay, 50 µL of cortisol standard and diluted fecal extract was added to each well based on the plate map. Immediately following this, 50 µL of HRP label was added to the wells. Plates were covered and incubated at room temperature for 2 hr. Following a wash, 50 µL of substrate buffer (0.01% tetramethylbenzidine and 0.004% H_2O_2 in 0.1 M acetate citrate acid buffer, pH 6.0) was then added to generate a color change. Color reaction was halted after 15 min using 50 µL of stop solution (50 µL of 0.5 mol/L H2SO4), and the plates were read primarily at 450 nm and secondarily at 630 nm on an ELx800 (BioTek) microplate reader. The sensitivity of the fecal cortisol FCM was calculated as the value 2 standard deviations from the mean response of the blank samples. The sensitivity of the fecal cortisol metabolite EIA was 1.2 ± 0.2 pg $well^{-1}$ (n = 3). Intra- and inter-assay coefficients of variation were determined by calculating the mean of CV within a plate (for intra-assay) and between the plates (for inter-assay) assayed during analysis. The intra- and inter-assay coefficients of

variation were 4.26 ± 1.10% (n = 3) and 6.42 ± 3.71% (n = 3) respectively.

Data processing and analysis

All FCM concentrations are expressed as (ng/g) on the basis of net dry feces weight. Analysis of monthly profiles of FCM secretion involved establishing a baseline level in order for significant peaks to be identified. FCM concentrations from individual koala joeys were analyzed using an iterative baseline method.[29] In this method, the baseline is calculated through an iterative process, excluding concentrations greater than "mean + 2 SD." In successive iterations, all concentrations greater than "mean + 2 SD" are excluded until no points fall above this threshold. The mean iterative baseline is the mean of the remaining concentrations at the completion of the iterative process. Concentrations that fall above the threshold (mean + 2 SD) of the final iteration are classified as significant peaks. The "mean + 2 SD" of the final iteration represents the "cut-off" (i.e., baseline threshold) value and all concentrations above this value are considered to be indicative of a significant acute stress response. Several measures were calculated to summarize fecal hormone profiles for each individual koala joey: (1) overall mean of all samples for the collection period, (2) baseline mean that excluded all values greater than "mean + 2 SD" of the final iteration, and (3) peak mean that only included all values > 2 SD above the baseline mean. These values are highlighted in Table 1. The iterative baseline calculation can be performed using the R package hormLong derived by Fanson and Fanson[30] for large datasets and/or when monitoring multiple hormonal profiles. However, given the nature and scope of the current study, these calculations were performed in Microsoft Excel 2021©.

Statistical analysis

Statistical analysis was performed in GraphPad Prism version 9.3.1. The parametric assumptions of normality and homoscedasticity were tested, and a single factor ANOVA, followed by Tukey's multiple comparison test, was run to determine whether overall mean, baseline mean, baseline threshold, and peak mean FCM concentrations differed ($P < 0.05$) among individuals.

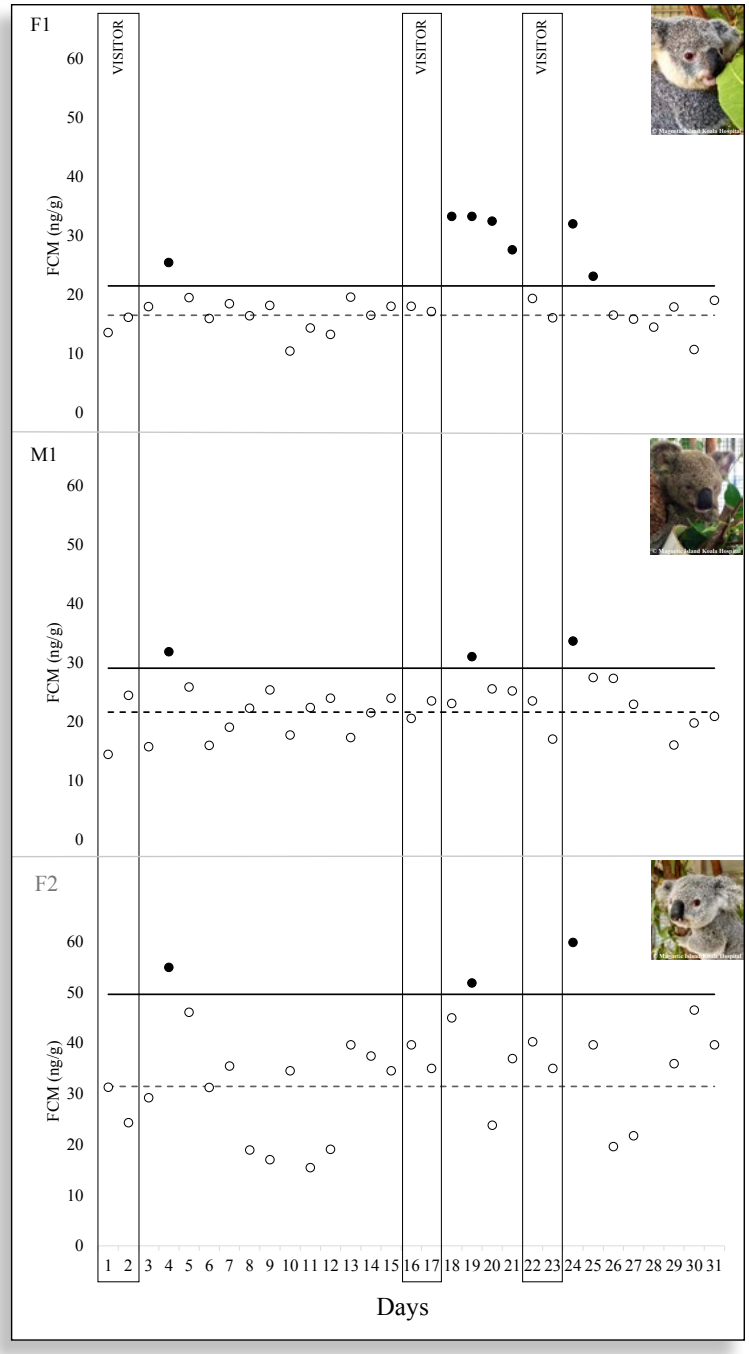


FIGURE 1. FCM profiles of koala joeys undergoing rehabilitation at the Magnetic Island Koala Hospital. All open circles illustrate baseline FCM concentrations, whereas closed circles illustrate significant peaks in FCM concentrations as identified by the iterative baseline method. Vertical boxes delineate visitor events as identified by the staff at Magnetic Island Koala Hospital. Solid horizontal line represents the baseline threshold value. Dashed horizontal line represents the mean iterative baseline.

Results

Identification of stressors

The staff at the Magnetic Island Koala Hospital identified visitors as the only potential stressor for the three koala joeys undergoing rehabilitation. Based on the list of potential stressors faced by marsupials in captivity (see methods), the hospital staff confirmed that no other stressors were present throughout the duration of the study. A visitor event was defined as any occurrence where human visitors were present inside the enclosure and in the vicinity of the koala joeys. These events mainly included journalists and media presence for interviews or photography, where the primary aim was to document and publicize the rehabilitation efforts of the hospital. In addition, families of donors were also allowed to take pictures inside the enclosure, to acknowledge their contributions and encourage continued support. All visitor events followed strict

no-contact protocols. Visitors were confirmed as a stressor based on the FCM profiles of all three koala joeys, indicating that all individuals displayed a significant rise in FCM concentrations within 1–3 days after each visitor encounter. These results are presented in the following section.

FCM profiles and acute stress response of koala joeys to visitors

The daily FCM concentrations of the three koala joeys (Fig. 1) ranged from 10.41 to 33.27 ng/g dry feces for individual F1, 14.42 to 33.65 ng/g dry faeces for individual M1, and 15.41 to 59.82 ng/g dry feces for individual F2. There were significant differences in the overall mean ($F_{2,88} = 7.689$, $p < 0.05$), baseline mean ($F_{2,75} = 13.21$, $p < 0.05$), baseline threshold ($F_{2,9} = 1.104$, $p < 0.05$), and peak mean ($F_{2,9} = 0.503$, $p < 0.05$) FCM concentrations between the three koala joeys over the one-month sampling period (Table 1). The overall mean FCM concentrations of F1 (19.36 ± 6.22 ng/g dry feces) and M1 (22.81 ± 4.76 ng/g dry feces) were significantly different ($p < 0.05$ for both comparisons) from F2 (34.02 ± 11.12 ng/g dry feces), but not amongst each other ($p = 0.24$) (Table 1). The baseline mean FCM concentrations of all three koala joeys were significantly different from each other ($p < 0.05$ for all comparisons) (Table 1). The baseline threshold FCM concentrations of F1 (21.48 ± 4.67 ng/g dry feces) and M1 (29.07 ± 1.40 ng/g dry feces) were significantly different ($p < 0.05$ for both comparisons) from F2 (49.6 ± 2.86 ng/g dry feces), but not amongst each other ($p = 0.09$) (Table 1). The peak mean FCM concentrations of F1 (29.59 ± 4.16 ng/g dry feces) and M1 (32.19 ± 1.33 ng/g dry feces) were significantly different ($p < 0.05$ for both comparisons) from F2 (55.52 ± 4.03 ng/g dry feces), but not amongst each other ($p = 0.78$) (Table 1). There were no consistent patterns in FCM profiles of koala joeys in relation to their age and/or sex (Fig. 1).

All koala joeys displayed a significant increase (above baseline threshold) in FCM concentrations within 1–3 days after each visitor event. After the 1st visitor event (Days 1–2), all koala joeys exhibited a significant peak in FCM concentrations (Day 4) followed by an immediate return to baseline concentrations. The FCM concentrations of all koala joeys remained within the baseline threshold limit (Days 5–14) until the next visitor event (Days 15–16). Following the 2nd visitor event, all koala joeys exhibited a significant peak in FCM concentrations (F1: Day 18; M1: Day 19; F2: Day 19). The FCM concentrations of M1 and F2 immediately returned within the baseline threshold concentrations until after the next visitor event. It is important to note that for F1, the FCM concentrations decreased consistently over the following days, but remained above the baseline threshold. Following the 3rd visitor event (Days 22–23), all koala joeys exhibited a significant peak in FCM concentrations (Day 24). The FCM concentrations of M1 and F2 immediately returned within baseline threshold, however, the return to baseline was slightly delayed for F1 (Fig. 1). The FCM concentrations of all joeys remained within the baseline threshold until they were released on Day 31.

