INTERNATIONAL WILDLIFE REHABILITATION COUNCIL

Volume 37, Number 1, 2017

JOURNAL OF WILDLIFE REHABILITATION



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Adding to methods of detecting the presence of algal toxins from HABs in marine birds

Comparing efficacy of commonly used milk replacer formulas in desert and eastern cottontails

Study of endangered South African vultures in rehabilitation reveals challenges and successes

ABOUT THE JOURNAL

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 onsite 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.



Left:
Barbary macaques ***(Macaca sylvanus).***
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On the cover:
Green heron (*Butorides virescens*).
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IWRC

Providing science-based education and resources on wildlife rehabilitation to promote wildlife conservation and welfare worldwide.

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The *Journal of Wildlife Rehabilitation* is published by the International Wildlife Rehabilitation Council (IWRC), PO Box 3197, Eugene, OR 97403 USA. ©2017 (ISSN: 2166-9198). All rights reserved.

JOURNAL OF WILDLIFE REHABILITATION

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EDITORIAL

Practicing Leadership

I have recently had the opportunity to reflect on leadership through conversations with a mentor. The conversations we have had throughout the fall and related readings have helped me expand and strengthen my abilities as a leader. As I was contemplating what to write in this first issue of the 2017 Journal, my mind turned back to what I have been learning. I hope my short summary of these takeaways will be of use to you, your staff, your volunteers, and your wildlife center.

- **Provide staff with the tools to solve their own problems,** including training, technology, and organizational knowledge. I am fairly certain this is central to leading a healthy growing organization, although it is something I am still learning.
- **All staff should excel at the basics.** Spend reflective time identifying where each individual needs support, training, or change. Ensure staff are aware of their strengths; tailor the job to those strengths. Tie this into your mission and have them define how their job fits in. Make this explicit. Do not assume. Think strategically about the skill sets your staff and volunteers have and use them. If a needed skill is missing, seek it out.
- **Create a culture of why and what if**. Once your staff and volunteers are onboard, and in fact even before, encourage them to ask why something is done a particular way and offer alternatives. This always creates an excellent learning opportunity - sometimes for the individual who questioned why, sometimes for yourself as the words 'because that's how I learned it' come out of your mouth. Use their fresh perspective to either reaffirm your practice or get the organization out of a time-sink rut.
- **Communication is central to good function**. When board, staff, and volunteers are siloed into specific duties, locations, or times a disconnect occurs between groups. They do not see the whole picture so it's harder to understand and appreciate a leader's efforts and to care about the entire organization. This sort of 'us vs. them' thinking impedes the function of an organization. It fosters unconstructive dissent and dissatisfaction with work—paid or volunteer. Fortunately, there's a solution…
- **Begin with an open discussion of each person's role in the organization** not just their position title but how they strengthen your organization's mission. Explicitly communicate how the skills of specific people led to an outcome. This is where having previously worked on awareness of strengths and personal goals can forward the discussion. We are all in it for the collective mission, each using our own strengths.

It's the leader's job to facilitate each person understanding themself, their teammates, and their larger organizational community and also to ensure that people are in positions that play to their strengths while growing their abilities.

I have enjoyed stretching my leadership skills and learning more about people management although the process is far from finished. As I grow, as IWRC grows, what's needed will change and change again. And that's the beauty of being open to learning—whether it is growing as a leader, the informal training you receive every time you observe an animal, or the insight contained in the hard-won knowledge shared in the following peer reviewed papers.

—Kai Williams
Executive Director

Impact of Outdoor Recreation on Wildlife

FORT COLLINS, CO (December 8)—Newly published research in the journal PLOS ONE by scientists at WCS (Wildlife Conservation Society), Colorado State University (CSU), and University of California-Berkeley finds that human recreation activities in protected areas are impacting wildlife, and more often than not, in negative ways.

Nature-based, outdoor recreation is the most widespread human land use in protected areas and is permitted in more than 94 percent of parks and reserves globally. Inspiring an estimated eight billion visits per year to these areas, outdoor recreation is typically assumed to be compatible with conservation. Increasingly, however, negative effects of recreation on wildlife are being reported.

"People generally assume that recreation activities are compatible with conservation goals for protected areas," said Courtney Larson, CSU PhD student and lead author of the study. "However, our review of the evidence across wildlife species and habitat types worldwide suggests otherwise."

The authors reviewed 274 scientific articles published between 1981 and 2015 on the effects of recreation on a variety of animal species across all geographic areas and recreational activities.

More than 93 percent of the articles reviewed indicated at least one impact of recreation on animals, the majority of which (59 percent) were negative. Hiking, for example, a common form of outdoor recreation in protected areas, can create a negative impact by causing animals to flee, taking time away from feeding and expending valuable energy.

Surprisingly, studies of hiking and other non-motorized activities observed negative effects on wildlife 1.2 times more than motorized activities. Larson explained:

"The findings do not mean that everyone should hop on an all-terrain-vehicle instead of hike. Since motorized activities generally cover a larger area, their influence on animals, while less intense, is more widespread. In addition, motorized activities can result in other environmental impacts such as soil loss and vegetation disturbance, which were not analyzed in the study."

Among the negative impacts observed were decreased species diversity; decreased survival, reproduction, or abundance; and behavioral or physiological disturbance (such as decreased foraging or increased stress). Negative effects were documented most frequently for reptiles, amphibians, and invertebrates.

Positive effects of recreation on wildlife were most often observed on birds in the crow family and mammals in the rodent order. These effects included increased abundance and reduced flight responses.

WCS Associate Conservation Scientist Sarah Reed said, "The harmful effects of recreation are a growing concern for land managers who must balance goals for recreation and conservation, as protected area visitation rates increase. Results of this study are critical to inform science-based solutions to avoid or mitigate those impacts."

The study also suggests that snow sports may impact wildlife more frequently than other activities popular in the summer, such as hiking and boating. Determining exact reasons for this result were beyond the scope of the study, though the authors said this may be an important area to explore for future research.

In other results, the team found that the majority of the research on recreation impacts is conducted in North America and Europe. Most of the articles reviewed focused on impacts to mammals (42 percent) or birds (37 percent) while studies of amphibians, reptiles, and fish, were lacking.

CSU Professor Kevin Crooks said, "Clearly, there is still much to know about the impacts of recreation on wildlife; however, we must start by simply acknowledging that recreation and conservation are not always compatible for all species, in all locations. This will make it easier to justify additional research on this topic, establish limits on public access to protected areas, and encourage changes in the behavior of recreationists, leading to improved conservation outcomes."



Hikers at Selawik National Wildlife Refuge, Alaska.

PHOTO © USFWS. CC BY-NC-ND 2.0 LICENSE.

The authors say that the results of the study present an opportunity for a broader discussion on balancing the accommodation of increased numbers of protected area visitors with those of wildlife.

Ways to help minimize recreational impacts to wildlife in protected areas mentioned in the literature include: staying on existing trails, respecting seasonal closures, minimizing noise, not approaching wildlife, and reducing speeds of motorized vehicles.

"Effects of recreation on animals revealed as widespread through a global systematic review," appears in the current edition of *PLoS ONE.* Authors include Courtney L. Larson of Colorado State University, Sarah E. Reed of WCS and Colorado State University, Adina M.

Merenlender of University of California Berkeley, and Kevin R. Crooks of Colorado State University.

Puerto Rican Amazon Reintroduction Marks Third Population on Island

PUERTO RICO (November 30)—At dawn on November 30th two dozen people watched from behind a blind as 31 Puerto Rican amazons (*Amazona vittata*) were released. The birds have spent the past year residing in the wire mesh and steel 16-foot-tall prerelease cage, acclimatizing to the site.



PHOTO © TOM MACKENZIE, USFWS. CC BY-NC-ND 2.0 LICENSE.

Puerto Rican amazon parrot *(Amazona vittata).*

The Puerto Rican parrot is an endemic species of Puerto Rico, and the only native parrot in the United States. Conservation professionals have been working toward the parrot's reintroduction to the Maricao Forest for more than 40 years. This reintroduction begins a new chapter in the history of the Puerto Rican parrot recovery program. During pre-Columbian times the parrot was abundant, but through the years, deforestation, predation, diseases and poaching caused the population to crash. In the 1970's, chicks and eggs were captured from the wild, and a collaborative effort between state and federal agencies began. Today, the population has more than 500 birds that are distributed among state and federal facilities and, until today, only two wild locations in the El Yunque National Forest and Rio Abajo Commonwealth Forest.

These released parrots were born and cared for at the other locations. The parrots were transferred from the José L. Vivaldi Aviary located at Rio Abajo Commonwealth Forest and the Iguaca Aviary located at El Yunque National Forest; two facilities exclusively dedicated to captive breeding and veterinary care for Puerto Rican parrots.

Monitoring the parrots' dispersal, survival rate, and habitat use will be a priority for the coming weeks. The parrots are fitted with radio-collared transmitters. Four people embedded throughout the forest will track the parrot's signal for the next year. In anticipation, an old trail system was rehabilitated to allow biologists access to secluded areas.

Monitoring recruitment of breeding birds and other population attributes will be the next priority in 2017. Artificial nests were installed throughout the forest to provide viable nesting sites for the first group of parrots that may start reproducing here. Within the next few years, state and federal biologists will seek to control predators and competitors, monitor the size of the wild population, maximize parrot reproduction in the wild, continue release of captive-reared parrots, monitor all releases of Puerto Rican parrots to identify mortality factors and to reduce their impacts and assess habitat use, and develop and implement plans to expand the release program. The big picture, long term goal moving forward is to establish at least three interacting populations of parrots in the wild and document sustained population growth for 10 years. Initiating the third location is a key milestone towards recovery. It minimizes the risks of the parrots' extinction because it is less likely that hurricanes and disease outbreaks, and other threats like predation will affect equally and simultaneously three spatially segregated populations.

The future looks bright for the Puerto Rican parrot. The Maricao Commonwealth Forest, commonly known as only the Monte del Estado, which refers to the mountain of that name located in the forest, is surrounded by privately-owned forested lands of abandoned coffee plantations which have reverted back to secondary forest. Many of the landowners have conservation agreements with the Service's Partners for Fish and Wildlife Program and the Puerto Rico Department of Natural and Environmental Resource's Auxiliary Forests. These private lands are expected to provide landscape connectivity between the Maricao Forest and other major forested areas, such as the Susúa Commonwealth Forest, the Guilarte Commonwealth Forest, forested regions of the Cordillera Central, and potentially even the northern Karst region where another population of Puerto Rican parrots is currently thriving.

Jaguar Survival Requires Landscape Level Conservation

NEW YORK (November 23)—The Wildlife Conservation Society (WCS) reports the publication of a plan to help guide multi-institutional efforts in conserving the jaguar (*Panthera onca*) in the Amazon basin. A region known to conservationists as the central Amazon Jaguar Conservation Unit is the largest jaguar stronghold in the world. It encompasses parts of Brazil, Bolivia, Peru, Ecuador, Colombia, Venezuela, Guyana, French Guyana, and Suriname. However, even in this vast area, changes in biological diversity and jaguar populations can come quickly.

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The use of blood collection cards for assessing presence of microcystin in marine and estuarine birds

Corinne M. Gibble, Kendra Hayashi, and Raphael M. Kudela

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Ruddy duck *(Oxyura jamaicensis).*

ABSTRACT:

Microcystis aeruginosa blooms and production of associated toxins, microcystins, are a common occurrence in freshwater systems throughout California, and microcystins have recently appeared in nearshore marine environments along the central coast of California. Because nearshore feeding birds may be especially vulnerable to harmful algal blooms (HABs), we investigated the use of Whatman® FTA® blood sample collection cards for the detection of microcystins in estuarine and marine birds admitted to rehabilitation in Monterey, CA between 2011 and 2015. Blood cards were analyzed via competitive enzyme-linked immunosorbent assay (ELISA), and results indicated that a large volume of blood (0.5mL) was necessary to detect a realistic level of toxin (0.5ppb). This may not be obtainable from sick, dehydrated, or injured birds in rehabilitation, however this method was shown to have utility for postmortem analysis and large die off events. Blood cards collected in this study were binned by species, and two groups tested positive for microcystin toxin. Current available data on the effects of HABs on marine birds is lacking, and better detection techniques are needed.

KEYWORDS: blood collection cards, ELISA, seabirds, marine birds, estuarine birds, waterbirds, microcystin, *Microcystis aeruginosa,* harmful algal blooms, HABs, cyanoHABs

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Introduction

Harmful algal blooms (HABs) are naturally occurring phenomena in freshwater and marine environments, and are found worldwide. Some algae that produce these blooms have the ability to release powerful toxins to the environment. Recently, toxins (microcystins) associated with the blooms of a common freshwater cyanobacterium, *Microcystis aeruginosa*, were detected in the near-shore marine environment of central California in Monterey Bay, and have been confirmed to be entering these environments through freshwater outflows.[1,2]

M. aeruginosa has been shown to tolerate salt water environments and microcystin toxins can persist in saltwater and freshwater habitats,[3,4,5,1] making marine and estuarine wildlife feeding near outflows at high risk for exposure. Microcystins are known hepatotoxins that have the ability to cause impairment at many levels of the food web. In addition to direct toxic effects, exposure of aquatic organisms to elevated concentrations of microcystins have been shown to negatively impact primary consumers like herbivorous zooplankton, secondary consumers such as fish and invertebrates, as well as upper trophic levels such as birds and mammals.[6,7,8,1] In 2007, numerous sea otters were found dead in Monterey Bay with signs of liver failure and microcystin was found in outflow areas where sea otters had stranded.[1] During necropsy investigations, liver tissue tested positive for microcystin toxin and correlated lesions were also discovered. Miller et al.[1] also successfully validated the uptake of microcystins in common prey items during experimental trials. However, at this time, potential population-level impacts of these biotoxins on otters and other coastal wildlife remain undetermined.

