INTERNATIONAL WILDLIFE
REHABILITATION COUNCIL

Volume 33, Number 3, 2013

JOURNAL OF WILDLIFE REHABILITATION

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Implications and management of pathogen exposure in wildlife translocation

Measuring effects of human disturbance on heart-rate response in wild penguin flocks

Behavioral comparisons between wild, captive and inbred red-crowned cranes

ABOUT THE JOURNAL

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

On the cover:
Bighorn sheep (*Ovis canadensis*) and caribou (*Rangifer tarandus*), Washington State, USA.

Left:
Eastern gray squirrel (*Sciurus carolinensis*).

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JOURNAL OF
WILDLIFE
REHABILITATION

Volume 33(3)

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DEPARTMENTS

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Oh, the humanity . . .

Replacing one's calendar with a more current version offers an opportunity beyond choosing a new photographic theme for the kitchen wall. Annually, we are encouraged--by the media, by self-help gurus, by friends and family--to take stock, to reflect, and even to consider the more existential aspects of life.

My personal half-century mark soared past a few years ago and, in the words of my departed and inimitable mom, when asked to share her New Year's resolutions, "Honey, if I were going to change, I'd have done it long before now." In place of personal growth goals, therefore, I've been thinking about my species and its role in the diverse community of Earthlings.

The mental pump may have been primed, at least subconsciously, by the three peer-reviewed papers included in this issues, all of which feature people as significantly, if not as prominently, as the species named in their titles. Because without humans, the concepts of translocation (Boyce et al., page 7), domestication and captivity (Chen et al. page 15), and anthropogenic disturbance (Viblanc et al., page 21) are moot.

But I didn't recognize how strongly the theme of human impact on wildlife runs through this entire issue until, having finished a volunteer shift at the St. Louis Art Museum, I decided to make a detour as I hurried toward the parking lot. Isabella Kirkland's Taxa series had opened that morning and, since the exhibition consists of only six paintings, it was easy to convince myself I wouldn't be there long.

I underestimated Ms. Kirkland.

The paintings capture nearly 400 species of flora and fauna. Moving slowly around the small gallery, I slipped into each scene and into classification mode, murmuring common names as I recognized members of the cast: black-capped vireo, golden toad, cattle egret, brown tree snake, golden-headed lion tamarin, atlas beetle, princess parrot, golden hamster, barn owl, black-footed ferret, passenger pigeon, and Tasmanian tiger. Then, shifting my focus from macro to wide-angle, my eyes settled on the titles of each work, displayed in small square white marquees placed unobtrusively beside each frame.

Descendant. Ascendant. Trade. Collection. Back. Gone.

The paintings are a visual roll call: of declining native and increasing introduced species; plants and animals of interest for legal and illegal trade, study, or exhibit; of creatures saved from the brink of extinction or rediscovered . . . and the ones we've lost forever.

I've heard biologists argue that wildlife rehabilitation interferes with the natural order, weakening the fitness of a species by offering a helping hand to individuals who would not otherwise have survived to reproduce. This viewpoint would warrant more respect, in my opinion, if works such as Isabella Kirkland's did not offer proof that there were human fingerprints all over wildlife populations long before anything remotely resembling rehabilitation took place. Homo sapiens is a native and dominant species on Earth--how could it be otherwise?

Five of the Taxa paintings are as sad as they are stunning. The sixth canvas, a chronicle of success stories, is significantly less crowded than the others, yet it provides ballast against the current of despair that runs through its companion pieces. The next time I'm called upon to defend the practice of wildlife rehabilitation, I'll think of the faces depicted there and the role wildlife rehabilitators have played in ensuring their continued presence on my beloved planet.

Kieran Lindsey
Editor

Southern Elephant Seal Recovers 5000 km Away from Home

ADELAIDE, New South Wales, Australia (December 11, 2013)—"Ellie," the southern elephant seal, is reportedly recovering well from a wrong-turn incident that ended when she was discovered in Port Elliot and moved to the Australian Marine Wildlife Research and Rescue Organisation (AMWRRO). Caregivers are helping the marine mammal return to a healthy weight by feeding her 18-19 kg of salmon per day, and slowly increasing her portion size to over 30 kg per day.

Aaron Machado, President of the AMWRRO, explained that Ellie is at the rehabilitation facility on Torrens Island. The elephant seal is believed to have strayed from a group of islands south of New Zealand and Australia where the species is commonly found, approximately 5,000 km from her current location.

Once Ellie has fully recovered she will be released south of Adelaide, Machado said. A previous elephant seal released by AMWRRO near Mount Gambier was seen two weeks later on the southeastern tip of Tasmania.

Tri-State Bird Rescue and Research Expands

NEWARK, New Jersey, USA (December 11, 2013)—Construction has started on the new Wildlife Response Annex at TriState Bird Rescue and Research. The building will contain a 1,200-square-foot oiled animal area and a large rehabilitation area, as well as laboratory, exam, training, and office space. The new building will greatly expand the center's ability to treat wildlife affected by large scale events such as 2012's Hurricane Sandy.

Pilot Whales Die of Malnutrition

SNIPE POINT, Florida, USA (December 10, 2013)—Eleven dead pilot whales were found lying near each other on an island chain near Key West, Florida, on Sunday, December 8 2013. Necropsies revealed they were emaciated and suffering from malnutrition. The young ones were especially emaciated, according to Julia Zaias, a veterinarian with the Marine Animal Rescue Society. Two of the females were pregnant.

National Oceanic and Atmospheric Administration stranding coordinator Blair Mase said the whales' empty bellies could be a result of disease or of being out of the deep, cooler waters that form their natural habitat. "Really, we don't know how long they've been in the area and we don't have any clues of why they got here in the first place," she said in a conference call with the media. It could be weeks to months before the results of the necropsies come in, she said, and "there is a possibility there could be more whales in the area."


PHOTO © ALEXANDRE ROUX, FLICKR.COM. CREATIVE COMMONS.

Pilot whale *(Globicephala melas).*

Samples taken during the necropsies may help researchers discover any illnesses or anything else that could help them understand why the whales had beached. "We'll be testing for viruses," Zaias said. "We're looking to see if they have any there, and then we'll be looking for all other possible reasons."

The whales are believed to be part of a pod of 51 whales originally found stranded on a remote Everglades beach several days earlier. The whereabouts of 29 whales remain unknown.

Cold Snap Strands Sea Turtles

CORPUS CHRISTI, Texas, USA (December 10, 2013)—Cold fronts along the Texas coast have killed fish and left over 140 green sea turtles stranded along beaches and in the bays. At the time of the report, Donna Shaver, director of the National Park Service Division of Sea Turtle Science and Recovery, had documented 144 cold-stunned sea turtles and 18 turtle deaths. Most of the stunned turtles were found by seashore staff along Padre Island in the Upper Laguna Madre and Lower Laguna Madre; one was found at Corpus Christi Bay.

The sea turtle team at Padre Island National Seashore documented and warmed the animals at the Sea Turtle Lab. Most were then taken to the Animal Rehabilitation Keep at the University of Texas Marine Science Institute in Port Aransas, Texas. Shaver said her staff is trying to verify unconfirmed reports of an additional 20 cold-stunned turtles reported dead in the Lower Laguna Madre. Those found alive should have an excellent chance of recovery, Shaver said.

Biologists said the overall impact of the sudden temperature drop appears to be less deadly compared with previous coastal freezes. In 2011, for example, approximately 1,600 cold-stunned sea turtles were stranded along the Texas coast, and 230 of these died.

Storm Brings Influx of Seal Pups

NORFOLK, United Kingdom (December 10, 2013)—Recent flooding in the UK has resulted in admissions of over 100 grey and common seal pups to East Winch Wildlife Centre.

There had been fears that the Norfolk pups, which were not yet capable of surviving on their own, would fall victim to high tides that deluged communities on December 5-6. At one breeding ground in Horsey, whery grey seals breed between November and January, volunteers counted 440 pups on the beach prior to the surge and only 177 afterward. National Trust rangers spent the weekend searching for pups at one of the large colonies at Blakeney Point, which is commonly home to approximately 1,000 seals and pups.

The majority of seals and pups were able to reach higher ground on the sand dunes. Those who made it to the rehabilitation facility are expected to remain there for five months before release back into Norfolk waters.

Long-eared Owl Gets Second Chance Thanks to Duluth Firefighters

DULUTH, Minnesota, USA (December 10, 2013)—Duluth firefighters recently rescued a long-eared owl that had flown into the second-story window of a downtown building. Peggy Far of Wildwoods, a nonprofit wildlife rehabilitation organization, said the owl suffered head trauma from hitting the window. "It's not in very good shape," she said. "If it makes it through the next 24 hours, then we'll transfer it to the Raptor Center (at the University of Minnesota's St. Paul campus). It's very lethargic right now."

Long-eared owls nest near Duluth and often are banded at Hawk Ridge Bird Observatory, according to Laura Erickson, a Duluth birder. This bird may have been a migrant moving through the area, she explained.

Mice Used to Trap Tree Snakes

GUAM (December 3, 2013)—The U.S. Department of Agriculture (USDA) is testing tree deployment of dead mice laced with acetaminophen (an NSAID) to eradicate an invasive snake called the brown tree snake. Scientists from the USDA's Animal and Plant Health Inspection Service (APHIS) have been experimenting in the jungles near Anderson Air Force Base in Guam in an attempt to eradicate some of the estimated 2 million brown tree snakes.