Discussion

The primary aim of this study was to monitor FCMs in koala joeys within the context of potential stressors. The staff at the hospital could identify visitors as the only potential stressor affecting koala joeys during the study period. However, as hypothesized, potentially stressful stimuli (i.e., visitors) resulted in an increase in FCM concentrations on all three encounters, across all koala joeys. These findings confirm that visitors elicit an acute stress response in koala joeys. To our knowledge, this is the first study to report on the acute stress response of koala joeys to visitors.

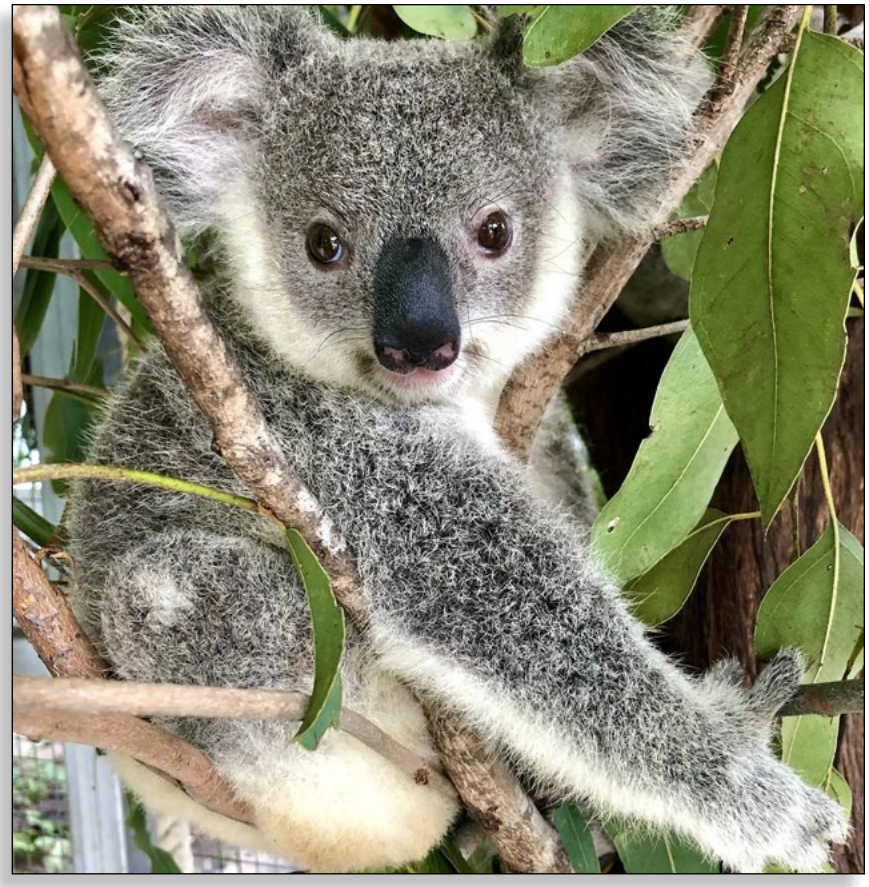
Assay validation is a crucial step in reliably assessing the endocrine status of any animal.[12,31] Validation typically refers to testing if the assay system in use can detect/capture/reflect biologically relevant changes in the adrenocortical activity of an animal.[31] In an ideal scenario, a complete validation involves analytical, physiological, and biological validation. Physiological validation generally involves pharmacologically stimulating (or suppressing) the circulating levels of GCs by using a standardized dose of synthetic ACTH (or dexamethasone, commonly known as the dexamethasone suppression test).[32] This circumvents the other components of the HPA axis and results in a direct stimulation of the adrenals to produce (or inhibit) the synthesis and release of GCs in the bloodstream.[12,33] Upon administration of ACTH, a spike in plasma GCs can be observed, and a peak increase in FCM levels occurs after a species-specific delay time.[34] If the assay in use can detect these changes, the assay is successfully validated. The primary objective is to test if the assay can detect biologically relevant changes in circulating GCs. However, physiological validation is not always feasible (or ethically justified), especially in

TABLE 1. Iterative baseline method representing the overall mean, baseline mean, baseline threshold and peak mean for each individual koala joey involved in the study.

INDIVIDUAL	APPROXIMATE AGE (IN MONTHS)	OVERALL MEAN (ng/g)	BASELINE MEAN (ng/g)	BASELINE THRESHOLD (ng/g)	PEAK MEAN (ng/g)
F1	14	19.36 ± 6.22 [a]	16.51 ± 2.48 [a]	21.48 ± 4.67 [a]	29.59 ± 4.16 [a]
M1	15	22.81 ± 4.76 [a]	21.61 ± 3.72 [b]	21.61 ± 3.72 [b]	32.19 ± 1.33 [a]
F2	12	34.02 ± 11.12 [b]	31.44 ± 9.07 [c]	49.6 ± 2.86 [b]	55.52 ± 4.03 [b]

Note: For each comparison, different superscripts ([a, b, c]) within the same column indicate significant mean differences.

endangered animals such as koala joeys, since it involves subjecting recuperating joeys to further undue stress. As an alternative, biological validation serves the same function as that of a physiological validation technique, except that it uses naturally occurring stressors (instead of pharmacologically manipulating GC concentrations).[31] Biological validation of an assay has been performed in several species using naturally occurring stressors such as capture, anthropogenic disturbances, and veterinary procedures. Although the R4866 assay has previously been validated for adult koalas,[17] the results from this study further reinforce the suitability of this assay for measuring "stress" in koala joeys. Humans are an obvious known stressor for wild animals (such as koalas).[24] Since all koala joeys evoked a significant increase in FCM concentrations after each visitor event, this study serves as a natural biological validation of the R4866 EIA, wherein the assay could detect and capture biologically relevant changes in FCM concentrations in response to a known stressor.



Orphaned koala joey in care at Magnetic Island Koala Hospital, Horseshoe Bay, QLD, Australia

Individual differences in the FCM profiles of koala joeys were not an unexpected finding. These differences are reflected in their overall mean, baseline mean, peak mean, and baseline threshold FCM concentrations. GCs are known to reflect inter- and intra- individual variation which can be mediated by several factors such as age, sex, body condition, genetics, season, habituation, temperament, and personality.[35,36] When considering baseline FCM concentrations, F2 had significantly higher baseline FCM concentrations relative to F1 and M1. As highlighted by Guindre-Parker,[36] baseline GCs are likely to be strongly correlated with the energetic balance of the animal. Since F2 is the youngest animal, it is possible that the elevated baseline FCM concentrations of F2 are a result of its high energetic demands. However, it is also possible that since F2 had spent the least amount of time under rehabilitation, it was habituated to a lesser degree to the captive environment, resulting in elevated baseline FCM concentrations (see Hogan et al).[14] Therefore, it is impossible to pinpoint a single factor that leads to the individual variation in FCM profiles, since it is likely to be a cumulative effect of several factors.

All three visitor events evoked a significant acute stress response in all joeys. It has been documented previously that visitors can evoke a stress response from adult koalas subjected to photography regimes.[24] Although in our study all three visitor events evoked a significant acute stress response in all joeys, the magnitude of stress response and the time taken to return to baseline concentrations is different for each individual and for each visitor event. Incorporating these results into the reactive scope model (RSM) [as proposed by Romero et al.][37] highlights new avenues about the physiological coping mechanisms of koalas. The RSM proposes that the physiological mediators associated with the stress response exist in four distinct ranges: (i) predictive homeostasis, (ii) reactive homeostasis, (iii) homeostatic failure, and (iv) homeostatic overload. The predictive range encompasses physiological mediator levels in response to predictable environmental change (i.e., seasonal and circadian variation). The reactive range encompasses physiological mediator levels in response to unpredictable changes (i.e., response to stressors). The predictive and reactive ranges comprise the normal reactive scope of an animal and is essential for the day-to-day functioning of the animal. The homeostatic overload range encompasses physiological mediator levels above the normal reactive scope (i.e., chronic stress). The homeostatic failure range encompasses physiological mediator levels below the normal reactive scope (i.e., insufficiency to respond to stressors). The homeostatic overload and failure serve as two extremes of the RSM and comprise ranges consistent with pathological effects.[37]

In our study, the baseline concentrations (represented by open circles in Figure 1) can be presumed to be indicative of the predictive homeostasis range reflecting the natural fluctuation in FCMs. After the 1st visitor event, the FCM concentrations of all individuals elicited a significant increase, indicating a classic activation of the HPA axis, followed by an immediate return to

baseline concentrations indicating the deactivation (via the negative feedback loop) of the HPA axis once the stressor had passed. The significant increases in FCM concentrations (represented by closed circles in Figure 1) can be presumed to be indicative of the reactive homeostasis range wherein the rise in FCM concentration allows the animal to cope with the stressor and maintain homeostasis.

Following the 2nd visitor event, the FCM concentrations of F1 were elevated for a longer duration relative to M2 and F2, which followed a similar pattern of immediately returning to baseline concentrations. Two scenarios are possible in this case: (i) presence of a prolonged stressor (i.e., visitors were present on successive days); (ii) delayed termination of stress response. Both of these scenarios make the animal susceptible to enter the homeostatic overload range that can have deleterious impact on the animal.[37] Since the staff at the Magnetic Island Koala Hospital confirmed the absence of visitors on successive days, the presence of a prolonged stressor can be ruled out. Thus, it could be presumed that the termination of the stress response was delayed due to weaker efficacy of the negative feedback loop. Delayed termination of stress response and its impact on the animal has been discussed in detail by Romero[38] by highlighting the example of Galápagos marine iguanas (*Amblyrhynchus cristatus*). Essentially, a weaker negative feedback loop extends the stress response and mimics a prolonged and sustained stressor, such that the FCM concentrations are elevated into the reactive homeostasis range for a much longer period. This results in a progressive shrinkage in the threshold between the reactive homeostasis range and homeostatic overload range.[39] For F2, this could mean that an additional stressor is likely to push this animal into the homeostatic overload range and the pathological consequences associated with it. This obviously depends on duration and magnitude of the additional stressor.[37]

During the 3rd visitor event, across all koala joeys, the stress response was elicited more immediately relative to the first two visitor events. It is possible that this could be due to the relatively shorter recovery time between the 2nd and 3rd visitor events. Frequent repeated stressors do not provide enough time for the recovery of threshold between the reactive homeostasis range and homeostatic overload range.[39] This means that visitors that elicit an acute stress response from the koala joeys, if repeated frequently over several days (without recovery time) could lead to chronic stress (unless the joeys get acclimatised to visitors). This, again, obviously depends on the rate of decline of the threshold between the homeostatic overload and the reactive homeostasis.[39] We would like to reiterate that the discussion about both of the aforementioned scenarios is based on presumptions, and there is no evidence to confirm this. However, there are real-life examples (see Romero[38] and Durant et al.[40]) that incorporate the reactive scope model to justify the physiological response to stressors. Future research on koalas involving a variety of acute stressors may help to get a deeper understanding of the reactive scope model.

