

JOURNAL OF WILDLIFE REHABILITATION

Volume 42, Number 2, 2022

IWRC
INTERNATIONAL WILDLIFE
REHABILITATION COUNCIL

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



LEFT:

Chimpanzee *(Pan troglodytes)*. In 2021, The Lwiro Primates Rehabilitation Center saw its highest number of intakes since its inception.

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ON THE COVER:

Red-shouldered hawk (*Buteo lineatus*), one of a number of raptor species found with chlamydia in rehabilitation.

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INTERNATIONAL WILDLIFE REHABILITATION COUNCIL

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

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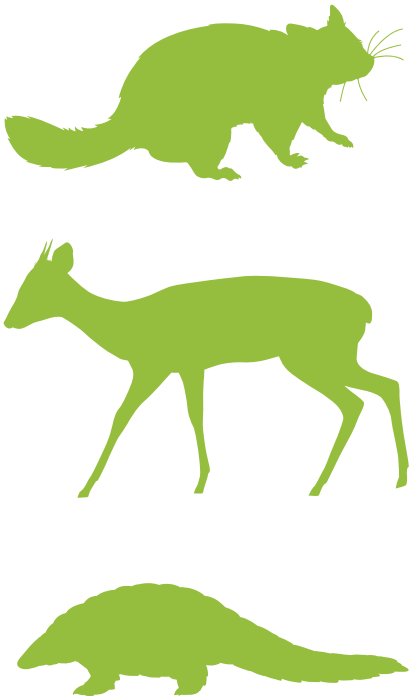
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EDITORIAL

Hello new logo



Our lovely new emblem evokes the animals of wildlife rehabilitation, from desert to sea. We had quite some fun working with the designer to draw up a list that represents the breadth of taxa commonly rehabilitated and the global nature of our work.

Surprisingly, when our staff first saw the proposed image that became our final choice, we disagreed on the identification of the mammal. It became something like those ambiguous images that show both a young and an old person, or a duck and a rabbit in the same picture. Some people saw a wolf or coyote, whereas others saw a woodchuck or marmot.

What mammal do YOU see in the logo? Let us know by tagging @theiwrc on social media and/or #IWRCnewlogo.

—Kai Williams
Executive Director

INTERNATIONAL WILDLIFE REHABILITATION COUNCIL



Elk Calves Found in Record-breaking New Mexico Fires

ESPAÑOLA, NEW MEXICO, USA *(June 9, 2022)*—A young elk calf was found in the midst of a 486 square mile burn in New Mexico. The burn was part of one of New Mexico's several major fires this spring, the Calf Canyon/Hermits Peak fire. As of June 9th the fire is at 318,599 acres and 65% contained. The elk is currently with a surrogate parent at Cottonwood Rehab in northern New Mexico. Cottonwood's Facebook page reported a second fire-affected calf is currently in intensive care for smoke inhalation.

Proposed ESA Change May Allow for Introduction of Species Outside Historic Range

FALLS CHURCH, VIRGINIA, USA *(June 6, 2022)*—In the first Endangered Species Act (ESA) interpretive rule produced under the Biden–Harris administration, the U.S. Fish and Wildlife Service is proposing to revise section 10(j) regulations under the ESA to better facilitate recovery by allowing for the introduction of listed species to suitable habitats outside of their historical ranges. The proposed change will help improve the conservation and recovery of imperiled ESA-listed species in the coming decades, as growing impacts from climate change and invasive species cause habitats within their historical ranges to shift and become unsuitable.

The Service notes that foundational conservation policy must keep pace with corresponding science, which has shown that climate change and invasive species are pushing plants and animals into completely new geographic areas for the habitat needed for their continued survival. Improving the ESA's experimental population regulations will prevent more species from becoming stranded when conditions change in their current habitat, and help establish them in more suitable habitats given these rising threats.

"Recovering species and preventing their extinction will require innovative, proactive, science-based policies and conservation actions that address the growing impacts from climate change and invasive species before it is too late," said Martha Williams, Service Director. "The Service remains committed to working with our diverse state, local, and Tribal partners to meet these growing challenges, and appreciates how vital locally driven, partnership-based solutions will be in the coming years."

The Service uses experimental populations as a recovery tool when there is a need to establish a new population of an ESA-listed species outside of its *current* range. This tool furthers the conservation of the species by establishing more populations, while also providing fewer regulatory restrictions for affected partners. Establishment of an experimental population requires a rulemaking process, including publishing the rule in the Federal Register and providing the public an opportunity to review and submit comments. Experimental populations have been used to help advance the recovery of numerous listed species, including but not limited to California condors, whooping cranes, and Sonoran pronghorns. In addition, the service is considering introducing the Guam kingfisher outside its historical range. The species currently cannot be reintroduced to its former habitat on Guam because of the presence of brown tree snakes.

Under the proposed revisions, the Service would be able to introduce an experimental population of an ESA threatened or endangered species into suitable habitat outside of its current range and probable historical range.

Influx of Brown Pelicans into Rehabilitation

CALIFORNIA, USA *(June 8, 2022)*—Since mid-May, several Southern and Central California wildlife rehabilitation facilities from San Luis Obispo County south to San Diego County have been admitting hundreds of California brown pelicans. The pelicans have been found emaciated and frequently with secondary injuries or broken wings. Many of these birds died shortly after arrival at a facility. The California Department of Fish and Wildlife (CDFW), the U.S. Fish and Wildlife Service (USFWS), local wildlife rehabilitation facilities, and other state partners are coordinating to assess the ongoing situation.

CDFW is conducting postmortem examination and testing on pelicans that have been brought into rehabilitation facilities. Results indicate that pelicans are succumbing to starvation-related problems. Currently, there are no indications of disease or unusual parasites. CDFW is unable to provide information on the underlying cause of this event at this time. According to an International Bird Rescue Facebook post, as of June 7th agencies and wildlife rehabilitation centers are still looking for the underlying cause(s), even as the first group of affected birds are released.

Wildlife Center Calls for Rodenticide Ban

KILLINGWORTH, CONNECTICUT, USA *(June 6, 2022)*—Connecticut wildlife rehabilitation center A Place Called Hope takes the lead in urging a ban on anticoagulant rodenticides in their state. A recent post on their Facebook page includes a sample letter for contacting the state legislature. The problem of unintentional poisoning and its effect on wildlife seen at the center has been highlighted by local news outlets.

HPAI Found In Red Foxes

MADISON, WISCONSIN, USA *(June 9, 2022)*—The Wisconsin Department of Natural Resources (DNR) received confirmation in early May that three wild red fox kits tested positive for a strain of highly pathogenic avian influenza (HPAI) that is currently affecting domestic and wild bird populations in North America. These cases represent the first detections of HPAI in wild Wisconsin mammals. As of June 9th similar cases for HPAI H5N1 have been confirmed in foxes from Iowa, Minnesota, Michigan, New York, Ontario, Estonia, Ireland, and the Netherlands.

"We don't have any evidence that foxes are a significant source of transmission for the virus," said Lindsey Long, DNR

Wildlife Health Veterinarian. "The three foxes in these cases most likely contracted the H5N1 strain of HPAI after eating infected wild birds."

The three Wisconsin fox kits were from three different counties and showed advanced neurological symptoms after being brought to Dane County Humane Society's (DCHS) Wildlife Center. Testing by the Wisconsin Veterinary Diagnostics Laboratory confirmed that HPAI was present in all three animals.

People should avoid approaching any wild mammal that appears sick or injured. Do not approach a wild mammal that is behaving abnormally; examples may include neurological symptoms such as pacing or walking in circles, unbalanced posture, or displaying head and body tremors.

Neurologic symptoms in mammals can signify many different conditions, including exposure to environmental contaminants, parasitic infections, and diseases such as canine distemper and rabies.

Wildlife Care Continues During War in Ukraine

KHARKIV, UKRAINE *(May 20, 2022)*—Wildlife rehabilitation and research continue during the current war in Ukraine. Ukrainian Bat Rehabilitation Center, Eco-Halych, and others are continuing their work. Research happening this spring on bats and nesting storks will contribute to the global information on the effects of human conflict on wildlife. Previous studies on war and wildlife have been published in *Nature*, *Smithsonian*, *Science Daily*, *Nature Scientific Reports*, and *Conservation Biology*, among others.

HPAI outbreak leads to NA center restrictions and closures

EUGENE, OREGON, USA *(May 15, 2022)*—Wildlife centers across North America adjusted their intake policies and protocols as the current strain of HPAI H5N1 moved across the continent this winter. Centers from British Columbia to Maryland have added additional strictures to their protocols and/or stopped intake of certain species. The methods needed to minimize risk vary based on each wildlife rehabilitator's situation (quarantine space availability, community risk, etc).

A May 9 article in *Wisconsin State Farmer* summarizes the current outbreak in North America, including the variation in numbers reported by different agencies and wildlife rehabilitation data for several states.

2021 Chimpanzee Intakes Unusually High at Lwiro

LWIRO, DEMOCRATIC REPUBLIC OF THE CONGO *(May 4, 2022)*—Intakes are on the rise at Lwiro Primates Rehabilitation Center but safe release sites are rare with armed humans blocking access to appropriate forest locations.

Speaking with Reuters, center director Itsaso Velez del Burgo states, "It's horrible because the year 2021 was the worst year in the history of our centre, we had 15 arrivals. And you have to think that for every chimpanzee that arrives here at the sanctuary, there are ten more that died in the forest."

Intakes come from hundreds of miles away and are mostly unweaned youngsters that were victims of poaching for bushmeat or the illegal pet trade.

City Searches for Location to House Proposed Wildlife Center

LOS ANGELES, CALIFORNIA, USA *(April 6, 2022)*—Los Angeles City Council voted unanimously to pursue establishing a permanent location for LA Wild. The city council acknowledged the large number of wildlife intakes by local animal services agencies and the lack of resources to care for them. Supporting the proposed permanent nonprofit wildlife rehabilitation center would provide a public service to the citizens of Los Angeles.

US Proposes Ban on Killing Contests on Public Lands

WASHINGTON, D.C. *(April 5, 2022)*—HR7398, legislation to ban wildlife killing contests on public lands, was introduced into the US House of Representatives April 5 by Rep. Steve Cohen (D-Tenn.). It is under consideration in the Natural Resources and Agriculture committees. Cosponsors include Reps. Earl Blumenauer (D-Ore.), Gerry Connolly (D-Va.), Jim Cooper (D-TN), Peter DeFazio (D-Ore.), Adriano Espaillat (D-N.Y.), Ruben Gallego (D-Ariz.), Raúl Grijalva (D-A.Z.), Sara Jacobs (D-Calif.), Andy Levin (D-Mich.), Grace Meng (D-N.Y.), Jerrold Nadler (D-N.Y.), Eleanor Holmes Norton (D-D.C.), Mark Takano (D-Calif.), Dina Titus (D-Nev.), and Cori Bush (D-M.O.).

The bill states within its findings, Section 2(3), that "Scientific evidence does not support claims that wildlife killing contests permanently reduce populations of targeted species, increase populations of deer or other game species, or prevent conflict between native carnivores, humans, pets, and livestock."

Additional information can be found at the projectcoyote.org site.

Powerful Quote on World Wildlife Day

GENEVA, SWITZERLAND *(March 7, 2022)*—World Wildlife Day occurred March 3. The theme of "Recovering key species for ecosystem restoration" identified individual species, like the Saiga antelope and the California condor, as well as groups of species—including corals and seaweed—as the glue that holds their ecosystems together. These species' wellbeing is thus crucial to conserve the biodiversity we have left and restore what has been lost, which is the focus of this year and part of the U.N. Decade on Ecosystem Restoration.

A CITES press release quotes the U.N. Development Programme Administrator, Achim Steiner. "In 2022, we must finally dispel the false dichotomy that there is a "zero-sum game" of wildlife versus the economy; or protecting ecosystems versus the economy," said Steiner. "Indeed, the world is waking up and recognising the true value of nature which holds many of the solutions we need to address the climate crisis and achieve the Global Goals." ■

A systematic review of factors affecting wildlife survival during rehabilitation and release

Holly R. Cope,[1] Clare McArthur,[2] Christopher R. Dickman,[2] Thomas M. Newsome,[2] Rachael Gray,[1] and Catherine A. Herbert[2]

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In the study by De La Cruz et al.[40] oiled rehabilitated **surf scoters** (*Melanitta perspicillata*) showed 14.3% survival, while unoiled rehabilitated scoters had 77.2% survival.

Introduction

Wildlife rehabilitation is practiced in many countries, resulting in the rescue, care, and release of millions of animals every year.[1] Wildlife commonly require rehabilitation due to collisions with motor vehicles, abandonment and domestic animal attack (among others), while many targeted rescues are in response to environmental disasters such as oil spills or wildfire.[2,3] Wildlife rehabilitation has been defined as "the act of providing temporary care for injured, sick or orphaned wildlife with the goal of releasing them back into the wild."[4] Although there is limited evidence of the fate and contribution of released animals to the conservation of populations or species,[1,5] there are other reasons why rehabilitation can be valuable, or valued. For example, release of rehabilitated animals may help to supplement and maintain existing populations.[1,3,6–8] Wildlife care and rehabilitation often garner attention from the community and media and serve as effective education and fundraising tools.[2,9] The knowledge and experience gained while rehabilitating commonly encountered species can also support the care of threatened species.[2] Rehabilitation can also be supported for ethical, legal and welfare concerns in certain situations, varying by country.[10,11] For these reasons, wildlife rehabilitation is likely to continue, and assessments of the factors affecting wildlife survival during rehabilitation and release can help to inform future directions.

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ABSTRACT: Millions of native animals around the world are rescued and rehabilitated each year by wildlife rehabilitators. Triage and rehabilitation protocols need to be robust and evidence-based, with outcomes consistently recorded, to promote animal welfare and better understand predictors of wildlife survival. We conducted a global systematic review and meta-analysis of 112 articles that reported survival rates of native mammals and birds during rehabilitation and after release to determine intrinsic and extrinsic factors associated with their survival.