PHOTO © USDA /APHIS, FLICKR.COM. CREATIVE COMMONS LICENSE.

Brown tree snake on barbed wire *(Boiga irregularis).*

An 80 milligram dose of the NSAID (lethal to snakes but harmless to most other animals) is injected into each dead mouse. Paper streamers attached to the rodents act as weak parachutes when they are dropped from helicopters into the trees. The $8 million project is a joint effort of the U.S. Department of Interior and the U.S. Department of Defense.

Tiny radios were implanted in some of the mice, according to the Pacific Daily News. USDA then attempts to track the snakes' activity. Crews searched two 136-acre areas in an attempt to locate the radios and discover whether the snakes have eaten the mice or if the mice simply decompose.

The brown tree snake is credited with causing the extinction of nine avian species native to Guam.

Wolf Hollow Celebrates 30-Year Anniversary

SAN JUAN ISLAND, Washington, USA (December 5, 2013)—Founded in 1983 as a licensed wildlife rehabilitation facility, Wolf Hollow Wildlife Rehabilitation Center began as a vet clinic in Friday Harbor on San Juan Island. The center now cares for about 500 animals per year but, oddly enough, has never admitted a wolf for care (the center got its name when the founder fell in love with the animals during a veterinary internship).

Many in the area who are familiar with the center believe it to be government-funded; however, Wolf Hollow is a nonprofit organization that relies on donations from individuals, families, and businesses, along with grants from foundations, and a variety of fundraising events.

To date, Wolf Hollow has cared for more than 200 different species, including California sea lions, eagles, owls, swans, swallows, and even painted turtles. Center staff members are on call 24 hours a day, seven days a week.

Poached Hawks Prompt Utah DWR Investigation

SMITHFIELD, Utah, USA (November 25, 2013)—Utah Division of Wildlife Resources (UDWR) officers are looking for those responsible for the shooting deaths of at least eight hawks. The UDWR began receiving reports of hawks being shot in mid-November. In all, eight hawks have been shot with a small-caliber firearm; none has survived.

There is not much a rehabilitator can do to repair a bullet through the spine. DaLyn Erickson, executive director of the Wildlife Rehabilitation Center of Northern Utah, explained that euthanasia is the only real treatment option. "It's a very, very hard thing to do, and it's very frustrating."

Conservation officer Chris Schulze said, "The people who are doing this of course are not profiting from it. They're not getting a big set of antlers or getting any meat from it. They're just shooting these animals and leaving them." Hawks are considered a migratory species so they are protected under federal law. Those responsible for shooting the hawks could be charged with a class B misdemeanor and face fines and jail time. The restitution value for each hawk is $100, Schulze added. ■

Wildlife translocation: the conservation implications of pathogen exposure and genetic heterozygosity

Walter M. Boyce[1*], Mara E. Weisenberger, M.[2] Cecilia T. Penedo,[3] Christine K. Johnson[1]

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Bighorn sheep juveniles *(Ovis canadensis).*

Background

Innate and adaptive immune responses evolved in vertebrates as a first and secondary line of defense, respectively, against a diverse and changing array of pathogenic organisms. The effectiveness of these immunologic responses, and hence the fitness of individuals, populations, and species, is driven by pathogen exposure history and the immunogenetic repertoire of major histocompatibility complex (MHC) genes and non-MHC genes (Acevedo-Whitehouse and Cunningham 2006; Smith *et al.* 2009). Novel, highly virulent pathogens can overwhelm host immune responses not primed to their exposure, and such pathogens can be a strong selective force, reducing the distribution and abundance of a species over short time-frames (1-2 generations) through effects on survival and reproductive success (Smith *et al.* 2009). Over multiple generations, a history of ongoing pathogen exposure theoretically should select for more resistant immunogenotypes that limit fitness impacts by responding effectively upon initial exposure (innate immunity) or re-exposure (adaptive immunity).

Bighorn sheep (*Ovis canadensis*) are a useful model for examining this interplay between disease, demography, and genetics. They are a polygynous, highly philopatric species found in small, fragmented populations in the mountainous regions of western

ABSTRACT: Background: A key challenge for conservation biologists is to determine the most appropriate demographic and genetic management strategies for wildlife populations threatened by disease. We explored this topic by examining whether genetic background and previous pathogen exposure influenced survival of translocated animals when captive-bred and free-ranging bighorn sheep (*Ovis canadensis*) were used to re-establish a population that had been extirpated in the San Andres Mountains in New Mexico, USA.

Results: Although the free-ranging source population had significantly higher multi-locus heterozygosity at 30 microsatellite loci than the captive bred animals, neither source population nor genetic background significantly influenced survival or cause of death. The presence of antibodies to a respiratory virus known to cause pneumonia was associated with increased survival, but there was no correlation between genetic heterozygosity and the presence of antibodies to this virus.

Conclusions: Although genetic theory predicts otherwise, increased heterozygosity was not associated with increased fitness (survival) among translocated animals. While heterosis or genetic rescue effects may occur in F1 and later generations as the two source populations interbreed, we conclude that previous pathogen exposure was a more important marker than genetic heterozygosity for predicting survival of translocated animals. Every wildlife translocation is an experiment and, whenever possible, translocations should be designed and evaluated to test hypotheses that will further improve our understanding of how pathogen exposure and genetic variability influence fitness.

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Reprint: Boyce et al., *BMC Ecology* 2011, 11:5


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North America (Buechner 1960). They are highly susceptible to infectious disease, and outbreaks of disease regularly cause high morbidity and mortality (Buechner 1960; Cassirer and Sinclair 2007). The history of population die-offs dates back to European settlement of the western United States over 200 years ago [3], indicating that novel pathogens were likely introduced by contact with domestic sheep (*Ovis aries*). Pneumonia epizootics appear to be driven by density-dependence, serving to constrain population size (Monello *et al.* 2001), and presumably selecting for the most fit genotypes. However, small populations of bighorn sheep also are prone to inbreeding and genetic drift, making it difficult to understand the relative importance of pathogen-mediated selection, drift, and inbreeding on genetic variability and fitness.

We approached this problem by testing whether genetic background and previous pathogen exposure influenced survival when animals from two different founder populations were simultaneously translocated into the San Andres Mountains (SAM) in New Mexico, USA. The SAM once supported the largest population of native bighorn sheep in the state. However, by the late 1990s, a combination of disease, mountain lion (*Puma concolor*) predation, and drought had reduced this population to the point of extinction, and translocation from captive and/or free-ranging herds was necessary to reestablish a self-sustaining population in the SAM. The two founder populations chosen for reintroduction were a genetically diverse free-ranging herd in the Kofa National Wildlife Refuge (KNWR), Arizona, and a less diverse captive herd in the Red Rock Wildlife Area (RRWA) that was originally derived from the native SAM population. In November 2002, 51 bighorn sheep were translocated into the SAM from the KNWR (*n* = 20) and the RRWA (*n* = 31), and 30 more bighorn were translocated from KNWR in November 2005. We examined genetic variation at 33 microsatellite loci to compare genetic variability, and we conducted population health analyses at the time of capture to assess infectious disease exposure. Radio-collared sheep released into the SAM were then monitored through February 2007 to determine survivorship and cause-specific mortality.

Methods

Study animals

The SAM mountain range represents the largest amount of high quality bighorn sheep habitat in New Mexico, and the native SAM bighorn sheep population exceeded 200 animals prior to a psoroptic scabies epizootic that began in the late 1970s. The population remained around 30 animals through the early 1990s and then declined to a single ewe that was temporarily brought into captivity in 1999 for less than two weeks. Nine animals sampled just prior to extirpation of the population had low mean heterozygosity at MHC (0.075) and microsatellite (0.359) loci (Boyce *et al.* 1996). Immediately prior to the reintroduction effort that began in November 2002, the SAM population consisted of four rams that had been translocated from the RRWA as part of a sentinel disease study, and the single native ewe that was released along with the sentinel rams (Boyce and Weisenberger 2005). A total of 81 bighorn sheep were captured, sampled, and translocated to the SAM in 2002 and 2005 from RRWA and KNWR. In 2007, nine offspring from these animals were captured and sampled for genetics and disease surveillance in the SAM. The survival analysis presented in this paper focuses only on animals translocated to the SAM, and data from these nine offspring born in the SAM are presented for descriptive purposes only.

The RRWA is a 500-hectare enclosure for bighorn sheep located in central New Mexico. The RRWA population was initiated in 1972 with founders from the SAM, and has served as the source for >260 bighorn sheep translocated within New Mexico since 1979 (Rominger *et al.* 2004). This population has been managed as a closed herd (no immigrants), and has low genetic diversity (0.36) relative to other free-ranging populations in the desert southwest (0.44-0.63) (Gutiérrez-Espeleta *et al.* 2000). Genetic and disease samples for this study were collected when bighorn sheep were captured for translocation to the SAM in 2002 (*n* = 31). The size of the RRWA population was <100 animals at the time of capture and translocation.