For all koala joeys, the concentration of FCMs decreased within 1–3 days after visitor events, and remained within baseline until the next visitor event, suggesting that joeys were not chronically stressed. Instead, these results strongly suggest that the koala joeys are physiologically responding to environmental cues (visitors in this case) to maintain homeostasis. Care must be taken not to overstate the case for the effect of visitors identified in this study. It was evident that visitors were aversive enough to activate the HPA axis, but there was no direct evidence that this translated into a detrimental effect on the welfare of the joeys. In this study, all visitor encounters were acute, resulting in only temporary elevations of FCM concentrations, and there was no evidence of chronic stress in any animal.

Limitations

A significant limitation of this study is the small sample size, consisting of only three koala joeys. However, since each koala joey was exposed to visitors thrice in the study, this provides a total of nine confirmatory events where visitors elicited the physiological stress response in koala joeys. Although the multiple events per joey help to strengthen the reliability of the observed responses, they may not fully compensate for the small number of subjects. The inclusion of only three koala joeys may not adequately represent the broader population's variability in physiological stress responses to visitors. Furthermore, novel environments, such as rehabilitation centres, are likely to present a suite of biotic and abiotic stressors. The absence of any other stressors (except visitors) could be an artifact of a short (one-month) study period. Future research should focus on longitudinal (ideally year-long) monitoring of FCM in koala joeys.

We acknowledge that our study lacks a true control (i.e., an individual subjected to a single stressor (visitor) event to observe a rise in FCM concentrations, followed by no subsequent visitor events and no stress response), to confirm that in fact visitors were the cause of this increase in FCM concentrations. However, for all koala joeys, after each visitor event, the concentration of FCMs remained within baseline until the next visitor event, which serves as an internal/self- control and suggests that visitors evoked a significant physiological stress response from these koala joeys. It is also important to note that this research was not designed as an experiment; it instead relied on routine functioning of the hospital. The research did not intend to interfere with the normal rehabilitation procedures followed by the hospital, and as a result, intentionally subjecting or exempting joeys from stressors was deemed unsuitable.

Conclusion

This study has provided a better understanding of koala joey stress physiology. There was a significant variation in the FCM profiles of the three koala joeys, which can be attributed to the natural variation between individuals. Daily profiling of FCM secretion revealed that visitors serve as an acute stressor for rehabilitating koala joeys and evoke a significant stress response. While the study provides preliminary evidence that koala joeys

may exhibit physiological stress response to visitors, the findings should be interpreted with caution, and further research with a larger sample size is needed to confirm these results. Whether or not koala joeys would induce a similar response when encountered by familiar people (i.e., husbandry staff) remains to be investigated. The results of this study have established a basis for the future monitoring of wellbeing in koala joeys and have provided insight into how current stress management strategies can be improved. It is important that visitor encounters be kept to a minimum, and perhaps avoided altogether, especially for young rehabilitating joeys.



Supplementary Materials

The material has been provided as a supplementary file at the online portal.

Author Contributions

Conceptualization, E.N. and H.P.; methodology, E.N. and H.P.; formal analysis, E.N. and H.P.; investigation, E.N. and H.P.; resources, E.N. and H.P.; data curation, E.N., H.P, A.B. and I.B.; writing—original draft preparation, H.P.; writing—review and editing, E.N., H.P., A.B. and I.B.; supervision, E.N.; project administration, E.N. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement

The study was conducted according to the guidelines of the Native or Exotic Wildlife and Marine Animals (NEWMA) Animal Ethics Committee and approved by the Institutional Review Board (or Ethics Committee) of The University of Queensland (approval number: AE000035).

Informed Consent Statement

Not applicable.

Data Availability Statement

Data are contained within the article or supplementary material.

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Conflicts of Interest

The authors declare no conflict of interest.

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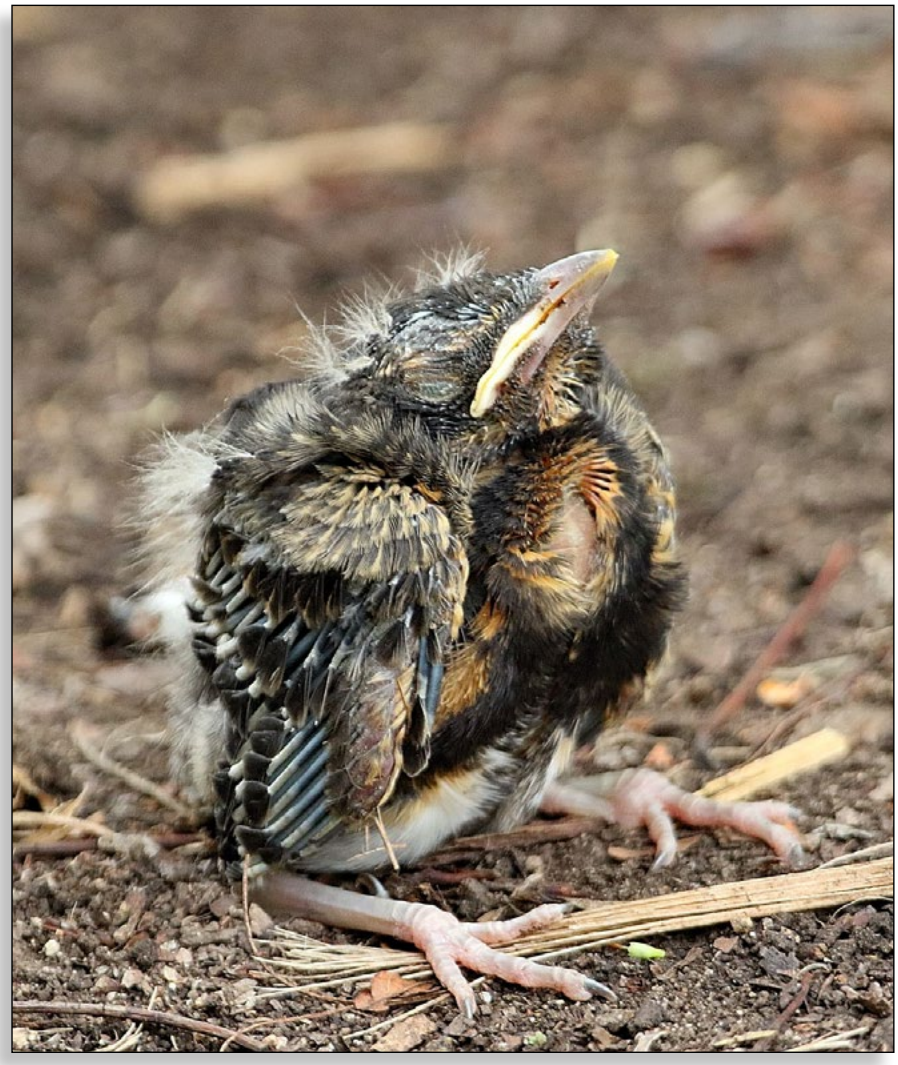
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Spatial analysis of common neonate avian species found locations and implications for zoonotic disease transmission and wildlife care in Champaign County, Illinois, USA (2015–2020)

Emma Prybylski,[1] William Sander,[2] Csaba Varga[3,4]

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American Robin *(Turdus migratorius)*

Introduction

Human–wildlife interactions, including those driving wildlife rehabilitation facility intakes,[1] are increasingly common as urban sprawl extends into rural areas.[2] While the proximity of wildlife to dense human populations can lead to conflicts, such as the potential spread of zoonotic diseases,[2] it is also important to acknowledge the benefits of wildlife presence. Wildlife species in urban areas can contribute to ecosystem services and provide health and well-being benefits through daily human–wildlife interactions, especially in mental and spiritual aspects.[3,4] However, the encroachment of urban areas into rural regions can alter wildlife populations, change disease dynamics, and introduce new reservoirs of infections to new hosts.[5] Zoonotic

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ABSTRACT: Wildlife neonate avians are frequently received by wildlife rehabilitation centers when members of the public mistakenly assume they have been abandoned by their parents. From 2015 to 2020, we assessed the distribution of the four most common avian neonates presented to the University of Illinois Wildlife Medical Clinic in Champaign County, Illinois, USA: American robins (*Turdus migratorius*, n = 152); European starlings (*Sturnus vulgaris*, n = 83); house sparrows (*Passer domesticus*, n = 54), and mallard ducks (*Anas platyrhynchos*, n = 50). A Geographic Information System was employed to illustrate and analyze spatial connections between the environment and the location of neonate avians. Compared to mallard ducks, only found in developed areas, some European starlings and American robins were located in undeveloped areas. House sparrows were primarily located in highly developed areas. All bird species were found in areas with a low percent canopy. American robins (OR = 2.06; $p = 0.003$) and mallard ducks (OR = 3.63; $p < 0.001$) had higher odds of survival when compared to European starlings and house sparrows. This assessment identified habitats linked to increased human–wildlife interactions and offers avenues for educational efforts.

KEYWORDS: American robin, common mallard duck, environment; European starling; house sparrow, Illinois; neonate; spatial analysis; zoonotic.

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diseases, or zoonoses, are transmitted from animals to humans and encompass many bacteria, viruses, fungi, parasites, and other pathogens.[6] Our analysis identifies environments with significant human–wildlife interactions to develop opportunities for targeted educational outreach about neonate avians to prevent the spread of zoonotic diseases.

Neonates compose many cases admitted to rehabilitation facilities.[7] "Neonate" comes from the Latin word "natus," which means "to be born," and it describes a newborn in the first few weeks of life as it undergoes physiological changes.[8] Because wild animal neonates require specialized care and diets to grow and develop properly, raising and releasing neonates from wildlife rehabilitation facilities is challenging and often unsuitable.[1] Well-intentioned people often rescue wildlife neonates, believing they are abandoned. However, most of these neonates are not abandoned and do not require critical attention.[1,9,10]

Due to growing human populations and limited resources, there is a need to reduce the number of neonates admitted to rehabilitation centers to utilize funds for animals that require special care and to reduce the possible transmission of emerging and re-emerging zoonoses.[6,11,12] Emerging zoonoses are newly evolved or recognized diseases and typically develop with increased human–animal interactions or geographical expansion.[6,13] At least 250 zoonoses affecting humans were deemed emerging and re-emerging in the last 70 years, and wildlife species can act as reservoirs.[14,15] To better locate areas of increased human–wildlife contact and reduce the potential spread of zoonoses, we can survey the geographical relationship of where neonate wildlife are found.