We assessed survival during rehabilitation and in the short- and long-term post-release, with the hypothesis that survival will vary as a function of species body size, diel activity pattern, trophic level, and study location (region of the world). We aimed to determine the direction of effect of these factors on survival to assist in decision-making during triage and rehabilitation. Results showed that mammals and birds were equally likely to survive all stages of rehabilitation, and survival rates varied between locations. Birds in North America had the poorest survival rates post-release, particularly long-term, as did diurnal and carnivorous birds in the short-term post-release. Anthropogenic factors such as motor vehicle collisions and domestic

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To identify factors influencing the success of wildlife rehabilitation, it is first necessary to define "success," and this may vary among stakeholders. From a wildlife rehabilitator's perspective, success could constitute recovery from initial injuries and release back into the wild.[12] Success for the individual animal could entail recovery from injuries and long-term survival in the wild with successful reproduction.[13] Success at the population level could constitute persistence of populations where rehabilitated animals are released, with released animals maintaining individual territories and contributing to the reproductive population, without introducing deleterious genetic alleles or disease, or pushing the population beyond the carrying capacity of the habitat or exacerbating intraspecific competition.[6,7] Such measures of success at the population level likely mirror those for the success of a conservation reintroduction, which has been defined as the creation of a self-sustaining population.[14] In this review, we consider success in terms of individual animal survival during care, and short- and long-term survival post-release. However, the potential impact of released rehabilitated animals at the population level remains a knowledge gap for many species.

Communities and native animals rely on volunteer wildlife rehabilitators to rescue, rehabilitate and release injured or orphaned wildlife.[5,15,16] However, few studies have used an experimental approach to assess rehabilitation methods or factors associated with survival to release. Consequently, rehabilitators rely largely on an evolution of methods through trial and error, shared knowledge, and guidelines developed by wildlife authorities[1] (see examples).[17,18] For example, habituation to humans can reduce survival in the wild if animals do not display appropriate predator avoidance behaviours.[6,19] However, few studies have quantified the effect of different measures employed by rehabilitators to avoid habituation.[5] Similarly, limited numbers of studies have monitored survival outcomes post-release in relation to the rehabilitation methods used.[19] Given the likelihood of increased frequency and severity of natural disasters in the future, including wildfires,[20,21] combined with increased threats of urbanization, such as motor vehicle collisions, dog attack, and entanglement in netting or wire,[16] it is likely that wildlife rescue, rehabilitation, and release will play an increasingly important role in conservation efforts over time.[6,22,23] As such, it is valuable to assess current survival rates and factors associated with the success of rehabilitation of rescued wildlife.

We used a systematic approach to review survival rates of native mammals and birds during rehabilitation and post-release to determine factors associated with survival. We focused on mammals and birds as these classes are commonly rescued and rehabilitated, with survival data subsequently reported in the literature. The effects of a range of intrinsic and extrinsic factors on survival were evaluated to develop a framework of key considerations for wildlife rehabilitation, and to guide future research on best-practice rehabilitation methods. Specifically, we hypothesised that survival rates during rehabilitation and post-release will vary as a function of species traits that could impact susceptibility to anthropogenic and environmental threats, such as body size, trophic level and diel activity pattern, and survival will vary between regions of the world. Thus, survival likelihood will be species- and context-specific.

Materials and Methods

Systematic review scope

A standard systematic search strategy, as outlined by Pullin and Stewart,[24] was used to identify peer-reviewed and grey literature reporting mammal and bird survival during wildlife rehabilitation around the world. Search results were recorded using a Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram.[25] Online databases Scopus and Web of Science were searched along with relevant conference proceedings, reference lists of selected articles (backwards search), and Google Scholar. The literature search was completed by H. Cope over a three-month period from January to March 2021, and included 50 journals, three of which were particularly relevant: *Animal Welfare*, *Journal of Raptor Research*, and *Journal of Wildlife Rehabilitation*. Additionally, 16 relevant books, symposium proceedings, reports, and theses were used. We used search terms relating to the location of the study, focal taxa, rehabilitation intervention, and survival, without date limits (S1 Table). The online systematic review tool, SysRev, (sysrev.com) was used to reduce the returned articles based on title, abstract, and keywords. As thousands of articles were returned, they were sorted by relevance then searched until there were 50 consecutive non-selected articles (an arbitrary number, usually representing two pages of search results, past which relevant articles were unlikely to be found). Full texts were then reviewed against selection criteria as follows: research was conducted on native mammals or birds that entered care for any reason; the sample size and a survival measure and timeframe (e.g. number of mortalities, annual survival rate, minimum known alive) during rehabilitation or post-release were reported; and the article (or abstract) was available online and in English. Few studies included a control group or intervention other than rehabilitation, so this was not a requirement for inclusion. Where studies included a control group, this involved monitoring a sample of wild counterparts, uninjured animals, or known baseline survival rates for the resident population.

Abstract

CONTINUED FROM PAGE 7

or feral animal attack contributed to morbidity and post-release mortality in 45% (168 of 369) of instances. The reasons for rescue and associated severity of diagnosis were commonly reported to affect the likelihood of survival to release, but factors affecting survival were often species-specific, including bodyweight, age, and characteristics of the release location. Therefore, evidence-based, species-specific, and context-specific protocols need to be developed to ensure wildlife survival is maximised during rehabilitation and post-release. Such protocols are critical for enabling rapid, efficient rescue programs for wildlife following natural disasters and extreme weather events which are escalating globally, in part due to climate change.

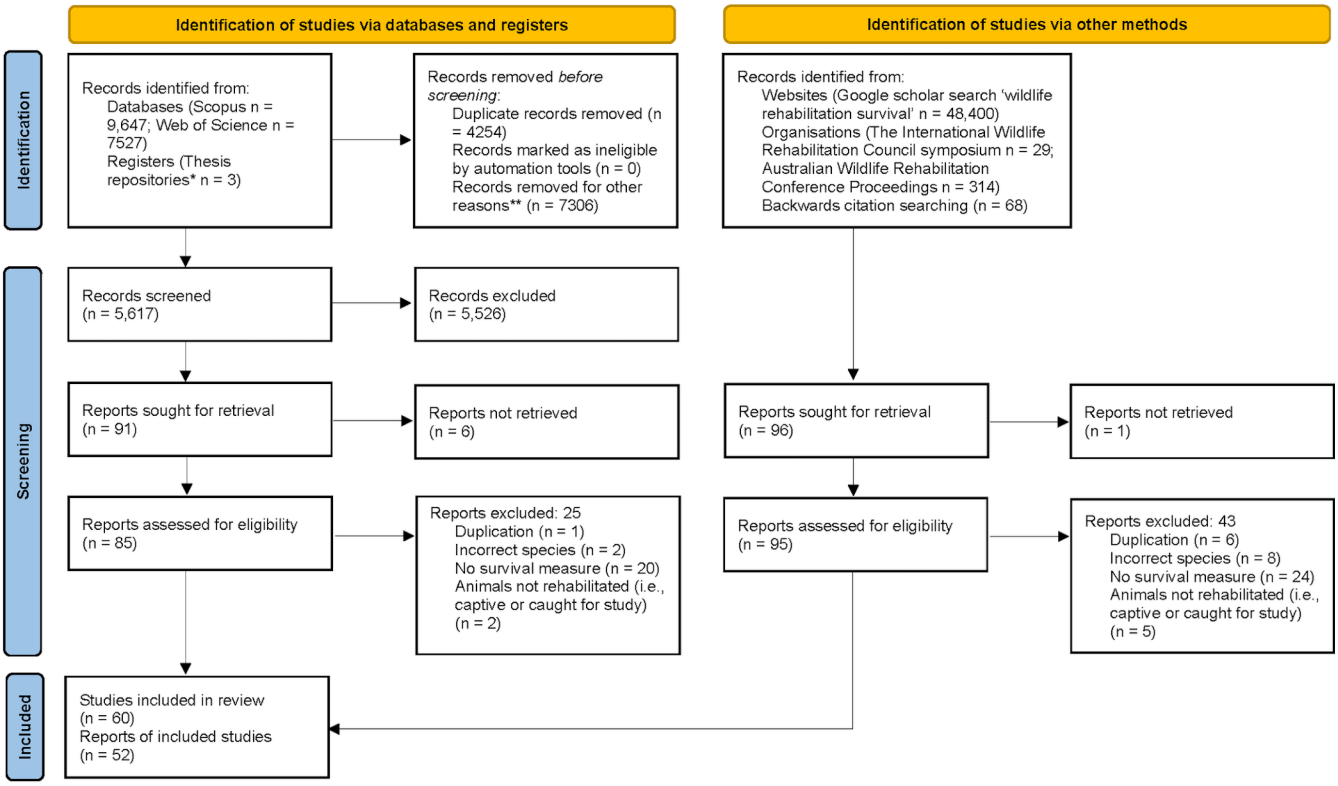


FIGURE 1. PRISMA flow diagram of systematic search strategy and results. Adapted from Page et al.[25]
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Statistical analyses

Definition of variables

One reviewer created a summary of study characteristics for each article detailing study species, sample size, study location, reason for entry into care, percentage of unassisted deaths in care (i.e. excluding euthanasia), percentage survival to release from care, short- and long-term survival post-release, factors reported as affecting survival, and causes of mortality. Post-release survival was categorised as short-term (< six months) or long-term (> six months) to minimise bias between study outcomes. These time frames were selected because some studies reported survival at multiple time points, and monitoring varied from 14 days to six years. We considered six months to be a reasonable time for animals to settle into their environment and forage independently in more than one season. Location was also included in the analyses to disentangle the potential effects of the suite of species found at a location, varying rehabilitation policies and practices around the world, or other abiotic processes from the biological characteristics of species.

We used the Encyclopedia of Life (https://eol.org/) to categorise species according to class (Aves, Mammalia), diel activity pattern (any time, crepuscular, diurnal, nocturnal), average adult weight (small < 5.5 kg, medium 5.5–100 kg, large > 100 kg) and trophic level (herbivores [primary consumers], omnivores and carnivores/pescatarians [secondary and tertiary consumers], apex carnivores). The adult weight classes were designed to separate species according to the Australian critical weight range, i.e. 35 g–5.5 kg,[26] from other species within the same order and trophic level as we expected smaller species to be more susceptible to predation and mortality. The largest weight class encompasses mammals that have a greater probability of being threatened than the medium weight class.[27] There were no avian species in the largest weight class. Trophic levels were designed to separate dietary niches such as predators from prey species. These sub-groups were considered sufficient to reduce risk of bias from individual studies. There were insufficient samples to further stratify studies based on the methods used.

The reasons for entry into care and causes of post-release mortality were grouped into three categories—anthropogenic, environmental, and non-specific (those that could not be attributed)—and reported as frequencies. Factors affecting survival during rehabilitation or after release were categorised as being related to the event that precipitated entry into care (e.g. severity and type of injury), intrinsic or individual traits (e.g. body size, behaviour and age), intervention (e.g. rescue protocols, choice of diet and pre-release training), release environment (e.g. timing of release, release method and habitat quality), and human–wildlife interface (e.g. hunting activity and urban expansion), and summarised. These factors were shown statistically or observationally to affect survival in the reviewed articles.

Statistical methods

Statistical analyses were performed in R (version 4.0.5).[28] A mixed-effects meta-regression model in the METAFOR package was used to assess the relationship between survival and characteristics of the study species. Species was included as a random effect to account for multiple studies on the same species. Effect sizes were weighted by the sample size due to a lack of reported error measures for most articles (survival was generally reported as percentage known alive), based on the expectation that variance will decrease with larger sample sizes. Survival rates and sample sizes were then used to calculate log-odds of survival. Survival was initially compared between Aves and Mammalia for each stage of rehabilitation, being the unassisted death rate in care (i.e. deaths not resulting from euthanasia), survival to the end of rehabilitation (i.e. release to the wild or long-term captivity), short-term survival post-release, and long-term survival post-release. Each class was then assessed separately to determine the effect of factors hypothesised to affect survival including study location (Oceania [and Asia], North America, Europe, Africa, and Others [Middle East, Southern America]) and species' diel activity pattern, adult weight class, and trophic level at each stage of rehabilitation. Strength of association was first assessed between all paired combinations of predictors using a Fisher's exact test, and predictors with a relationship ($p < 0.05$) were not included together in models. All combinations of predictors were modelled and Akaike's Information Criterion (AIC)[29] was used to select the best model with the lowest AIC value by ≥ 2 points. Where no model satisfied this criterion, the most parsimonious model (least number of predictors) within two points of the lowest AIC value was selected. Between-study heterogeneity was reported as I^2.[30] Probabilities of survival were calculated as a back-transformation of log-odds for single predictor models for simplicity of interpretation.

Publication bias can exist where small studies with small effect sizes are not published or there is selective reporting within studies.

TABLE 1. Number of published articles from each country or region reporting survival of wildlife during rehabilitation, post-release or during both phases.

REGION	REHABILITATION	POST-RELEASE	BOTH	TOTAL*
Oceana (and Asia)				
Australia	7	11	6	24 (21.4)
East Asia	1	0	0	1 (0.9)
New Zealand	1	0	0	1 (0.9)
South Asia	0	1	1	2 (1.8)
North America				
Canada	2	0	0	2 (1.8)
North America	10	14	5	29 (25.9)
Several northern hemisphere countries	0	1	0	1 (0.9)
Europe	15	14	3	32 (28.6)
Africa				
Southern Africa	6	6	5	17 (15.2)
Others				
Middle East	2	0	0	2 (1.8)
South America	0	1	0	1 (0.9)
Grand Total	**44**	**48**	**19**	**112 (100)**

*Values presented as total (percentage of grand total).