The KNWR is located in southwestern Arizona and contains a free-ranging population of bighorn sheep that has significantly higher heterozygosity (0.60) than the RRWA captive population (Gutiérrez-Espeleta *et al.* 2000). Like the RRWA population, the KNWR herd has been the source for transplants of hundreds of animals within Arizona and to other western states. Genetic and disease samples for this study were collected at capture from animals translocated to the SAM in 2002 (n = 21) and 2005 (n = 30). The population at KNWR has averaged about 700-800 animals since 1981, but dropped to <400 animals by 2006. Since 1979, > 560 bighorn sheep have been translocated from KNWR.

Genetic analysis

Genomic DNA was extracted from blood and genetic variability was assessed by examining 33 microsatellite loci (OarFCB128, MAF209, OarFCB304, MAF33, MAF48, MAF65, OarFCB11, MAF36, OarFCB266, ETH152 (= D5S2), DRB3 (MHC-linked loci), BMC1009, CELJP23, BM203, OARCP026, TGLA94, FCB193, IRBP, CELB9, BM6506, CELJP15, BM4107, CSRD247, HSC, INRA023, INRA063, INRA105, MAF214, McM527, OarAE129, OarCP49, OarFCB20, SPS113). Many of these loci have been examined in previous publications (Boyce *et al.* 1996; Gutiérrez-Espeleta *et al.* 2000; Whittaker *et al.* 2004), and we have used them to examine heterozygosity in different populations of bighorn sheep across their range in North America (unpublished). Briefly, using fluorescently labeled microsatellite primers, microsatellites present in genomic DNA were amplified by PCR (polymerase chain reaction). PCR products were separated by capillary electrophoresis using an Applied Biosystems 3730 DNA Analyzer (Applied Biosystems, Foster City, California, USA), with a fluorescent-labeled base pair size standard in each lane. Image analysis and fragment size determination were carried out using STRand software [11]. Deviations from Hardy-Weinberg equilibrium (HWE) and allele frequencies were examined with GenePop (Raymond and Rousset 1995).

Multi-locus heterozygosity (MLH) values were calculated for each animal as the preferred metric of genetic variability. Chapman *et al.* (2009) showed that different genetic metrics (MLH, SH, IR) are highly correlated and non-independent, and they advocated the use of the simplest metric, MLH, in future studies of heterozygote-fitness correlations (HFC). For our multivariate analyses, MLH values were categorized into two categories using a cutoff >0.5 to indicate a generally low or high level of genetic diversity.

Assessment of Health and Disease Exposure Status

Antibodies are a marker of previous exposure and priming of the adaptive immune response to specific pathogens. Sera from blood collected at the time of capture were tested for the presence of antibodies to three viruses known to cause respiratory disease and pneumonia in bighorn sheep: bovine respiratory syncytial virus (BRSV), bluetongue virus (BTV), and parainfluenza-3 virus (PI3). Assays were performed at the California Animal Health and Food Safety laboratory in Davis, California, and results were classified as positive or negative. Results for BTV were reported as positive or negative; titers ≥1:20 were considered positive for BRSV, and titers >1:16 were considered positive for PI3.

Post-release Monitoring

A VHF radiocollar (Telonics Inc., Lotek Wireless Inc., Advanced Telemetry Systems, Inc., and Telemetry Solutions) with mortality sensor was placed on animals to be translocated at the time of capture to facilitate monitoring after their release in the SAM. Animals were transported to the San Andres National Wildlife Refuge in the SAM and released within 48 hrs of capture. Resight of individual animals by radio-signal was conducted by state and federal wildlife biologists through February 2007 to detect mortalities and determine specific causes of death. Mortalities were investigated immediately and a field investigation and necropsy typically performed within 72 hrs of death. Mortalities were classified as lion predation based on criteria of Hayes et al. (Hayes *et al.* 2000), or as pneumonia based on gross lesions present in the lungs. All mortalities that could not be classified as predation or pneumonia based on field examination were categorized as unknown, but “other” than predation and pneumonia. The number of days each animal survived was calculated as the difference between the date of release and the date of death, or the date sheep were last observed. For individuals lost to follow up, the end date was the date individuals were last observed, and for sheep that were confirmed alive at the end of the study period, this date was February 1, 2007.

Statistical Analysis

We used independent univariate and multivariate approaches to explore correlation within our dataset, and to identify factors most influencing pathogen exposure, number of days until death (or end of study period if survived) and death due to suspected pneumonia. Independent variables evaluated for their impact on these three outcomes included sex, age-class (<3 yr, 3 to <6 yr, ≥6 yr), source population (RRWA vs KNWR), year released (2002, 2005), exposure status to each pathogen (BRSV, BTV, PI3) at time of release, and genetic variability (MLH). Significant associations with outcomes variables, confounding, and effect modification were evaluated by stratified univariate analyses using exact categorical tests and the student’s t test. The relationship between MLH (as a continuous variable) and age and sex class were evaluated by the two-way ANOVA in order to adjust for source population. Independent categorical variables were also examined for their relationship to loss to follow-up by the two-sided Fisher exact test to determine whether variables were related to censorship, and therefore could not be included in the survival analyses.

Binary logistic regression was used to evaluate whether independent variables were related to death due to pneumonia, which was the most common cause of death detected during the study period. Only sheep that died of suspected pneumonia or were confirmed alive at the end of the study were included in this analysis (*n* = 50). Sheep that died from predation or unknown causes were excluded from this analysis. Variables were selected by backward stepwise elimination (likelihood ratio test $P < 0.1$) and confidence intervals for the logistic model evaluating risk factors for pneumonia were calculated using conditional exact inference due to low sample size. Hosmer-Lemeshow goodness of fit test was used to estimate overall fit of the final logistic model.

For the survival analyses, median survival time was calculated for all risk factors of interest measured at time of translocation. The Kaplan-Meier product-limit estimator was used to estimate the survivor function, and the log-rank test (Mantel and Haenszel 1959) was used to test the equality of survivor function for each independent variable with groups considered significantly different if $P < 0.1$. Variables significantly associated with survival in these univariate analyses were evaluated for their relationship to failure rate (days until death) using the semi-parametric Cox proportional hazards regression. Sex was significantly associated with censorship so this variable was excluded from survival analyses. Variables were selected for the Cox proportional hazards model by manual backward elimination using a selection criterion of $P \leq 0.1$ for terms to stay in the model, and all categories of any significant variable were retained in the model. The Breslow approximation method was used to handle tied failure times (Breslow 1974). The proportional hazards assumption based on Schoenfeld residuals was evaluated to determine if the relative risk for each variable of interest was the same in time for the duration of the study. All statistical analyses were performed using STATA SE 11.1 software (STATACorp, 4905 Lakeway Drive, College Station, Texas 77845 USA).

Results

Characteristics of Source Populations

Bighorn sheep translocated from RRWA were predominantly male (18/20), while 72% (34/47) of the sheep translocated from KNWR were female (Fisher exact $P < 0.001$). Age class was not significantly associated with source population (Fisher exact test $P = 0.068$), but twice as many sheep in the 3-6 yr old range were captured in KNWR (23/36) compared to RRWA (11/31). All sheep relocated to SAM in 2005 were from KNWR, but these newly introduced sheep were demographically similar and did not differ with regard to pathogen exposure or genetic diversity from sheep introduced from KNWR in 2002.

Serologic evidence of previous pathogen exposure varied substantially by source population for all pathogens, except PI3 (Table 1). Sheep from KNWR were more likely to be exposed to BRSV (14/36) compared to sheep from RRWA (4/31, Fisher exact $P = 0.026$). None of the sheep captured in KNWR had evidence of exposure to BTV, while 12/31 sheep from RRWA were seropositive to BTV (Fisher exact $P < 0.001$). Results from the univariate analysis stratified by source population indicated that age was a significant confounder related to BTV exposure for sheep from RRWA. In fact, older sheep (≥6 years) captured at RRWA were 12 times more likely to be exposed to BTV than younger age classes (two-sided Fisher exact test, $P = 0.012$). Significant associations with age class were not detected for the other pathogens after adjusting for the effect of source population.

Three of the 33 microsatellite loci tested were monomorphic and were excluded from further analyses (BM4107, INRA063, TGLA94). Complete genotypes were determined for all animals at each of the remaining 30 loci with the exception of a single animal at a single locus. Allele frequencies did not significantly differ from HWE expectations across loci, and null alleles were not detected. The genetic diversity parameter, MLH (Table 1), was significantly higher in sheep captured in KNWR (mean = 0.579, 95% CI 0.555-0.603) compared to sheep captured in RRWA (mean = 0.394, 95% CI = 0.340-0.449). Mean MLH did not differ by sex or age class once comparisons were adjusted by source population.

Specific Causes of Mortality

Cause of death could be determined by field post-mortem examinations for 25 sheep. Pneumonia was suspected as a cause of mortality in 17 sheep, and mountain lion predation was identified as the cause of death in 8 sheep. Specific cause of death could not be determined for 9 sheep, but predation and pneumonia were excluded as their cause of death. Deaths due to lion predation occurred in 2002, 2003, and 2006, while deaths due to pneumonia occurred every year (2002-2007). Most deaths due to pneumonia (65%; 11/17) occurred in the fall from September through November, and seven of these occurred in a cluster in the fall of 2006. Pneumonia impacted all age classes with 27% (4/15) of 1-3 year old sheep, 35% (9/26) of 3-6 year old sheep, and 44% (4/9) of >6 year old sheep dying of pneumonia. Mean MLH was not significantly different among the 33 sheep that survived (mean = 0.50, 95% CI 0.45-0.55) compared to the 17 sheep that died of pneumonia (mean = 0.44, 95% CI 0.37-0.51), but these analyses lacked power due to low sample size (two-sided t test $P = 0.161$, power = 0.31). Similarly, risk factors were not significantly associated with death due to pneumonia in the multivariate analyses using exact conditional logistic regression.