European starlings are native to western Europe and have expanded to most of North America and are also found in Australia, New Zealand, South Africa, and Argentina.[16,17] They are frequently located in human-dense landscapes such as agricultural, urban, and suburban areas.[16,18] Typically, European starlings are not found in large stretches of forest or desert but are located in areas close to water sources and cavities for nesting.[19] Based on the literature, we hypothesized that most European starlings would be found in highly developed areas with low tree cover.

American robins are a popular songbird species in North America that construct mud-lined nests in manufactured structures, shrubs, and tree trunks.[20,21] American robins breed in various habitats across North America, ranging from cities to rural areas, and can be seen in orchards, gardens, parks, and backyards.[22,23] Due to their nest building and breeding habits, we expected them to be found in highly developed areas with low tree cover similar to European starlings.

House sparrows are one of the most common avian species in the world.[24] They live in various environments, from deserts to communities near the Arctic circle, near humans.[25,26] House sparrows are considered anthro-dependent, meaning that some populations have gone extinct due to nonexistent human populations.[25,27] Their human dependence is clear from their expansion into Northern Europe, Eastern Europe, and Central Asia in the 1800s as agriculture and urbanization spread.[25] Based on their colonization locations, house sparrows will build nests in pre-built cavities and on roofs or walls of manufactured structures.[25] Because house sparrows are dependent on human populations and colonize in extreme climates, we hypothesized that they would be found in highly developed areas with low tree cover, similar to European starlings and American robins.

Mallard ducks are the most common waterfowl species in the Northern Hemisphere.[28] They are an essential avian species in the management and conservation of wetlands because they are a significant game species.[28] Mallards are commonly found in wetlands such as ponds, parks, and estuaries.[29] Because rapid urban sprawl is infiltrating habitats of wildlife species, people in urban areas visit their local parks and ponds and are inclined to feed animals such as ducks.[5,30,31] Due to their nature, we hypothesized that mallard ducks would be commonly found in highly developed areas with low tree cover, the same as European starlings, American robins, and house sparrows.

Illinois is an agricultural and urbanized-dominated state where only a small percentage is considered suitable for wildlife.[32] Significant losses of forests and wetlands are evident across the state, and the development of urban areas continues to expand,



American Robin nestlings in care

while wildlife habitat has declined. According to an Illinois Natural History Survey from 1976 in which forests in Illinois were evaluated for their ability to support avian populations, results demonstrated a significant decrease in avian wildlife habitats over the preceding several decades.[33] As increasing urbanization to support a growing population results in forest fragmentation, wildlife is negatively impacted because substantial habitat loss follows.[32]

While there is a greater risk of decreased biodiversity and reduced habitats for some species because of urban sprawl, some can thrive in densely populated areas.[34] This could be because they have adaptations to urban conditions, can manage to live in more artificial environments, or can live in a wide array of habitats.

Tree cover is a tool used to assess urban forests and their associated benefits.[35] Tree cover has been decreasing by 175,000 acres per year in urban and community settings, while solely urban areas have been experiencing a loss of 138,000 acres per year.[35] Recent projections conclude that urban land in the United States is expected to increase from 67.6 million acres (in 2010) to 163.1 million acres by 2060; this is a 95.5 million acre increase over 50 years and equates to urban land more extensive than the state of Montana.[35] While there have been efforts to expand tree cover through planting and natural regeneration, tree cover loss ultimately exceeds tree cover gains in most states.[35,36]

Since the avian species examined are commonly found near urban and suburban areas, we hypothesized (H1) that all four avian species are found in highly populated and developed areas with a lower percentage of tree canopy in Champaign County.

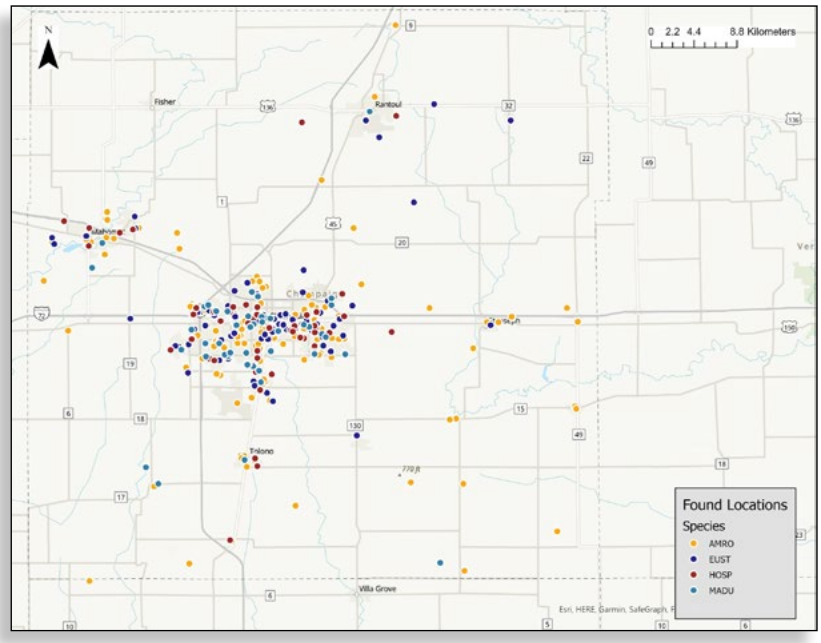


FIGURE 1. Distribution of neonate avian species submissions to the Wildlife Medical Clinic at University of Illinois from 2015–2020 in Champaign County, Illinois, USA. **LEGEND: AMRO** (American robin), **EUST** (European starling), **HOSP** (house sparrow), and **MADU** (mallard duck).

Materials and Methods

The most frequent neonate avian species admitted into the University of Illinois Urbana-Champaign Wildlife Medical Clinic (WMC) were verified using RaptorMed (RaptorMed.com LLC, 2011). RaptorMed is an electronic medical records program for wildlife rehabilitation centers, zoos, and aquariums. A complete list of avian species received as "neonate" to the WMC was produced from 2015 to 2020. The four most abundant avian species were the American robin (*Turdus migratorius,* AMRO), European starling (*Sturnus vulgaris,* EUST), house sparrow (*Passer domesticus,* HOSP), and mallard duck (*Anas platyrhynchos,* MADU). This five-year range was evaluated because electronic medical records were fully utilized in WMC and provided more detailed records.

Relevant data such as patient ID, species, age, status, admission date, final date, injury case, and found address including city, county, state, and zip code was exported into an Excel file (Microsoft Excel for Microsoft 365, 2022). Results were narrowed down to the four most common avian species. Only subadult patients were included in the final data. Cases found outside of Champaign County and those with no correlated found address were removed. Multiple entries from avians admitted as a group were removed (i.e., a clutch from the same nest). The final dataset comprised 339 animals: 152 AMRO, 83 EUST, 54 HOSP, and 50 MADU.

RaptorMed addresses were converted to exact GPS coordinates from a geocoding platform called Geocodio (Copyright © 2014–2023 Dotsquare LLC, Norfolk, Virginia). Dotsquare produced an accuracy score for each converted address, ranging from 0 to 1, with 1 being the most accurate. Addresses with a score lower than 1 were identified for further analysis and manually corrected. A second and third run of the data verified correct conversion before spatial analysis.

Density-based clustering was utilized to visually depict the clustering of submissions for all four avian species in Champaign County, IL. This approach facilitated grouping the locations where the four species were found based on their density and proximity. The primary objective was to identify regions from which a high concentration of submissions was received for the four species, and to distinguish them from other areas (Fig. 1).

Environmental factors (percent canopy and land cover class) were extracted at each found location and examined. The study used land cover classes from the National Land Cover Database (NLCD). NLCD data is developed by the United States Geological Survey (USGS) from satellite imagery using a complex modeling process. The environmental categories were analyzed by high and low groups for statistical analysis.

High canopy cover was defined as ≥ 15%. Landover classes 21–24 (Developed–Open Space, Developed–Low Intensity, Developed–Medium Intensity, and Developed–High Intensity)

were defined as high development. Classes were separated into high development, which represents all developed areas, and low development, which represents forest, hay/pasture, and crops. Land cover classes Mixed Forest, Hay/Pasture, and Cultivated Crops were defined as low development. Tables were created dividing the cases into these sections for statistical analysis.

Stata software (version 18.1, 2023, StataCorp LLC) was used to run a two-proportion Z test, which compared the proportion of each bird species found in areas with high proportion versus low tree canopy, and undeveloped land areas versus developed land areas. The null hypothesis assumed that the proportion of birds found in an area (e.g., undeveloped area) is identical with the proportion of birds found in another area (e.g., developed areas). A statistically significant ($p < 0.05$) Z test signified that the two proportions were not equal.

In addition, logistic regression models were built to compare each species' (e.g., mallard ducks vs. house sparrows) odds of survival after admission into the wildlife clinic.

Discussion

This study identified areas in Champaign County, Illinois, USA, with increased human–wildlife interactions. We focused on the four most frequently presented neonate avian species at the Wildlife Medical Clinic in Urbana, IL. It was hypothesized that the four species would be found in highly developed areas with a lesser percentage of tree canopy. The results confirm that these four species were commonly found in highly developed areas with a low percent canopy; only a few European starlings and American robins were found in undeveloped areas. While this study focused on neonate avian species found and presented to a rehabilitation facility in Champaign County, IL, a similar method can be utilized for other regions.

Interpreting which environments in Champaign County, IL, are correlated with appreciable human–wildlife interactions can aid in decreasing the intake of healthy neonates to wildlife rehabilitation clinics through targeted educational outreach, which synonymously reduces the inherent spread of zoonotic diseases.

A substantial limitation of this study is that neonate avians presented to the University of Illinois Wildlife Medical Clinic are restricted by what animals are found and brought to WMC by community members. The results of this analysis are also limited by the wildlife finders' capability to report the exact found locations of the avian species presented to the wildlife rehabilitation center. Intakes without an exact found address were removed from the data set, which could skew the conclusions made. Another limitation of this study is that more developed areas with higher human populations increase the likelihood of finding and admitting animals into rehabilitation centers; this can skew the finding towards developed areas. Furthermore, socioeconomic factors, such as family income and education level, could impact admissions. A recent study conducted in Krakow, Poland, identified that urban residents generally agree that injured wildlife should be offered care by professionals and treated appropriately before their release.[37] Despite these constraints, the findings of this study exemplify the significance of employing spatial analysis in pinpointing regions with heightened human–wildlife interactions.