The freshwater to marine transfer of this toxin to Monterey Bay waters as described by Miller et al.[1] and Gibble and Kudela[2] has the potential to cause major environmental harm and adverse impacts to coastal wildlife like estuarine birds and seabirds. Bays, rocky areas, and sandy

J. Wildlife Rehab. 37(1): 7-12. © 2017 International Wildlife Rehabilitation Council.

beaches along the central California coast provide critical habitat for many species of marine and estuarine birds. These nearshore feeders may be especially vulnerable to toxin-producing HABs, particularly for toxins such as microcystin that bioconcentrate in the invertebrate and fish prey items.[9,10,11,12,13,1] Additionally, most marine and estuarine birds are comparatively small-bodied, and therefore may be at increased risk for intoxication. Because sea otters use the same nearshore environment as many estuarine birds and seabirds, they may be serving as sentinels for as-yet-unrecognized impacts of microcystins on nearshore-feeding waterbirds. These birds not only share habitat with sea otters, but also consume many of the same foods.

Although HABs are a regular occurrence along the central California coast, potential temporal and spatial links with mortality events in seabirds have lagged far behind similar studies in sympatric marine mammals.[14] These bird populations may have been heavily impacted in the past by HABs, although systematic biochemical testing is needed to confirm potential population-level impacts. Blood collection cards have been used in previous studies to assess exposure to HAB toxins in marine and terrestrial mammals,[15,16,17,18,19,20,21,22] and also chemical pollutants in the blood of animals[23,24] but have not previously been used to detect microcystin toxin in birds or other animals. Because microcystin is a hepatotoxin that has the ability to remain in the blood stream post exposure, [25] blood cards may be a cost-effective means for analyzing microcystin toxicity in birds. Other potential metrics of detection include post mortem analysis or analytical methods such as liquid chromatography/mass spectrometry (LCMS) testing. Testing during necropsy negates the chance to treat birds while still in rehabilitation, while the latter limits applicability because of the need for specialized equipment and high per-sample costs. In addition to being affordable, blood cards are easy to use and easy to store, making them a potentially viable protocol for use at wildlife rehabilitation centers.[26,24] Our objectives in investigating blood cards were two-fold. Our first objective was to determine if blood cards could be used in tandem with commercially available ELISA kits. Our second objective was to determine if marine and estuarine birds in the Monterey Bay area test positive for microcystins in blood samples.

Methods

Blood Card and ELISA Compatibility Trials

To evaluate viability of our proposed method, Whatman™ FTA blood sample collection cards (Whatman; GE Healthcare Bio-sciences, Pittsburg, PA, USA) were used in conjunction with Abraxis Microcystins ELISA for Serum (Abraxis LLC; Warminster, PA, USA) kits. This particular ELISA assay has demonstrated satisfactory cross-reactivity with all cyanobacterial cyclic peptide toxin congeners, and has 100% cross reactivity with microcystin-LR, the most common variant of microcystin (Abraxis 2016). According to a study by Heussner et al.,[27] neither storage time nor pipetting impacted results for human serum run on ELISA, so it was assumed losses due to storage or pipetting were negligible.[27]

Blood was collected from live marine birds (brown pelicans) at the Society for the Prevention of Cruelty to Animals for Monterey County (MSPCA) Wildlife Center, in Monterey, CA and International Bird Rescue (IBR) in Fairfield, CA. Brown Pelicans were used due to the large amount of blood available from this large bird. In the lab, blood was spiked with Calbiochem InSolution™ MCY-LR standard to a final concentration of 100ppb and applied to blood cards in 0.005mL applications. To provide a control, whole blood was also applied to blood cards in 0.005mL applications. This volume was chosen because it is a reasonable amount of blood to be taken from a bird in rehabilitation. Blood on blood cards was extracted using methods adapted from Maucher and Ramsdell[17] and Maucher et al.[18] Prior to analysis by ELISA, blood spots were cut from cards, and Reagent A (0.25mL) provided in the ELISA kit was used to hydrate the blood spots. The blood spots were then sonicated using a sonic dismembrator (Model 100; Thermo Fisher Scientific, Massachusetts, USA) for 30s at ~10W and centrifuged at 3600 rpm for 10 minutes. The supernatant was used as the sample, and triplicate samples were analyzed by ELISA following the exact specifications provided by the manufacturer. A standard curve was created by using the standards provided with the ELISA kit. The serum matrix microcystin standards provided are as follows: 0, 0.1, 0.25, 0.5, 1.0 ppb. The known standards were used to create a standard curve, subsequently applied to the unknown samples. Heussner et al.[27] noted that the amount of solvent MeOH used could affect specific congeners of microcystin in human blood serum analyzed on ELISA. They cited that 5% MeOH had a noticeable effect on recovery while 80% MeOH had no discernable effect on recovery. For this reason, 50% MeOH and also Milli-Q de-ionized water were used to assess cross reactivity and false positives, and were also used to assess recovery during evaporation trials. Ultimately, 50% MeOH was deemed most appropriate for both assessments.

The limit of detection was calculated and evaluated using standard additions of the MCY-LR standard. While we acknowledge the use of a spiked sample to determine recovery and method sensitivity does not always precisely reflect what happens in wild animals, the spike-recovery results provide a conservative estimate of likely recovery in unknown samples. The use of ADDA-based ELISA should ensure detection of the most common microcystins, but we acknowledge that without directly testing all possible compounds the results may be biased to the most common congeners, and may not include biologically transformed (e.g. covalently bound) microcystins.

Evaporation Trials

To determine if blood cards could be pooled to examine multiple individuals at once, blood cards were also evaluated with the addition of an evaporation/concentration step prior to ELISA analysis. Blood was spiked with a known concentration at 50ppb and applied to blood cards to assess recovery. This facilitated determination of how much microcystin was lost during the evaporation step. Individual blood spots were cut from the blood cards, sonicated for 30s at ~10W, and centrifuged at 3600 rpm for 10 minutes. The supernatant was evaporated in two different solutions using either 0.5mL 50% MeOH, or 0.5mL 100% Milli-Q de-ionized water, using a Caliper TurboVap II (Caliper Lifesciences, Hopkinton, MA) nitrogen evaporator at 40°C. After evaporation the dehydrated pellet was rehydrated using Reagent A, provided by the ELISA kit, and used

as the sample. ELISA was performed with replication in triplicate.

Application of Methodology on Birds

Detection of microcystin toxin in marine and estuarine birds was achieved through a collaboration with the MSPCA Wildlife Center. A subset of marine and estuarine birds admitted to the wildlife center between the summer of 2011 and the winter of 2015, were sampled for blood. These birds were live stranded in Monterey County, CA (Figure 1), and strand location was recorded for each individual. Blood was collected via Fisherbrand™ microhematocrit capillary tubes (capacity = 70 µL; Fisher Scientific; Thermo Fisher Scientific, Massachusetts, USA) and administered to blood sample collection cards. Blood cards were stored frozen (-20°C) until analysis. Blood cards were later binned by species and by date to evaluate multiple individuals simultaneously, and to obtain the appropriate amount of blood needed for detection (Table 1). A total of eight bins were created as follows: Common murres (*Uria aalge;* COMU) 2011-2012, COMU 2013-2014, COMU 2015, Brandt's cormorants *(Phalacrocorax penicillatus)*, Ducks (surf scoters [*Melanitta perspicillata*]; ruddy ducks [*Oxyura jamaicensis*]), American coots *(Fulica americana)*, grebes (western grebes [*Aechmophorus occidentalis*]; Clark's grebes [*Aechmophorus clarkii*]; eared grebes [*Podiceps nigricollis*]), loons (common loons [*Gavia immer*]; Pacific loons [*Gavia immer*]). After binning, blood cards were processed following the evaporation procedure described above, using 50% MeOH evaporation solution exclusively.

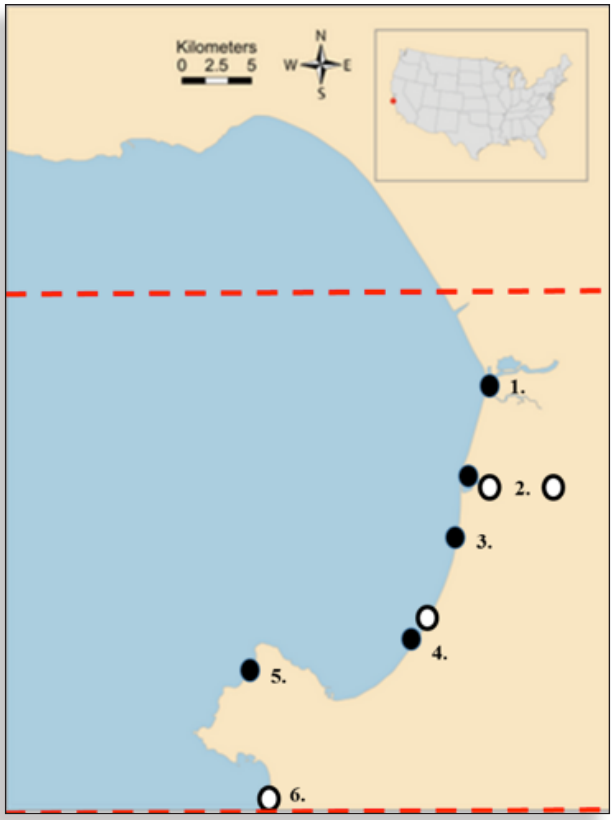
Results

Blood Card and ELISA Compatibility Trials

There was a clear delineation between spiked blood and control blood during the initial trials. All spiked blood was well above non-detect levels at approximately 100ppb for each replication. The control blood was below the limit of detection. The lowest detectable concentration of microcystin using this method was calculated conservatively as 0.15 ppb. This was calculated based upon the lowest standard (0.1 ppb) to give us a conservative working point. For a conservative amount of blood (0.005 mL) to be taken from a live bird, the concentration of microcystin in the blood would have to be at least 31.2 ppb for detection. This is a very high level of toxin. To detect microcystin in bird blood at a realistic level (0.5 ppb), the blood sample would have to be at least a volume of 0.5 mL (Table 2). There was no cross reactivity detected between the ELISA kits and 50% MeOH or Milli-Q, and these solutions were deemed appropriate for use in evaporation trials.

Evaporation Trials

Due to results from the compatibility trials, we determined that blood spots would be pooled to obtain the appropriate amount of blood. When blood spots were pooled, the evaporation technique was employed, and 50% MeOH was determined to be a superior extraction solution due to length of time required to evaporate completely. Pooling and evaporating blood cards was a successful means of examining multiple individuals simultaneously, and recovery after the evaporation method was applied was at 70%.



MAP USED WITH PERMISSION: TRACEY SAXBY, KATE BOICOURT, INTEGRATION AND APPLICATION NETWORK, UNIVERSITY OF MARYLAND CENTER FOR ENVIRONMENTAL SCIENCE (IAN.UMCES.EDU/IMAGELIBRARY/).

FIGURE 1. Map of Monterey Bay stranding locations. Red lines denote boundaries of stranding locations for birds examined in this study. Strand locations are labeled as follows: 1. Moss Landing State Beach, 2. Salinas River State Beach, 3. Marina State Beach, 4. Del Monte Beach, 5. Carmel River State Beach, 6. Asilomar State Park. Black dots indicate live stranding locations for birds binned in the group, COMU 2015; white dots indicate live stranding locations for birds binned in the group, Ducks.

Application of Methodology on Birds

The results of our findings for species-specific detection of microcystin are summarized in Table 1. Only COMU had sufficient sample density to evaluate temporal trends; for all other bins, data include the years 2011-2015. Two groups tested positive for microcystin toxin presence in the blood. The first group was Ducks (<2.56ppb), and the second group was COMU 2015 (<1.06ppb). Both groups had a very low level of microcystin detection; all other groups were non-detect. Of groups that tested positive for microcystin, the most common areas of stranding were Del Monte Beach and Moss Landing State Beach. Of the group COMU 2015, 63% were found at Moss Landing State Beach; 50% of the Duck group were found stranded at Del Monte Beach.

Discussion

Microcystin toxin has been implicated in the deaths of birds worldwide, and is an emerging issue in the nearshore coastal environment in California. Other studies have examined the internal effects of this toxin in birds during necropsy via biochemical testing in the organs,[28,29] in the feathers,[30] and measured presence and absence through microscopy in gut contents.[31] However, no blood values have been recorded and the lower limit for microcystin sensitivity for waterbirds remains unknown.[32] Means for better investigating contact with and effects of

TABLE 1. Species-specific detection of microcystin on binned birds.

SPECIES BIN	YEARS	NUMBER OF BIRDS	MICROCYSTIN IN BLOOD (PPB)
COMMON MURRE	2011-2012	14	ND
COMMON MURRE	2013-2014	18	ND
COMMON MURRE	2015	16	<1.06
BRANDT'S CORMORANTS	2011-2015	26	ND
AMERICAN COOTS	2011-2015	7	ND
GREBES	2011-2015	8	ND
LOONS	2011-2015	26	ND
DUCKS	2011-2015	7	<2.56

*ND = NON-DETECT

TABLE 2. Limit of detection for volume of blood tested and levels of detectable microcystin (MCY) in blood samples.

VOLUME OF BLOOD (ML)	DETECTABLE MCY (PPB)
0.005	31.2
0.01	15.6
0.05	3.12
0.1	1.56
0.5	0.312

microcystin on live birds remain largely undeveloped. Our results show that blood cards, while effective, have some caveats for appropriate use. They may be best for large birds, or when large amounts of blood are available, or if several blood cards are binned to obtain the appropriate amount of blood. They can also be used for post-mortem analysis, during necropsy, or retrospectively to investigate a large die-off event, as this method provides the means to test many birds at once. While we did not test other animals, the same methods should be applicable to organisms for which more blood is available (e.g. dogs, cattle). It is important to note that, when binned, information about specific birds will not be discernable. Rather, birds will be analyzed as a group and the results will be representative of the group only. Positive cases have the potential to be diluted by negative cases, leading to an inability to estimate toxin concentration in individuals, and a conservative estimate of toxin concentration for the group.