Risk Factors Associated with Survival

Year of release was not associated with overall survival in univariate or multivariate analyses and survival time did not differ significantly by source population. However, age class at time of release was marginally associated with survivorship (log rank test $P = 0.079$) in the univariate analyses. More than half of the sheep released at 1 to <3 years old survived the entire study period (1,509 days), while median survival time was 1,234 days for sheep 3 years to <6 years old and only 475 days for sheep released when 6 years or older. Genetic diversity and pathogen exposure were not related to survival time, even after stratifying by source population.

Risk factors found to be significantly associated with time to death in the Cox proportional hazards multivariate framework were the oldest age class and previous exposure to BRSV (Table 2). Based on the hazards ratios of the Cox proportional hazards model, we found that sheep with serological evidence of previous exposure to BRSV had approximately one-third the risk of death compared to sheep that had not been exposed to BRSV prior to release. Age was a significant confounding factor in the survival analyses and, as expected, older age class was associated with increasing risk and a shorter time to death. Sheep released at ages ≥6 years had 3.4 times the hazard rate of sheep released at 1 to 3 years of age. Source population, MLH as a continuous and binary variable, year of release, and previous exposure to other pathogens were not associated with survival once we accounted for the influence of age and exposure to BRSV on time to death. Associations with sex could not be evaluated in the modeling procedure because males were more likely to be lost to follow up. Evaluation of the proportional hazards assumption for the final model based on a test of Schoenfeld residuals indicated that the relative risk for each variable of interest was the same in time for the duration of the study ($P = 0.322$).

Discussion

A key challenge for conservation biologists is to determine the most appropriate demographic and genetic management strategies for wildlife populations threatened by disease (Table 1).

Bighorn sheep in North America provide a useful model for examining this issue because they are highly susceptible to infectious diseases, and they are frequently translocated to re-establish or augment populations. In this study, we took advantage of a management effort—the restoration of bighorn sheep to the SAM—to test whether genetic background and previous pathogen exposure influenced the survival of translocated bighorn sheep.

Variability and local adaptation are considered to be key genetic factors influencing the persistence of small populations of bighorn sheep (Whittaker *et al.* 2004; Singer *et al.* 2000; United States Fish and Wildlife Service 2000), and this strongly influenced the choice of source animals for the SAM translocation. Since the RRWA herd was founded with animals from the SAM, their translocation presumably maximized the retention of locally adapted gene complexes. However, the RRWA herd had low genetic variability (mean MLH = 0.39; Table 1) because it had been managed for >20 years as an inbred population. In contrast, the KNWR population was much more diverse (mean MLH = 0.57-0.60; Table 1), ensuring that the newly established SAM population would be more variable than one established only with animals from the RRWA captive population.

While the genetic management goal of achieving increased heterozygosity in the new SAM population was accomplished, it was not associated with enhanced survivorship of the individual sheep that were translocated. Bighorn sheep from the KNWR had significantly higher genetic diversity than those from the RRWA, but neither source population nor genetic background(MLH) influenced their survival in the SAM. Our failure to detect a significant relationship between genetic diversity and adult survival is consistent with the Chapman et al. [(Chapman *et al.* 2009) meta-analysis of HFC studies. In their comprehensive analysis, Chapman *et al.* (2009) concluded that the effects of such correlations are very weak, and that other proposed measures of genetic variation (SH, IR) are no more powerful than MLH for detecting relationships. We acknowledge that a larger sample size and more marker loci would have increased the power of our analysis, but any such effect is apparently very weak. Our failure to detect a fitness effect with neutral markers (microsatellites) is perhaps not surprising given that Gutierrez *et al.* (2001) failed to find a strong association between MHC variation and disease resistance in bighorn sheep.

Previous examination of genetic variability among bighorn sheep suggests that microsatellite diversity has been influenced primarily by neutral factors, and MHC diversity by balancing selection (Boyce *et al.* 1996; Gutiérrez-Espeleta *et al.* 2000; Gutierrez-Espeleta *et al.* 2001; Forbes *et al.* 1995). These studies have shown that genetic distances between populations are

TABLE 1: Multi-locus heterozygosity (MLH) and prevalence of antibodies to pathogens among bighorn sheep from the Kofa National Wildlife Refuge (KNWR) in Arizona, the Red Rock Wildlife Area (RRWA) in New Mexico, and the San Andres Mountains (SAM) in New Mexico, USA.

	RRWA[a] 2002 $n = 30$	KNWR[a] 2002 $n = 20$	KNWR[a] 2005 $n = 31$	SAM[a] 2007 $n = 9$
PATHOGEN EXPOSURE[b]				
BTV	38.7%	0%	0%	11%
PI3	74.2%	33.3%	34.0%	22%
BRSV	19.4%	42.9%	34.0%	11%
GENETIC DIVERSITY[c]				
MLH	0.39	0.57	0.60	0.59

[a] Bighorn sheep from RRWA and KNWR were sampled at the time of their translocation into the SAM (2002, 2005), and offspring from these animals were sampled in 2007 in the SAM.
[b] BTV = bluetongue virus, PI3 = parainfluenza-3 virus, BRSV = bovine respiratory syncytial virus.
[c] Based on analysis of 30 microsatellite loci.

TABLE 2: Factors significantly related to time to death in the final Cox proportional hazards model of survival of bighorn sheep translocated to the San Andres Mountain, New Mexico, USA.

FACTOR	HAZARD RATIO	STD. ERR.	Z	P>|Z|	95% CONF. INTERVAL
Age 3 to <6 yr[a]	1.47	0.664	0.85	0.397	0.60 - 3.56
Age ≥6 yr[a]	3.37	1.75	2.34	0.019	1.22 - 9.32
BRSV antibodies[b]	0.37	0.18	-2.03	0.043	0.14 - 0.97

[a] Youngest age class (1 to <3 years old) designated as reference category. [b] BRSV = bovine respiratory syncytial virus.

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roughly proportional to geographic distance, and that most genetic variability is apportioned within, rather than between, populations. These results are best explained by isolation by distance (reduced gene flow), and provide little genetic evidence for local adaptation. Our results are consistent with this view—the RRWA animals derived from the original SAM population did not have increased survivorship. Conversely, inbreeding within the RRWA population could have reduced fitness by increasing the accumulation and expression of deleterious recessive alleles (Hedrick *et al.* 2001; Tallman *et al.* 2004). We found no evidence of such an effect among translocated adult sheep (survival was not associated with source population). Although we did not detect fitness differences among translocated animals, heterosis or genetic rescue effects may occur in F1 and later generations as the two source populations interbreed (Tallman *et al.* 2004; Hogg *et al.* 2006). While we were able to sample nine offspring born in the SAM for genetic and pathogen analyses (Table 1), we were unable to monitor their relative fitness and they were not included in any of our survival analyses.

Pneumonia was the most frequent cause of death, accounting for 50% (17/34) of the documented mortalities. This mirrors what has been reported for many other populations, illustrating the ongoing importance of pneumonia as a cause of morbidity and mortality across the range of bighorn sheep (Cassirer and Sinclair 2007, Singer *et al.* 2000, Gross *et al.* 2000, George *et al.* 2008). Several bacteria (*Pasteurella* and *Manheimia* spp.) and viruses (BRSV, PI3) have been implicated as primary or secondary pathogens in enzootic and epizootic pneumonia, and we associate these organisms with pneumonic disease because they can be detected in sick and dead animals. However, we know far less about causal relationships. The multifactorial etiology of pneumonia, coupled with the complex interplay of host, pathogen, and environment, has made it very difficult to identify a single, specific cause of pneumonia.

Instead of focusing on identifying pathogens after death, we looked at how previous pathogen exposure influenced the future survival of healthy bighorn sheep. The presence of antibodies was used as a marker of pathogen exposure, indicating that viral transmission and infection had occurred at some point in the past. Animals translocated into the SAM showed evidence of previous exposure to BTV (RRWA only) as well as BRSV and PI3 (both RRWA and KNWR; Table 1). All animals were healthy at the time of translocation, yet previous BRSV exposure was associated with increased survival regardless of age class. This suggests that antibodies to this virus provided some level of protection against pneumonia after animals were released into the SAM.

Age class at the time of release was significantly related to survival, and younger animals lived longer than those older than six years of age (Table 2). This intuitive result illustrates the demographic advantage of selecting young animals for reintroductions. Singer *et al.* (Singer *et al.* 2000) found that translocation success was directly related to the number of animals—larger translocations resulted in populations that were more likely to persist over time. However, pneumonia epizootics appear to be driven by density-dependence, and thus disease may constrain population growth and size (Monello *et al.* 2001). Lethal removal of mountain lions was conducted in the SAM throughout our study period (Rominger *et al.* 2004), likely influencing the relative proportion of mortality caused by predation versus pneumonia. In the absence of controlled studies, we have no way of inferring whether lion control increased overall survivorship. Regardless, there were no significant relationships between mean MLH and death due to lion predation or pneumonia.