TABLE 2. Frequency of studies reporting various anthropogenic, environmental and non-specific causes for entry of wildlife into care, and mortality post-release.*

ORIGIN OF CAUSE FOR REHABILITATION OR MORTALITY	CAUSE OF ENTRY INTO CARE	CAUSE OF MORTALITY POST-RELEASE
Anthropogenic	**117 (48.0)**	**51 (40.8)**
Collision—motor vehicle	13 (5.3)	16 (12.8)
Gunshot or poaching	8 (3.3)	15 (12.0)
Domestic or feral animal attack or predation	11 (4.5)	11 (8.8)
Oil spill	21 (8.6)	0 (0)
Toxicosis or poisoning	12 (4.9)	1 (0.8)
Electrocution/collision with powerlines	8 (3.3)	3 (2.4)
Collision—structure	10 (4.1)	0 (0)
Confiscated	10 (4.1)	N/A
Relocated or displaced	4 (1.6)	1 (0.8)
Entanglement	3 (1.2)	1 (0.8)
GPS or VHF collar injury	N/A	1 (0.8)
Other (e.g. capture myopathy, trap, tree felling, human interference	17 (7.0)	2 (1.6)
Environmental	**24 (9.8)**	**36 (28.8)**
Disease	19 (7.8)	6 (4.8)
Predation by native predator	1 (0.4)	19 (15.2)
Misadventure (burrow collapse, drowning, ingested wasp, killed by elephant)	0 (0)	6 (4.8)
Fire, flood or storm	3 (1.2)	2 (1.6)
Natural mortality—age-related	N/A	3 (2.4)
Heat stress	1 (0.4)	0 (0)
Non-specific	**103 (42.2)**	**38 (30.4)**
Generic trauma or unidentified illness (i.e. Sick, injured, trauma, exhaustion)	41 (16.8)	7 (5.6)
Orphaned or stranded juvenile	46 (18.9)	N/A
Malnutrition	8 (3.3)	5 (4.0)
Unresolved initial ailment	N/A	7 (5.6)
Unknown causes	8 (3.3)	19 (15.2)
Studies that did not cite a specific reason	**10**	**11**

*Values presented as number of studies, not number of individuals, with percentage of the total in parentheses.

We tested for bias in METAFOR by creating a funnel plot of effect size versus sampling variance of the effect size for each survival measure.[31] Egger's test was used for funnel plot asymmetry and trim-and-fill analysis[29] was used to estimate magnitude of publication bias.

Results

The literature search yielded 5,617 publications, of which 187 were initially selected; after reviewing the full texts, 112 articles satisfied all inclusion criteria (Fig. 1). Several articles presented independent survival results for more than one species or population, and these results were analysed separately and hereafter referred to as studies, totalling 125. Articles were published between 1981 and 2021. Sample sizes ranged from 2–22,344 (mean 1076, median 63). Eighteen articles included a control group with sample sizes ranging from 3–5726 (mean 684, median 23). Retrospective studies using wildlife rescue databases contributed to the large mean sample sizes. Research was mostly conducted in Australia, Europe, North America, and Southern Africa (Table 1). Funnel plot analysis showed an estimated lack of 13 studies with large effect sizes for unassisted death in care ($p = 0.0006$) resulting in possible underestimation in our results, six missing studies with small effect sizes for both rehabilitation survival ($p = 0.0038$) and post-release short-term survival ($p = 0.0328$) resulting in possible overestimation, and no publication bias in long-term post-release survival ($p = 0.1397$; S2 Table).

TABLE 3. Summary of mixed-effects meta-regression models with the best fit and significant predictors of survival for bird and mammal classes for each survival stage

	UNASSISTED DEATH LOG-ODDS					
Aves (I^2 = 87.36)[a]	**Estimate**	**s.e.**	**Z-value**	**Lower**	**Upper**	**P-value**
Intercept	–1.76	0.13	–13.62	–2.02	–1.51	<0.001*
locationEurope	0.35	0.12	2.97	0.12	0.59	0.003*
locationNorthAmerica	0.75	0.13	5.96	0.50	1.00	<0.001*
locationOceania	–0.43	0.22	–1.92	–0.87	0.01	0.055
locationOther	0.14	0.26	0.55	–0.37	0.65	0.582
Mammalia (I^2 = 97.56)	**Estimate**	**s.e.**	**Z-value**	**Lower**	**Upper**	**P-value**
Intercept	–2.30	1.14	–2.02	–4.54	–0.07	0.043*
trophic_levelHerbivore	0.36	0.33	1.09	–0.28	1.00	0.274
tropic_levelOmnivore	1.12	0.33	3.36	0.47	1.78	0.001*
adult_weight_classMedium	0.79	1.12	0.71	–1.40	2.98	0.479
adult_weight_classSmall	0.31	1.11	0.28	–1.86	2.48	0.779
	POST-RELEASE SHORT-TERM SURVIVAL LOG-ODDS					
Aves (I^2 = 40.30)	**Estimate**	**s.e.**	**Z-value**	**Lower**	**Upper**	**P-value**
Intercept	2.56	0.71	3.63	1.18	3.95	<0.001*
trophic_levelCarnivore	–1.95	0.59	–3.30	–3.10	–0.79	0.001*
trophic_levelHerbivore	0.80	0.94	0.85	–1.04	2.64	0.394
trophic_levelOmnivore	–0.31	0.74	–0.42	–1.76	1.13	0.671
diel_activityNocturnal	2.16	0.50	4.29	1.17	3.14	<0.001*
locationEurope	–2.16	0.52	–4.14	–3.18	–1.14	<0.001*
locationNorthAmerica	–2.01	0.51	–3.93	–3.01	–1.01	<0.001*
locationOceana	–0.62	0.80	–0.78	–2.18	0.94	0.438
	POST-RELEASE LONG-TERM SURVIVAL LOG-ODDS					
Aves	**Estimate**	**s.e.**	**Z-values**	**Lower**	**Upper**	**P-value**
(I^2 = 98.29) (AIC 198.69) Intercept	**1.78**	**2.09**	**0.85**	**–2.32**	**5.88**	**0.395**
diel_activityDiurnal	–0.85	2.02	–0.42	–4.81	3.11	0.674
diel_activityNocturnal	–1.26	1.99	–0.63	–5.17	2.64	0.527
locationEurope	–1.60	0.87	–1.85	–3.30	0.09	0.064
locationNorthAmerica	–3.70	1.03	–3.59	–5.72	–1.68	<0.001*
locationOceania	–0.17	1.04	–0.16	–2.21	1.87	0.871
(I^2 = 97.77) (AIC 197.87) Intercept	**0.92**	**0.49**	**1.90**	**–0.03**	**1.88**	**0.058**
trophic_levelHerbivore	0.93	0.86	1.07	–0.76	2.61	0.283
trophic_levelOmnivore	1.49	1.90	0.78	–2.23	5.21	0.433
locationEurope	–2.01	0.76	–2.62	–3.50	–0.51	0.009*
locationNorthAmerica	–3.59	0.93	–3.88	–5.41	–1.78	<0.001*
locationOceania	–0.80	0.87	–0.92	–2.52	0.91	0.357

[a] I^2 reports between-study heterogeneity. [b]The mixed-effects meta-analysis function treats the first alphabetical factor level as a baseline with an estimate of zero; i.e., locationAfrica, trophic_levelApexPredator, diel_activityAnytime, adult_weight_classLarge. *P-values <0.05 indicate factor levels that are significantly different from zero.

Reason for entry into care and causes of mortality

Most reasons for entry into care were anthropogenic in origin, followed by non-specific causes, with a small proportion attributed to natural environmental causes (Table 2). Most mortalities in care occurred as a direct result of the initial reason for admission, either by euthanasia or unassisted death. In eight articles, secondary complications caused death, as sequelae of the initial reasons

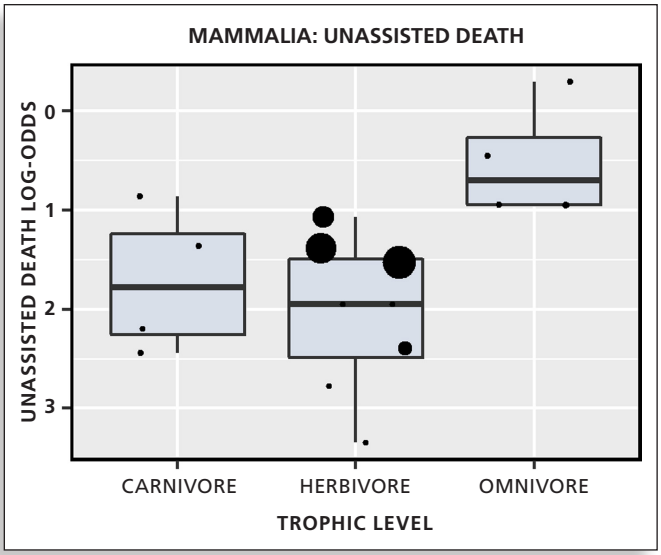


FIGURE 2. Distribution of log-odds across factors affecting unassisted death rates for mammalian species. Note that each point represents the log-odds of survival of one study-species combination, and the size of the point is proportional to the inverse variance of the log-odds (i.e. larger points have more weight). https://doi.org/10.1371/journal.pone.0265514.g002

for admission or resulting from rescue procedures, treatment, or captivity. The mean unassisted death rate after entry into care was 17.9% (2.4) and 18.2% (4.0) for birds and mammals, respectively (overall 17.9% (2.1); presented as mean (SE)). The most common known causes of post-release mortality were predation (by domestic, feral, native, and conspecific predators, equalling 24% of all causes), vehicle collision, and illegal shooting or legal hunting.

Factors associated with survival

There were no differences between classes for unassisted death ($p = 0.20$) or survival during ($p = 0.08$) or after rehabilitation (short-term $p = 0.38$, long-term $p = 0.40$); however, not all levels of predictors were present in both classes at all survival stages, so we assessed classes separately for effects of study location, diel activity pattern, trophic level, and adult weight class (referred to as the full model). No factors were significant for either birds or mammals for survival to the end of rehabilitation (S3 Table). The log-odds of unassisted death during care for mammals was best explained by trophic level and adult weight class; omnivores had a significantly higher ($p < 0.0001$) death rate than carnivores (Table 3; Fig. 2) (there was a significant association between trophic level and diel activity pattern ($p = 0.025$) and between trophic level and location ($p = 0.046$), so these combinations were excluded from models). Short-term post-release survival of mammals was best explained by two models including trophic level, location, and diel activity with support based on AIC values, although no factors were significant (there was a significant association between adult weight class and diel activity ($p = 0.0001$), adult weight class and location ($p < 0.001$), and diel activity and location ($p = 0.046$), so these combinations were excluded). For long-term post-release survival of mammals, the full model had the best fit, although no predictors had a significant effect on survival (S3 Table).

Study location affected the unassisted death rate of birds; North America had the highest log-odds of death and Oceania had the lowest log-odds of death (Table 3). Mean probabilities of unassisted death were 10% Oceania, 15% Africa, 17% Others, 20% Europe, and 27% North America (Fig. 3A). For short-term

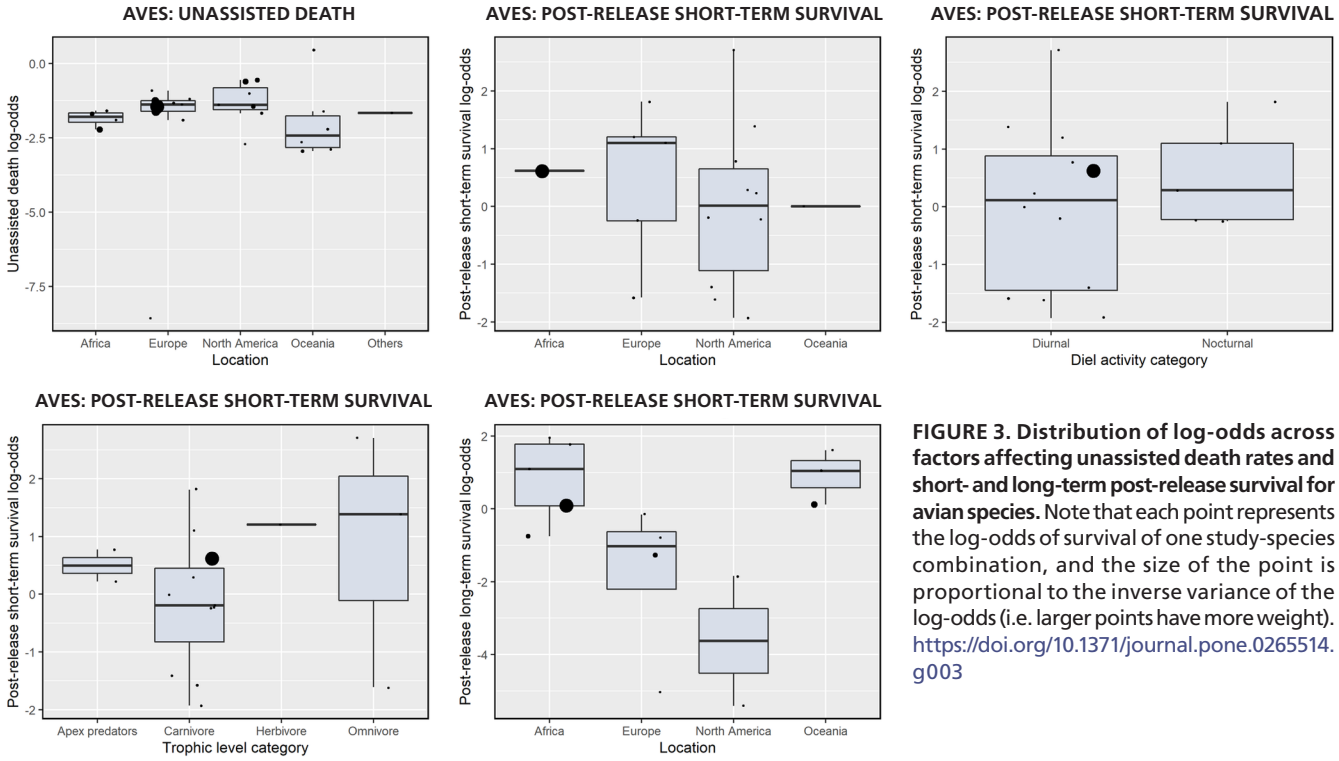


FIGURE 3. Distribution of log-odds across factors affecting unassisted death rates and short- and long-term post-release survival for avian species. Note that each point represents the log-odds of survival of one study-species combination, and the size of the point is proportional to the inverse variance of the log-odds (i.e. larger points have more weight). https://doi.org/10.1371/journal.pone.0265514.g003

post-release survival, the model with trophic level, diel activity and location had the best fit for birds, although a large proportion of the variation came from sampling variation ($I^2 < 50\%$; Table 3). Studies in Africa had a higher survival probability than Europe, North America and Oceania (65%, 49%, 55% and 50%, respectively; Fig. 3B), diurnal birds had lower survival probability than nocturnal birds (51% and 64%, respectively, Fig. 3C), and carnivorous birds had lower survival probability than apex predators, herbivorous and omnivorous birds (45%, 63%, 77%, and 66%, respectively; Fig. 3D). Two models including diel activity, location and trophic level had support based on AIC values for long-term survival of birds, and study location had a significant effect on survival ($p < 0.0001$; Table 3); North America had the lowest survival probability (6% North America compared with 31% Europe, 65% Oceania and 72% Africa; Fig. 3E).