Because this method allows for collective testing, it has the potential to provide a cost effective evaluation of microcystin exposure in birds that may otherwise go unnoticed. Generally, attempts to detect toxin in live birds in rehabilitation can be cost prohibitive. Outsourcing testing to labs often can cost several hundred dollars per sample. Alternatively, blood collection cards are inexpensive, easy to use, easy to store, and easy to transport making them an excellent option for some rehabilitation centers and researchers. LCMS was deemed inappropriate for this particular study because it requires a column extraction including several cleaning steps during sample preparation. More dilution with a higher extraction volume is necessary to use this method, and more cleaning steps would likely dilute the microcystin toxin, making LCMS less sensitive than ELISA. Use of inexpensive metrics such as the use of blood collection cards may become increasingly important if this toxin, formerly found exclusively in freshwater environments, continues to be a problem in the coastal and marine environment, as seen in Monterey Bay, California.[1,2]

Monterey Bay supports the largest density and biomass of seabirds in the entire California Current System[33] and has a high abundance of birds inhabiting the land–sea interface, including threatened and endangered species, making this region especially important as critical habitat.[34] The bay also forms the centerpiece of the Monterey Bay National Marine Sanctuary, and is considered the second most important area for sea ducks (*Melanitta* spp.) and other nearshore species that winter along the Pacific coast of the United States.[35] This region also serves as an important molting and foraging area for migratory birds, so local microcystin production may also impact wider-ranging species.

Our results reveal at least two groups of birds in Monterey Bay that had contact with microcystin toxin between 2011-2015. Other groups that were tested may also be in contact with this toxin, but were not captured in our study due to sampling constraints. However, because we had so few detections, contact with microcystin toxin may not have been a persistent problem in in the areas of Monterey Bay inhabited by the birds from this study, during the time they were tested. Birds encountering the toxin may be doing so through acute exposure during periods of elevated microcystin toxin within their environment. Stranding location data taken during intake and admittance provided information about potential areas where birds may have encountered microcystin toxin, if exposure was acute. The MSPCA responds to strandings of marine and estuarine birds for Monterey County only (Figure 1). The birds included in this study ranged from Carmel, CA to Moss Landing, CA, and birds from the group COMU 2015 were most commonly stranded at Moss Landing State Beach; however, they were also found at Asilomar State Park, Del Monte Beach, and Marina State Beach (Figure 1). Birds from the group Ducks were most commonly stranded at Del Monte Beach, but also found stranded near Salinas River State Beach and Carmel River State Beach. These strand locations are at or near areas known to be freshwater outflows of microcystin to the coastal ocean[1,2] making acute exposure a possibility.

The groups identified as positive for microcystin by this study are likely candidates for microcystin intoxication in Monterey Bay because of diet and foraging locations. Surf scoters and ruddy ducks regularly eat invertebrate species[36,37,38,39,40] that are known to uptake microcystin toxin and depurate it slowly[9,10,11,12,13,1,41] and these species of ducks are found in estuarine and nearshore marine environments. These foraging habits provide the potential for a higher likelihood of contact if microcystin is present in the area where prey species are being consumed. Common murres commonly eat fish, but also consume invertebrates in some instances.[42] Both of these prey types have been shown to take up microcystin toxin.[9,12,7,8] However, perhaps a more important factor for this group of birds, is the year in which they stranded. In 2015, the west coast of the US experienced unprecedented intensity, duration and frequency of harmful algal blooms. Pinto Lake in Watsonville, CA is known as a local area "hot spot" for microcystin toxin production, and has been implicated in microcystin production

that caused the death of sea otters feeding at freshwater outflows in Monterey Bay.[1] In 2015, Pinto Lake had numerous large blooms of microcystin producing *Microcystis aeruginosa* (Kudela unpublished data). Other freshwater sources of microcystins in the Monterey Bay area may have experienced similar bloom conditions and toxin production as that found at Pinto Lake in 2015. If there were several blooms producing toxin, there would be a greater likelihood of trophic transfer through prey items to species in upper trophic levels, like common murres.

The magnitude and frequency of HABs have increased significantly in recent years,[43] and the interaction between HABs and birds has likely increased contemporaneously. Our findings suggest that some bird species were in contact with microcystin in Monterey Bay between 2011 and 2015. However, these results may not directly translate to mortality for these birds. Outcomes from this study can be used as a starting point to investigate the interaction between microcystin and species that tested positive. However, further testing and development of new tools and techniques is required to gain a better understanding of the role this toxin may be playing in the morbidity and mortality of waterbirds. The scant data available on the effects of toxic algae on marine and estuarine birds coupled with the results of this study show the need for better surveillance and detection techniques for HABs, and the development of better tools to evaluate algal toxins in birds.[14] Following further investigation, a systematic approach should be taken to develop a monitoring program for HAB-related marine and estuarine bird mortality. This type of monitoring could provide better detection of toxic events and subsequent exposure incidents for marine and estuarine birds.

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Acknowledgements

We would like to thank the MSPCA Wildlife Center and International Bird Rescue with great appreciation to Jessica Shipman, Rosanna Leighton, Dr. Rebecca Duerr, and Michelle Bellizzi who made this work possible. Salary and tuition assistance came from the NOAA Nancy Foster Scholarship program (CMG) NOAA award number: NA10SEC481003.

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Comparison of outcomes using two milk replacer formulas based on commercially available products in two species of infant cottontail rabbits

Gabriele Paul and Daniel G. Friend

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Desert cottontail *(Sylvilagus audubonii).*

ABSTRACT: Infant lagomorphs (rabbits and hares) are considered difficult to rehabilitate, with gastrointestinal disease being a major cause of mortality in this group. Wildlife rehabilitators use a variety of different milk replacers in an effort to improve the outcomes for orphaned or injured individuals that come into rehabilitation. To test whether different milk replacers can influence survival of infant desert and eastern cottontails in rehabilitation, this study compared the performance of two milk replacer formulas derived from commercial sources. The results show that one formula outperformed the other, resulting in fewer incidences of gastrointestinal disease and significantly higher release rates.

KEYWORDS: cottontail, milk replacer, rabbit, wildlife rehabilitation

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Introduction

Infant lagomorphs (rabbits and hares) are considered difficult to rehabilitate,[1,2,3,4] with gastrointestinal disease being a major cause of morbidity and mortality in this group.[5] A lack of suitable milk replacers is often cited as a leading cause of poor outcomes for infant lagomorphs in a wildlife rehabilitation setting.[1,4] Many different commercial brands and formulas for milk replacers are used by wildlife rehabilitators in an attempt to improve survival of infant rabbits (Table 1). However, only a few studies have been published that compare milk replacer outcomes,[1,4] and most of these have

J. Wildlife Rehab. 37(3): 13-19. © 2017 International Wildlife Rehabilitation Council.

TABLE 1. Examples of Milk Replacers used for Cottontails in Rehabilitation (where FV refers to Fox Valley Animal Nutrition).

REFERENCE	MILK REPLACER USED OR CITED
Schott, 2016 (6)	1 part FV 32/40 + 2 parts water
Knight, 2015 (7)	¾ parts FV 32/40 + ¼ part FV Ultra Boost + 2 parts water + FV LA-200 (1/4 tsp per cup of liquid)
Knight, 2015 (7)	1.5 parts PetAg Exotic Multi-Milk + 1 part PetAg Milk Matrix 42/25 blended together; then 1 part blended powders +1 part water
Oberly, 2015 (1)	Starter/Ultra (composition undefined)
Oberly, 2015 (1)	Goat's Milk Formula (composition undefined)
Hentz, 2014 (4)	1 part FV 30/50 + 2 parts raw goat's milk, by weight
Hentz, 2014 (4)	1 part FV 32/40 + 2 parts spring water (or distilled water)
Leggett & Farver, 2013 (8)	1 part 42/25 (FV or PetAg or Zoologic) (or 1 part 40/25(FV or PetAg or Zoologic)) + 1 part FV Ultraboost + 2 parts water
Fosco, 2012 (9)	1 part FV 32/40 + 2 parts water
Fosco, 2012 (9)	1 part PetAg KMR + 1 part FV Ultraboost + 2 parts water
Farr, 2010 (10)	1 part FV 32/40 + 2 parts water
Gode & Ruth, Eastern Cottontail, 2010 (11)	1 part PetAg Esbilac (or PetAg KMR) + 1 part PetAg Multi-Milk + 2 parts water
Gode & Ruth, Eastern Cottontail, 2010 (11)	1 tbsp PetAg KMR (or Zoologic 42/25) + 3 tbsp boiled, spring, or filtered water + 1 capsule Bovine colostrum 25% IgG + 3 drops Flax oil + 1 capsule Vibra Gest (or 1 pinch Prozyme Plus) + sprinkle of L-glutamine + 1/8 tsp Primal Defense
King, 2007 (3)	1 part FV 32/40 + 2 parts water + 1/8 tsp FV LA 200
King, 2007 (3)	1 part PetAg KMR + 1.5 parts PetAg Multi-Milk + 1/3 parts Optimum 100% Egg Protein Powder natural vanilla flavor + 1 pinch FV Lactobacillus Acidophilus + 3 parts water



An Eastern (left) and desert (right) cottontail in rehabilitation.

been small, with poor experimental controls. In addition, little is known about whether different species respond differently to various milk replacers, with most available data pertaining to eastern cottontails.[1, 3]

Colorado, where this study was conducted, is home to eight species of lagomorphs: four rabbits, three hares, and one pika.[12, 13] The Colorado Wild Rabbit Foundation (CWRF) has used two milk replacers (KMR plus Ultra Boost and FV 32/40) with some success over several years, in five species of lagomorphs (desert cottontail, Eastern cottontail, mountain cottontail, white-tailed jackrabbit, and snowshoe hare). However, only two of these (desert cottontail *Sylvilagus audubonii* and eastern cottontail *S. floridanus*) are received for rehabilitation at this facility in sufficient numbers to make a short-duration (one season) study feasible. As a starting point for further research, the authors chose to use the two milk replacers derived from commercial sources with which they were familiar to see if there are measurable differences in outcomes in the two most commonly received species of lagomorphs at their facility.

Some reports from cottontail rehabilitators have suggested that higher concentrations (e.g., KMR plus Ultra Boost mixed 1:1 with water[9]) or larger amounts per feeding (e.g. 13–15 % of body weight per feeding[4] or 13–15 ml of formula per 100 g of body weight) may produce better results due to the higher amounts of daily nutrients provided. Prior to beginning this study, the authors conducted several small pilot studies to test these ideas. One group of subjects was fed a more concentrated formula (KMR plus Ultra Boost mixed 1 part to 1.25 parts water; KMR plus Ultra Boost mixed 1 part to 1 part water; or Fox Valley 32/40 mixed 1 part to 1.5 parts water), twice daily, up to 10% of body weight. Another small group received larger amounts per feeding (KMR plus Ultra Boost mixed 1 part to 1.5 parts water or Fox Valley 32/40 mixed 1 part to 2 parts water), twice daily, up to 12% of body weight. These studies resulted in poor outcomes, so these protocols were rejected for further study.

Prior to conducting our research, the CWRF reviewed protocols and concluded that the planned study complied with the State of Colorado Chapter W-14, Wildlife Rehabilitation requirement regarding Care, Treatment and Disposition of Wildlife,[14] as well as the Wildlife Rehabilitators Code of Ethics.[15]

Materials and Methods

All unweaned desert and eastern cottontails received at our facility between March and July of 2016 that were candidates for rehabilitation were included in this study. However, subjects that weaned or died (or were euthanized) within three days of inclusion into the study population were removed from the study.

Additionally, any subjects that died (or were euthanized) during the study due to causes clearly unrelated to the milk replacer (e.g. spinal injury, infection, etc.; *N*=7) were also removed. This yielded a total sample size of 150 individuals who were enrolled in the study: 116 desert cottontails (DCTs) and 34 eastern cottontails (ECTs). All individuals in this sample remained in the study group until final disposition—either death or release.

In early August, it became apparent that one milk replacer in the study yielded better results than the other, so the study was terminated and rabbits switched to the milk replacer that showed better results.

Subjects were divided into two groups using a matched-pairs experimental design as described below. Each group was fed one of two milk replacers, MR1 or MR2, also described below. Of the DCTs, 62 (53%) received MR1 and 54 (47%) received MR2. Of the ECTs, 19 (56%) received MR1 and 15 (44%) received MR2.

Outcomes were divided into two categories: "Released" and "Died" (the latter including those requiring euthanasia). Criteria for release included a minimum weight of 150 g, steady weight gain, appropriate behavior (e.g., capture avoidance, hiding, etc.), and, for nestlings at intake, a minimum of three full days spent in outdoor pre-release caging with weight gain.

Milk Replacers Used

The two milk replacers used in this study (Table 2) were chosen based on the authors' prior experience and training and, in the authors' experience, are commonly used for cottontails within the wildlife rehabilitation community (Table 1). These formulas were consistently used, without dilution, during all stages of the rehabilitation process. (Note that all formula ratios referred to herein are based on volume measurements unless otherwise noted; based on the authors' experience this is common practice in the field of wildlife rehabilitation).

According to the published data, these two milk replacers differ somewhat in the amount of solids, protein, fat, and carbohydrates they contain, as shown in Table 3. It is important to note that the emphasis of this study was to compare the rehabilitation outcomes of two cottontail milk replacers as they are applied in practice, not on nutritional comparisons. A detailed nutritional comparison of the two milk replacer formulas is outside our scope.

However, Casey and Casey[16] conducted an extensive analysis of a wide array of milk replacers used in wildlife rehabilitation, along with comparisons to wild mothers' milk, that reveals differences between the two formulas used in this study (see Table 3). According to this nutritional analysis, MR1 provides more nutrients than MR2 per volume; comparisons on a mass basis can be obtained in Ref. 16 as well; the confounding issues of powder packing, humidity, evaporation of prepared product, etc. have not been addressed in this paper.

TABLE 2. Milk replacers used in study.

MILK REPLACER	PARTS POWDER	PARTS WATER
MR1	1 part PetAg Kitten Milk Replacer (KMR) + 1 part Fox Valley Ultraboost	3
MR2	1 part Fox Valley 32/40	2

As seen in Table 3, neither formula closely matches the reported composition of cottontail milk providing only roughly half of the amount of solids, protein and fat, but higher amounts of carbohydrate. However, our pilot studies with formula concentrations/amounts intended to more closely replicate mothers' milk suggested this approach was not beneficial. Also, while there are a number of possible formulas that could have been compared in this study and which may more closely match cottontail milk (see Table 1), there are few published studies documenting the nutritional composition or performance of these formulas. Therefore, the authors chose MR1 and MR2 based on their personal experience of having used them in practice for many years.