Results

The final dataset comprised 339 admitted neonate avians: 152 American robins, 83 European starlings, 54 house sparrows, and 50 mallard ducks (Table 1). The four avian species analyzed were commonly found by human populations in the Champaign-Urbana, IL area (Fig. 1) in highly developed areas ($p < 0.001$) (Table 2) with a low percent canopy ($p < 0.001$) (Table 3).

TABLE 1. ADMITTED NEONATE AVIAN SPECIES.

ANIMAL (N)	ADMITTED
MADU	50
HOSP	54
EUST	83
AMRO	152

LEGEND: MADU (mallard duck), HOSP (house sparrow), EUST (European starling), AMRO (American robin). The final dataset consisted of 339 neonate avians: 152 AMRO, 83 EUST, 54 HOSP, and 50 MADU.

TABLE 2. LAND COVER CLASS TABLE.

ANIMAL (N)	LAND COVER CLASS	% (N)	95% CONF. INT.	P-VALUE
MADU (94)	Developed Undeveloped	100 (47) 0 (0)	100–100 0–0	< 0.001
HOSP (94)	Developed Undeveloped	100 (47) 0(0)	100–100 0–0	< 0.001
EUST (164)	Developed Undeveloped	97.56 (82) 2.439 (82)	94.22–100.9 –0.8998–5.778	< 0.001
AMRO (276)	Developed Undeveloped	97.83 (138) 2.174 (138)	95.39–100.3 –0.2592–4.607	< 0.001

LEGEND: MADU (mallard duck), HOSP (house sparrow), EUST (European starling), AMRO (American robin). Two-sample T-test of proportions within each species group showing distribution of species found in areas of high land cover class versus low land cover class. Higher land cover class (≥ 15%) is associated with more developed areas. N = total reports; n = number in a category; % = n/N * 100. Only EUST and AMRO had been found in undeveloped areas, and for that reason, we have proportion comparisons. For MADU and HOSP, all proportions are 100% in developed areas. The proportion of EUST and AMRO found in developed areas is statistically significantly different than in undeveloped areas.

TABLE 3. PERCENT CANOPY.

ANIMAL (N)	PERCENT CANOPY	% (N)	95% CONF. INT.	P-VALUE
MADU (100)	Low	98 (50)	94.12–101.89	< 0.001
	High	2 (50)	–1.881–5.881	
HOSP (208)	Low	88.46 (104)	82.32–94.60	< 0.001
	High	11.54 (104)	5.398–17.68	
EUST (166)	Low	92.77 (83)	87.20–98.34	< 0.001
	High	7.22 (83)	1.658–12.80	
AMRO (304)	Low	90.74 (152)	86.19–95.39	< 0.001
	High	9.21 (152)	4.613–13.81	

LEGEND: MADU (mallard duck), HOSP (house sparrow), EUST (European starling), AMRO (American robin). Two-sample Z-test of proportions within each species group showing distribution of species found in areas of high percent canopy (≥ 15 %) versus low percent canopy (0–14.99%). N = total reports; n = number in a category; % = n/N * 100. The proportion of each species found in low canopy areas is significantly higher than in high canopy areas.

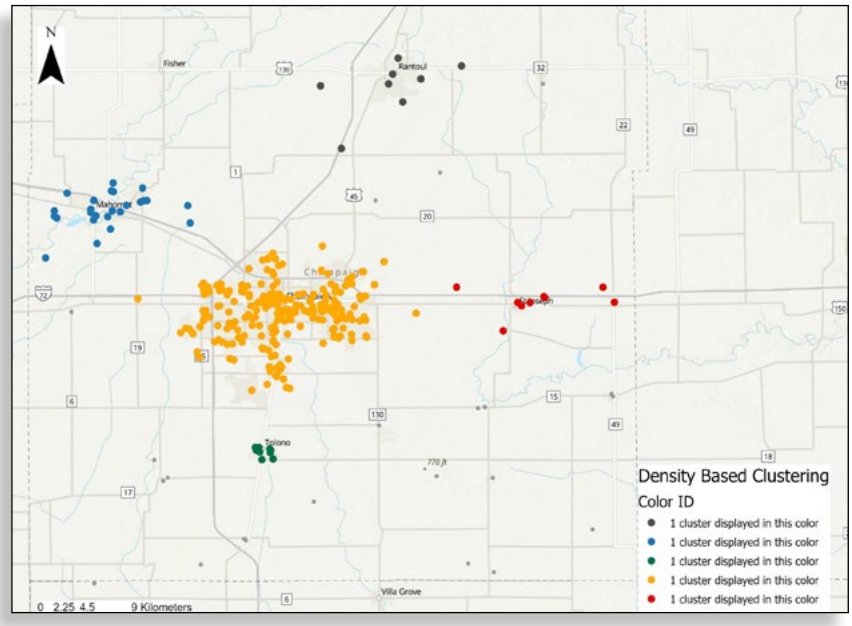


FIGURE 2. Density based clustering of neonate avian submissions from 2015–2020, Champaign County, Illinois, USA.

The density-based clustering of all species submissions is described in Figure 2. Five clusters were identified, which were located around the main population centers (Champaign-Urbana, Tolono, St. Joseph, Mahomet, and Rantoul).

Compared to European starlings and house sparrows admitted to the wildlife clinic, the odds of survival of American robins (OR = 2.06, 95% CI:1.29 - 3.30, p = 0.003) and mallard ducks (OR = 3.63, 95% CI:1.96 - 6.73, p < 0.001) were significantly better. When comparing American robins to mallard ducks, American robins had lower odds of surviving (OR = 0.498, 95% CI: 0.261–0.952; p = 0.035) when compared to mallard ducks.

Conclusions

The findings of this study can help wildlife rehabilitation centers target urban areas for education and outreach because most submissions were clustered around main population centers (Fig 2). In urban areas, there needs to be more knowledge and information regarding handling wildlife, which continues to increase as wildlife habitats decrease. Targeted education and outreach programs could include raising awareness about the ecological roles of local avian species, and the possible health risks associated with improper wildlife handling. This study investigated the relationships between where neonate avian species were found in relation to land cover class, percent canopy, and their odds of survival after admission to a wildlife clinic. As the results of this study and other relevant literature exploring these relationships demonstrate, increasing urban developments have the potential to increase human–wildlife interactions. These relations can increase the chances of humans finding neonate wildlife and spreading zoonoses. European starlings, American robins, house sparrows, and mallard ducks are abundant and close to human populations. These four avian species can carry and distribute certain species of enteric bacteria, viruses, and fungi, such as *Escherichia coli*, *Campylobacter jejuni*, and some avian influenza strains, such as influenza A virus.[16,38] Additionally, the spread of zoonoses in birds can be linked to potential impacts on livestock production, so further research can be conducted to investigate the environmental impacts of avian pathogens on small- and large-scale livestock systems. Future studies can use the same method to study other counties in Illinois and other states. By understanding the areas where neonate avian species are frequently found, rehabilitation centers can better allocate resources and anticipate the species they are likely to admit. This could lead to improved preparation regarding specialized care, appropriate housing, and medical treatments tailored to the needs of the most commonly admitted species. Further research can also be conducted to investigate the potential underlying factors, such as public perception and attitudes, contributing to the differential admission rates of various avian species to wildlife rehabilitation centers.

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A case study of knemidokoptic mange in a wild Eurasian eagle owl (*Bubo bubo*)

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ABSTRACT: A wild adult Eurasian eagle owl was presented to a wildlife rehabilitation center and diagnosed with knemidokoptic mange, lice, and blood parasites. Clinical presentation included emaciation and dehydration coupled with skin edema, crusts, and feather loss on the legs. Knemidokoptiasis is rarely reported in wild owls, with only one instance documented in a closely related species, the great horned owl (*Bubo virginianus*). Although mange is sporadically encountered in wildlife facility patients, a thorough examination looking for a possible underlying immunosuppressive state or the presence of an additional parasitemia is not always conducted. This case was treated with oral ivermectin combined with a topical steroid and antibacterial–antifungal cream in an essential oil and green clay medium for the mite infestation and doxycycline for the treatment of blood parasites. Full recovery was noted 22 days post-admission, notably a shorter time in care than similar case studies described in the literature. This case demonstrates the importance of updating treatment protocols and arriving at a full clinical diagnosis in wild animal patients, as well as the need for performing a detailed parasitic examination to address any underlying issues and raising questions regarding the etiology of the condition. To the author's knowledge, this case study is the only reported instance of medically treating a wild owl of this species.

KEYWORDS: Eurasian eagle owl, *Knemidokoptes*, *Bubo bubo*, skin disease, scaly legs, hyperkeratosis, ivermectin, doxycycline, Dermacombin, clay ointment, ectoparasite, endoparasite, Strigiformes, *Hemoproteus*.

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Introduction

An approximately three-year-old[1] adult Eurasian eagle owl (*Bubo bubo*) presented in poor body condition along with dehydration, scaly and swollen legs encased in crusts, and an unpleasant odor. The bird had been rescued from a backyard in the Golan Heights after a farmer witnessed a wet owl on the ground struggling to fly (Fig. 1). The farmer contacted a wildlife center volunteer from Israel Nature and Parks Authority, who brought the owl to the Agamon Wildlife Rehabilitation Center (AWRC) clinic.



FIGURE 1. Adult Eurasian eagle owl. (A) On the ground in a farmer's backyard at the time of rescue. (B) In a rehabilitation cage at Agamon Wildlife Rehabilitation Center a few days before release.