Conclusions

We conclude that increased heterozygosity did not increase survival following translocation, nor did it reduce individual susceptibility to dying specifically from pneumonia. Instead, we found that previous pathogen exposure was more important than genetic heterozygosity as a marker for predicting survival of translocated animals. In their review of more than 600 published HFC effects, Chapman *et al*. (2009) reported a publication bias with no-significant effects being under-reported in the literature. We acknowledge that confounding factors, such as age, and small sample sizes likely limited our ability to detect significant relationships between genetic variability, disease and fitness. These limitations often plague investigators who conduct observational field studies in natural settings. We encourage others to test the theoretical advantages of increased genetic variability using a variety of genetic markers in long term field studies that also evaluate ecological factors, such as disease, that are likely to influence survival. Every wildlife translocation is an experiment, but many translocations lack an experimental design. Whenever possible, translocations should be designed and evaluated to test hypotheses that will improve our understanding of how pathogen exposure and genetic variability influence fitness.

Acknowledgements

This project was supported by the U.S. Fish and Wildlife Service, the New Mexico Department of Game and Fish (NMDGF), the White Sands Missile Range, and the National Fish and Wildlife Foundation. We appreciate the support and cooperation of Elise Goldstein, Eric Rominger, and Darrel Weybright from NMDGF; Grace Lee from the University of California, Davis; as well as personnel from the Arizona Department of Game and Fish, the Arizona Desert Bighorn Sheep Society, and the New Mexico Chapter of the Foundation for North American Wild Sheep. This study was performed in compliance with state and federal regulations.

The findings and conclusions in this article are those of the author(s) and do not necessarily represent the views of the U.S. Fish and Wildlife Service.

Authors' contributions

WMB and MEW designed and implemented the study. MCTP and CKJ conducted genetic and statistical analyses, respectively. All authors have approved the final manuscript.

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Behavioral alterations in domestication process: Comparative studies between wild, captive, and inbred red-crowned cranes (*Grus japonensis*)

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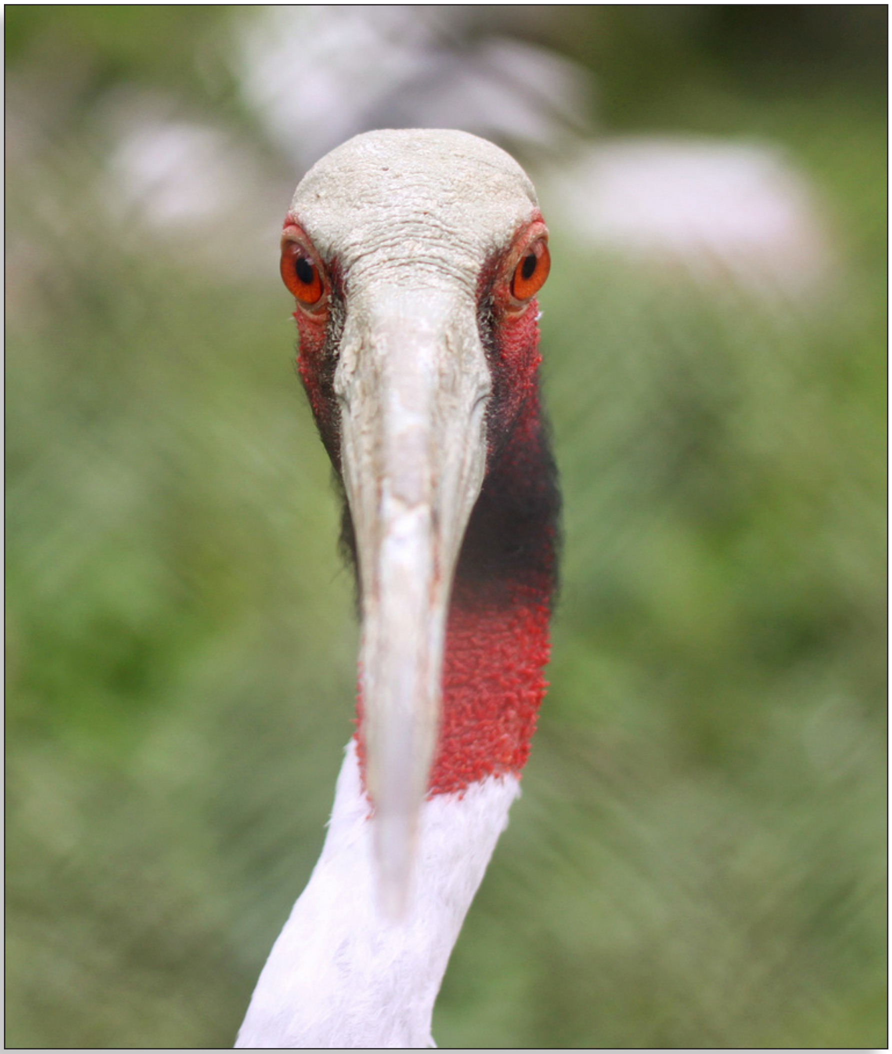

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ABSTRACT: The red-crowned crane *(Grus japonensis)* is one of the largest birds in East Asia and is among the rarest and most endangered cranes in the world. To enhance population management and further conservation, researchers compared behavioral changes from wild to captive populations. Meanwhile, researchers also compared the artificial inbred population with the natural normal populations. In this research, five main behavioral patterns of red-crowned cranes, including resting, moving, preening, feeding, and alerting were identified by all occurrence sampling and instantaneous scanning sampling methods with 5-10 min intervals. The referred wild and captive populations were concluded from the previous publications and the artificial inbred populations were observed in Hangzhou Wildlife Park (China) during August and September, 2009. Researchers found the difference ($p>0.05$) between wild and captive populations is not significant. However, preening in the captive population is higher than in the wild population. The wild population spent more time resting compared with the captive population, yet feeding is the most time-spent behavior for both populations. Furthermore, behavioral patterns between normal and inbred populations are significantly different ($p<0.05$). Alerting and resting behaviors are significantly higher in the normal population than in the inbred population. Moreover, moving is the dominant behavior of the inbred population but feeding takes the most time in the normal population.

KEY WORDS: Red-crowned cranes, *Grus japonensis,* behavioral patterns, artificial selection, wild, captive, inbred population

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Reprint: *Journal of Animal and Veterinary Advances* 11(15): 2711-2715, 2012.
ISSN: 1680-5593 © Medwell Journals, 2012

Introduction

Animal domestication probably began thousands of years ago coming along with human demands. Through efficient means of artificial selection, animals are domesticated with clear uses. From wild to captive, from captive to artificial breeding, the processes of behavioral alterations are extremely interesting to the scientific community as well as to the public. The red-crowned crane *(Grus japonensis)* is a large crane that is the second rarest crane in the world. A small number of populations each with 1,700-2,000 individuals are living in East Asia and were listed on the IUCN red list of threatened species in 1994 (Bird Life International, 2009). This migratory bird ranges from Russian Siberia to North Korea and Southern China and adapts to the wetland environments (Higuchi *et al.,* 1998). Its behavioral studies started in the early 1990s; most of those were focused on time budgets and activity rhythms (Wu *et al.,* 2002; Zhou *et al.,* 2002; Tian *et al.,* 2005, 2006; Lu

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and Cheng, 2006; Li *et al.,* 2007), reproduction behavior (Wu *et al.,* 2002; Qiu *et al.,* 2002; Zou *et al.,* 2003a), feeding behaviors (Zou *et al.,* 2003a, 2007a, b), and habitat selections (Ding and Zhou, 1982; Lu and Zhou, 1990; Ma *et al.,* 1999). Wildlife domestication is one of the most useful strategies to sustain species. As one of the endangered cranes, this approach shows applicable access to maintain diversity of the cranes. However, in advance, it is important to understand its physiology and the behavior of the targeted wildlife. Comparative studies regarding reproductive behavior of semi-wild and wild populations of red-crowned crane behavior showed that all behavioral styles were similar with no significant differences. Yet, the fluctuation of reproduction behavior is much more severe in a semi-captive population than in a wild population (Ji *et al.,* 2008). Zou *et al.* (2007a) reported that the semi-domestic population had the same behavioral patterns as wild populations, but the feeding behavior was different due to the availability of food resources. Wang *et al.* (2010) compared semi-captive and wild populations of red-crowned cranes which indicated that the semi-captive population had a lower requirement of nest-site selection. Despite many studies that have been performed comparing crane behaviors, there is still a large gap of information that has not been illustrated. In this study, researchers aim to demonstrate behavioral differences between captive and wild populations of red-crowned cranes. Meanwhile, researchers will compare the behavioral differences between normal and inbred populations of red-crowned cranes. Finally, researchers will depict a clear view of how the domestication process from wild to most human-selected inbred populations can alter cranes' behaviors. This study will also contribute to other wildlife rearing management and conservation knowledge.