Factors associated with survival in individual studies

In the reviewed articles, 18 included an experimental design to assess the outcomes of different methods of rehabilitation (e.g. hand-rearing diet, treatment regime, and soft versus hard release). Fifty-eight studies statistically assessed the effect of various factors on survival, and 38 included observations of factors that authors believed affected survival rates in their study (Table 4). Most factors were intrinsic to the individual or species (e.g. body size, age, and sex), or related to the interventions enacted by humans in the rescue, rehabilitation and release process (e.g. rescue protocol, habituation to humans, and release location) (Table 4).

Post-release survival of rehabilitated and control animals

Only 18 studies incorporated a control group, which was usually a wild cohort that did not require rehabilitation. Most studies showed either reduced survival for rehabilitated animals compared with control groups, or similar outcomes (Table 5). Oiling in particular appears to cause a large decrease in survival after rehabilitation, highlighted in the study by De La Cruz et al.[40] where oiled rehabilitated surf scoters (*Melanitta perspicillata*) showed survival of only 14.3% compared with 49.8% for unoiled non-rehabilitated scoters at five months, while unoiled scoters rehabilitated for other reasons had 77.2% survival.

Discussion

This systematic review supports our hypothesis that wildlife survival during rehabilitation and post-release is species- and context-specific. Most studies in this review were from Australia, Europe, North America, and southern Africa. Meta-analysis demonstrated effects of species' diel activity type, trophic level, and location of the study on survival, but not adult weight, supporting some but not all our hypotheses. Study location was a strong predictor of death in care and survival short- and long-term post-release for birds. There are various potential explanations for the effect of location, including differing triage protocols and therefore frequency of euthanasia, impacts of different threats in the environment, and varying perceptions towards the value of wildlife around the world.[3,118,119] The reason for rescue and associated severity of diagnosis were strong predictors of survival to release, and in some cases, post-release survival. Our results synthesised five clear categories of factor that can impact survival outcomes for rescued wildlife and that must be addressed in rescue, rehabilitation, and release protocols. These factors relate to the event, individual animal, intervention, release environment, and the human–wildlife interface. Oil spill events appear to have stimulated global wildlife rehabilitation research efforts, with 15 articles published from five countries, and generally result in low rates of survival. Only two articles (both in Australia) assessed the survival of rehabilitated wildfire-affected animals, and showed that they had low to moderate rates of survival.[22,109] Overall, the number of studies that included an experimental approach or control to assess factors affecting survival was low.

Factors affecting survival during and after rehabilitation

Unassisted deaths in care can act as an indicator of ineffective triage criteria and appropriate treatment and husbandry protocols, as these are animals that die without euthanasia. The rates of unassisted death varied depending on the study location for birds, and by trophic level for mammals. This variation could indicate that the decision to euthanise is made sooner in Oceania compared with North America, or that threats in North America are more likely to result in unpredictable death in care. The only omnivorous mammals with unassisted death rates recorded in the meta-analysis were raccoon dogs (*Nyctereutes procyonoides*) and European hedgehogs (*Erinaceus europaeus*), both with relatively high death rates. Hedgehogs that were admitted due to trauma, parasite infections, and vehicle collisions had very low recovery rates, and road casualties died very quickly in care.[56,61] Raccoon dogs treated in Japan for severe *Sarcoptes scabiei* infections experienced 57.4% and 38.9% unassisted death rates for two groups given different treatment regimens,[86] allowing the researchers to determine the best treatment to reduce future unassisted deaths. The greatest death rate recorded in the review was 61.3% for little penguins (*Eudyptula minor*) rescued after an oil spill event in Australia.[35] Most mortalities occurred within the first 12 days, and were attributed to the degree of oiling, the amount of oil ingested, the low body weight of penguins on arrival, and inappropriate cleaning techniques used by inexperienced and unsupervised volunteers.[35] An oil spill five years later showed greatly improved survival rates for little penguins, with only 5% unassisted deaths,[34] highlighting the importance of evaluating outcomes and refining protocols over time.[77]

In the reviewed articles, there were several large mammalian species with high short-term post-release survival rates. In our meta-analysis, large species included Asiatic black bears (*Ursus thibetanus*), American black bears (*U. americanus*) and brown

CONTINUED ON PAGE 16

TABLE 4. A summary of factors associated with wildlife survival during rehabilitation and after release, with the direction of effects (higher or lower probability of survival), and factors categorised into event-related, intrinsic, or individual (inter-specific and intra-specific), intervention-related, release environment, and human-wildlife interface (see S5 Table for full list of species referenced).

FACTOR	PHASE AFFECTED	HIGHER PROBABILITY OF SURVIVAL AFTER RESCUE	LOWER PROBABILITY OF SURVIVAL AFTER RESCUE
EVENT-RELATED			
Reason for admission and associated diagnosis and severity	Survival to release[32–58]	Less severe reason for rescue or diagnosis, e.g. uninjured orphans[a]	More severe reason for rescue or diagnosis, e.g. fracture[a]
	Survival post-release[34]	Less extensive oiling[a]	More extensive oiling[a]
Size of disaster	Survival to release[33,59]	Major oil spill event i.e. greater search and recovery effort[a, 59]	Widespread events (e.g. heat stress) can overwhelm rehabilitator capacity[b, 33]
Season of admission	Survival to release[54]	N/A[c]	Coincides with physiologically taxing events, such as breeding or moulting[a]
INTRINSIC OR INDIVIDUAL—INTER-SPECIFIC			
Body size	Survival to release[60]	Larger body size[b]	N/A
Migratory or not	Survival post-release[60]	Non-migratory (i.e. no strain of migration soon after release)[b]	Migratory (i.e. become oiled far from breeding localities)[b]
Behaviour	Survival to release[61]	Resilient and easily adapts to captivity	N/A
	Survival post-release[62]	Easily adapts to post-release environment[b]	N/A
INTRINSIC OR INDIVIDUAL—INTRA-SPECIFIC			
Age	Survival to release [6,33,36,37,44,50,53,57,59,63–66]	Juveniles may present with less severe injuries such as orphaning, so have greater survival than adults[a,6,33,36,37,44,50,53,57,66]	Juveniles can have lower survival than adults,[a] often due to characteristics of their age such as moult, presence of an umbilicus (susceptible to infection) or differing fitness requirements[59,63–65]
	Survival post-release[67,68]	Juvenile pelicans had better survival than adults[a, 68]	Juvenile (hand-reared) possums did not survive as long as adults[a, 67]
Sex	Survival to release[47,51,69]	Male polecats were more likely to be released than females[a, 69]	Male sparrowhawks were less likely to be released than females,[a, 51] male raptors were more susceptible to unassisted mortality than females[47]
	Survival post-release[34]	Male little penguins had higher survival rates than females[a]	N/A
Bodyweight	Survival to release [37,54,57,59,64,70–72]	Heavier bodyweight at time of rescue[a]	Lower rescue mass and poor rescue condition[b]
	Survival post-release[34]	As above[a]	As above[b]
Individual personality	Survival post-release[73]	More exploratory individuals[a]	Less exploratory individuals[a]
Brood size	Survival to release[72]	Improved juvenile survival when larger brood is rescued[a]	N/A
Physiological parameters	Survival to release[41,54]	Higher body temperature, higher packed cell volume and higher total plasma protein[a, 41]	Low total plasma protein, low haematocrit[a, 54]
Activity pattern	Survival to release[44]	Young diurnal raptors were admitted more frequently and had greater release rates than adults[a]	Adult nocturnal raptors were admitted more frequently than young birds.[a] Diurnal birds were more often treated for fractures than nocturnal birds[a]
INTERVENTION-RELATED			
Personnel and facilities for rescue, transport, care, and release	Survival to release [35,36,59,60,74–78]	Readily available and adequately equipped care facilities, trained personnel, and refined protocols[b]	Birds delivered by the public to the wildlife care centre (versus an animal collection officer or veterinarian),[b] time delay between event (e.g. oil spill) and rescue or veterinary treatment[b]
	Survival post-release[75,79]	As above[b]	N/A

TABLE 4 (CONT)

FACTOR	PHASE AFFECTED	HIGHER PROBABILITY OF SURVIVAL AFTER RESCUE	LOWER PROBABILITY OF SURVIVAL AFTER RESCUE
Wildlife care centre	Survival to release [36,80]	Some centres had higher survival,[a] possibly due to increased levels of experience and appropriate triage and treat ment regimes	Some centres had lower survival,[a] possibly due to lower levels of experience and ineffective or inappropriate triage.
Choice of hand-raising diet	Survival to release[71,81]	Artificial milk-replacer had greater survival than fish- formula,[a] and a commercial milk-replacer was better than another brand[a]	N/A
Maintenance of bodyweight	Survival post-release[74, 82–85]	Sufficient fat reserves or heavier mass on releasea[b]	Weight loss in migratory birds[b]
Treatment regimens	Survival to release [6,35, 66, 86]	Individuals with a better response to treatment;[a] disease management and ancillary treatment[a]	Treatment based on clinical signs rather than diagnostic tests;[b] incorrect drug dosages given by rehabilitators,[b] inappropriate treatments[b]
	Survival post-release [66,68,87–89]	Appropriate disease management[b]	Inadequate oil removal procedures;[b] failure to eliminate pathogen before release[b]
Habituation to humans	Survival to release[6]	Less human-imprinted animals are more likely to survive[a, 6]	N/A
	Survival post-release [6,34,67,73,85,90–93]	Shorter periods of rehabilitation may be better[b,67], although duration did not affect little penguin survival[a,34]	Loss of wild behaviours such as predator avoidance and disruption of social development due to human habituation[a,b] although habituation was not related to survival in deer[a, 90]
Hunting and wild behaviour training	Survival to release[78]	Construction of a pre-release flight tunnel for raptors[b, 78]	N/A
	Survival post-release	Provision of suitable hunting training[b] [91,94–97]	Lack of pre-release training to navigate situations likely to be encountered in the wild;[b] the mother-fawn relationship is essential, so hand-reared fawns lacked traits required for survival[b, 97]
Formation of social groups in captivity	Survival post-release [93,98,99]	Formation of social groups and surrogate mentor females provided for juveniles[ab]	Lack of group cohesiveness prior to release[b]
Readiness for release	Survival post-release [40,68,79,84,85,89,100,101]	Yearling age improves survival in bear cubs;[a] adequate waterproofing and bouyancy for water birds[b]	Released with unresolved ailments or long-term effects from reason for rescue[b]
RELEASE ENVIRONMENT			
Timing of release	Survival post-release [67,76,79,82,100]	Release during the non-breeding season;[a] mild weather;[b] high prey or food availability	Majority of hedgehog mortalities occurred during spring when they are most active[a, 82]
Release method	Survival post-release [60,62,82,97,98,101–104]	Release to a wild flock or known congregation area;[b] release of female macropods in groups with other female rearing companions,[b] soft release;[b, 97,101,104,a] release close to breeding locations[b]	Hard released Asiatic black bears had shorter survival than soft-released bears[a, 104]
Familiarity of habitat	Survival post-release [13,34,67,90,105–107]	Familiar release habitat may not be necessary for all species[34, 67, 105, b]	Unfamiliar release habitat[b]

continued, page 16

TABLE 4 (CONT)

FACTOR	PHASE AFFECTED	HIGHER PROBABILITY OF SURVIVAL AFTER RESCUE	LOWER PROBABILITY OF SURVIVAL AFTER RESCUE
Habitat quality	Survival post-release [67,92,95,107–113]	Unfamiliar habitat may be suitable while there are sufficient food trees and the carrying capacity has not been exceeded[b, 67, 108]	At or near carrying capacity;[b] reduced canopy continuity following bushfires;[b] competition and attacks from conspecifics;[b] the need to immediately travel long distances;[b] the presence of illegal hunting activity and proximity to dwellings and roads
Predators	Survival post-release	Control or absence of predators[b] [8,22,95]	Presence of predators[b]
HUMAN–WILDLIFE INTERFACE			
Increasing human population and habitat fragmentation	Survival post-release [65, 114]	N/A	Stress associated with bushfires and habitat fragmentation may be contributing to disease in koalas[b]
Hunting activities	Survival post-release[84]	N/A	Survival rates of bears reflect their permitted hunting pressure[b]
Urban expansion	Survival post-release[84]	N/A	Increasing encounters in recolonised areas results in more illegal kills[b]

[a,b] Factors identified by statistical or other inference (denoted by superscript a and b, respectively) that affected survival to release or survival post-release. [c]N/A indicates fields where no relevant data were presented. If survival to release or survival after release do not appear under phase affected for a given factor this indicates that no studies assessed that phase.
https://doi.org/10.1371/journal.pone.0265514.t004

bears (*U. arctos*) in North America and white rhinoceros (*Ceratotherium simum*) in South Africa. Their high rate of survival could be associated with the success of the captive rearing process, as most of these animals entered rehabilitation as orphans,[84,108,120] or a reduced risk of predation conferred by their size. In the long-term post-release, birds in North America had particularly low rates of survival, relating to studies of the peregrine falcon (*Falco peregrinus*) and brown pelican (*Pelecanus occidentalis californicus*). be associated with the success of the captive rearing process, as most of these animals entered rehabilitation as orphans,[84,108,120] or a reduced risk of predation conferred by their size. In the long-term post-release, birds in North America had particularly low rates of survival, relating to studies of the peregrine falcon (*Falco peregrinus*) and brown pelican (*Pelecanus occidentalis californicus*). Anderson et al.[68] determined that rescue and treatment after oiling did not restore pelicans to normal survivability; however, the 14% survival rate of peregrine falcons was similar to non-rehabilitated peregrines in the same population.[117] As stated by Morris et al.,[13] "rehabilitation cannot confer immortality" (pg. 65), and released rehabilitated animals are susceptible to the same threats as their wild counterparts,[91] yet not always equally. For example, rehabilitated and wild ringtail possums (*Pseudocheirus peregrinus*) in Australia faced the same predation pressures, however, translocated rehabilitated possums were at a disadvantage in unfamiliar territory and initially had lower survival rates.[106]

Few studies have experimentally assessed factors influencing wildlife survival during rehabilitation and release,[1] yet many of the reviewed articles retrospectively assessed or made observations of factors that influenced survival in their study. The reason for admission and the associated severity of diagnosis were both predictors of survival to release in many studies across a broad range of species, particularly birds.[42,44,58] However, this was not always the case. For example, the initial cause for rescue had no effect on wombat (*Vombatus ursinus*) survival during rehabilitation, where age and response to treatment were predictive of survival instead.[6] In many avian studies, especially on raptors, the main reasons for admission to care were trauma and orphaned young, with trauma resulting in lower release rates, while raising orphaned young was relatively successful.[48] The large proportion of carnivorous/pescatarian birds rescued due to oiling may have contributed to the low short-term post-release survival of birds revealed by our meta-analysis.