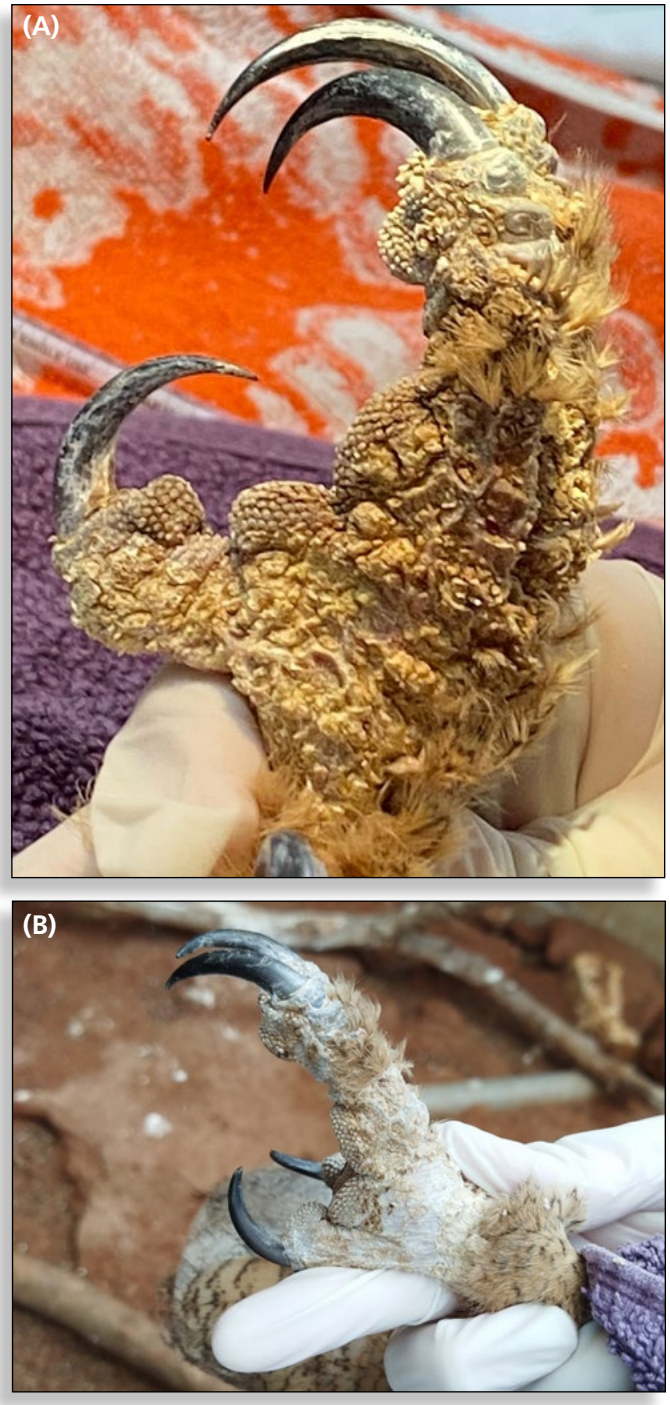


FIGURE 2. Legs of the Eurasian eagle owl. (A) Distal portion covered with crusts at presentation and before treatment. (B) Significant clinical improvement in the appearance of the crusts after treatment.

Methods

Upon initial clinical examination, the owl had a keel score of 2 (mildly skinny)[2] and weighed 1.7 kg. Both legs were covered in crusts (Fig. 2) and emitted an intense, strong, and unpleasant odor suggestive of a secondary bacterial or yeast infection with accompanying dermatitis. Feather loss was observed extending distally from the tarsus to all affected areas. Additionally, ecto-parasitic lice, most likely from the suborder Mallophaga, were observed between the feathers. The owl received subcutaneous fluids (Ringer's lactate solution) equivalent to 5% of its body weight as rehydration therapy. The owl's legs were bathed with an antiseptic scrub (4% chlorhexidine gluconate). After being dried, the owl was housed overnight in an isolated unit. Four mice were provided as food, but the owl refused to eat and required hand-feeding the following day.

One day after arrival, the owl was presented to a veterinarian who took skin scrapings from three different sites on each leg (Fig. 3) to check for the presence of burrowing mites. The skin samples were macerated in one drop of 0.9% saline solution.

Blood was drawn from the owl's lateral tarsus vein with a hypodermic needle and collected into a capillary tube for the following diagnostic tests: blood cytology, packed cell volume (PCV), and total solids (TS) by spectrophotometry.



FIGURE 3. Skin scraping.

Examination of the owl's blood film revealed the presence of *Hemoproteus* gametocytes[3] within blood cells (Fig. 4). Microscopic examination of the initial skin scrapings revealed numerous mites from the genus *Knemidokoptes* (Fig. 4).[4] The PCV was normal at 40%, but TS was slightly high at 5 g/dl on the refractometer, most likely because the owl was still dehydrated.[2] On day two, apart from additional fluid therapy, the owl received one initial dose of 1% ivermectin (0.2 mg/kg PO) (Noromectin®, Norbrook Laboratories, UK), and the dose was repeated 14 days later. Also on day two, one-quarter of a 100 mg doxycycline tablet (12.5–25 mg/kg PO) (doxycycline, Dexcel Pharma, Israel)[5] was given, and this dose was repeated once a day hidden in the owl's food for a total five successive days. Both medications were given for the general treatment of parasites. Caretakers wearing gloves spread a thick herbal cream over the owl's legs so that both legs were entirely covered. The cream was made with four drops each of four different essential oils (lavender, *Commiphora myrrha, Boswellia carteri,* and *Copaifera langsdorffii*) mixed with one teaspoon of the generic antibacterial–antifungal skin cream Dermacombin (neomycin sulfate, gramicidin, nystatin, triamcinolone acetonide; Taro Pharmaceutical Industries, Haifa Israel) and one teaspoon of a green clay ointment.Four days after application, the cream hardened and fell off independently. It was reapplied to both legs in the same manner as the first time. The owl seemed improved in general: It was no longer dehydrated and was eating by itself with an improved appetite. The edema and redness of the legs seemed to decrease.

On the 22nd day after arrival, another microscopic examination showed no mites. The appearance of the crusts on the legs was also significantly improved (Fig. 2).

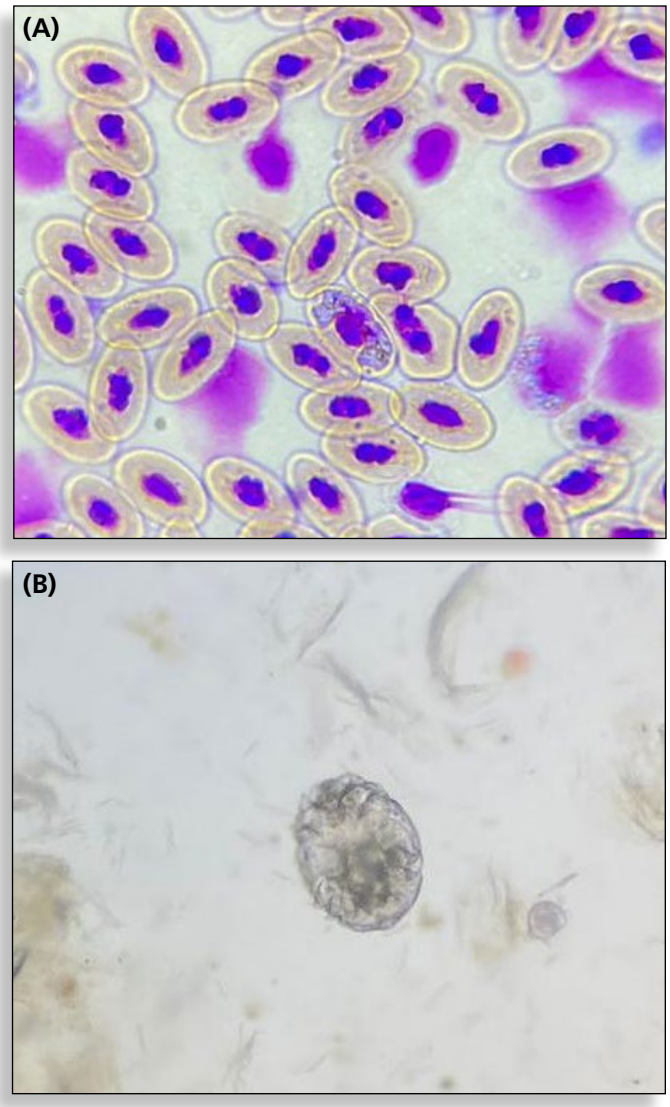


FIGURE 4. Microscopic examination. (A) A blood film revealed the presence of one intracellular organism consistent with a *Hemoproteus* gametocyte, which is known to be transferred from one infected host to another by a blood-sucking insect. (B) A mite suspected to be *Knemidokoptes* sp.

During the entire period of the bird's hospitalization and recovery, the eagle owl stayed in a cage isolated from the rest of the avian patients since these mites may be transmitted from bird to bird through direct or prolonged close contact.

The cage was designed according to the needs of the eagle owl species. The staff kept the environment as dry and hygienic as possible due to the preference of secondary pathogens (i.e., certain bacteria and fungi) for moist environments. The staff also wore gloves in any interaction with the eagle owl. All the center's enclosures and equipment used for the owl were thoroughly sterilized. All other materials that came in contact with the owl during his entire stay at the center were discarded directly into the trash.

After the owl's full clinical recovery (Fig. 1), a release day was determined. The chosen release site was where the owl had been found, an important decision due to the species' natural territorial instinct.

Discussion

Knemidokoptes mites burrow into the epithelium, feed on the host's tissue, or eat the feather quill's pith. This can cause hyperkeratosis or an infection known as scaly leg or scaly face with macroscopically visible crusty lesions.[6] Only a few cases have been reported on wild birds,[7] and only one case was documented in a captive nocturnal raptor.[8] This is the first publication of a case of knemidokoptic mange in a wild Eurasian eagle owl. Intracellular blood parasites are common in wild birds and are transmitted by blood-sucking insect vectors, such as the *Hippobosca* flies and midges of the genus *Culicoides*.[3] The single blood parasite in Figure 4 fits the description in the literature of *Hemoproteus* sp.[3] Its presence can indicate that the bird's immune system was already compromised by this type of blood parasite. *Hemoproteus* is known to be associated with anemia, anorexia, and depression. Mild signs of anorexia and depression were consistent with the findings in this case study. Miller described the difference between the male and female *Knemidokoptes* mite; the male has long pedicles and legs in contrast to the short legs of the female mite. Our microscopic specimen was suspected to be a female, based on Miller's description.[3,9] However, the mite in this discussion was not molecularly analyzed, and the matter of species is still undetermined.