Experimental Design

This study used a matched-pairs experimental design in order to minimize the effects of confounding variables (e.g., cause of admission and age at admission). Subjects were grouped into matched pairs, with each pair matched as closely as possible based on our pre-defined matching criteria. Within each pair, one individual was randomly assigned (via a coin toss) to one of the two milk replacer groups, MR1 or MR2. The second individual in the pair was assigned to the other milk replacer group. Pairs were matched according to the following priority order:

- Member of the same litter (Many pairings were from a common litter.)
- Species (All pairs were of the same species.)
- Stage at intake (All pairs were same stage at intake.)
- Weight at intake
- Injury/cause of admission

TABLE 3. Macronutrient Content of Milk Replacers Used in Study.[16,17]

MILK REPLACER	% SOLID IN PREPARED FORMULA	FORMULA COMPONENTS AS % OF COTTONTAIL MILK	% PROTEIN IN PREPARED FORMULA	FORMULA COMPONENTS AS % OF COTTONTAIL MILK	% FAT IN PREPARED FORMULA	FORMULA COMPONENTS AS % OF COTTONTAIL MILK	% CARBOHYDRATE IN PREPARED FORMULA	FORMULA COMPONENTS AS % OF COTTONTAIL MILK
MR1	19.8	55	7.4	52	8.3	51	2.8	150
MR2	16.2	46	6.2	44	6.9	43	2	106

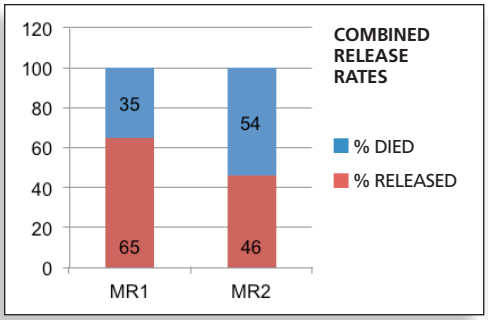


FIGURE 1. Combined release rates.

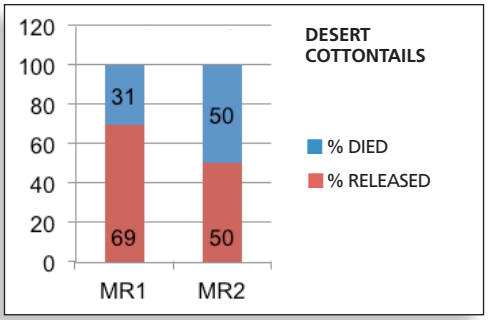


FIGURE 2. Release rates for desert cottontails.

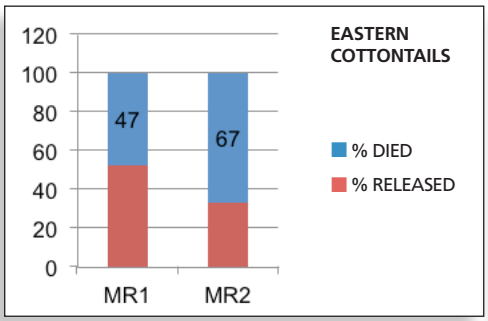


FIGURE 3. Release rates for eastern cottontails.

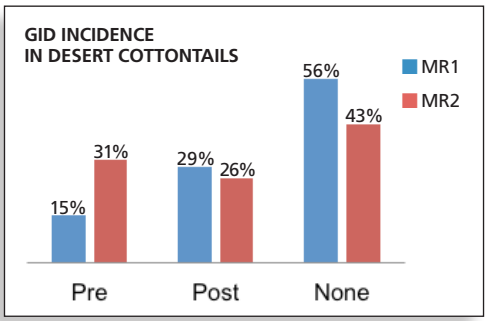


FIGURE 4. GID incidence in desert cottontails.

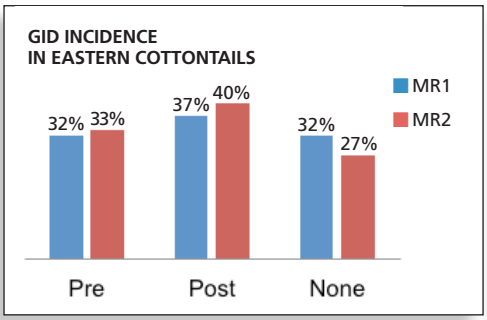


FIGURE 5. GID Incidence in Eastern cottontails.

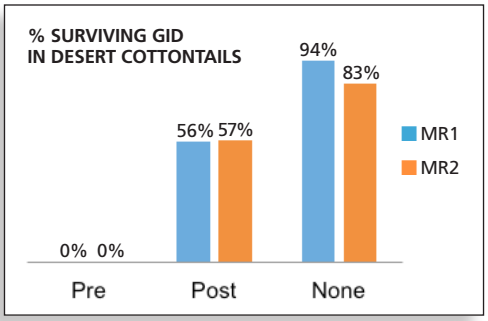


FIGURE 6. Desert cottontails surviving GID.

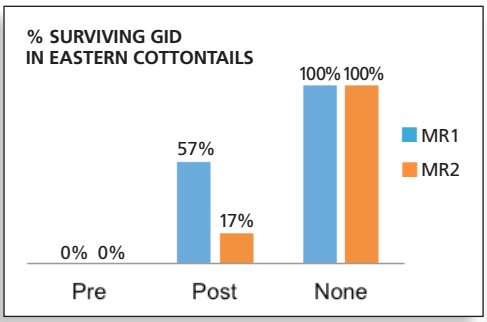


FIGURE 7. Eastern cottontails surviving GID.

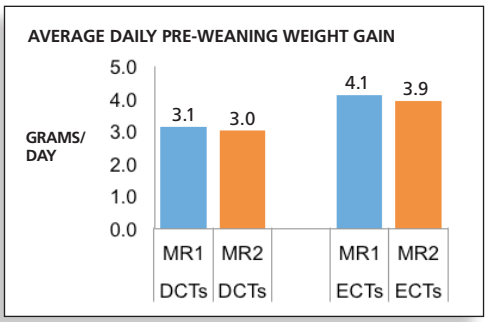


FIGURE 8. Average daily weight gain.

If a member of a matched pair was subsequently excluded from the study as described above, e.g., weaned within three days, the remaining individual was retained for our statistical comparisons. This is the reason for the slightly different numbers in each group.

Feeding Protocol

Subjects were syringe-fed twice a day with the assigned milk replacer, in an amount up to 10% of their body weight. Individuals were allowed to eat as little or as much (up to 10%) as they wanted. All other care and husbandry given (housing, non-milk diet, weaning protocols, etc.) were the same for all individuals, except for medication, which was given based on need. In particular, once their eyes opened, fresh water, grass, and greens were provided to all subjects. The amount of formula and/or number of feedings offered during weaning took into consideration the previous amounts consumed, weight gain, age, and behavior.

Results

Survival

Of the 150 individuals in this study, 57% survived to release and 43% died (N_R= 85, N_D= 65). Individuals in the MR1 group had significantly higher release rates ($p < 0.02$ [18]) than those in the MR2 group, with 65% of the MR1 group being released and 35% (N = 81, N_R= 53, N_D= 28) died, versus 46% of the MR2 group released and 54% died (N = 69, N_R= 32, N_D= 37) (Figure 1).

Among DCTs, 69% of the MR1 group were released (N = 62, N_R= 43, N_D= 19) versus only 50% of the MR2 group (N = 54, N_R= 27, N_D= 27) (Figure 2). These differences are statistically significant, with $p < 0.04$.

Among ECTs, the difference in release rates between the two groups also seemed pronounced, with 53% of the MR1 group being released (N = 19, N_R= 10, N_D= 9) versus only 33% of the MR2 group (N= 15, N_R= 5, N_D= 10) (Figure 3). However, these differences are not statistically significant, probably due to the smaller sample size of ECTs.

Gastrointestinal Disease

Gastrointestinal disease (GID), which manifests as GI disturbances such as diarrhea, bloat, GI stasis, cecal dysbiosis, or a combination thereof, was the leading cause of death during the study, responsible for 91% (N_{GID}= 59) of all deaths (N_D= 65) in our sample. GID with onset prior to weaning consisted mostly of diarrhea. GID with onset after weaning consisted mostly of cecal dysbiosis. This study was not designed to address weaning protocols, which can be complex, except to ensure that they are consistent across all individuals in this study; we hope to return to

this subject in future research efforts. Study subjects that experienced both pre- and post-weaning GID are included in the pre-weaning category.

Half (50%) of the DCTs (N_{DCT} = 116) and the majority (71%) of the ECTs (N_{ECT}= 34) developed either pre- or post-weaning GID during the study; of these, 69% of the DCTs and 79% of the ECTs died.

In DCTs, pre-weaning GID was significantly more common in the MR2 group (p<0.03), with twice as many developing it as in the MR1 group (31%, N_{MR2}= 17 vs. 15%, N_{MR1}= 9) (Figure 4). In ECTs, a similar proportion of both groups developed pre-weaning GID (32%, N_{MR1}= 6 vs. 33%, N_{MR2}= 5) (Figure 5).

Post-weaning GID was somewhat more common in DCTs in the MR1 group than in the MR2 group (29%, N_{MR1}= 18, vs. 26%, N_{MR2}= 14), whereas in ECTs it was somewhat more common in the MR2 group (40%, N_{MR1}= 6 vs. 37%, N_{MR2}= 7). However, these differences are not statistically significant.

In both species, pre-weaning GID resulted in 100% mortality, regardless of which milk replacer was given. While a few of these individuals appeared to recover and survived into the post-weaning stage, they all ultimately developed post-weaning GID and died.

Of all DCTs that developed post-weaning GID, 44% died, whereas 62% of the ECTs with post-weaning GID died. The death rates from post-weaning GID for DCTs were similar for both milk replacers (MR1 = 44%, MR2 = 43%). In ECTs, death rates were 43% in the MR1 group, but were much higher (83%) in the MR2 group. However, these differences are not significant, probably due to the smaller numbers in each group of ECTs (Figure 6, Figure 7).

In all cases, individuals that did not develop GID had better overall survival rates. However, DCTs in the MR1 group that did not develop GID had a better release rate (94%) than those in the MR2 group (83%); this difference is not statistically significant. For ECTs, those that did not develop GID had a release rate of 100% regardless of which milk replacer they received.

TABLE 4. Stage at Intake.

	RELEASED				DIED				
STAGE AT INTAKE	MR1		MR2		MR1		MR2		Total
Eyes Closed / Partly Closed	27	59%	15	41%	19	41%	22	59%	83
Eyes Open	26	74%	17	53%	9	26%	15	47%	67
Total	53		32		28		37		150

TABLE 5. Weight at Intake.

	RELEASED				DIED				
WEIGHT AT INTAKE (GRAMS)	MR1		MR2		MR1		MR2		Total
40–49	6	67%	2	25%	3	33%	6	75%	17
50–59	10	43%	7	44%	13	57%	9	56%	39
60–69	26	74%	17	53%	9	26%	15	47%	67
70–79	8	57%	6	46%	6	43%	7	54%	27
80–89	8	89%	7	70%	1	11%	3	30%	19
>90	4	100%	1	50%	0	0%	1	50%	6
Total	53		32		28		37		150

Stage and weight at intake

The differences between outcomes for the MR1 and MR2 groups were consistent over all stages and weights at intake; see Table 4 and Table 5. For all categories, we observed higher death rates in the MR2 group with a single exception. For cottontails weighing between 50 g and 59 g at intake, there was a similar release rate regardless of milk replacer.

Average daily weight gain

The average daily weight gain prior to weaning of cottontails that survived to release was similar in both groups, with those in the MR1 group showing only slightly larger weight gains. DCTs in the MR1 group averaged 3.1 ± 1.4 g/day, in MR2 group 3.0 ± 1.6 g/day; ECTs in the MR1 group averaged 4.1 ± 2.0 g/day, in the MR2 group 3.9 ± 1.2 g/day (Figure 8). These averages encompass the entire growth range from intake to weaning for all released cottontails in the study.

Palatability

The authors did not see any substantial differences in the palatability of the two milk replacers, although rabbits in the MR1 group were more likely to suckle, whereas rabbits in the MR2 group were more likely to lick from the end of the syringe.

Storage

On two occasions prior to the initiation of this study (in 2013 and in 2015), the authors experienced major die-offs of infant rabbits that were being fed the KMR/Ultra Boost combination (MR1). The rabbits developed severe pre-weaning diarrhea and died within seven days. The authors strongly suspect rancidity/spoilage of the KMR powder to be responsible. In both cases, 28 oz. cans of KMR powder purchased at local pet stores had been used. The unopened powder containers had been stored at room temperature (per package instructions) and were well within their expiration dates. Once the powder was replaced, the die-off ceased. Prior to discarding the "bad" powder, the authors noticed a slightly sour smell and slightly yellowish color to the powder, but these were subtle and were not detected prior to the die-offs. Subsequently, the authors made several changes that have appeared to resolve this issue. We began purchasing only bagged KMR (5 lbs.) online, storing unused powder in the freezer. Only small amounts (approximately two weeks' worth) of the powder are kept in the refrigerator between uses. Also, formula is now reconstituted just prior to feeding and unused portions are discarded.

Package instructions for the storage of KMR are:

> *Unopened powder should be kept in a cool, dry place. Reconstituted KMR must be kept refrigerated for up to 24 hours. Opened powder can be refrigerated for up to 3 months, or can be frozen for up to 6 months to preserve freshness.*[19]

The authors have had no such problems with any of the Fox Valley products (32/40 and Ultra Boost), but the above protocols have been used for all milk replacers since the die-offs.

Fox Valley 32/40 and Fox Valley Ultra Boost contain no storage instructions on the label, but private communication with Fox Valley staff indicates that both can be stored at room temperature and that freezing will extend the shelf life of the product.