Materials and Methods

Studying location and animals

This research was conducted at Hangzhou Wildlife Park (119°59′ E, 30°09′ N), Fuyang County, Hangzhou City, Zhejiang Province, China. The average temperature in this region is 16.2°C annually with average temperatures of 28.6 and 3.8°C in summer and winter, respectively. The average rainfall is 1,435 mm year^{-1}. During the time of this research, the average temperature range was from 36.3°-42.4°C. A small inbred population from two nests of red-crowned cranes (*N*=6) was observed in this study. They were kept in a grass-bamboo-made enclosure with an area of 100 m^2 in a roof-netted captivity during the night, and open grassland with an area of 2,000 m^2 was used for daytime activities. In the middle of the grassland, there is a 20 m^2 pond that is 15–30 cm in depth. They were fed twice a day with dry fodder, chicken eggs, and fresh vegetables in the morning around 10:00 AM and with live loaches (*Cypriniformes*) in the afternoon around 3:00 PM. All animals were in good health and the experimental protocol met the regulations of animal care and uses (Law of Wildlife Protection, China in 1988, and Regulations for the Administration of Affairs Concerning Experimental Animals, China in 1988) and also received approval from the Veterinary Committee of the Wildlife Park.

Methods and processing

During observations, observers always hid in front of the enclosure, kept a distance of 100-200 m, and telescope monitoring was assisted to check the behavioral patterns. The observation persisted 4 weeks from 9:00 AM to 5:00 PM during August and September in 2009. The behavioral patterns were identified by all occurrence sampling and instantaneous scanning sampling methods with 5-10 min intervals. The observation items recorded in this study mainly included resting, feeding, moving and alerting. Resting was defined as standing or lying quiescently on the ground or in the water without moving and feeding. Feeding was defined as initiative eating either on the ground or in the shallow lakes. Moving was defined as walking or pacing in or out of the water without any other relevant target actions including the reproduction actions. Alerting primarily meant looking around and making sounds or behavioral alarms to the population. The wild population means natural population in the original habitats. The captive population is kept in the captivities with natural mating selection. The artificial inbred population are the offspring (F3 generation) bred domestically by human selection normally accompanied by physiological and morphological faults. Moreover, their F1 (grandparents) and F2 (parents) are both captive inbred populations. The normal population includes captive and wild populations, excluding the inbred population.

Statistics analysis

Data analysis is based on the software SPSS Statistics 18 and Microsoft Office Excel 2003. The total efficient data were 2,835 sampling observations. Behavioral data was calculated by temporal percentage of defined behaviors (resting, feeding, moving and preening, and alerting behaviors) in defined observing time. The ratio (full ratio is equal to 1) of activity meant the occurring frequency of defined behavior. The one-way ANOVA test was used to compare the mean percentages of behavioral patterns between

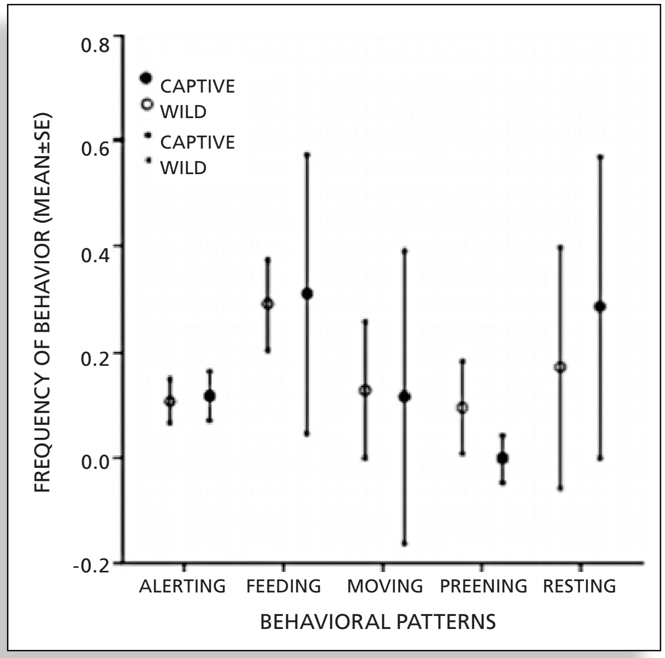

FIGURE 1: Frequencies of behavioral patterns between wild and captive crane populations

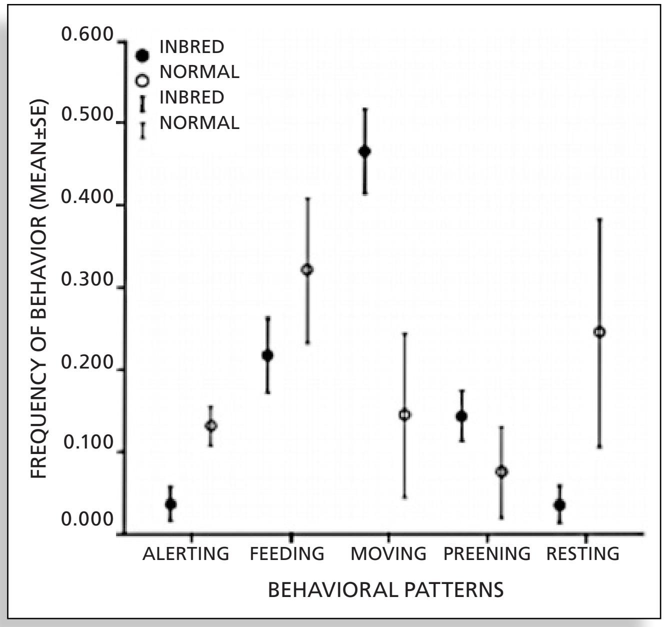

FIGURE 2: Frequencies of behavioral patterns between normal and inbred crane populations

TABLE 1: AVAILABLE DATASET OF BEHAVIORAL TIME BUDGETS OF DOMESTIC AND WILD POPULATIONS

Population	Resting (%)	Moving (%)	Preening (%)	Feeding (%)	Alerting (%)	Origins
Domestic	4.36	33.15	21.17	28.31	13.01	Zhang et al. (2007)
	7.40	12.30	14.72	46.65	18.92	Zhang et al. (2007)
	18.43	21.50	11.40	32.03	16.64	Tian et al. (2006)
	23.30	12.33	16.47	37.73	10.13	Tian et al. (2006)
	54.73	3.27	1.47	28.19	12.34	Li et al. (2007)
Wild	27.83	12.17	6.88	65.09	10.79	Cui and Li (2005)
	52.12	3.48	1.04	27.77	15.59	Zou et al. (2003b)
	48.77	3.27	0.34	27.36	18.84	Zou et al. (2003a)
	9.13	44.11	1.95	29.14	15.67	Zhou et al. (2002)

captive and wild populations and between normal and inbred populations. Chi-square statistics were adopted for the significant test of paired-behavioral patterns between captive and wild populations and between normal and inbred populations. Significant level was set as 0.05. The data was represented as mean ± SE.

Results and Discussion

Animal behaviors are susceptible to different human selection forces under evolution. Hereinto, domestication is an evolutionary process which may cause various behavioral variances. Price (1984) summarized domestication closely connects to adaptation in a captive environment and it is often achieved by genetic changes over generations as well as by environment-induced changes in development that recur in each generation. Such domestication enforces that animals which are reared in captivity are greatly stressed by artificial selection. After long-term adaptation and evolution, they changed their lifestyles and genetic basis according to human demands.

Comparisons of behavioral patterns between captive and wild populations

In Figure 1, the ratio of each behavioral pattern of the captive population is not significantly different from the wild population (F = 0.050, *df* = 1, $p > 0.05$). Feeding behavior is the dominant behavior for both the captive (34.58 ± 3.48%) and the wild populations, (37.34 ± 9.26%). Preening behavior is much less in the wild population (2.55±1.48%) compared with the captive population (13.05 ± 3.30%). Alerting is slightly higher in the wild population (15.22 ± 1.66%) than in the captive population (14.21 ± 1.58%). The captive population takes less time for resting (21.64 ± 8.97%) than does the wild population (34.46 ± 10.01%) (Table 1).

The free-living wild populations normally spend more time food searching, while food provision for the captive population is always controlled within defined times and places. Food provisioning and human control over the breeding process have reduced competition for important resources and thus have permitted selection for the retention of juvenile characteristics (Price, 1984) (Fig. 1). The results also support that the wild population has a higher rate of feeding behavior than the captive population and might be threatened by unpredictable environments and other potential risks (Anholt and Werner. 1995). Alerting behavior of the wild population should be higher than that of the captive population, which also meets the results (Zhang *et al.,* 2007; Tian *et al.,* 2006; Li *et al.,* 2007; Cui and Li, 2005; Zou *et al.,* 2003a, 2002). Interestingly, preening behavior is higher in the captive population than in the wild population. Birds remove dust, dirt, and parasites from their feathers and adjust their feathers in the optimum position while preening (Delius, 1988; Martin-Platero *et al,* 2006; Lewis *et al.,* 2007; Tripet and Heinz, 1999). Most birds will preen several times a day to keep themselves healthy. It is probably that captivity or a cage is a closed environment in which the hygienic condition is not as healthy as in natural habitats. Birds have no free rights to select their habitats, instead of adapting themselves into it. Resting behavior is less in captivity than in the wild. This resting strategy may be related to the energy-based socio-economic principle (Bryant. 1997). Fernie *et al.* (2000) compared behaviors between free-ranging and captive American kestrels which shows captive kestrels are more active than the control kestrels. It is important to understand that better resting could turn out more feeding. Domestication has influenced the quantitative animal movements rather than qualitative nature of the behavioral response. Price (1984) concluded the postulated loss of certain behavior patterns under domestication is a heightening of response thresholds above normal levels of stimulation. Within the process of domestication, responsiveness is reduced to changes in the animal environment.