Our review found that intrinsic traits of species or individuals can affect survival outcomes, yet traits of importance vary with the species and type of injury they sustain. For example, the large body size and non-migratory nature of Cape gannets (*Morus capensis*) may have contributed to higher release rates after oiling than for smaller oiled bird species.[60] Age and body-weight upon entry to care were often correlated with survival to release, and in some studies, survival differed by sex. Larger body sizes may contribute to higher release rates in some cases by conferring a degree of robustness to the animal, or through increased effort contributed to rehabilitating larger species considered to be charismatic megafauna.[121] Although some physiological parameters were associated with survival, it can be impossible to define a cut-off measure to guide triage protocols.[70]

The reviewed studies reported many intervention-related aspects of rescue, transport, treatment, and release methods that

TABLE 5. Post-release survival of rehabilitated and control groups of various wildlife species grouped by survival pattern between the two groups.

SPECIES	REHABILITATED GROUP SURVIVAL	CONTROL GROUP SURVIVAL
	REHABILITATED GROUP SURVIVAL LESS THAN CONTROL GROUP	
Common ringtail possums *(Pseudocheirus peregrinus)*[106]	101 days	182 days
Little penguins *(Eudyptula minor)*[34]	59% (Ninth Island), 44% (Low Head)	77% (Ninth Island), 50% (Low Head)
Brown pelicans (*Pelecanus occidentalis californicus*)[68]	9% at 2 years	53% at 2 years
Common murres (*Uria aalge*)[79]	45% at 60 days	92% at 60 days
Common murres[12]	39 days	485 days
Cape vultures (*Gyps coprotheres*)[7]	74.8% annual survival	91.3% annual survival
American coots (*Fulica americana*)[115]	49% at 4 months	76% at 4 months
Surf scoters (*Melanitta perspicillata*)[40]	14.3% at 5 months	49.8% at 5 months
	REHABILITATED GROUP SURVIVAL GREATER THAN CONTROL GROUP	
Hedgehogs (*Erinaceus europaeus*)[83]	73.1%	63.6% at 8 weeks
Surf scoters[40]	77.2% at 5 months	49.8% at 5 months
	REHABILITATED GROUP SURVIVAL SIMILAR TO CONTROL GROUP	
Sea otters (*Enhydra lutris*)[93]	71% at 1 year	75% at 1 year
Koalas (*Phascolarctos cinereus*)[22]	58% annual survival	67% annual survival
Carnaby's cockatoos (*Zanda latirostris*)[98]	73% annual survival	61%–69% annual survival
Hedgehogs[82,116]	57% at 38 days	50% at 38 days
Shorebirds[74]	50% at 6 months	52% at 6 months
Western gulls (*Larus occidentalis*)[76]	100% at 6 months	90% at 6 months
Cape gannets (*Morus capensis*)[60]	86% (Malgas Island), 88% (Bird Island) annual survival	88% (Malgas Island), 90% (Bird Island) annual survival
Peregrine falcons (*Falco peregrinus*)[117]	14% at 1 year	10–11% at 1 year

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affect survival pre- and post-release. Habituation to humans and the associated loss of wild behaviours such as predator avoidance can result in poor survival.[6,73,85,90–92] Therefore, shorter periods of rehabilitation may be better,[67] yet in this time it is critical to teach hunting, foraging, and wild behaviours to support survival.[91,94,95] Depending on species' social behaviour, it may be important to form and release social groups together.[112] The provision of mentor animals could also provide benefits for animals such as deer, as the mother–fawn relationship has been shown to be essential for survival.[97] A veterinary examination prior to release is important in assessing readiness in terms of appropriate age, physical fitness, independence, and recovery from disease or injury, and requirements will vary between species for optimising survival.[68,89,101]

Several factors relating to the release environment were found to influence survival of rehabilitated wildlife, including the timing of release, release method, quality of the release habitat, and presence of predators. Responses varied among species. For example, soft release improved survival of Asiatic black bears,[104] but not kangaroos and tawny owls.[102,122] Habitat familiarity and quality also can be critical for some species' long-term survival.[108] Thus, an unfamiliar environment contributes to low survival rates for possums, hedgehogs, and deer.[13,90,106,107] For koalas, habitat quality is more important than habitat familiarity,[67] possibly due to their specialist feeding habits. A few studies identified effects of the human–wildlife interface on survival.[44] For example, survival rates of bears reflected hunting pressures, with increased numbers of encounters in recolonised areas resulting in more illegal kills by local residents.[84]

Limitations of the papers in this review

It is possible that relevant articles were missed in our search, particularly if they were published in another language, which may be why some regions were not represented in our results. Our analysis also indicated some publication bias. However, asymmetry in funnel plots does not always reflect publication bias and can result from other factors, such as poor methods leading to exaggerated effects in smaller studies.[31] Few studies in this review included a control group, even though comparing survival with a control group is beneficial to assess whether rehabilitated wildlife is disadvantaged post-release. Lunney et al.[22] found that if they

had examined only rehabilitated burnt koalas, their project would have been determined a failure due to the low survival rates, yet survival was similar to that of unburnt koalas in the same area. Another confounding factor was the different post-release monitoring methods, durations, and measures of survival presented by reviewed articles. Some authors presented minimum percentages of animals known to be alive, while others calculated an annual survival rate or mean days survived, and there were often large numbers of individuals unaccounted for due to emigration from the monitoring area, failure of tracking collars, or early conclusion of fieldwork.[102] Several retrospective studies utilising wildlife rescue centre and rehabilitation databases acknowledged the poor quality and inconsistency of the data recorded.[2,3,32,33] We note that some zoos contribute to rehabilitation research through their wildlife hospitals and other partnerships. However, these outcomes were not specifically searched for via zoo webpages, as relevant articles could have been detected in Google Scholar and conference proceedings searches (outlined in S1 Table).

Lessons from reintroduction biology

There is a wealth of published studies on conservation translocation and reintroduction programs with varying levels of success,[5,14] which could be used to improve release protocols after rehabilitation. Some wildlife rehabilitation standards and guidelines include a requirement that rescued wildlife be returned to the location where they were found, if possible. Yet, with areas of suitable habitat diminishing,[123,124] or when the reason for rehabilitation is habitat loss (for example, catastrophic bush fires), policies for the translocation of rescued wildlife may need to be considered where survival will not be negatively affected.

Batson et al.[125] synthesised 30 techniques that have been used in translocation programs to influence post-release survival, separated into Animal Focused Tactics and Environmental Focused Tactics. The factors associated with survival presented in Table 4 align with many of these tactics, and as such could be used as a checklist prior to release of rescued wildlife and as a guide for future research priorities in rehabilitation programs. Research should be conducted to support best practice recommendations for each of these tactics, and we recommend that wildlife rescue organisations ensure that they educate their rehabilitators on these tactics (where data are available) for species in their region.

Environmental preconditioning in the form of predator control is an important consideration, particularly for translocation programs, given the large number of failures attributed to predation.[14] Our results show that introduced and native predators also played a role in mortalities of released rehabilitated wildlife. There is evidence to support the benefits of protection from predators (via wildlife training or use of a fence), predator control efforts (e.g. baiting or shooting), and absence of predators.[126–129] It would be beneficial for environmental managers to engage with wildlife rehabilitation organisations and provide data about introduced predator and conspecific densities in surrounding habitat, and any intended control programs, so that suitable release locations can be appropriately identified.

Conservation translocations follow guidelines set by the International Union for Conservation of Nature,[130] which state that post-release monitoring is an essential part of a responsible conservation translocation with data collected on survival, reproduction, and dispersal. However, post-release monitoring by wildlife rehabilitators is often limited due to lack of funds, lack of expertise, and onerous requirements for state permission.[19] This is where collaboration between university and government researchers and wildlife rehabilitation organisations can provide great benefits. GPS tracking technology would assist post-release monitoring, and rapid advancements in technology now allow access to devices of smaller size and greater battery duration at low cost.[5]

Recommendations for wildlife rehabilitation and future directions

Adequate resources for rapid rescue responses are key to improving survival rates of wildlife, particularly after severe or widespread incidents.[33,59] After the Black Summer bushfires in Australia there were cases where wildlife rescuers could not access fire grounds due to safety concerns or lack of support,[15] highlighting the need for appropriate emergency response plans and resources.[23] It is also likely that veterinarians will encounter a larger volume and diversity of wildlife than they are accustomed to during disaster events. As such, it is vital to develop advice and support services for veterinarians.

Whether or not wildlife rehabilitation contributes to conservation outcomes is debated and lacks evidence,[1] yet it will continue to be practiced around the world and likely play a role in the persistence of local wildlife populations following increasingly frequent and severe environmental disasters.[20,21] The need to incorporate wildlife rescue into broader disaster response plans is gaining traction, with some organisations facilitating improvements to disaster preparedness[131] and developing wildlife first aid guidelines.[132] Rescued wildlife are exposed to the stress of the initial adverse event, in addition to stress occasioned by transport, treatments, captivity, and release.[114] The potential distress experienced by animals needs to be pragmatically weighed with the benefits of survival for the individual, population and species. If animals are released with a reduced likelihood of survival this presents a potentially serious welfare concern if they are unable to adapt or are more susceptible to threats than wild counterparts. It is vital that research continues to develop our understanding of basic biology and husbandry requirements of native wildlife,[133] along with factors associated with survival at all stages of rehabilitation. Our comparison of post-release survival rates between study and control groups has highlighted the value of including a comparison with a wild cohort in future studies. The factors highlighted by this review and summarised in Table 4 should be used as a framework to guide the development and revision of species-specific and evidence-based rescue and treatment protocols globally. With these robust protocols, veterinarians and rescue organisations can continue

to minimise animal suffering and maximise the effectiveness of rehabilitation programs in an environment affected by climate change and urban expansion. Threat mitigation must also be prioritised to reduce the need for wildlife rescue in the first place.

Supplementary files

S1 Checklist. (DOCX)

S1 Table. Summary of the systematic search methods and numbers of articles returned. (DOCX)

S2 Table. Publication bias funnel plot analysis, eggers test output and trim-and-fill analysis output. (DOCX)

S3 Table. Summary of mixed-effects meta-regression models with the best fit but no significant predictors of survival. (DOCX)

S4 Table. A summary of reviewed articles and the survival measures reported for mammals and birds during care, and in the short- and long- term post release. (DOCX)

S5 Table. A summary of factors associated with wildlife survival during rehabilitation and after release, including relevant species and references. (DOCX)

S6 Table. Systematic review article data used for meta-analysis. (XLSX)

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Chlamydia buteonis in birds of prey presented to California wildlife rehabilitation facilities

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Cooper's hawk (*Accipiter cooperii*).

Introduction

Chlamydiae are obligate intracellular, gram-negative bacteria responsible for a variety of diseases in multiple species, including humans.[1] One example, *Chlamydia psittaci*, is a cosmopolitan chlamydial species and important zoonotic pathogen. This organism has a global distribution and has been reported in > 469 bird species encompassing 30 orders.[2,3] Currently, there are 14 recognized and 3 candidate species in the genus *Chlamydia*,[4–9] including the newly identified *C. buteonis*.[10]

There are relatively few reports of chlamydial infections in raptors in the United States.[11–14] In 1983, *C. psittaci* was isolated from four red-tailed hawks (*Buteo jamaicensis*; RTHA) in northern California (CA) and in 1992, *C. psittaci* was cultured from a RTHA in Louisiana with respiratory distress and diarrhea.[11,12] A recent study reported a 1.37% prevalence of chlamydial DNA in free-ranging raptors in northern CA, although anti-chlamydial antibodies using an elementary body agglutination (EBA) assay were not identified in any bird.[14] In Oregon, 3.6% of raptors at rehabilitation centers were positive for *C. psittaci* DNA.[13] In 2019 a red-shouldered hawk (*Buteo lineatus*; RSHA), one of 12 birds in an enclosure, developed conjunctivitis and died. Antemortem and postmortem testing including whole genome sequencing identified a novel chlamydial organism with *C. psittaci* and *C. abortus* as closest relatives and it was proposed that this novel organism be identified as *C. buteonis*, RSHA strain.[10]

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ABSTRACT
Chlamydial infections, caused by a group of obligate, intracellular, gram-negative bacteria, have health implications for animals and humans. Due to their highly infectious nature and zoonotic potential, staff at wildlife rehabilitation centers should be educated on the clinical manifestations, prevalence, and risk factors associated with *Chlamydia* spp. infections in raptors. The objectives of this study were to document the prevalence of chlamydial DNA shedding and anti-chlamydial antibodies in raptors admitted to five wildlife rehabilitation centers in California over a one-year period. Chlamydial prevalence was estimated in raptors for each center and potential risk factors associated with infection were evaluated, including location, species, season, and age class. Plasma samples and conjunctiva/choana/cloaca swabs were collected for serology and qPCR from a subset of 263 birds of prey, representing 18 species. Serologic assays identified both anti-*C. buteonis* IgM and anti-chlamydial IgY antibodies. Chlamydial DNA and anti-

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Chlamydiaceae species have significant impacts on human and animal health worldwide.[15,16] Recent discoveries of new species such as *C. buteonis* highlight major gaps in our understanding of host range, diversity and pathogenicity of *Chlamydiaceae* family members. Continued characterization of novel chlamydial organisms provides evidence that additional species may be pathogenic and pose zoonotic risks.[15,17] Recent expansions in their known host range highlight the risk for spill-over infections with increasing contact between wildlife, livestock, and humans because of greater wildlife habitat encroachment, intensification of livestock production, and pet ownership.[15,17] Most human cases of *C. psittaci* have been linked to companion birds, but some are linked to free-living birds.[18,19] A *C. psittaci* genotype identified from a raptor at a Belgian refuge was also cultured from three human contacts; one person reported symptoms consistent with chlamydiosis.[19] *Chlamydia psittaci* can be transmitted via ingestion or inhalation of ocular and nasal discharges and droppings from infected birds leading to risk of outbreaks at rehabilitation centers.[20–22] However, for many chlamydial species, modes of transmission are still unclear.