Van Velden observed that measures of parasite prevalence or intensity, often distributed unevenly within host populations, differ across age groups, genders, or plumage colors.[7] This suggests that certain individuals may be more vulnerable to parasitic infections than others, potentially due to various physiological or environmental factors. In both birds and mammals, males are more likely to have a higher parasite intensity due to biological and behavioral differences.[7] Differences in immune system responses between males and females may be due to testosterone, which is essential for male development but may also inhibit the production of certain immune cells. These findings suggest that testosterone may play a critical role in shaping immune system function and may explain gender differences in immune response. Males may be more susceptible to *Knemidokoptes* due to increased energy costs during breeding or greater exposure to infected prey. During the breeding season, males may undergo significant behavioral changes, such as displaying aggression towards their rivals or reducing grooming habits. These changes in behavior may create a male vulnerability to infection, causing them to become contagious to the females during the critical breeding period.[7]

The owl described here arrived at the AWRC in November. From October to December, the male Eurasian eagle owl commences vocal activity, mainly for territorial purposes, as preparation for attracting a mate.[10] Additionally, Van Velden and Latta found that habitat was a significant determinant in infection rates, and knemidokoptic infections were more prevalent in dry desert habitats than in moist or high-elevation habitats.[7,11] November 2022 was a very dry month in Israel, with lower-than-average precipitation. These facts describe conditions that may contribute to the bird's susceptibility to a variety of parasites. As we mentioned earlier, the level of parasites in an individual may depend on their age. Older individuals generally have higher parasite burdens, possibly due to differences in hormonal balance or simply due to their increased exposure to potential parasites or vectors.[7] In this case study, we can assume that the owl was in its third year based on the differences in color of its primary feathers (Fig. 5).[1]

Latta conducted one of the few studies investigating the impact of *Knemidokoptes* infection in wild birds. The study focused on the Dominican Republic's prairie warblers (*Setophaga discolor*) and palm warblers (*S. palmarum*). The results revealed that mite infection caused a significant reduction in the birds' muscle mass scores.[11] This study corroborated our clinical observation that the owl arrived slightly underweight with a keel score of 2 (mildly skinny)[2] and had been infected by knemidokoptic mites.

Two other cases of eagle owls described to us by the Israeli Wildlife Hospital at Safari Park Zoological Gardens, Ramat-Gan, Israel (personal correspondence, January 26, 2023) were admitted during the same year with a similar parasitic skin condition



FIGURE 5. Calculating the owl's age. The dark feather primary P7 indicates the owl is an adult. The dark P3 and P4 primaries show they are third-generation adult feathers (third calendar year).[1]

(diagnosed as *Knemidokoptes* spp.). Detailed descriptions of diagnosis and treatment were not suitable for publication. Therefore, our case is the first published scientific report of *Knemidokoptes* infection in an Eurasian eagle owl and may represent a larger number of similar cases. These confirmed cases support Van Velden's research hypothesis that male owls may infect female owls with Knemidokoptes parasites during courtship and breeding seasons.[7]

In this case, the treatment protocol involved an antiseptic scrub bath (4% chlorhexidine gluconate) of both legs, followed by drying. Subsequently, the infected areas on the legs were treated with an herbal cream of four essential oils mixed with antibacterial (Dermacombin) cream and green clay. Systemic ivermectin and doxycycline were also administered. Within four days, a slight improvement was observed after the first application of the herbal cream had dried and fallen off. In other cases of knemidokoptic mange in wild and captive birds, the treatments were limited to only several administrations of systemic antiparasitic medications.[8,9,12]In the case of a captive juvenile great horned owl reported in 1989, treatment was reported to have been a single dose of ivermectin (0.2 mg/kg). This nocturnal raptor's lesions were completely healed within 42 days post-treatment.[8] The adult Eurasian eagle owl in our study fully recovered within 22 days post-treatment, when a microscopic examination revealed no *Knemidokopte*s parasites seen and lesions on both legs had healed.

In *Current Therapy in Avian Medicine and Surgery*, Beaufrère and Laniesse write in their chapter on strigiform medicine, "Owls are distributed worldwide, and approximately 10 to 15% of the species are threatened with extinction, and most species are experiencing a decrease in population numbers."[13] The Eurasian eagle owl (*Bubo bubo*) is the largest of this taxonomic group.[13] Although a growing body of literature addresses the role of parasites and disease in the limitations observed in some breeding populations,[11] more research should be conducted regarding this parasite in breeding nocturnal raptors, given the hypothesis that host populations with parasites might be expected to have a lower equilibrium or lower population growth rate than those without parasites.

Conclusion

This is the first documented case study of a mite infection from the genus *Knemidokoptes* in a wild Eurasian eagle owl species. This case presents a classic clinical picture of skin manifestations associated with an underlying immunosuppressive state. It necessitates a deeper investigation into the etiology, both for this individual and the wider population. The treatment protocol used in this case appears to offer a shorter duration and more effective form of medical care. However, in this case, the skin manifestations were confined to the owl's legs, whereas other avian case reports of *Knemidokoptes* infection have described additional areas of the skin being affected. A full recovery within 22 days (in our case, possibly due to our specific protocol) suggests that this protocol warrants further testing in birds with more severe, widespread knemidokoptic mange. This case offered an opportunity to improve previous treatment protocols for knemidokoptiasis in wild raptors.

Understanding the parasite's occurrence and its effects on a wild raptor species may also prove useful should future epizootic events threaten raptor populations.

Acknowledgments

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European hedgehog (*Erinaceus europaeus*)

Potential drivers of vector-borne pathogens in urban environments: European hedgehogs (*Erinaceus europaeus*) in the spotlight

Springer A et al. One Health. 2024;18:100764. https://doi.org/10.1016/j.onehlt.2024.100764

Vector-borne diseases (VBDs) are considered as (re-)emerging, but information on the transmission cycles and wildlife reservoirs is often incomplete, particularly with regard to urban areas. The present study investigated blood samples from European hedgehogs (*Erinaceus europaeus*) presented at wildlife rehabilitation centres in the region of Hanover. Past exposure to *B. burgdorferi* sensu lato (s.l.) and tick-borne encephalitis virus (TBEV) was assessed by serological detection of antibodies, while current infections with *Borrelia* spp., *Anaplasma phagocytophilum*, *Rickettsia* spp., *Neoehrlichia mikurensis*, *Bartonella* spp., *Babesia* spp. and *Spiroplasma ixodetis* were investigated by (q)PCR. Of 539 hedgehogs tested for anti-*Borrelia* antibodies, 84.8% (457/539) were seropositive, with a higher seropositivity rate in adult than subadult animals, while anti-TBEV antibodies were detected in one animal only (0.2%; 1/526). By qPCR, 31.2% (168/539) of hedgehog blood samples were positive for *Borrelia* spp., 49.7% (261/525) for *A. phagocytophilum*, 13.0% (68/525) for *Bartonella* spp., 8.2% for *S. ixodetis* (43/525), 8.0% (42/525) for *Rickettsia* spp. and 1.3% (7/525) for *Babesia* spp., while *N. mikurensis* was not detected. While further differentiation of *Borrelia* spp. infections was not successful, 63.2% of the *A. phagocytophilum* infections were assigned to the zoonotic ecotype I and among *Rickettsia* spp. infections, 50.0% to *R. helvetica* by ecotype- or species-specific qPCR, respectively. Sequencing revealed the presence of a *Rickettsia* sp. closely related to *Rickettsia felis* in addition to a *Bartonella* sp. previously described from hedgehogs, as well as *Babesia microti* and *Babesia venatorum*. These findings show that hedgehogs from rehabilitation centers are valuable sources to identify One Health pathogens in urban areas. The hedgehogs are not only exposed to pathogens from fleas and ticks in urban areas, but they also act as potent amplifiers for these vectors and their pathogens, relevant for citizens and their pets.

Survey of antimicrobial and probiotic use practices in wildlife rehabilitation in the United States

Miller EA, Amato R, Ponder JB, Bueno I. PloS one. 2024;19(8):e0308261. https://doi.org/10.1371/journal.pone.0308261

Antimicrobial resistance is a global health concern. As such, there have been increased efforts to monitor and standardize antimicrobial prescribing practices in humans and domestic animals. In contrast, there is relatively little known about specific prescribing practices in wild animals despite the wide use of antimicrobials and other microbial interventions, such as probiotics to treat captive wildlife. Therefore, the goal of this study was to examine current antimicrobial and probiotic use from a cross-section of wildlife rehabilitation facilities in the United States. An anonymous electronic survey was sent to 105 United States permitted wildlife facilities to collect information about admissions, current antimicrobial and probiotic use practices, and current staff knowledge and attitudes surrounding antimicrobial resistance and probiotic effectiveness. Respondents from over 50% of facilities participated in the survey (54/105), including 45 facilities that treated birds. All facilities reported using antimicrobials, including some from groups considered critically important for human medicine, for a wide range of medical conditions and prophylaxis. Among antibiotics, enrofloxacin and amoxicillin-clavulanic acid were the most commonly used. Antifungals were not as widespread, but itraconazole was the most commonly used. Over 75% of respondents said that their facilities would benefit from having standardized antimicrobial guidelines in place. Probiotics were also used in more than 50% of facilities, but there was notable disparity in opinions regarding their efficacy. The results of this survey are a first step towards understanding antimicrobial and probiotic use practices in the treatment of captive wildlife and developing an antimicrobial stewardship program for wildlife rehabilitation.

Trends in vervet monkey admissions to a wildlife rehabilitation centre: a reflection of human-wildlife conflict in an urban-forest mosaic landscape

Pillay KR, Streicher JP, Downs CT. Mamm Biol. 2024 Aug 29:1–17. https://doi.org/10.1007/s42991-024-00447-x



PHOTO ©LIONMOUNTAIN. CC0 VIA PIXABAY.

Vervet Monkey (*Chlorocebus pygerythrus*)

Despite the common destructive effects of urbanization on biodiversity assemblages, certain species thrive in urban environments. One mammalian species that has persisted in the urban-forest mosaic landscape is the vervet monkey (*Chlorocebus pygerythrus*). It is a common resident primate in Durban, eThekwini Municipality, KwaZulu-Natal Province, South Africa, with frequent contact with humans and domestic wildlife. Due to their adaptability in using human-altered landscapes, the interactions between humans and vervet monkeys have increased and are often negative. Our study determined trends in the admission cases of vervet monkeys to a Durban wildlife rehabilitation center and assessed the main factors contributing to these admissions to mitigate human-vervet monkey conflict. Our analyses were conducted on recorded admission data for vervet monkeys at a wildlife rehabilitation centre from 2011 to 2018. Members of the public (90.0%) mostly reported vervet cases, with admissions mostly recorded from the central district (46.8%) of the municipality. The number of admitted vervet monkeys increased significantly over the years and months, with a mean (± SE) of 127.3 ± 21.34 and 84.8 ± 5.37, respectively. Only 34.3% of vervet monkeys were alive at the end of the admission process from the 83.7% that were admitted alive at the rehabilitation center. The high number of deaths resulted from anthropogenic activities, primarily motor vehicle strikes (30.8%) and domestic dog (*Canis lupus familiaris*) attacks (13.9%). We modeled survivability for vervet monkeys, and our results showed that season, age category, and the cause of admission were all significant factors influencing survival after admission. The sex of vervet monkeys had no significant effects on survivability after admission to the rehabilitation center. We suggest that wildlife rehabilitation centers in priority areas use these findings in education to improve human coexistence with vervet monkeys. The trends also serve as a foundation for human-vervet conflict resolution programes. The advantages of publishing records from rehabilitation centers are that they will raise awareness of the challenges posed by vervet monkeys in urban environments, where they are sometimes perceived as a nuisance because of anthropogenic influences in the urban mosaic landscape.