Discussion

This study suggests that differences between milk replacers may have a significant impact on survival of infant cottontails in rehabilitation. GID, both pre- and post-weaning, was the largest cause of death of infant cottontails in the study. Significant differences between the two milk replacers tested were seen in the occurrence of the most fatal kind—pre-weaning onset GID—in desert cottontails. This suggests that the choice of milk replacer alone can influence outcomes for infants of this species. However, conclusive findings for eastern cottontails were somewhat weak given the small sample size, and should be repeated with a larger sample. Differences in the occurrence of post-weaning onset GID were not statistically significant between the two groups of milk replacers, suggesting that other factors must be considered, such as the method for introducing solids or novel food items, stressors, etc. This is an area where further research is needed.

This study does not shed light on the cause of the different outcomes between the two milk replacers, i.e., whether the slightly higher nutrient density of MR1, as documented by Casey and Casey, [16] (see Table 3) played a role. Alternatively, micronutrient content, manufacturing processes, or something else may account for the difference. PetAg's KMR includes probiotics[20] whereas the two Fox Valley products used in this study do not,[21,22] but it is not known whether the bacterial species included in KMR have any effect on rabbits.[6] This would be yet another important area for further research.

Finally, future research should consider possible differences in the dietary requirements of different species of infant lagomorphs.

Conclusion

Infant cottontails are considered by many wildlife rehabilitators to be difficult to rehabilitate, often having low survival rates. Since cottontails are one of the most common species entering wildlife rehabilitation facilities, even small differences in outcomes can have large cumulative effects. Given the variety of commercial products, home-made recipes, and combinations thereof used to raise cottontails, science-based comparisons of their performance are vital. This study found that the KMR plus Ultra Boost formula (MR1) was superior to Fox Valley 32/40 for raising the infant desert and eastern cottontails enrolled in this trial.

The selection of milk replacer can have a significant impact on survival rates, and further research is needed in this area to improve survival of these wild animals in rehabilitation.

Disclaimer

The authors received no financial support from any manufacturer or organization mentioned or cited in this paper for the research, authorship, and/or publication of this article and assert that no conflicts of interest are present.

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Vulture rescue and rehabilitation in South Africa: An urban perspective

Vinny Naidoo, K. Wolter, Ian W. Espie, and Antoinette Kotze

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Cape Griffon vulture *(Gyps coprotheres).*

ABSTRACT: South Africa is home to nine vulture species, of which seven are endangered. While the cause of the population declines remains largely speculative, a vast amount of effort has been dedicated towards the protection of populations by ensuring sustainable and safe food sources for the various colonies. Limited focus was placed in the past on efforts related to the rescue and/or rehabilitation (R&R) of injured birds and the release of these birds back into the wild. This paper provides an overview of the causes, the impact and success of three organizations involved in R&R efforts of vultures in the Magaliesberg mountain range and surrounding areas over a period of 10 years. Study material included 162 Cape griffon (CGV) and 38 African white-backed (AWBV) vultures. Datasets include the number, sex and age of birds received, the reason the vultures were brought in for R&R, surgical interventions performed and outcomes of rescue efforts. The CGV dominated the rehabilitation attempts. Results further show that a large number of apparently healthy birds were presented for veterinary treatment. The R&R data clearly indicate that the major cause of injuries was birds colliding with overhead pylons, as a high number of soft tissue and skeletal injuries were observed. The study also shows that successful releases of rescued birds are possible. It is concluded that urbanization has had a major negative impact on vultures around the Magaliesberg mountain range.

KEYWORDS: African white-backed vulture, Cape griffon vulture, *Gyps*, Magaliesberg, rehabilitation, rescue, vultures

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Introduction

Vultures are characterized by their large size, large featherless heads, curved beaks and are known for being high fliers.[17] They are further differentiated from other raptors by being predominantly carrion feeders that only resort to predation in times of extreme food shortages.[5,13,17] Being scavengers, vultures are important components of healthy ecosystems where their major contributory ecological role is to devour carcasses thereby preventing the spread of diseases.[8,16,17,24] The family is made up of the old-world (*accipitrid*) and the new-world (*cathartid*) vultures with the former, also known as griffon vultures, closely related.[28] Unconfirmed reports from Namibia suggest interbreeding between the two species to produce hybrids (Rare and Endangered Species Trust [REST], Namibia, pers. comm.). The only major difference between the two species is their nesting sites as the CGV nests on cliff faces while the AWBV prefers nesting in trees.

Reprint: *Jl S. Afr. Vet. Ass.* 2011;82(1): 24–31.

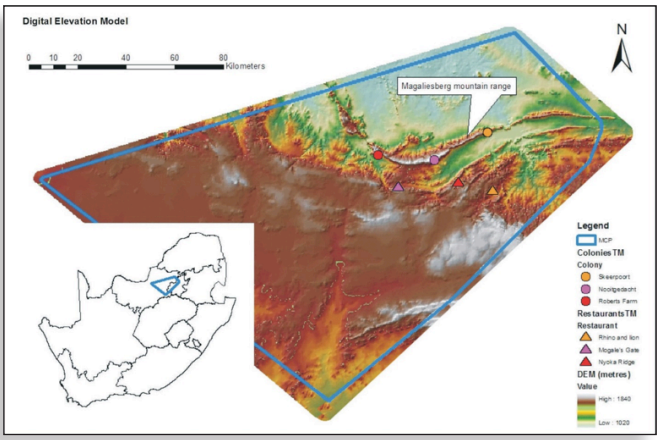


FIGURE 1. Illustration of the three colonies of Cape griffon vultures that have featured widely in rehabilitation efforts. The three colonies are found along the Magaliesberg mountain range. The three restaurants are areas where the birds are routinely fed. The Magaliesberg mountain range runs into Pretoria city, which lies approximately 20 kilometers due east of the vulture colonies.

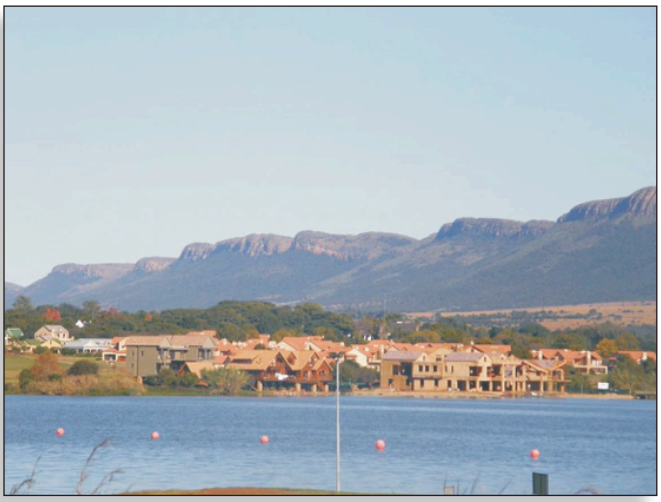


FIGURE 2. Photograph showing the development adjacent to the cliffs of the Magaliesberg, where the vulture colony resides.

Both species face major threats within the southern African region that include poisoning, electrocution, power-line collisions, habitat destruction, insufficient food resources, disruption of breeding sites and the illegal collection of body parts for traditional medicine.[2,6,10,11,17,20] In an effort to prevent the decline in the number of birds, various initiatives to contribute to the conservation of the species were put in place. One of these initiatives is the so-called vulture restaurants, which in collaboration with the local farming community provide carcasses to vultures to supplement their food resources.[17,20] Other efforts include educational material such as posters and booklets advising farmers on the use of being the true descendants of the vulture species. The latter, such as the condor and black vulture, have evolved from the stork family.[1,30] Despite their important role in ecosystem health, vulture numbers throughout the world have declined at a rapid rate with southern Africa being no exception, having seven of the nine species listed in the Red Data Book.[9,12,14,15,18,24,26]

Of the nine vulture species in southern Africa, the Cape griffon vulture *(Gyps coprotheres)* (CGV) and the African white-backed vulture *(G. africanus)* (AWBV), are the most abundant, with the former being near-endemic only to this region.[9,30] The CGV is currently listed as vulnerable and the AWBV near-threatened with the latest estimates placing the AWBV at 270,000 individual birds in Africa and the CGV at approximately 4,000 breeding pairs in southern Africa.[9] While the AWBV and CGV are of markedly different size (5.5 kg versus 9 kg), the species share a number of similarities that include lifetime pairing, breeding once a year with on average the production of one egg per year.[17] Phylogenetic studies suggest that the two species are genetically eco-friendly products and creating awareness around vulture poisonings, attempts by the national energy provider (ESKOM) to research and erect vulture-friendly pylons, the promotion of vulture eco-tourism and the rescue and/or rehabilitation (R&R) of injured or poisoned birds collected in the field. This paper focuses on the causes, the impact and successes of rescue and/or rehabilitation (R&R) efforts of birds as a valid component of vulture conservation in southern Africa in a semi-urban environment.

Materials and Methods

Study Area

The study area in Pretoria and the Magaliesberg mountain range is semi-urban and under intense housing development, e.g., a major golfing estate is present 1.5 km from a major breeding area on the Magaliesberg cliffs (Figs. 1 and 2). This area is home to 10% of the total CGV population and includes approximately 400 breeding pairs on the cliffs.[7] AWBVs are not known to breed within the study area.

Participating Organizations

The three organizations that contributed data to this study are the De Wildt Cheetah and Wildlife Trust (De Wildt), the National Zoological Gardens of South Africa (NZG), and the Vulture Programme of the Rhino and Lion Wildlife Conservation NPO (R&L). While undoubtedly vulture rehabilitation is occurring throughout the country, there was a concerted effort by these organizations to dedicated vulture conservation in the region. For the purposes of this study, rehabilitation was defined as the process of restoring sick or injured birds to a normal life by therapy and ensuring fitness prior to final release back into the wild. Rescue is similarly defined as rehabilitation, except that these birds were not released into the wild. The former was ascertained as a result of the tagging process which allowed for the resighting of released birds. To obtain an understanding of the management of the birds during rehabilitation, a description of each of the facilities follows:

De Wildt Cheetah and Wildlife Trust (De Wildt)

De Wildt focuses on cheetah conservation and breeding. In addition, endangered species such as the African wild dog and vultures are part of the collection. Vultures at this facility include CGVs (n=50) and AWBVs (n=8) that are housed in five 5m x 10m x 3m (L x B x H) aviaries. The birds are mainly grounded

with limited flight. Artificial perches comprising tree stumps and wooden logs are provided. The facility has housed birds for nearly 20 years, with animal recordkeeping extended back to 2002. Data are captured into a purpose-built Office Excel (MS Office) spreadsheet. Individuals of both vulture species were presented to the facility for rehabilitation and were either collected by De Wildt staff from the Magaliesberg colony and surrounding areas or received from outside organizations where more advanced veterinary care was lacking.

Veterinary care of injured birds was provided by a full-time veterinarian at an on-site veterinary clinic. Injured birds were housed in individual pens until released into the wild. For specialized veterinary procedures, assistance was provided by the Exotic Animal Clinic at the Onderstepoort Veterinary Academic Hospital (OVAH) run by the Faculty of Veterinary Science of the University of Pretoria. In most cases, after receiving specialist care, vultures were returned to De Wildt facility for after-care.

National Zoological Gardens of South Africa (NZG)

The NZG has a record of housing, conserving and exhibiting wildlife that extends over 100 years. The facility has a large aviary (30m× 20m×5 m) (L×B× H) housing both CGV and AWBV. The aviary mimics a natural environment with a number of natural trees as well as a raised cliff with ledges suitable for breeding. Birds presented to the facility for rehabilitation originated from collections made by NZG staff and the public from the Magaliesberg colony and surrounding areas. In addition, the organization accepted AWBVs as well as CGVs from their satellite facility in Lichtenburg which has an active vulture restaurant frequently used by both vulture species.

An on-site animal hospital and three full-time wildlife veterinarians are responsible for the daily health care of the animal collection. It also has an extensive animal record-keeping system linked to the International Species Information System (ISIS). As with De Wildt, the more specialized veterinary procedures were referred to the OVAH whereafter birds were returned to the facility for after-care. Rehabilitated birds were either included in the animal collection or traded within the zoo community. The NZG has been successful with the captive breeding of both vulture species since 2002 and in particular the artificial incubation of eggs and hand-rearing of nestlings.

Vulture Programme of the Rhino and Lion Wildlife Conservation NPO (R&L)

The R&L was established in 2006 with the aim of adding an important conservation component to vulture rehabilitation especially investigating the survival rate of newly released rehabilitated vultures. The facility has a number of different enclosures of various sizes, all of which are fitted with perches. The largest of the enclosures (40 m × 9 m × 7 m) (L×B×H) was sponsored by Eskom and enables the birds to fly to some extent to increase their fitness prior to release. Birds presented to the facility for rehabilitation were either collected by staff from the Magaliesberg colony or surrounding areas whereas mainly AWBVs requiring more advanced veterinary care were accepted from other organizations.



FIGURE 3. Adult Cape griffon vulture with a patagial tag on the right wing, metal ring on the right leg and a telemetric tracker on the back. The picture illustrates the ease of identification that the patagial tags offer over conventional rings, especially at distance.

The facility also has 6 enclosures (3 m ×3 m×6 m) (L×B×H) for the housing of individual injured or sick birds while recuperating. All veterinary treatments are provided by the OVAH or the Veterinary Department at the Johannesburg Zoological Gardens. All birds kept or released are identified using patagial tags on each wing (Fig. 3). The tags have visible numbers as well as contact details to facilitate monitoring. This facility follows a strict release protocol by returning birds they personally collect to the wild within seven days of recovery after receiving treatment.

Data Collection

Data collected include the number, sex and age (adult, subadult and juvenile, nestlings) (Table 1)17 where possible for all birds received for rehabilitation. Injuries were classified as soft tissue or skeletal injuries (fractures), exhaustion or poisoning. Surgical interventions where necessary were described as amputations (removal of 1 or both wings), fracture repair (surgical pinning), birds that died during surgery (anesthetic death) and birds euthanized for ethical or welfare reasons. The outcome of rehabilitation was assessed as the number of birds released. Data were collated from the patient records maintained by the respective organizations over the specified 10-year period and presented as the number and percentage of birds handled by each organization.