Comparisons of behavioral patterns between normal and inbred populations

Comparing the wild population with the captive population, the differences between normal and inbred populations are much fluctuated (Fig. 2). Behavioral patterns are significantly different between normal and inbred populations (F = 32.876, *df* = 4, $p<0.05$). Alerting (Chi-square = 37.351, *df*= 23, $p<0.05$) and resting (Chi-square = 32.876, *df* = 20, $p<0.05$) behaviors are significantly different between normal and inbred populations (Fig. 2). The normal population uses 28% of time to rest but the rest time of the inbred population is much lower with only 3.69%. Alerting has a higher ratio in the normal population (14.72%) than in the inbreeding one (4.23%). Moving is the dominant behavior of the inbred population (51.47%) which is much higher than that of the normal population (16.14%). Inbreeders (15.95%) prefer preening twice as much as the normal population (7.8%). Feeding behaviors of both populations are relatively high but the normal population (35.95%) is much more willing to eat than the inbred populations (24.66%).

Inbreeding may be unavoidable to relatively small, closed, captive populations (Fuller and Thompson 1960). Basically, natural selective pressures are much reduced in captive populations. Inbreeding should typically cause a reduction of genotypic variability and fitness. Species facing inbreeding depression can seriously lead to extinction (Brook *et al.* 2002). In this study, inbred birds are the third generation of captive inbred population. Their parents and grandparents should have adapted to captivity from long-term rearing. Inbreeding is always accompanied with body flaws, genetic vulnerabilities and behavioral abnormalities (Brook *et al.* 2002). The studies confirm that inbreeding may affect behavioral patterns quantitatively (Margulis and Altmann 1997). Moving behavior of the inbred population is greatly higher than the natural population which is firstly published. Alerting behavior of the inbred population is much lower than in the natural population which should be in relation with long-term domestication. A high rate of alerting behavior also can further imply an abnormal development of a bird's nervous system. A low rate of preening behavior in the inbred population can be similarly explained as in the captive population. In general, the inbred population is very much different from the captive and wild population. Despite those inbred birds belonging to the captive population, there is still a large difference from the normal captive population. Researchers demonstrate that quantitative behavioral alterations from the wild population to the captive and inbred populations are reduced by the process of domestication. Again, domestication is one of the good reasons for species decline and genetic loss. Therefore, abnormal behaviors from the inbred population should be given special attention which could promote better wildlife conservation and management.

Conclusion

In this research, researchers compared behavioral changes according to the process of domestication. The results show no significant differences are found between wild and captive populations. In general, preening behavior of the captive population is higher than the wild population; the wild population spends more time resting compared with the captive population; feeding is the most time-spent behavior for both wild and captive populations. However, for the normal population and inbreeding population, alerting and resting behaviors are significantly more in the former rather than latter. Moving is the dominant behavior of the inbreeding population and feeding was taking the most time of the normal population. In general, inbreeding is confirmed to be able to affect behavioral patterns.

Acknowledgements

The researchers gratefully acknowledge the assistance of all veterinarians and animal keepers in Hangzhou Wildlife Park (HWP), and special thanks to Vice-manager Mr. Zhang for data collection. The research is supported by Zhejiang Provincial Natural Science Foundation of China (No. Y3090613) and Key Project of Zhejiang Provincial Education Department of China (No. Z200906965).

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Coping with continuous human disturbance in the wild: insights from penguin heart rate response to various stressors

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ABSTRACT: We questioned the extent to which anthropogenic disturbances might impact wildlife and affect the systems under study. From a research perspective, identifying the effects of human disturbance caused by research activities is crucial in order to understand potential biases and derive appropriate conclusions from data.

We document a case of biological adjustment to chronic human disturbance in the king penguin (*Aptenodytes patagonicus*), breeding on remote, protected islands of the Southern ocean. Using heart rate (HR) as a measure of stress response, we show that, in a colony exposed to the continuous presence of humans for over 50 yr, penguins have habituated to some, but not all, types of stressors. The birds in areas of high chronic human disturbance were found to exhibit attenuated HR responses to acute anthropogenic stressors of low-intensity (i.e., sounds or human approaches) to which they had been subjected intensely over the years. However, such attenuation was not apparent for high-intensity stressors (i.e., captures) which only a few individuals experience each year.

Conclusions: Habituation to anthropogenic sounds/approaches could be an adaptation to deal with chronic innocuous stressors, and beneficial from a research perspective. Alternately, whether penguins have habituated to anthropogenic disturbances over time, or whether human presence has driven the selection of human-tolerant phenotypes, remains an open question with profound implications, and emphasizes the need for more knowledge on the effects of human disturbance on long-term studied populations.

KEYWORDS: stress, heart rate, habituation, selection, seabird, human disturbance, long-term monitoring

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Reprint: *BMC Ecology* 2012 12:10.

Background

Whereas considerable knowledge in ecology and animal behavior has been gained from scientific research on wild animal populations (e.g. [1-17], reviewed in [18]), continuous exposure to humans can have profound effects on the biology of wild species, (e.g. [15, 1-21]). Thus, a crucial aspect of ecological research is to investigate and identify those effects (especially that of chronic disturbance), in order to understand and account for potential biases when deriving conclusions from the data yielded by studies in the wild [17]. Several authors have questioned how the exposure to anthropogenic disturbance might affect the biology of species under study [19-21]. For instance, some species have been shown to habituate to (i.e., tolerate) [22] frequent human disturbance (e.g., marine iguanas, *Amblyrhynchus cristatus*; [19]; Magellanic penguins, *Sphenicus magellanicus*; [20,23]; Jackass penguins, *Spheniscus demersus*; [24]). In contrast, other species have been shown to sensitize to human stressors (e.g., yellow-eyed penguin, *Megadyptes antipodes*; [25]), and others still have been shown to exhibit different responses depending on their developmental stage (e.g., nestling or juvenile hoatzin chicks, *Opisthocomus hoazin*; [26]). Frequent anthropogenic disturbance is also known to drastically alter behavior patterns, (e.g., in threatened killer whales intense boat traffic results in a 14% decrease in the animals' foraging time [21]), and to affect reproductive output [25,27], or offspring provisioning [28].

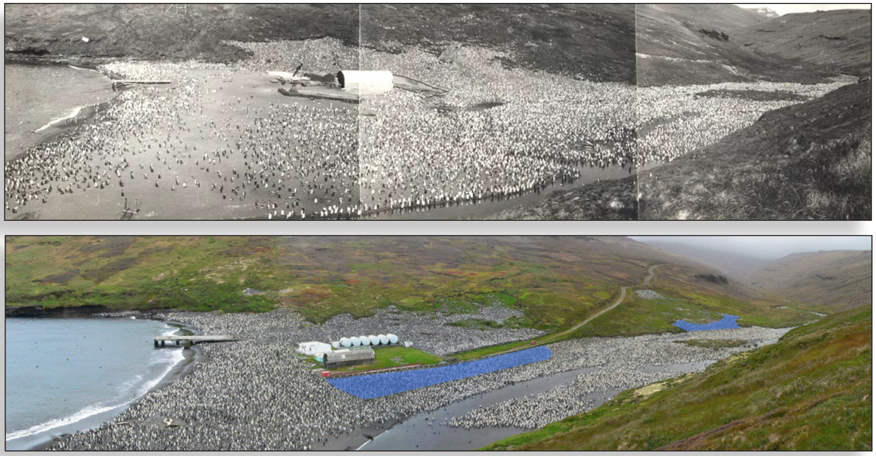

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Figure 1. Chronic human disturbance in the king penguin colony of “La Baie du Marin” on Possession Island (Crozet Archipelago, 46°25′S, 51°45′E). In 1961–1962, a first camp was installed on the beach side of the colony (top picture taken in 1967). Since then, logistic facilities have remained and scientific facilities have been installed at the same place. After 1967, a road was built in order to facilitate logistic operations and transits of material (food, equipment) to the permanent station situated some 500-m above (bottom picture). Thus, part of the colony has been subjected to the regular presence of humans and their activities (scientific or other), whereas another part far from the facilities was left relatively undisturbed. This study is based on the comparison of the HR stress response of birds located within the areas of high (HD) or low (LD) human disturbances (blue-shaded areas).

A major complication of assessing the consequences of human disturbance on wildlife is that those consequences are not always directly visible. For instance, even if seemingly unaffected (i.e., behaviorally calm), animals might undergo profound physiological changes in response to anthropogenic disturbances, or even to the mere presence of human observers (e.g., changes in heart rate, [29-32]).

So what can be said about the continuous presence of humans in specific wildlife populations for the purpose of long-term monitoring and scientific research? To what extent do researchers affect natural processes? There is a need for more data in order to evaluate the impacts of anthropogenic disturbances on wildlife, especially for protected species in pristine environments. Such studies are essential not only because they enable to establish guidelines for the conduct of scientists towards studied species and the management of tourism and recreational activities in natural habitats [26,29-34], but especially because of their implications on the way we think about scientific experiments in the wild, and the inferences we derive from those experiments [17].