Diagnosis of chlamydial infections is challenging due to the absence or variability of clinical signs; multi-modal diagnostic testing is recommended for confirmatory diagnosis.[23] Isolation in cell culture was considered the gold standard until recently; now PCR-based detection is considered the gold standard for chlamydial diagnostics.[24,25] Samples are collected from epithelial surfaces that shed *C. psittaci* (e.g., conjunctiva, choana, and cloaca) or from infected tissues such as liver, lung, air sac, and spleen.[23,26,27] Additional testing includes serology using paired serum samples collected from acute- and convalescent-phase patients obtained at least 2 weeks apart and tested in the same laboratory at the same time.[23] Commercially available EBA assays detect *C. psittaci* IgM, while indirect fluorescent antibody tests (IFA) detect *Chlamydia* spp. IgY at the genus level.[23] As a result, serology can lead to conflicting results based on chlamydial species and time of sample collection.

Abstract CONTINUED

chlamydial antibodies were detected in 4.18% (11/263) and 3.14% (6/191) of patients, respectively. Chlamydial DNA was identified in raptors from the families Accipitridae and Strigidae while anti-*C. buteonis* IgM was identified in birds identified in Accipitridae, Falconidae, Strigidae, and Cathartidae. Two of the chlamydial DNA positive birds (one Swainson's hawk (*Buteo swainsoni*) and one red-tailed hawk (*Buteo jamaicensis*) were necropsied, and tissues were collected for culture. Sequencing of the cultured elementary bodies revealed a chlamydial DNA sequence with 99.97% average nucleotide identity to the recently described *Chlamydia buteonis*. Spatial clusters of seropositive raptors and raptors positive for chlamydial DNA were detected in northern California. Infections were most prevalent during the winter season. Furthermore, while the proportion of raptors testing positive for chlamydial DNA was similar across age classes, seroprevalence was highest in adults. This study questions the current knowledge on *C. buteonis* host range and highlights the importance of further studies to evaluate the diversity and epidemiology of *Chlamydia* spp. infecting raptor populations.

To our knowledge there are no studies assessing detection of chlamydial DNA and seroprevalence in raptors admitted to wildlife rehabilitation centers in the US. Additionally, investigations of infection risk factors are lacking in wild birds. The objectives of this study were to evaluate the prevalence of chlamydial DNA shedding and seroprevalence in raptors admitted to five wildlife rehabilitation centers throughout CA over a one-year period and to explore risk factors associated with infection.

Materials and Methods

Ethics statement

This study was approved by the Institute of Animal Care and Use Committee of the University of California, Davis (protocol #19119).

Sample collection

All raptors presenting to five CA wildlife rehabilitation centers from April 2016 to May 2017 were sampled. The following centers participated in the study and represented northern, central and southern CA: (1) California Raptor Center (CRC; Davis, northern CA), (2) Pacific Wildlife Care (PWC; Morro Bay, central CA), (3) Monterey SPCA (MSPCA; Monterey, central CA), (4) Living Desert Zoo Rehabilitation Center (LD; Palm Desert, southern CA), and (5) California Wildlife Center (CWC; Calabasas, southern CA). Criteria for inclusion included permissions from California Department of Fish and Wildlife to collect diagnostic samples from raptors, an on-site veterinarian for sample collection and an average intake of 200+ raptors/year. Veterinarians performed a full physical examination on each raptor at admission and recorded their findings, admission date, location found, species, sex, age class, and reason for admission.

Conjunctival, choanal, and cloacal (c/c/c) mucosal swabs were obtained using a sterile, rayon-tipped swab (Puritan, 25–806 1PR). The swab tip was placed in a sterile 1.5 ml Eppendorf tube and stored at -20˚ C until shipment. Every three months, swabs were shipped overnight to the Infectious Disease Laboratory, University of Georgia (IDL) for quantitative real-time polymerase chain reaction (qPCR) to detect *Chlamydia* spp. DNA. Whole blood was collected from the jugular or medial metatarsal veins. Blood samples were placed into lithium heparin tubes and centrifuged at 3800 × g for 6 minutes. Plasma was collected into two cryovials and stored at -20˚C pending shipment. Every three months, plasma was shipped overnight to the University of California, Davis and stored at -80˚C until overnight shipment to the IDL. One aliquot of plasma was tested for chlamydial antibodies using elementary bodies purified from a RTHA (IDL17_4553_RTH) and Swainson's hawk [*Buteo swainsonii;* SWHA]) (IDL16-5840_SH) hawk *Chlamydia buteonis* isolate at the IDL. A second aliquot was shipped to the Avian and Wildlife Laboratory, University of Miami (AWL) for *Chlamydia* genus-specific IFA.

Two birds (one RTHA [(IDL17_4553_RTH)] and one SWHA [IDL16-5840_SH]) admitted to the CRC exhibited clinical signs suggestive of chlamydial infection. Both birds were

positive by *Chlamydia* spp. DNA via qPCR and subsequently euthanized. Necropsies were performed within 12 hours. Lung, liver, spleen, and air-sac tissues were collected for culture using aseptic technique. Tissues were placed in cryovials with sterile saline, and shipped overnight on ice to the IDL for chlamydial culture.[27,28]

Culture

Tissue samples from two birds were disrupted on the Mini Bead Mill (VWR) using 1.5ml tubes pre-filled with 2.4 mm metal beads (VWR 10158–598). Filtration using a 0.45 um filter syringe was performed on tissue homogenates and inoculated on Vero-cell monolayers with DMEM (Corning 10-013-CV) supplemented with non-essential amino acids and 10% fetal calf serum according to previously published protocols.[3,27] Cell cultures with suggestive cytopathic effects (CPE) were tested for chlamydial DNA via qPCR. Briefly, 1uL of heat-inactivated cell culture supernatant was tested for *Chlamydia* spp. DNA via the ompA qPCR described previously.[27,28] Positive cultures from the SWHA (IDL16-5840_SH) and RTHA (IDL17_4553_RTH) went through six passages to obtain nearly 100% CPE to maximize the concentration of infectious elementary bodies (EB) for isolation. DNA was extracted from purified EBs using a nucleic acid purification extraction (QuickExtract™, Epicentre Biotechnologies, Madison, WI) according to manufacturer's instructions and frozen at -80˚C until sequencing.

Sequencing and bioinformatic analysis

Chlamydial DNA was submitted for high throughput sequencing to the Centers for Disease Control and Prevention. The NEB Next Ultra DNA library prep kit for Illumina (New England Biolabs, Ipswich, MA) was used to prepare gDNA libraries according to the manufacturer's protocol.

Genome sequencing was performed on the isolates using the Illumina MiSeq desktop sequencer (Illumina, San Diego, CA). Illumina sequencing read quality was evaluated using FastQC program (v0.10.1).[29] Processing was performed with Cutadapt (v1.5)[30] and reads were removed from the data set if they met one of the following criteria: (a) had low quality (<25) sequence bases; (b) trimmed adapter sequences; (c) had an error rate above 0.03; or (d) less than 75 base pairs in length. De novo assembly was performed with VelvetOptimiser v2.2.5 and Velvet v1.2.10.[31]

Long read sequencing was performed using the Pacific Biosciences (PacBio) RS II (Pacific Biosciences, Menlo Park, CA). One or two SMRT cells were used for each isolate with a movie time of 240 minutes. The sequences were assembled with SMRT Analysis v2.2 Hierarchical Genome Assembly Process version 2 (HGAP 2) or 3 (HGAP 3) protocol. The Illumina sequencing data associated with each isolate was aligned to the long read PacBio assembly for nucleotide accuracy comparison. Average nucleotide identity (ANI) was determined using program Pyani (v0.2.10) with default settings.[32] A maximum-likelihood phylogenomic tree was computed using IQTree[33]+ ModelFinder[34] with 1000 iterations of bootstrapping from a pan-genomic analysis using Anvi'o[35] based on aligned protein sequences from all shared single copy gene clusters. Anvi'o software identified 742 single copy genes that were distinguishable as homologous among all genomes and used for further phylogenetic analysis. Sequences (SWHA (IDL16-5840_SH) and RTHA (IDL17_4553_RTH) were submitted to NCBI BioProject accession number PRJNA613727.

Quantitative PCR

Swabs were processed for DNA extraction by boiling samples for 5 minutes in buffered saline solution containing dithiothreitol (DTT). Initially, standard target DNA amplification of the *ompA* sequence for *C. psittaci* qPCR with melting curve analysis was executed using the LightCycler 480 System and probe detection as previously described.[27,36] Although positive samples were amplified using *C. psittaci* primers, the melting curve analysis of the amplicon showed that the curve Tm and shape was unique from *C. psittaci* positive-control samples, thus the samples were determined as "atypical." To further identify the "atypical" positive samples, extracted DNA was amplified by targeted PCR for *C. buteonis* using primers 17473F (AGCTCACATCAT CGCTCTCG) and 17937R (TGCGTGTTGTCGAACTAGCT) followed by gel electrophoresis. Further, Sanger sequencing was performed by Genewiz (South Plainfield, NJ) to confirm the identity of *C. buteonis* PCR products, which aligned to *C. buteonis* with 100% identity.

Serology

All plasma samples were evaluated for antibodies using isolate-specific EBA serology.[36] The EBs from RTHA (IDL17-4553) were obtained by centrifuging infected Vero cells at 46,467 x G for 1 h. The pellet was washed in Opti-MEM (Thermo Fisher, Grand Island, NY) and centrifuged at 1,200 x G for 5 min. The EBs were inactivated, layered on renografin, and centrifuged at 60,226 x G for 90 min. The band containing EBs was attained, pelleted, and resuspended in diluent to a working concentration as described previously.[36] A suspension of EBs was mixed with unknown plasma samples on a slide by a mechanical rotator for 3 minutes at 150 rpm.[37] Samples were seropositive if agglutination was present.[37] Antibody titers were recorded as the highest serum dilution with agglutination, if there was no agglutination in control wells. EBA titers ≥1:10 were considered positive.

The second aliquot of plasma was shipped to AWL for IFA IgY testing. Fixed slides with the *C. trachomatis* inclusion stage (previously grown in McCoy cells on IFA slides under 5% CO_2 and 37˚C in Modified Eagle's medium (MEM) with supplements) were stored at -70˚C and evaluated using a previously published method.[38] A titer >1:25 was considered positive.[38]

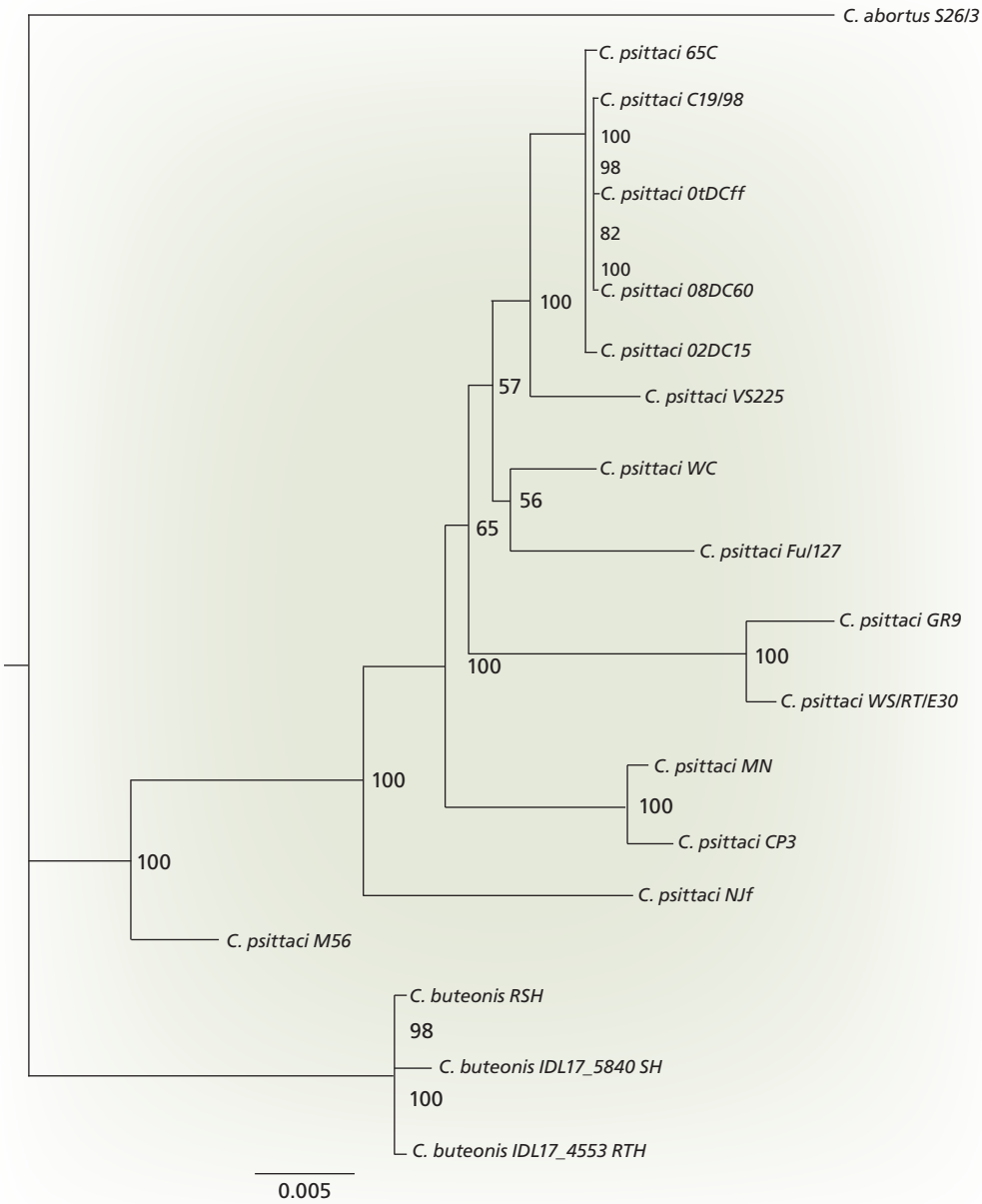
Statistical analysis

The prevalence of raptors with antibodies against *Chlamydia* spp. and chlamydial DNA detection by qPCR were estimated overall and for each center separately. Associations between *Chlamydia*

spp. infection, serological status and putative risk factors for infection were analyzed using Chi-square tests of independence. Risk factors evaluated included: (1) species; (2) age class: nestling (nestling, pre-fledge, and branchling), hatch year/juvenile (fledglings and 1st year birds), and adult, (3) season of admission—fall (September–November), winter (December–February), spring (March–May), and summer (June–September), (4) rehabilitation center, and (5) region (northern, central, and southern CA). Demographic and environmental variables associated with infection or seropositivity ($p \leq 0.1$) were further evaluated using multivariable logistic regression models. Sex was excluded from the multivariable analyses as it was undetermined for the majority (n = 196) of birds. Potentially confounding variables and interactions were evaluated in the models. A variable was considered to be confounding if there was a ≥10% change between the adjusted and unadjusted estimates of the odds ratios for other variables in the model. Final parsimonious models were selected by comparing Akaike's Information Criterion (AIC) among competing models. Adjusted odds ratios with 95% confidence intervals were estimated to assess the strength of the association between risk factors and chlamydial status. All statistical tests were performed with R statistical software version 3.4.2.[39]

Temporal and spatial statistical analyses

Locations at which the birds were found were manually geocoded from zip code data using Google Earth version 5.2 (Google Inc, Mountain View, California, USA). Latitude and longitude coordinates recorded using the WGS 84 geographic coordinate system were converted to decimal degrees. Clustering of seropositive birds and birds testing positive for chlamydial DNA was evaluated using the Bernoulli model and purely spatial and purely temporal scan statistics in SatScan™ version 9.0.[40] Locations of sampled birds and statistically significant clusters were mapped using ArcGIS® (ESRI, Redlands, California, USA).