The same method for determining the age of vultures widely used by the vulture fraternity (although not statistically validated), was applied by all three facilities. This method makes use of external characteristics listed in Table 1. The De Wildt and R&L birds

TABLE 1: Methods used to age vultures, based on external characteristics.

FEATURE	ADULT VULTURE	JUVENILE VULTURE	SUBADULT
		CAPE GRIFFON	
IRIS	Straw-yellow	Black	Black
NECK SKIN	Appears bluish in colour and naked for much of its length	Coloured pink to magenta	
HEAD	Facial skin has a hint of blue. Head is covered in short white hairy feathers which generally point backwards.	Head is covered in woolly down	
FEATHERS	Contour or body feathers are plain creamy-white. Long scapulars and tertiaries down the adult's back are also distinctive in this regard, each having a broad white edge and a central blackish-brown patch. The naked 'eye' patches have a bluish skin which is often tinged with red around the perimeter, and they are surrounded by white down.	The naked 'eye' patches on the either side of the buggy crop and highlighted by their surrounds of white down, having the same skin colour as the neck. Contour feathers are pointed, streaked and generally browner in colour.	Features of the adult plumage slowly appear, but not all at once. Contours have a central black patch and the secondaries acquire pale undersides. Later the streaking almost disappears.
		AFRICAN WHITE-BACKED	
IRIS	Black	Black	Black
NECK SKIN	They have long necks covered in thin downy feathers. Neck skin is black.	Thickly covered in downy white cotton wool. The skin is yellowish-green with number of black spots, with bare patches on either side of the crop.	
HEAD	The down on the head is hairy looking. Bill and head skin are black.	Thickly covered in downy white feathers. The skin is yellowish green with number of black spots which coalesce in front of the eye.	
FEATHERS	The adults are variable in colour, a few being off-white and the most being variations of dark brown. The down on the head is hairy looking. The contour feathers are uniformly coloured and usually plain, with the last row of upperwing coverts being plain blackish brown. The backs of the animals are pure white.	The body and wing contour feathers are slate coloured with each feather having a white midrib.	New feathers are rounded. The ruff disintegrates and the contours show a paler hue from the 3rd or 4th year. In the 4th year, white feathers also appear on the back to completely change by 6 years age.

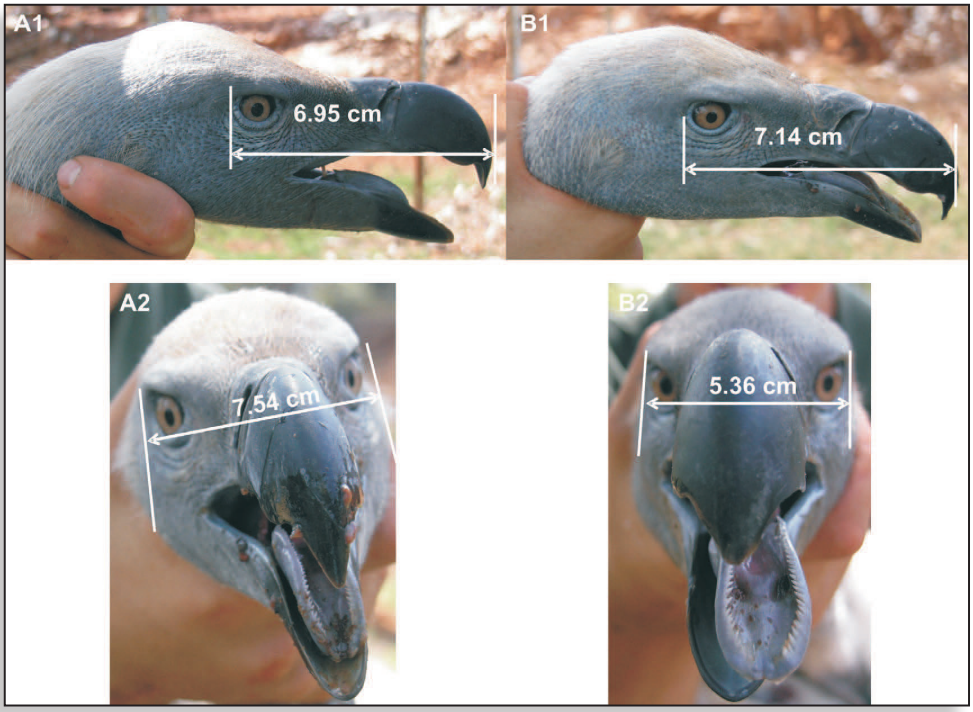


FIGURE 4. Illustration of the morphological differences between male (A1 & A2) and female (B1 & B2). Female heads are typically longer (B1) and narrow (B2), while the male heads are shorter (A1) and broader (A2).

were sexed according to the general morphological characteristics of the head (non-validated method). Females have a narrow head (as seen from the lateral canthus of the eyes) with egg-shaped dome at the top of the skull, while the males have a more triangular, flattened head with more prominent eye sockets (Fig. 4). The NZG makes use of laparoscopy and more recently DNA technology using Polymerase Chain Reaction (PCR) to determine sex.[7] Genomic DNA was isolated from blood collected in EDTA tubes

using the Qiagen DNeasy Blood and Tissue kit.® Amplification of the CHD1 gene was conducted using primer sets; 2550F: 5'-GTTACTGAT TCGTCTACGAGA-3' and 2718R: 5'-ATT GAAATGATCCAGTGCTTG-3'.27. PCR amplification was carried out in a total volume of 25 µ and included a no-template control as well as positive controls for a male and female bird of known sex. PCR was conducted with PR omega go Taq DNA polymerase®, which has a 1× buffer containing 10 mM Tris® HCl (pH 9.0), 50 mM potassium chloride (KCl), and 0.1 % Triton® X 100. The final reaction conditions were as follows: 1 x PCRbuffer, 1.5 mM MgCl2, 200 µ of each 2'deoxynucleotide triphosphate (dNTP), 5 pico mol (pmol) of each of the forward and reverse primer, 0.25 units (U) Taq DNA polymerase and 10 ng genomic DNA template. PCR cycling were as follows: Denaturation for 2 minutes (min) at 94 °C, followed by 30 cycles of denaturation for 30 seconds (s) at 94 °C, primer annealing for 30 s at 50 °C and extension for 2 min at 72 °C, followed by final extension at 72 °C for 10 min. PCR products were added to tracking dye and were separated by electrophoresis in a 2% agarose gel for 45 min at 100 volts in 1 x Tris-Borate-Edta buffer (TBE).

Results

A total of 163 CGVs and 38 AWBVs were received during the specified period by the three organizations. Age distribution for the CGV showed that 43.5 % were adults, 1.2 % subadult, 44.7 % juveniles and 9.4 % nestlings. The AWBV had a very similar distribution of adults and juveniles at 41.18 % and 47.6 % respectively. The sex ratios were evenly distributed for the CGV, and skewed towards males for the AWBV (Table 2).

De Wildt attempted rehabilitation of 85 (52.5 %) of the CGVs, mainly adult males (Table 2). The majority of the CGVs (n = 33) collected were recorded as power lines or pylon injuries which resulted in soft tissue injuries and in more severe cases, in fractures and ultimately amputations (Tables 3 and 4). In total, 26 of the 33 CGVs presented from power-line injuries required more advanced surgical intervention (Table 4). It is also interesting to note that a large proportion of birds were presented for minor reasons such as apparent exhaustion (n = 27) or birds picked up following heavy rain (n = 22). A total of 17 (52.5 %) AWBVs were collected, most of which were adult males (Table 2). Of these, 10 were presented as a result of power-line injuries and the rest were mainly healthy with the exception of 1 chick which had fallen from a nest (Table 3). A large percentage of the AWBVs presented with injuries that required surgical interventions. This high incidence is most likely attributable to birds requiring advanced veterinary care that were collected from outside the Gauteng and North West Provinces by rehabilitation agencies. One bird was brought in for organophosphate poisoning. This specific vulture was treated with fluids and atropine and eventually recovered. In total 40 (47.1 %) CGVs and three (17.6 %) AWBVs were ultimately released and the rest remained in captivity (Table 5).

The NZG received a total of 32 (19.8 %) CGVs comprising 94 % adult birds of which most were males. Injuries were mainly due to suspected pylon collisions as the birds were found on the ground in close proximity to power lines (Tables 2 and 3). The NZG released 2 CGVs following treatment (Table 5). The AWBVs presented to the NZG were mainly healthy birds received from welfare organizations (Tables 2 and 3). One of the AWBVs was diagnosed with skeletal abnormalities (cortical thinning with decreased radio density) that were consistent with a calcium deficiency. The other was brought in due to a pylon injury and the third was rescued after being kept as an exotic pet. The latter had its wings clipped and was not able to fly. There is no history as to how the wild bird ended up as a pet. None of the AWBVs required surgical intervention for their rehabilitation (Table 4).

The R&L accounted for 45 (27.8%) and 10 (26.3%) of the CGVs and AWBVs respectively, comprising mainly juveniles and nestlings. Of this, a large number were received after rainy conditions which also explains the high percentage of unknown sexes for the CGV at R&L (Table 2). Although 66 % of the CGVs were received due to an inability to fly, no detectable anatomical injuries were found on clinical examination (Table 3). These birds recovered within 2–3 days after having been fed. Seven birds had to be euthanized due to severe wound contaminations and severe complications (myiasis and bacterial infection) (Table 3). In one incident, six AWBVs were presented for suspected arsenic poisoning. These birds originated from a colony of 15 birds of which nine were found dead (dead birds are not included in this study). Arsenic poisoning was diagnosed from necropsy results which showed hemorrhagic gastroenteritis, clinical signs of excitability of the live birds and recovery following treatment with penicillamine. The R&L released a total of 21 of the 45 (46.7 %) rehabilitated CGVs and seven of the 10 AWBVs (70 %) (Table 5).

Discussion

The aim of this study was to describe the efforts to rescue and/or rehabilitate vultures in the Magaliesberg area of South Africa by three organizations. Although the AWBV is the most abundant vulture species in southern Africa, nine rehabilitation was dominated by the CGV within the three centers. This most likely reflects a difference in the behavior of the two species, in that the AWBV nests in trees and the CGV on cliffs. The Magaliesberg mountain range running through the greater Pretoria area and Gauteng region provides ample nesting opportunities for the CGV.

In total, 63 (38.9 %) CGVs and 10 (26.3 %) AWBVs were rehabilitated, while 68 (42.0 %) CGVs and 25 (65.8 %) AWBVs were retained in captivity. The reason for the greater skew towards retaining a bird in a captive environment stems from the huge responsibility and effort that rehabilitation requires. For rehabilitation, the major factor to consider is the fitness of the bird for free flight and survival, i.e. loss of muscle tone for flight and increase in body mass due to the deposition of fat from general inactivity. The R&L with a 51% release rate assesses the fitness level of birds by their ability to fly in a large training aviary before release. Another factor that had a major impact on rehabilitation was the

TABLE 2: The number, age, and sex of birds that were presented for rehabilitation. Values in brackets are percentages.

Gyps coprotheres (CGV)																
	N		SEX						AGE							
ORGANIZATION			MALE		FEMALE		UNKNOWN		ADULT		SUBADULT		FLEDGLING		UNKNOWN	
De Wildt	85	(52.47)	38	(63.33)	34	(61.82)	13	(27.66)	37	(52.11)	39	(70.91)	8	(22.86)	1	(100.00)
R&L	45	(27.78)	5	(8.33)	9	(16.36)	31	(65.96)	4	(5.63)	14	(25.45)	27	(77.14)	0	
NZG	32	(19.75)	17	(28.33)	12	(21.82)	3	(6.38)	30	(42.25)	2	(3.64)	0		0	
Total	162		60		55		47		71		55		35		1	
Gyps africanus (AWBV)																
	N		SEX						AGE							
ORGANIZATION			MALE		FEMALE		UNKNOWN		ADULT		SUBADULT		FLEDGLING		UNKNOWN	
De Wildt	17	(44.74)	9	(47.37)	3	(27.27)	5	(62.50)	7	(33.33)	8	(57.14)	1	(50.00)	1	(100.00)
R&L	10	(26.32)	5	(26.32)	4	(36.36)	1	(12.50)	3	(14.29)	6	(42.86)	1	(50.00)	0	
NZG	11	(28.95)	5	(26.32)	4	(36.36)	2	(25.00)	11	(52.38)	0		0		0	
Total	38		19		11		8		21		14		2		1	

R&L: Rhino & Lion Wildlife Conservation Non-Profit Organisation; NZG: National Zoological Gardens of South Africa.

TABLE 3: Conditions managed during rehabilitation by the respective organisations. Values in brackets are percentages.

Gyps coprotheres (CGV)												
ORGANIZATION	N		SOFT TISSUE INJURIES		SKELETAL INJURIES		EXHAUSTION		COMPROMISED*		POISONING	
De Wildt	85	(52.47)	7	(43.75)	26	(57.78)	27	(90.00)	22	(34.38)	3	(42.86)
R&L	45	(27.78)	0	(0.00)	11	(24.44)	3	(10.00)	31	(48.44)	0	(0.00)
NZG	32	(19.75)	17	(28.33)	12	(21.82)	3	(6.38)	30	(42.25)	2	(3.64)
Total	162		60		55		47		71		55	
Gyps africanus (AWBV)												
ORGANIZATION	N		SOFT TISSUE INJURIES		SKELETAL INJURIES		EXHAUSTION		COMPROMISED*		POISONING	
De Wildt	17	(44.74)	3	(50.00)	8	(80.00)	0		5	(38.46)	1	(11.11)
R&L	10	(26.32)	1	(16.67)	1	(10.00)	0		0	(0.00)	8	(88.89)
NZG	11	(28.95)	2	(33.33)	1	(10.00)	0		8	(61.54)	0	(0.00)
Total	38		6		10		0		13		9	

R&L: Rhino & Lion Wildlife Conservation Non-Profit Organisation; NZG: National Zoological Gardens of South Africa.
*Birds unable to fly due to unknown causes.

degree of injury, as evident from the poor release rate of birds with fractures—severe cases either required amputation or orthopedic surgery. For both cases flight is not possible, the former for obvious reasons, while the latter usually resulted in slight bone malapposition that subsequently interfered with the precise aerodynamics required for sustainable long-distance flight.[21] Unfortunately, available avian orthopedic techniques are still very inadequate to meet the precise requirements for flight.