Rehabilitation outcomes of bird-building collision victims in the Northeastern United States

Kornreich A, Partridge D, Youngblood M, Parkins K. PloS one. 2024:e0306362. https://doi.org/10.1371/journal.pone.0306362

Building collisions are a leading threat to wild birds; however, only those that are found dead or fatally wounded are included in current mortality estimates, with injured or stunned birds largely assumed to survive long-term. Avian building collision victims are often brought to wildlife rehabilitators for care, with the hopes they can be released and resume their natural lives. We examined the wildlife rehabilitation records of over 3,100 building collisions with 152 different avian species collected across multiple seasons to identify patterns of survival and release among patients. The number of admissions varied by season; fall migration had the highest number of cases and winter had the least number of cases, and summer having the lowest release proportion and winter having the highest. The most common reported injury was head trauma and concussion. Our logistic and Poisson models found that mass had a strong positive effect on release probability, and the season of summer had a strong negative effect on release probability. Mass and winter had a strong positive effect on treatment time, and age and the seasons of fall and winter had a strong negative effect on treatment time in these models. Ultimately, about 60% of patients died in care, either by succumbing to their injuries or by euthanasia. Patients that were released remained in care for longer than patients that died. This study reports different data than carcass studies and views bird-building collisions from the perspective of surviving victims to explore longer-term effects of these collisions on mortality. Increased communication and collaboration between wildlife rehabilitators and conservation researchers is recommended to better understand building collisions and how to respond to this leading threat to wild birds. These findings, along with our estimate of delayed mortality, suggest that overall collision mortality estimates based on carcass collection far exceed one billion birds in the U.S. each year.

Trypanosoma cruzi infection in black bears (*Ursus americanus*): A case report in a cub from California and serologic survey for exposure in wild black bears from several states

Hughes R et al. Vet parasitol: Regional Studies and Reports. 2024;(101129):101129. https://doi.org/10.1016/j.vprsr.2024.101129

Trypanosoma cruzi is an important cause of disease and death in humans and dogs, and although wildlife infections are common, less is known about disease manifestations. A 12-week-old male American black bear (*Ursus americanus*) cub with mild lethargy and anorexia presented to a wildlife rehabilitation center in Lake Tahoe, California. The cub continued to become increasingly weak and showed decreasing interest in play and other activities. The cub was anemic and had increased γ-glutamyltransferase (GGT) liver enzymes. A large number of trypanosomes were noted on a thin blood smear. *Trypanosoma cruzi* was isolated in culture from a subsequent blood collection. Proliferative bony lesions were noted on radiographs, but this finding was considered unrelated to the *T. cruzi* infection. The number of parasites observed in thin blood smears dramatically dropped over time, but it remained PCR positive until at least nine months. The cub continued to gain weight and became increasingly active. Serum samples from the cub were positive with three different serologic assays (IFA, ELISA, and ICT). The bear was not treated because of the decreasing parasitemia and the improvement in activity and appetite. Although the bear could not be released due to issues unrelated to *T. cruzi,* it remains healthy in a captive facility. Sequence analysis of the DHFR-TS and COII-ND1 gene sequences confirmed the bear was infected with DTC TcIV. Following the detection of this clinical case, a serologic survey was conducted to determine the prevalence of *T. cruzi* exposure of black bears in California, North Carolina, and Pennsylvania. Because no serologic assay has been validated for use in bears, three different assays were used. Marked differences in apparent seroprevalence range from 1% (requiring all three assays to be positive) to ~20.7% (requiring only one assay to be positive). Black bears are naturally exposed to *T. cruzi* across the United States. Future studies using PCR testing of tissues or blood would be needed to better understand the prevalence of *T. cruzi* in wild black bears, lineages most commonly associated with infection, and if *T. cruzi* represents a health threat to bears.

Anatomy, digital radiography and cone-beam computed tomography of Western European hedgehog (Erinaceus europaeus) skull

Ruszkowski JJ, Zdun M, Arciszewski MB. BMC Vet Res. 2024;20(1):420. https://doi.org/10.1186/s12917-024-04280-9

BACKGROUND: European hedgehogs (*Erinaceus europaeus*) are small insectivorous mammals common in Europe. With increased urbanization, the species become more frequent in the cities and near other human dwellings. The anatomy of the species with the use of diagnostic imaging techniques along with the classical anatomical methodology has not been researched before. In addition to the description of this species' skull, the data may contribute to progress in hedgehog medicine, delivering a basis for diagnosing and treating head trauma in this species. RESULTS: Cadavers of 30 European hedgehogs have been used to study the anatomy of the head of the species. Along with classical anatomical techniques - latex and corrosion cast specimens, x-ray, and cone-bean computed tomography scans were performed. The methods were then compared, and the detailed anatomy of the head was described. The skull of the Western European hedgehog was elongated and oval in shape, and could be divided into two distinct regions: one formed of neurocranial bones and the other of facial bones. The neurocranium was composed of the following bones: the occipital, interparietal, parietal, frontal, temporal, sphenoid, ethmoid, and pterygoid bones. The following facial bones were identified: the incisive, nasal, maxilla, zygomatic, palatine, vomer, and the mandible. The most important findings include the primitive tympanic bullae, the absence of a supraorbital foramen, and the lacrimal bones, which were indistinguishably fused with the maxillae. CONCLUSIONS: The results of the study may be used in future research on the comparative anatomy of the other members of the Eulipotyphla order. With the increase of hedgehogs in the cities, it is important to establish new diagnostic and treatment protocols for wildlife rehabilitation centers. Anatomical and radiological descriptions may be used as a base for such work. The anatomical features of the hedgehog skull described in the study may prove useful for further studies from a comparative anatomical perspective. Additionally, the data may serve as a basis for developing identification guidelines for archaeological studies and forensic research.

Oxidative status: A general but overlooked indicator of welfare across animal species?

Beaulieu M. BioEssays. 2024 Jun 4:e2300205. https://doi.org/10.1002/bies.202300205

Because of their ubiquity, plasticity, and direct effects on the nervous system, markers of oxidative status may be of great value to assess animal welfare across species and conditions in the wild. However, welfare biologists have not yet seized this opportunity, possibly because the validity of these markers as welfare indicators remains questionable. A validation process was, therefore, performed here using a meta-analytical approach considering three conditions assumed to impair the welfare of animals. With very few exceptions, two of the four considered markers consistently varied across these negatively-valenced conditions. By highlighting the current underrepresentation of markers of oxidative status in animal welfare studies, and by concretely illustrating that some of these markers can consistently reflect negative affective states, this article aims to encourage biologists to include these physiological markers in their toolbox to better measure, monitor, and perhaps also improve the welfare of animals in their natural habitat. ◆

TAIL ENDS



After just leaving for a nice afternoon flight, Avery looked back and thought, 'Did I leave the stove on?'

Indian Roller
Coracias benghalensis
IUCN Red List–Least Concern, Population Trend – Increasing
PHOTO ©HARI K PATIBANDA. USED WITH PERMISSION.

SUBMISSION GUIDELINES

POLICY Original manuscripts on a variety of wildlife rehabilitation topics (e.g., husbandry and veterinary medicine) are welcomed. Manuscripts that address related topics such as facility administration, public relations, law, and education are invited as well.

Associate editors and anonymous reviewers, appropriate to the subject matter, evaluate each submitted manuscript. Concurrent submission to other peer-reviewed journals will preclude publication in the Journal of Wildlife Rehabilitation (JWR). The International Wildlife Rehabilitation Council (IWRC) retains copyright on all original articles published in the JWR but, upon request, will grant permission to reprint articles with credit given to the IWRC–JWR.

SUBMISSIONS All submissions should be accompanied by a cover letter stating the intent of the author(s) to submit the manuscript exclusively for publication in the JWR. Electronic submissions are required; hard-copy manuscripts are not accepted. The manuscript file should be attached to the submission letter (which can be the body of your email) and sent to:

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MANUSCRIPT Manuscripts should be MS Word documents in either PC or MAC platform (no PDF files). Manuscript should be typed in Times Roman, 12 pt., double spaced throughout with one-inch margins.

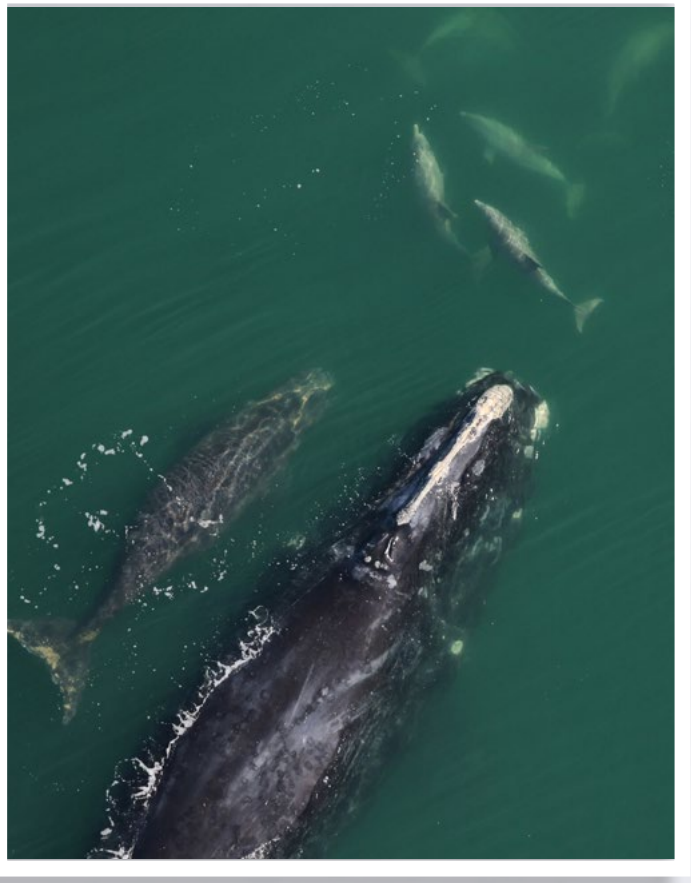
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North Atlantic right whale *(Eubalaena glacialis)* Right whale #1204 and her calf east of St. Catherines Island, Georgia, US.

IUCN Red List–Critically Endangered, Population Trend – Decreasing

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Providing evidence-based education and resources on wildlife rehabilitation to move the field of wildlife rehabilitation forward; to promote wildlife conservation and welfare; and to mitigate human-wildlife conflicts worldwide, through better understanding of wild animal ecology, behavior, and welfare.