Results

Chlamydial DNA was sampled from 263 raptors representing 18 species. The number of birds sampled from each rehabilitation center was: CRC (n = 43), MSPCA (n = 54), PWC (n = 79), CWC (n = 53) and LD (n = 34).

Sequencing and bioinformatic analysis

The sequencing results were nearly identical to the recently described *C. buteonis* sequence isolated from a RSHA.[10] Phylogenetic analysis revealed that the organisms cultured from SWHA (IDL16-5840) and RTHA (IDL17-4553) were most similar to the recently described *C. buteonis* sequence. This analysis supported the inclusion of the SWHA and RTHA isolates in the clade containing *C. buteonis* as determined by Laroucau et al.,[10]

FIGURE 1. Maximum likelihood phylogenetic tree showing that the SWHA and RTHA isolates were most similar to C. buteonis. The tree was computed using programs IQtree [33]+ ModelFinder [34] with 1000 iterations of bootstrapping from a pangenomic analysis using Anvi'o [35] based on aligned protein sequences from all shared sing copy gene clusters. https://doi.org/10.1371/journal.pone.0258500.g001

which is a phylogenetically intermediate between *C. psittaci* and *C. abortus* (Fig. 1); comparison between the recently described *C. buteonis* sequence[10] and the SWHA and RTHA isolates revealed 99.97% ANI.

Quantitative PCR

Swab specimens from 11/263 (4.18%) raptors were positive on *Chlamydia* spp. qPCR but were not characteristic for *C. psittaci* and were identified as "atypical positive;" all 11 positive chlamydial samples were also positive using *C. buteonis* primers (Table 1). Sanger sequencing from qPCR positive amplicons were identical to the recently described *C. buteonis* sequence.[10]

Positive qPCR samples were identified in 6.4% of qPCR positive birds taxonomically classified in the family Accipitridae and 2.0% in the family Strigidae (Table 1). Further, the most detected qPCR positive species was RTHA (n = 8), followed by SWHA (n = 1), Cooper's hawk (*Accipiter cooperii*; COHA) (n = 1), and great horned owl (*Bubo virginianus*; GHOW) (n = 1) (Table 1). Although positive qPCR results were more commonly detected in RTHAs, differences in prevalence across species and taxonomic families were not statistically significant (p = 0.798, p = 0.060, respectively). Additionally, positive qPCR prevalence was similar across age classes including 3.04% hatch years (n = 8), 0.38% juveniles (n = 1), and 0.76% adults (n = 2) (Table 2). However, significant seasonal differences for positive qPCR samples were identified (p = 0.002). The greatest number of positive birds were admitted in winter (n = 7), followed by spring (n = 2), summer (n = 2), and fall (n = 0) (Table 2). Birds admitted in the winter season were 8.1 times more likely to be qPCR positive than birds admitted during other seasons (OR = 8.1, 95% CI: 2.3–29.1).

Positive qPCR samples were identified from 3/43 (6.98%) birds admitted to CRC (2 RTHAs, 1 SWHA), 4/54 (7.41%) birds from MSPCA (4 RTHAs), 3/79 (3.80%) birds from PWC (1 Great Horned Owl [*Bubo virginianus*; GHOW], 2 RTHAs), and 1/34 (2.94%) birds from LD (1 Cooper's hawk (*Accipiter cooperii*; COHA; Table 2). No *C. buteonis* qPCR positives were detected in birds admitted to CWC. This corresponded to prevalence estimates of 6.98% (3/43) in northern CA, 5.26% (7/133) in central CA, and 1.15% (1/87) in southern CA; however, the differences across centers or regions were not statistically significant based on bivariate analyses (p = 0.318, p = 0.190, respectively).

A significant spatial cluster of raptors positive for *C. buteonis* DNA by qPCR (p = 0.002) was detected in northern CA, extending 24.5 km from its center at 38.310500 N, 121.901800 W and encompassing communities between Sacramento and San Francisco (Fig. 2). A significant temporal cluster of raptors testing positive for *C. buteonis* DNA was detected during the winter season lending further support to the seasonal pattern of infections.

TABLE 1. Results for chlamydial DNA and serology (elementary body agglutination; EBA and immunofluorescent antibody; IFA) by raptor species. Numbers of positive birds for each species are presented with the percentage testing positive in parenthesis below the number.
https://doi.org/10.1371/journal.pone.0258500.t001

		qPCR		EBA		IFA	
FAMILY	BIRD SPECIES	N	POSITIVE	N	POSITIVE	N	POSITIVE
Accipitridae	Bald eagle	1	0	1	0	1	0
	Cooper's hawk	43	1 (2.3%)	34	1 (2.9%)	34	0
	Rough-legged hawk	1	0	1	0	1	0
	Red-shouldered hawk	27	0	19	0	18	0
	Red-tailed hawk	70	8 (11.4%)	56	10 (17.9%)	55	9 (16.4%)
	Swainson's hawk	11	1 (9.0%)	5	1 (20.0%)	6	0
	White-tailed kite	2	0	2	0	2	0
	NA-hawk	1	0	1	0	1	0
	Subtotal	**156**	**10 (6.4%)**	**119**	**12 (10.1%)**	**118**	**9 (7.6%)**
Cathartidae	Turkey vulture	9	0	7	1	7	0
	Subtotal	**9**	**0 (0.0%)**	**7**	**1 (14.3%)**	**7**	**0 (0.0%)**
Falconidae	American kestrel	4	0	2	0	2	0
	Merlin	1	0	0	0	0	0
	Peregrine falcon	3	0	2	0	1	0
	Prairie falcon	1	0	1	1 (100.0%)	1	1 (100.0%)
	Subtotal	**9**	**0 (0.0%)**	**5**	**1 (20.0%)**	**4**	**1 (25.0%)**
Pandionidae	Osprey	1	0	0	0	0	0
	Subtotal	**1**	**0 (0.0%)**	**0**	**0 (0.0%)**	**0**	**0 (0.0%)**
Strigidae	Burrowing owl	2	0	0	0	0	0
	Great horned owl	46	1 (2.2%)	36	1 (2.7%)	35	1 (2.8%)
	Long-eared owl	1	0	0	0	0	0
	Western screech owl	1	0	1	0	1	0
	Subtotal	**50**	**1 (2.0%)**	**37**	**1 (2.7%)**	**36**	**1 (2.8%)**
Tytonidae	Barn owl	38	0	22	0	21	0
	Subtotal	**38**	**0 (0.0%)**	**22**	**0 (0.0%)**	**21**	**0 (0.0%)**

Serology

In total, 15 of 190 (7.89%) plasma samples were positive for isolate-specific anti-chlamydial EBA antibodies. Two birds positive via EBA had *Chlamydia* spp. qPCR positive results (Table 1). Complementary to the qPCR results, the EBA and IFA seroprevalences did not differ

by taxonomic family (p = 0.245, p = 0.229, respectively). In the family Accipitridae, 10.1% of EBA samples were positive and 14.3% in the family Cathartidae, 20.0% in the family Falconidae and 2.7% in the family Strigidae were also positive (Table 1). Meanwhile, IFA results showed 7.6% of EBA positive birds were classified in the family Accipitridae, 25% in the family Falconidae, and 2.8% in the family Strigidae (Table 1). The most seropositive species via EBA and IFA was the RTHA (n = 10 and 9, respectively), followed by SWHA (n = 1 and 0 respectively), COHA (n = 1 and 0 respectively), turkey vulture (*Cathartes aura*; TUVU) (n = 1 and 0 respectively), prairie falcon (*Falco mexicanus Schlegel*; PRFA) (n = 1 and 1 respectively), and GHOW (n = 1 and 1 respectively), (Table 1). Seroprevalence by EBA and IFA varied according to age (p = 0.006, p = 0.035, respectively) with the highest seroprevalence in adult birds (EBA: 50.0% adults, 42.8% hatch years/juveniles, 0% nestlings; IFA: 54.5% adults, 45.4% hatch years/juveniles, 0% nestlings). Adult birds were 3.5 times more likely to be seropositive by EBA (OR = 3.5; 95% CI: 1.1, 11.4) than juvenile birds (hatch years and nestlings). Age class was not statistically significant in the logistic regression model for seropositivity by IFA; however, age class was included in the final IFA model to adjust for confounding. Seroprevalence by EBA and IFA were not variable by season (p = 0.776, p = 0.412, respectively).

Eight of 28 (28.57%) birds from the CRC, 1/14 (7.14%) birds from MSPCA, 5/74 (6.76%) birds from PWC, 0/53 (0%) birds from CWC, and 0/21 (0%) birds from LD were EBA seropositive (Table 1). Differences in seroprevalence by EBA varied significantly across centers and regions (both p <0.001) with the highest seropositivity in northern CA (28.57%), then central CA (6.82%), and southern CA (1.35%). Birds admitted to centers in northern CA were 5.6 times more likely to be seropositive by EBA (OR = 5.6; 95% CI: 1.7, 18.9) than other regions. Eleven of 186 (5.91%) birds were seropositive by *Chlamydia* genus-specific IFA (Table 2). Positive IFA results from 6/11 samples correlated with positive EBA results and three positive IFA birds had positive *Chlamydia* spp. qPCR positive results (Table 2). One qPCR positive RTHA was also positive for both EBA and IFA antibodies (Table 2).

Seroprevalence of IFA by rehabilitation center was: CRC: 7/28 (25.00%), MSPCA: 2/14 (14.29%), PWC: 2/72 (2.78%), CWC: 0/51 (0%), and LD: 0/21 (0%). IFA seroprevalences were estimated to be 25% in northern CA, 4.65% in central CA and 0% in southern CA. Differences in prevalence of anti-chlamydial antibodies by IFA across centers and regions were statistically significant (both p <0.001). Birds in northern CA were 11.3 times more likely to be seropositive by IFA (OR = 11.3; 95% CI: 2.8, 45.3) than birds in other regions.

A significant spatial cluster of seropositive raptors (EBA and/or IFA) was detected (p = 0.001) in the same area (center at 38.310500 N, 121.901800 W) as the qPCR positive raptors, but encompassed a larger area, extending 42.40 km from its center (Fig. 3). A significant temporal cluster of raptors testing positive for chlamydial DNA was detected during the winter season lending further support to the seasonal pattern of infections.

TABLE 2. Comparative risk factors for raptors for positive qPCR (n = 263), elementary body agglutination antibodies (EBA; n = 190), or immunofluorescent antibodies (IFA; n = 186) with species, age class, wildlife rehabilitation center, and season of admission. Sex was excluded as this variable was undetermined for the majority (n = 196) of birds.