An interesting observation from this study was the large number of apparently healthy birds presented for treatment after heavy rains. The flight feathers of the birds appear to be water-logged and these birds are unable to take off after heavy rains, which is believed to have resulted from a combination of the birds being overweight from the constant feeding at vulture restaurants (14 kg has been recorded for this region for CGV dry weight) and the feathers soaked with water. The latter is not an oddity as the popular press commonly reports waterlogged feathers as a major obstacle to flight. When the increased weight from excessive caloric intake is combined with the flight behavior of vultures, an interesting aerodynamic challenge is faced by birds—already being heavy fliers, vultures are predominantly reliant on thermal currents for take-off, soaring and mobility in an attempt to conserve energy to compensate for their weight to energy/aerodynamic requirements for flight.[19,23] The added burden of being overweight in combination with the additional weight of the water interferes further with their flight characteristics. Unfortunately, this also translates to non-flight in the absence of thermal currents, which restricts soaring to mid-mornings when the first thermals arise, i.e. vultures are rarely seen soaring on days characterized by overcast and cool conditions. Healthy vultures presented on rainy days also

TABLE 4: Number of birds in which surgery was attempted by the different organisations. Values in brackets are percentages.

ORGANIZATION	N	AMPUTATIONS	FRACTURE REPAIR	DIED DURING SURGERY	EUTHANIZED DUE TO POOR PROGNOSIS
Gyps coprotheres (CGV)					
De Wildt	26 (60.47)	15 (83.33)	11 (57.89)	0	0
R&L	11 (25.58)	1 (5.56)	3 (15.79)	0	7 (100)
NZG	8 (18.60)	2 (11.11)	5 (26.32)	1 (100)	0
Total	43	18	19	1	7
Gyps africanus (AWBV)					
De Wildt	8 (88.89)	8 (88.89)	0	0	0
R&L	1 (11.11)	1 (11.11)	0	0	0
NZG	0	0	0	0	0
Total	9	9	0	0	0

R&L: Rhino & Lion Wildlife Conservation Non-Profit Organisation; NZG: National Zoological Gardens of South Africa.

TABLE 5: Different outcomes of the rehabilitated birds. Values in brackets are percentages.

ORGANIZATION	N	RELEASED	KEPT IN CAPTIVITY	TRADED	EUTHANIZED
Gyps coprotheres (CGV)					
De Wildt	85 (52.47)	40 (63.49)	38 (55.88)	6 (35.29)	1 (7.14)
R&L	45 (27.78)	21 (33.33)	10 (14.71)	2 (11.76)	12 (85.71)
NZG	32 (19.75)	2 (3.17)	20 (29.41)	9 (52.94)	1 (7.14)
Total	162	63	68	17	14
Gyps africanus (AWBV)					
De Wildt	17 (44.74)	3 (30.00)	14 (56.00)	0	0
R&L	10 (26.32)	7 (70.00)	2 (8.00)	1 (33.33)	0
NZG	11 (28.95)	0	9 (36.00)	2 (66.67)	0
Total	38	10	25	3	0

R&L: Rhino & Lion Wildlife Conservation Non-Profit Organisation; NZG: National Zoological Gardens of South Africa.

suggest that the birds were grounded and unable to fly as opposed to being injured or dehydrated. This highlights an added danger that vulture colonies have to face because of a close association with human habitation. It is likely that this would not occur in the wild and that the birds would normally return to their roosts if provided with sufficient time to fly again.

Another explanation for their non-flight may be mild poisoning that resulted from their feeding on contaminated carcasses. The one group of toxins that could explain the clinical signs observed are the organophosphorus, which are known to be inducers of muscle weakness. For the past 20 years the NZG has received a number of raptors, ranging from owls, hawks, eagles and one vulture (n = 28) which presented either in a state of collapse or with mild symptoms of incoordination and general weakness. Symptomatically, it was suspected that these birds had ingested poison, but this was never confirmed by laboratory tests. Some birds recovered well with treatment and feeding and others died, but post mortem results were inconclusive of poisoning. A white-backed vulture that had collapsed and had head tremors recovered dramatically after it was treated with atropine. It is therefore speculated that general weakness in vultures could be due to ingestion of low doses of a toxin.

The rehabilitation data clearly indicate that pylons were the major cause of injuries as demonstrated by the high number of soft tissue and skeletal injuries observed. It is speculated that this may be due to the inability of the birds to see power lines, as seen in other bird species, combined with the likelihood that they are unable to gain sufficient altitude rapidly enough to avoid power lines near their foraging sites.[3] In total, 61 (38 %) CGVs and 16 (42%) AWBVs were presented with these injuries. These observations therefore tend to support the earlier reported observations that the population declines of the CGV may be attributed to pylon injuries, but this is not necessarily the only cause.[27]

Concerning the higher number of releases of rehabilitated vultures by the R&L and De Wildt, it is clear that successful release of the rehabilitated birds is possible. More importantly, it emphasizes that contrary to popular belief, vultures do not immediately imprint on handlers. When the release data of R&L were evaluated for any correlation between duration within a capture enclosure to release (results not shown), none was found. Nonetheless, the release data need to be interpreted with care, as success is defined as the failure to find a vulture carcass soon after release, i.e. inaccuracies could result from jackal or other predators devouring dead vultures before the carcasses are found. In an attempt to better investigate the impact of rehabilitation and release for future projects if funding permits, it may be of value to attach telemetric trackers (cellular or satellite) at the point of release. For the birds on which the R&L had cellular trackers attached, the

death of the released bird was established by non-movement of the GPS tracker over a few days. The GPS trackers also to a large extent overcome the shortcomings of patagial tags that may not be sighted as a result of death, non-movement through a monitoring point, or loss of the tag.

Conclusions

With the huge inputs and dedication by various organizations, there is now a better understanding of the causes, impact and success of R&R of vultures. Of concern are the rehabilitation figures from De Wildt and R&L, which account for more than 120 birds out of a colony of just under 380 breeding pairs (17% of the total population required medical attention) recorded over the last 10 years. This study provides first evidence of the impact urbanization has had on vultures from the Magaliesberg and surrounding areas. While the data clearly indicate that rehabilitation efforts are required, they may not necessarily be having their desired impact, as the percentage of released birds remains relatively low (47.1 % for CGV and 17.6 % for AWBV). Nonetheless, the true value of rehabilitation efforts is their ability to protect the species, as even a single bird that is saved represents continued maintenance of genetic diversity within the threatened population.

Acknowledgements

Our sincere gratitude to our sponsors: Rand Merchant Bank, Computer Facilities, Tony & Lisette Lewis Foundation, Lomas Wildlife Protection Trust, Eskom, Aberdare Cables, and Natural Encounters Inc., as well as veterinarians and volunteers who have participated in the vulture rehabilitation efforts. We would also like to acknowledge the relevant provincial agencies for their support towards this effort.

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Huge extents of formerly unbroken forest, experts say, have already been lost to conversion to monoculture crops, cattle ranching, and hydro-electric, mining, and transportation projects. Development is inevitable in the Amazon, so the question becomes how to best ensure both sustainable development for local communities along with a secure future that includes the area's icon of functional ecosystems: the jaguar.

Recognizing the need to address that challenge, several leading Latin American conservation organizations working in the Amazon basin recently met in Quito, Ecuador, to review regional jaguar conservation efforts and outline priorities to maintain healthy jaguar populations in the Amazon in perpetuity. The result of the meeting is a document titled titled "Memorias Del Taller Internacional Planificando La Conservación Del Jaguar En La Amazonía."

The unique assemblage included experts from the Instituto Nacional de Pesquisas da Amazônia, Instituto de Desenvolvimento Sustentável Mamirauá, Instituto de Investigación de Recursos Biológicos Alexander von Humboldt, Fundación Omacha, Universidad Nacional de Colombia, Ministerio del Ambiente de Ecuador, Pontificia Universidad Católica del Ecuador, Universidad San Francisco de Quito, E.tech, TRAFFIC, World Wildlife Foundation (WWF), Panthera and WCS.

The participants drew on their extensive experience to develop a plan around five central themes to help secure the long-term future of jaguars: Landscape and Corridor Scale Conservation; Research and Monitoring; Conflict Management; Legislation, Policy and Administration; and Education, Training and Communication.

"The main recommendations to come out of the meeting were related to the importance of working at large landscape scales to conserve meaningful populations of jaguars," said Dr. Rob Wallace, Amazon Landscape Conservation Expert at WCS.

"This landscape approach requires an integrated threats-based strategy involving a series of long-term partnerships with territorial stakeholders such as protected areas, indigenous territories, municipal governments and others. WCS is proud of our long-standing conservation commitments to some of the most outstanding natural wilderness areas in the Amazon."



PHOTO © NATHAN RUPERT. CC BY-NC-ND 2.0 LICENSE.

Jaguar *(Panthera onca).*

Dr. Emiliano Esterci Ramalho, Monitoring Coordinator at the Mamiraua Sustainable Development Institute and a groundbreaking jaguar researcher in the flooded forests of central Amazonian Brazil underlined the importance of collective conservation efforts.

"This meeting encouraged us to create the Jaguar Conservation Alliance in Brazil, a multi-institutional initiative that aims to coordinate jaguar research and conservation efforts in the Amazon, and to ensure that our collective efforts amount to more than just the sum of their parts," Ramalho said.

The document also includes a post-workshop addition highlighting the emerging threat of hunting and illegal trade of jaguars in the Amazon and beyond. Forty years ago, jaguars benefited from international trade policy decisions such as the inclusion in 1975 of the jaguar in Appendix I of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) that prohibited all commercial trade in spotted cat skins for the international fur trade. Since then, in areas with effective conservation measures, jaguar populations have stabilized and in a number of cases, bounced back. However, conservation is an ongoing process of vigilance and actions to counter new threats. Jaguar hunting for trade has re-emerged, this time for teeth and other body parts for markets in Asia.

"Protecting and restoring critical habitats that also provide connectivity between habitats are the main goals for which we have to work together in order to ensure the survival of jaguars in the Amazon River Basin," said Diego Amorocho, WWF Species Program Coordinator Latin America & Caribbean.

"The globally significant Amazon jaguar population merits strong international cooperation not only to proactively maintain its habitat and prey, but also to actively and effectively counter any resumption in trade in jaguar parts," said Dr. John Polisar, Jaguar Conservation Program Coordinator for WCS.

U.S. Releases New Wildlife Harm Mitigation Plan

WASHINGTON, DC (November 18)—The U.S. Fish and Wildlife Service has announced a final revised Mitigation

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SELECTED ABSTRACTS

The bear circadian clock doesn't 'sleep' during winter dormancy

H Jansen, T Leise, G Stenhouse, K Pigeon, W Kasworm, J Teisberg, T Radandt, R Dallmann, S Brown and C Robbins. *Frontiers in Zoology.* 2016;13:42. doi: 0.1186/s12983-016-0173-x.

Most biological functions are synchronized to the environmental light:dark cycle via a circadian timekeeping system. Bears exhibit shallow torpor combined with metabolic suppression during winter dormancy. We sought to confirm that free-running circadian rhythms of body temperature (Tb) and activity were expressed in torpid grizzly (brown) bears and that they were functionally responsive to environmental light. We also measured activity and ambient light exposures in denning wild bears to determine if rhythms were evident and what the photic conditions of their natural dens were. Lastly, we used cultured skin fibroblasts obtained from captive torpid bears to assess molecular clock operation in peripheral tissues. Circadian parameters were estimated using robust wavelet transforms and maximum entropy spectral analyses.

Results: Captive grizzly bears housed in constant darkness during winter dormancy expressed circadian rhythms of activity and Tb. The rhythm period of juvenile bears was significantly shorter than that of adult bears. However, the period of activity rhythms in adult captive bears was virtually identical to that of adult wild denning bears as was the strength of the activity rhythms. Similar to what has been found in other mammals, a single light exposure during the bear's active period delayed subsequent activity onsets whereas these were advanced when light was applied during the bear's inactive period. Lastly, in vitro studies confirmed the expression of molecular circadian rhythms with a period comparable to the bear's own behavioral rhythms.

Conclusions: Based on these findings we conclude that the circadian system is functional in torpid bears and their peripheral tissues even when housed in constant darkness, is responsive to phase-shifting effects of light, and therefore, is a normal facet of torpid bear physiology.



Grizzly bear family *(Ursus arctos).*
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Everyday bat vocalizations contain information about emitter, addressee, context, and behavior

Y Prat, M Taub, and Y Yovel. *Scientific Reports 6.* 2016; Article number 39419. doi:10.1038/srep39419.

Animal vocal communication is often diverse and structured. Yet, the information concealed in animal vocalizations remains elusive. Several studies have shown that animal calls convey information about their emitter and the context. Often, these studies focus on specific types of calls, as it is rarely possible to probe an entire vocal repertoire at once. In this study, we continuously monitored Egyptian fruit bats for months, recording audio and video around-the-clock. We analyzed almost 15,000 vocalizations, which accompanied the everyday interactions of the bats, and were all directed toward specific individuals, rather than broadcast. We found that bat vocalizations carry ample information about the identity of the emitter, the context of the call, the behavioral response to the call, and even the call's addressee. Our results underline the importance of studying the mundane, pairwise, directed, vocal interactions of animals.

Associated article in *Nature*: http://tinyurl.com/zdouldx.

Effects of the social environment during adolescence on the development of social behaviour, hormones and morphology in male zebra finches *(Taeniopygia guttata)*

S Bölting and N von Engelhardt. *Frontiers in Zoology.* 2017;14:5. doi:10.1186/s12983-017-0190-4

Individual differences in behavior are widespread in the animal kingdom and often influenced by the size or composition of the social group during early development. In many vertebrates the effects of social interactions early in life on adult behaviour are mediated by changes in maturation and physiology. Specifically, increases in androgens and glucocorticoids in response to social stimulation seem to play a prominent role in shaping behaviour during development. In addition to the prenatal and early postnatal phase, adolescence has more recently been identified as an important period during which adult behaviour and physiology are shaped by the social environment, which so far has been studied mostly in mammals. We raised zebra finches (*Taeniopygia guttata*) under three environmental conditions differing in social complexity during adolescence—juvenile pairs, juvenile groups, and mixed-age groups—and studied males' behavioural, endocrine, and morphological maturation, and later their adult behaviour.

Results: As expected, group-housed males exhibited higher frequencies of social interactions. Group housing also enhanced song during adolescence, plumage development, and the frequency and intensity of adult courtship and aggression. Some traits, however, were affected more in juvenile groups and others in mixed-age groups. Furthermore, a testosterone peak during late adolescence was suppressed in groups with adults. In contrast, corticosterone concentrations did not differ between

rearing environments. Unexpectedly, adult courtship in a test situation was lowest in pair-reared males and aggression depended upon the treatment of the opponent with highest rates shown by group-reared males towards pair-reared males. This contrasts with previous findings, possibly due to differences in photoperiod and the acoustic environment.

Conclusion: Our results support the idea that effects of the adolescent social environment on adult behavior in vertebrates are mediated by changes in social interactions affecting behavioural and morphological maturation. We found no evidence that long-lasting differences in behaviour reflect testosterone or corticosterone levels during adolescence, although differences between juvenile and mixed-age groups suggest that testosterone and song behavior during late adolescence may be associated.

Start early! Does social instability during the pre- and early postnatal development prepare male wild cavies for social challenge later in life?

K Siegeler, L Lewejohann, K Failing, N Sachser, and S Kaiser. *Frontiers in Zoology.* 2017;14:2 doi:10.1186/s12983-016-0187-4.

Background: The social environment the mother experiences during pregnancy and lactation can powerfully influence the offspring's behavioural profile. Our previous studies in wild cavies show that two different social environments during pregnancy and lactation bring about different behavioural strategies of male offspring later in life: An unstable social environment leads to a behavioural camouflage strategy, hypothesised to be beneficial at times of socially challenging situations. A stable social environment during early phases of life, however, leads to an early reproduction strategy, expected to be more successful at times of social stability. In the present study, we observed the behavioural strategies of the two types of males in direct comparison in a socially challenging situation: Two adolescent males were placed simultaneously in an unknown social group consisting of one adult male and two females in a semi-naturalistic environment. Cortisol as well as testosterone concentrations and activity levels were compared. Furthermore, paternities were analysed after the males reached sexual maturity. We hypothesised that sons showing a behavioural camouflage strategy are better adapted to cope with this socially challenging situation compared to those displaying an early reproduction strategy.



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Common snipe *(Gallinago gallinago).*

Results: At the beginning of the experiment, no differences in plasma cortisol concentrations between the males were found, both showed a highly significant increase due to the challenging situation. From day five until the end of the experiment (duration = 40 days) sons showing an early reproduction strategy had significantly higher plasma cortisol concentrations compared with those showing a behavioural camouflage strategy. Plasma testosterone concentrations did not differ significantly. Activity levels decreased significantly over time independently of the male's behavioural strategy. Both types of males did not sire offspring during the observation period.

Conclusion: Higher cortisol values from day five until the end of the experiment in sons showing an early reproduction strategy indicate higher levels of stress in these males compared to those displaying a camouflage strategy. We conclude that the modulation of the males behavioural strategy due to an unstable social environment during early development facilitates the endocrine adaptation to a comparable social situation later in life.

Plumage quality mediates a life-history trade-off in a migratory bird

P Podlaszczuk, M Kamiński, R Włodarczyk, K Kaczmarek, T Janiszewski and P Minias. *Frontiers in Zoology.* 2016;13:47 doi:10.1186/s12983-016-0179-4

Background: Moult is one of the most costly activities in the annual cycle of birds and most avian species separate moult from other energy-demanding activities, such as migration. To this end, young birds tend to undergo the first post-juvenile moult before the onset of migration, but in some species the time window for the pre-migratory feather replacement is too narrow. We hypothesized that in such species an increased investment in the structural quality of juvenile feathers may allow to retain juvenile plumage throughout the entire migratory period and delay moult until arriving at wintering grounds, thus avoiding a moult-migration overlap.

Methods: The effect of juvenile plumage quality on the occurrence of moult-migration overlap was studied in a migratory shorebird, the common snipe. Ca. 400 of first-year common snipe were captured during their final stage of autumn migration through Central Europe. The quality of juvenile feathers was assessed as the mass-length residuals of retained juvenile rectrices. Condition of migrating birds was assessed with the mass of accumulated fat reserves and whole-blood hemoglobin concentration. Path analysis was used to disentangle complex interrelationships between plumage quality, moult and body condition.

Results: Snipe which grew higher-quality feathers in the pre-fledging period were less likely to initiate moult during migration. Individuals moulting during migration had lower fat loads and hemoglobin concentrations compared to non-moulting birds, suggesting a trade-off in resource allocation, where energetic costs of moult reduced both energy reserves available for migration and resources available for maintenance of high oxygen capacity of blood.

Conclusions: The results of this study indicate that a major life-history trade-off in a migratory bird may be mediated by the quality of juvenile plumage. This is consistent with a silver spoon effect, where early-life investment in feather quality affects future performance of birds during migration period. Our results strongly suggest that the juvenile plumage, although retained for a relatively short period of time, may have profound consequences for individuals' fitness.



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American badger *(Taxidea taxus).*

The occurence of pathogens in an endangered population of American badgers *(Taxidea taxus jacksoni)* in Ontario, Canada

D Ethier, J Sayers, C Kyle, J Nocera, D Ojkic, and D Campbell. *Journal of Wildlife Diseases.* January 2017;53(1)1:73-80.

American badgers *(Taxidea taxus jacksoni)* at the periphery of the species' range in Ontario, Canada, are listed as endangered because of an estimated population size of <200 mature individuals. The main threats faced by this population include habitat loss and road mortality. However, on 18 November 2013, a radio-implanted badger was found nonresponsive in an agricultural field with signs consistent with canine distemper virus infection, which was subsequently confirmed. This prompted our investigation into the occurrence of pathogens in this endangered carnivore to better quantify the level of risk infectious disease poses to population persistence. We examined serum samples from nine live-trapped individuals and 27 whole badger specimens submitted for postmortem examination. We found evidence of exposure to canine distemper virus, canine parvovirus, and leptospires. However, infection associated with disease was not the leading cause of mortality. Future research into the effects of disease on kit survival and a comprehensive understanding of disease severity and spread from reservoir populations (e.g., raccoons [*Procyon lotor*] and striped skunks [*Mephitis mephitis*]) to badgers will be of particular importance to the conservation of this endangered population.

Sarcoptic mange in endangered kit foxes *(Vulpes macrotis mutica)*: Case histories, diagnoses, and implications for conservation

B Cypher, J Rudd, T Westall, L Woods, N Stephenson, J Foley, D Richardson, and D Clifford. *Journal of Wildlife Diseases.* 2017;53(1): 46-53. doi: http://dx.doi.org/10.7589/2016-05-098.

The San Joaquin kit fox (*Vulpes macrotis mutica*) is a federally endangered small carnivore whose distribution is limited to the San Joaquin Valley in central California. Population decline is due to profound habitat loss, and conservation of all remaining populations is critical. A robust urban population occurs in the city of Bakersfield. In spring of 2013, putative cases of mange were reported in this population. Mites from affected animals were confirmed to be Sarcoptes scabiei morphologically and by DNA sequencing. By the end of 2014, 15 cases of kit foxes with mange had been confirmed. As with other species, sarcoptic mange in kit foxes is characterized by intense pruritus and dermatitis, caused by mites burrowing into the epidermal layers, as well as alopecia, hyperkeratosis, and encrustations, secondary bacterial infections, and finally extreme morbidity and death. Of the 15 cases, six foxes were found dead, six were captured but died during attempted rehabilitation, and three were successfully treated. We have no evidence that untreated kit foxes can recover from mange. Sarcoptic mange constitutes a significant threat to the Bakersfield kit fox population and could pose an even greater threat to this imperiled species if it spreads to populations in nearby natural lands.

Antimicrobial resistance of salmonella serovars and campylobacter spp. isolated from an opportunistic gull species, yellow-legged gull *(Larus michahellis)*

L Migura-Garcia, R Ramos and M Cerdà-Cuéllar. *J Wildl Dis.* 2017 Jan;53(1):148-152. doi: 10.7589/2016-03-051. Epub 2016 Oct 10.

Wildlife is a natural reservoir of Salmonella and Campylobacter, the most important human foodborne pathogens worldwide. Free-living birds have the potential to transport, over large distances, such zoonotic bacteria that may harbor antimicrobial resistance traits. On the northeastern Iberian coast, we assessed the role of yellow-legged gulls *(Larus michahellis)* as reservoirs of antimicrobial resistance in salmonella and thermophilic campylobacter isolates recovered from gulls at three colonies, with varying degrees of dependence on refuse dumps as food sources. Of the 39 salmonella isolates we tested, 17 were multiresistant (resistance

to three antimicrobial families), with eight being *Salmonella enterica* serovar *typhimurium*. Other clinically relevant salmonella serovars showing multiresistance included *Hadar, Redeney*, and *Virchow*. Relevant campylobacter antimicrobial resistances were detected among three *Campylobacter jejuni* isolates, of which all three showed resistance to nalidixic acid, two were resistant to ciprofloxacin, one was resistant to enrofloxacin, and one was resistant to tetracycline. Our results highlight the importance of free-living gulls with opportunistic feeding habits in the dissemination of enteric pathogens resistant to multiple antimicrobial agents of public health concern.

Abdominal cysticercosis in a red fox *(Vulpes vulpes)*

C Whipp, P Daoust, G Conboy, and H Gelens (2017). *Journal of Wildlife Diseases*. January 2017;53(1):197-199. doi: http://dx.doi.org/10.7589/2016-03-058

A large abdominal mass containing numerous cysticerci identified as those of *Taenia crassiceps* (*Cysticercus longicollis*) was found in the pelvic region of the abdominal cavity of a severely constipated and emaciated red fox (*Vulpes vulpes*) in Prince Edward Island, Canada. Cysticercosis has not previously been reported in a wild canid in North America.

Social status alters immune regulation and response to infection in macaques

N Snyder-Mackler, J Sanz, J Kohn, J Brinkworth, S Morrow, A Shaver, J Grenier, R Pique-Regi, Z Johnson, M Wilson, et al. *Science*. 2016;354(6315):1041-1045. doi:10.1126/science.aah3580 1041-1045.

Social status is one of the strongest predictors of human disease risk and mortality, and it also influences Darwinian fitness in social mammals more generally. To understand the biological basis of these effects, we combined genomics with a social status manipulation in female rhesus macaques to investigate how status alters immune function. We demonstrate causal but largely plastic social status effects on immune cell proportions, cell type–specific gene expression levels, and the gene expression response to immune challenge. Further, we identify specific transcription factor signaling pathways that explain these differences, including low-status–associated polarization of the Toll-like receptor 4 signaling pathway toward a proinflammatory response. Our findings provide insight into the direct biological effects of social inequality on immune function, thus improving our understanding of social gradients in health. ■

News

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Policy that will guide its review of potential impacts of land and water development projects on America's wildlife and their habitats. Through this policy, the Service will help others mitigate (avoid, minimize, and compensate) for a project's impacts to species and their habitats. This update of the Service's longstanding Mitigation Policy, which has guided agency recommendations since 1981, will provide a broad and flexible framework to facilitate conservation that addresses the potential negative effects of development, while allowing economic activity to continue.

The revisions implement a recent Presidential Memorandum directing certain federal agencies to adopt a common set of best practices to minimize the harmful impacts to wildlife and other ecological resources caused by land- or water-disturbing activities, and to ensure that any remaining harmful effects are appropriately addressed or mitigated. The revisions also implement a recent Secretarial Order on improving mitigation policies and practices within the Department of the Interior.

"Development without any consideration for the needs of wildlife and their habitats serves no one's long-term interests," said Service Director Dan Ashe. "But with robust mitigation, economic activity can move forward and still meet the highest conservation standards. As we look for ways to address the daunting challenges of the 21st century, this policy will help both us and project proponents successfully and strategically offset impacts to fish and wildlife and help maintain healthy ecosystems."

The revised policy is intended to be a single umbrella policy under which more detailed Service policies or guidance documents covering specific activities may be issued in the future. It expands the focus of mitigation to address larger-scale stresses such as climate change and invasive species that can work in tandem with development to adversely affect wildlife.

The policy applies to all authorities under which the Service can make mitigation recommendations, including the Service's authority to protect species listed under the Endangered Species Act, which was excluded from the 1981 policy. It also establishes a goal of achieving a net conservation gain, or at a minimum, no net loss, when recommending project mitigation, whenever the situation merits and doing so is allowed by law.

The revised policy establishes a new approach that will promote the most effective and efficient mitigation measures to be implemented across the landscape. This will require improved collaboration and coordination between all interested parties and effective integration of mitigation planning and landscape-level conservation strategies.

The revised policy became effective upon publication in the *Federal Register* on November 21, 2016. ■

TAIL END



Mom, she's touching me!

Red Squirrel *(Sciurus vulgaris).*

PHOTO © JAMES HAVARD, FLICKR. CC BY 2.0.

INSTRUCTIONS FOR AUTHORS

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:

Kieran Lindsey, Editor
jwr.editor@theiwrc.org

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Include the name of each author. Specify the corresponding author and provide affiliation, complete mailing address, and email address. The affiliation for all authors should be included in a brief (maximum of 100 words) biography for each that reflects professional experience related to rehabilitation or to the manuscript subject matter rather than personal information. Biographies may be edited due to space limitations.

Include an abstract that does not exceed 175 words and choose several (up to 14) key words.

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