PATIENT INFORMATION						TEST RESULTS		
BIRD ID	CENTER*	SPECIES	SEASON	SEX	AGE	QPCR	EBA	IFA
62	MSPCA	RTHA	W	NA	HY	+	NA	NA
63	MSPCA	RTHA	W	NA	HY	+	NA	NA
64	MSPCA	RTHA	W	NA	A	+	–	+
65	MSPCA	RTHA	W	NA	A	+	+	+
226	PWC	GHOW	S	NA	HY	+	–	–
232	PWC	RTHA	S	M	HY	–	+	–
236	PWC	RTHA	S	NA	HY	–	+	–
246	PWC	RTHA	W	NA	HY	+	+	–
254	PWC	GHOW	F	NA	HY	–	+	–
263	PWC	TUVU	F	NA	HY	–	+	–
303	PWC	RTHA	P	M	HY	–	NA	+
307	PWC	GHOW	P	M	HY	–	–	+
341	PWC	RTHA	W	NA	HY	+	–	NA
412	LD	COHA	S	NA	HY	+	–	–
644	CWC	SWHA	F	NA	A	–	+	–
804	CRC	COHA	S	NA	NA	–	+	–
811	CRC	RTHA	S	F	A	–	+	+
813	CRC	RTHA	S	NA	HY	–	–	+
815	CRC	RTHA	S	NA	HY	–	+	+
816	CRC	RTHA	S	M	A	–	+	+
824	CRC	RTHA	P	NA	A	–	+	+
826	CRC	RTHA	W	NA	A	–	+	+
861	CRC	PRFA	F	F	A	–	+	–
931	CRC	RTHA	P	NA	A	–	+	–
1001	CRC	RTHA	P	M	HY	+	–	+
1002	CRC	SWHA	P	F	J	+	NA	NA
1003	CRC	RTHA	W	M	HY	+	NA	NA

*MSPCA: Monterey SPCA, PWC: Pacific Wildlife Center, LD: Living Desert, CRC: California Raptor Center. RTHA: Red-tailed hawk; GHOW: Great horned owl; TUVU: Turkey vulture; COHA: Cooper's hawk; SWHA: Swainson's hawk; PRFA: Prairie falcon. F = Fall, W = Winter, P = Spring, S = Summer. HY = Hatch year, J = Juvenile, A = Adult. +: Positive result, -: Negative result, NA: Test was not performed/result was not determined. https://doi.org/10.1371/journal.pone.0258500.t002

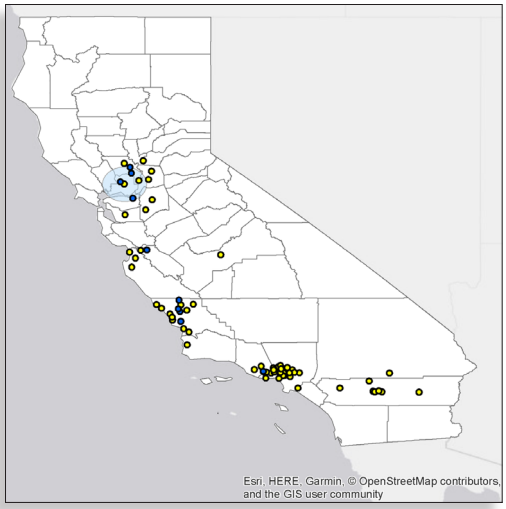


FIGURE 2. Map of the significant spatial cluster for raptors testing positive for *Chlamydia* spp. DNA by rt-qPCR in northern California. Distribution of raptors testing positive (blue) and negative (yellow; Center, 38.310500 N, 121.901800 W). https://doi.org/10.1371/journal.pone.0258500.g002

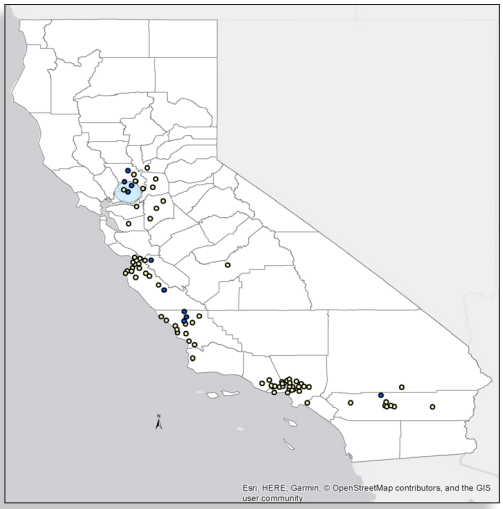


FIGURE 3. Map of the significant spatial cluster of *Chlamydia* spp. seropositive raptors by EBA and/or IFA in northern California. Distribution of seropositive raptors (blue) and seronegative raptors (yellow; Center, 38.310500 N, 121.901800 W). https://doi.org/10.1371/journal.pone.0258500.g003

Nine of 11 (81.82%) birds positive by qPCR had physical examination findings recorded upon presentation. Clinical manifestations in infected birds were non-specific and included dehydration (8/9 birds) and co-infection with feather lice (5/9 birds). Most EBA seropositive birds were dehydrated (9/14) and all seropositive birds had a variety of traumatic injuries (e.g., wing injuries) and co-infections including coccidiosis, trichomoniasis, and avian pox.

Discussion

Chlamydial DNA prevalence in raptors admitted to rehabilitation centers across CA was identified as "atypical" using *C. psittaci* primers and targeted PCR along with Sanger sequencing confirmed *C. buteonis* from the qPCR "atypical" positive DNA samples. Further, whole genome sequencing analysis performed on EBs from bacterial isolates from two raptors confirmed that the chlamydial isolate had a 99.97% ANI to *C. buteonis*, a newly identified species.[10] This is notable as the RSHA isolate was identified in France, whereas these isolates were identified in CA, USA. Isolate-specific EBA methodology was performed to investigate for IgM serological prevalence. One COHA and one GHOW had positive qPCR and antibody results; neither bird is in the genus *Buteo*. While previous research reported that C. *buteonis* has only been identified in the *Buteo* genus (RSHA), this study demonstrates that *C. buteonis* DNA was detected in other raptor hosts including a GHOW and detectable antibodies were identified in a TUVU, PRFA, and GHOW. These findings highlight the need for additional studies to evaluate diversity as well as pathogenicity and zoonotic potential of chlamydial species infecting raptors.

Chlamydial qPCR prevalence reported here (4.18%) is similar to data from raptors admitted to Oregon wildlife rehabilitation centers,[13] and is higher than that found in free-ranging *Buteo* spp. in CA.[14] Similarities in prevalence between these studies could be explained by similar geographical locations of study populations or prey species available in Oregon and northern/central CA. Chlamydial qPCR prevalence was similar between free-ranging raptors in Sweden (1.3%) and northern CA (1.37%).[14,41] The majority of raptors admitted to rehabilitation centers are sick or injured, thus chlamydial spp. infections would be expected to be higher than in free-ranging, presumably healthy birds. Meanwhile, a lower seroprevalence was detected in the study reported here than from raptors in Germany (63%) and previously from northern CA (44%);[11,42] however, different testing methodologies for *C. psittaci* were utilized in those studies. The findings reported here may also differ from previous studies due to time of infection, immune response or handling artifacts. Moreover, a final sample size of 250 birds/center was anticipated based on previous intake numbers. However, centers reported lower intake numbers during the study leading to a smaller sample size of approximately 25% of expected.

Most birds qPCR-positive for *Chlamydia* DNA had negative serological results. This could result from detection of exogenous DNA from environmental contamination, plasma samples acquired before an immune response could be mounted or immunosuppression due to co-infections, toxins, drought or lack of prey. Because a single swab from multiple mucosal surfaces was analyzed for chlamydial DNA, the primary source of the organism cannot be determined. Multiple birds that were EBA seronegative were IFA seropositive, possibly due to birds being in the chronic stage of infection.[28]

There were significant spatial clusters of *Chlamydia* DNA positive and seropositive birds detected in northern CA. It is possible that this chlamydial organism is confined to populations of raptors in northern, and to a lesser degree, central CA. An alternative explanation is that raptors are exposed to chlamydial organisms from infected dietary items that are more commonly found in these regions. In addition, chlamydial DNA prevalence and seroprevalence was highest in, but not exclusive to, RTHAs. Red-tailed hawks are one of the most abundant raptors within the CA central valley and constituted 26.6% of the sample population. The primary RTHA diet in the CA central valley consists of ground squirrels (*Spermophilus* spp.) black-tailed jackrabbits (*Lepus californicus*) and pocket gophers (*Geomys* spp., *Thomomys* spp.), although other mammals, small birds and reptiles have been reported.[43] It is possible that free-ranging rodents or rabbits may

be a source of a chlamydial organism capable of infecting raptors. To the authors' knowledge, there are no reports confirming chlamydial organisms in these prey species.

Chlamydial qPCR positive prevalence was highest in birds presented during the winter, and seroprevalence increased with age. The higher prevalence in winter months could be associated with environmental stressors leading to reduced immunity. For example, rehabilitators often receive juvenile raptors emaciated with secondary infections during the winter.[44] It is also possible that raptors are increasingly exposed to the organism due to prey shifts in winter months. Birds testing positive for chlamydial DNA presented with nonspecific clinical signs, and all seropositive birds suffered from traumatic injuries and/or co-infections. Oregon rehabilitation centers reported similar co-morbidities with positive chlamydial DNA birds.[13]

Positive birds reported here had common conditions such as pediculosis and dehydration; however, these conditions are common in ill raptors, regardless of the underlying condition. Correlations of clinicopathologic test results with *Chlamydia*-specific testing would be beneficial. Further epidemiological studies are needed to better understand hosts and transmission of this organism.

To our knowledge, this is the first prospective study assessing chlamydial DNA prevalence and seroprevalence in raptors admitted to wildlife rehabilitation centers in the US. Understanding the prevalence of this organism in rehabilitation centers is important because of its potentially high transmission rate to other birds and its relationship to other *Chlamydia* spp. with known zoonotic potential. Chlamydial infections in raptors can be problematic for individuals in contact with birds, including rehabilitation center staff, wildlife biologists, veterinarians and falconers.[20,21] Suspected zoonotic transmission of a chlamydial infection to volunteers at a rehabilitation center highlighted that wildlife rehabilitation workers were unaware of the symptoms of chlamydiosis.[19] Understanding the prevalence and risk factors associated with this pathogen aids in education and outreach to at-risk individuals regarding its zoonotic potential and prevention. The detection of atypical chlamydial organisms in wild raptors could also be of importance in the management of threatened or endangered species. Chlamydial bacteria can spread to other avian species, including sympatric avian populations such as SWHAs, a threatened raptor population in CA.

Conclusion

This study reports the prevalence and risk factors associated with a recently characterized chlamydial species in raptors presenting to wildlife rehabilitation centers in CA, USA. Previous research identified *C. buteonis* in a RSHA; however, this study shows that *C. buteonis* has a larger host range that expands to other raptor families in addition to Accipitridae including Falconidae, Strigidae, and Cathartidae. Information generated through this study will enhance awareness among wildlife rehabilitation staff of chlamydial infections in raptors and guide in assessments of admitted birds. These results demonstrate that the current understanding of chlamydial infections in raptors is limited and additional studies are needed to fully elucidate the diversity and epidemiology of *Chlamydia* spp. circulating among raptor populations in CA.

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Competing interests

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Evaluating the risk of SARS-CoV-2 transmission to bats in the context of wildlife research, rehabilitation, and control

JD Cook, EH Campbell Grant, Jeremy TH Coleman, JM Sleeman, MC Runge. *Wildl Soc Bull,* 2022; 46:e1262. https://doi.org/10.1002/wsb.1262

Preventing wildlife disease outbreaks is a priority for natural resource agencies, and management decisions can be urgent, especially in epidemic circumstances. With the emergence of SARS-CoV-2, wildlife agencies were concerned whether the activities they authorize might increase the risk of viral transmission from humans to North American bats, but had a limited amount of time in which to make decisions. We describe how decision analysis provides a powerful framework to analyze and reanalyze complex natural resource management problems as knowledge evolves. Coupled with expert judgment and avenues for the rapid release of information, risk assessment can provide timely scientific information for evolving decisions. In April 2020, the first rapid risk assessment was conducted to evaluate the risk of transmission of SARS-CoV-2 from humans to North American bats. Based on the best available information and relying heavily on expert judgment, the risk assessment found a small possibility of transmission during summer work activities. Following that assessment, additional knowledge and data emerged, such as bat viral challenge studies, that further elucidated the risks of human-to-bat transmission and culminated in a second risk assessment in the fall of 2020. We updated the first SARS-CoV-2 risk assessment with new management alternatives and new estimates of little brown bat (*Myotis lucifugus*) susceptibility, using findings from the fall 2020 assessment and other empirical studies. We found that new knowledge led to an 88% decrease in the median number of bats estimated to be infected per 1,000 encountered when compared to earlier results. The use of facemasks during, or a negative COVID-19 test or vaccination prior to, bat encounters further reduced those risks. Using a combination of decision analysis, expert judgment, rapid risk assessment, and efficient modes of information distribution, we provided timely science-based support to decision makers for summer bat work in North America.

Recommendations for the rehabilitation and release of wild-born, captive-raised cheetahs: the importance of pre- and post-release management for optimizing survival

EH Walker, S Verschueren, A Schmidt-Küntzel, L Marker. *Oryx,* 2022;56(4);495–504. https://doi.org/10.1017/S0030605321000235

Large carnivores are frequently released for conservation purposes, but early efforts struggled with inadequate monitoring and reporting, resulting in poor understanding of success. Although managers have improved release practice and monitoring, the use of orphaned, captive-raised large carnivores for release remains controversial because of the potential influence of captivity and the possible lack of natural behaviours in such individuals. Yet, rehabilitating orphaned individuals for release could help mitigate pressures on vulnerable wild populations. We present a case study on the rearing, rehabilitation and release of wild-born cheetahs *Acinonyx jubatus* that were rescued as orphans in Namibia. Our aim was to develop a methodological framework for cheetah release planning and post-release management based on the outcome of release trials conducted during 2004–2018. Between 2001 and 2012, we rescued 86 orphaned cheetahs from the wild. Of these, 36 (42%) were selected as release candidates. We found high success rates (75–96%) of selected individuals in achieving independence post-release. Additionally, annual survival estimates for rehabilitated individuals that reached independence were comparable to those of wild counterparts described by other studies, and some rehabilitated individuals reproduced with wild conspecifics. Our findings demonstrate the ability of wild-born, captive-raised cheetahs to transition back into the wild with strategic pre- and post-release management directed towards optimizing survival. This includes selecting appropriate release candidates, forming artificial coalitions, balancing habituation levels during captivity, choosing appropriate release sites, and providing strategic support during post-release monitoring. We encourage scientists and managers to implement and refine our protocol for rehabilitation throughout the cheetah's current and historic range.

Functional connectivity of the world's protected areas

A Brennan, R Naidoo, L Greenstreet, Z Mehrabin, N Ramankutty, C Kremen. *Science,* Jun 2022;376(6597);1101–1104. https://doi.org/10.1126/science.abl8974

Global policies call for connecting protected areas (PAs) to conserve the flow of animals and genes across changing landscapes, yet whether global PA networks currently support animal movement—and where connectivity conservation is most critical—remain largely unknown. In this study, we map the functional connectivity of the world's terrestrial PAs and quantify national PA connectivity through the lens of moving mammals. We find that mitigating the human footprint may improve connectivity more than adding new PAs, although both strategies together maximize benefits. The most globally important areas of concentrated mammal movement remain unprotected, with 71% of these overlapping with global biodiversity priority areas and 6% occurring on land with moderate to high human modification. Conservation and restoration of critical connectivity areas could safeguard PA connectivity while supporting other global conservation priorities. ■

TAIL ENDS



Sheesh, the honey-do lists never end.

Asiatic black bear
Ursus thibetanus

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The captive-raised **Guam kingfisher** (*Todiramphus cinnamominus*), now extinct in the wild, is a USF&W candidate for reintroduction outside its historic range.

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