Here, we report a case of biological adjustment to human disturbance in a wild king penguin (*Aptenodytes patagonicus*) colony of the Crozet Archipelago, which has been exposed to the continuous presence of humans for over 50 years. In 1961–1962, a permanent camp was established on Possession Island [35] (Fig. 1, top) both within and close to one of its major king penguin colonies: the ‘Baie du Marin’ (BDM) colony. As part of an international scientific effort to understand polar ecosystems, research in this penguin colony has been on going since the early 1960s. This has provided us with a unique opportunity to investigate the effects of continued human presence on the physiology of breeding penguins. We specifically question how breeding king penguins cope with chronic anthropogenic disturbance and consider whether heart rate (HR) responses to acute human stressors may be influenced by a history of close contact with humans. Using HR-loggers (see [36]) to monitor the stress response of penguins, we tested whether HR responses differed between birds holding breeding territories in colony areas subjected to very frequent (daily or more, see methods) human disturbance and birds breeding in relatively undisturbed (weekly or less) areas. Three different acute human stressors were applied (i.e., a loud metal sound, a distant approach, and a capture). In the BDM colony, loud metal sounds typically occur during the logistic operations that take place close to disturbed areas several times a year (e.g., cranes and trucks used during stevedoring for island supply). Distant approaches occur when scientists/tourists observe birds from the edges of the colony, whereas a limited number of captures are performed annually by scientists for research. HR provides a highly sensitive measure of stress responses that may be modulated independently of hormonal pathways [37], and allows greater insight than hormonal responses (such as corticosterone) on how stress responses are shaped depending on the specific nature of various stressors [37,38](Fig. 1). Using this method gave us the possibility to investigate how stress responses were shaped by chronic exposure to humans, and how this shaping might have varied according to stressor type and intensity, and potential risk for the animal.

Methods

Study population and location, bird marking and pre-disturbance follow up

Fieldwork was conducted on Possession Island, Crozet Archipelago (46°2′S, 51°4′E) in February-March 2011. Penguins belonged to the BDM colony, which is host to over 24,000 breeding pairs. This colony is located in the vicinity (some 500 m) of a permanent station (Alfred Faure) and is adjoining a beach that has been regularly used for logistical operations over the last 50 years, and where scientific shelters and technical buildings have been installed (Fig. 1).

Twenty pairs of king penguin were randomly selected from the colony and flipper-banded at the onset of incubation (semi-rigid P.V.C. Darvic bands; 25.8 mm wide, 1.9 mm thick, 7.4 g) to allow their identification and follow-up. This size sample complied with permits to manipulate birds in the BDM colony (see below). Ten pairs were located in a part of the colony adjoining permanent buildings and also very close (5–10 m) to a permanent road used daily by pedestrians and in some occasions by motorized vehicles (Fig 1: HD-area). Throughout the year and over the last 50 years, this part of the colony has been visited daily at a short distance (<5 m) by at least one human, and on some occasions by several groups of up to 10 visitors over a day. In addition, this is also the part of the colony where intensive scientific research has been conducted over the last 20 years, which implied approaching/entering the colony several times a day, including for bird capture purposes, almost all year round. The other 10 pairs were located in a remote part of the colony (about 300 m away from the beach; Fige 1: LD-area), not exposed to anthropogenic noises and where human visitations were much less frequent (around one visit per week over the last 10 years).

Due to time constraints with fieldwork, we subjected 33 out of the 40 birds to three types of acute stressors (see below) 50–80 days after banding. Males king penguins start to incubate after the female has laid their only egg, and partners subsequently alternate between incubation/brooding duties on land and foraging trips at sea throughout the season [39,40]. The specific breeding phenology of king penguins allowed us to determine the date of the onset of each incubation and brooding shift (mean duration of 15 and 12 days for incubation and brooding shifts, respectively), and to ensure that all birds (females at shift 4 and 6 of breeding, and males at shifts 5 and 7) used in this study were in a similar breeding status: birds brooding a non-thermally emancipated chick aged from 2 days to 1 month. This was important, as animals may perceive specific stressful stimuli differently depending on their life-history stage. In addition, stress responses may also be under seasonal variation [41,42]. Comparison of responses should thus be made within life history stages [42]. Eighteen of the stressed birds were located in the part of the colony with a very low rate of chronic human disturbance (the LD-area) and 15 of them were in the part subjected to a very high rate of chronic disturbance (the HD-area).

Heart rate monitoring

Prior to being stressed and usually within three days after the onset of a brooding shift, penguins were equipped with externally mounted HR-loggers (Polar® model RS800, Polar Electro Oy, Kempele, Finland), within the colony and on their breeding territory (see details in [36] for equipment, logger technology and accuracy of HR measurement). Each bird was equipped only once. At capture, the bird's head was covered with a hood to keep it calm. The logger transmitter (weighing less than 1% of total body mass) was attached to the middle of its back with Tesa® tape, and the receiver fixed on a metal pole within a 5-m distance of the animal. Such a set-up prevented the equipment from hindering the movements of the birds. This was confirmed by the fact that we never observed birds trying to remove electrodes or transmitters, nor did we observe any adverse effects of the equipment on the birds' health or behavior. Most animals developed a tachycardia due to handling (up to 165 beats per minute on average), from which they usually recovered within 15–30 min following release. Handling lasted between 5 and 10 min and this procedure never resulted in chick abandonment. Birds resumed normal activity (i.e., resting, comfort behavior or aggressive interactions with neighbors) within minutes after release. HR-loggers were set to store the sampled data for up to three days and sampling was set at a rate of one data point every two seconds. Following equipment, birds were left to recover for at least 12 hour (i.e., one night) before stressors were applied. We retrieved all equipment from the birds 2–3 hours following the last stress protocol. It is important to note that all individuals in this study were manipulated for a similar amount of time before stressors were applied. Differences in HR stress responses are thus not likely to be related to any prior manipulation undergone in order to band the birds and deploy the HR-loggers.

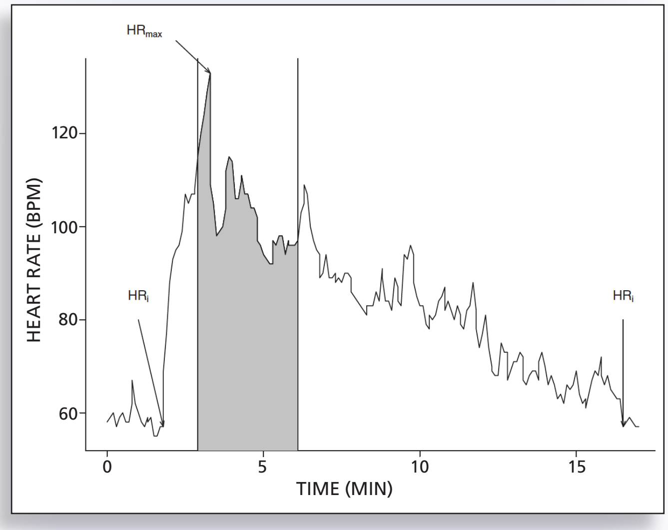

Figure 2. Typical heart rate (HR) response of a brooding king penguin to a human stressor. In this case, the bird was submitted to a capture-immobilization stress, being approached, captured and held captive for 3 minutes (grey area; see text). The approach occurred out of the grey area. HRi is the initial HR before HR started to increase in a constant way, and the HR reached after full recovery; HRmax is the maximum HR reached during the stress. HR is expressed in beat per min (bpm).

Stress protocols

Three different acute stressors were applied in a standardized manner to each bird: a human approach up to 10 m from the bird, a capture-immobilization, and a sound. The approach and sound stressors were chosen as representative of those to which penguins are regularly submitted in the part of the colony with a high rate of human disturbance, the capture stress being in contrast only occasionally applied to few individuals. Stressors were applied in a random order, over two days and with at least five hours separating stressors. The order in which stressors were applied did not affect the corresponding HR response (LMMs; t = 0.60 and 1.17, p = 0.55 and 0.24, n = 76, N = 33 birds; for HR excess and maximum HR increase, respectively). Observations on the focal bird at approximately 30–35 m distance started several minutes before stressors were applied to ensure that it was not sleeping and thus could both see and/or hear the experimenter or the sound, respectively. Moreover, we ensured that the birds were in a resting state for several minutes before proceeding with the test so that they maintained a baseline HR (see Fig. 2). While stressing the birds, their behavior and the distances from which the experimenters found themselves from the focal subject (estimated visually after training) were recorded in real time using a digital audio recorder (VN5500® Olympus Europa, Hamburg, Germany). Behavioral observations continued several minutes after the stressor was applied. These observations were done in order to account for the potential effect of routine bird behavior on HR (i.e., physical activities: aggressive interactions with neighbors, comfort behavior, chick care, or feeding). Indeed, physical activities not directly related to the stress response risked inducing significant HR increases, and thus bias the calculation of some parameters allowing us to characterize the response to a given stressor. The specific protocols for each type of stress were as follows:

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10-m approach stress: Penguins were approached from the front from a starting distance of at least 30 m within the bird's visual field and at an average speed of 0.5 m/sec. The starting distance was chosen from preliminary tests showing that in the BDM colony, the physiological detection distance of penguins when approached by humans (i.e., the distance at which HR started to increase) was around 20–25 m. Thus at 30 m, birds did not exhibit behavioral signs of vigilance towards the experimenter and HR remained at resting levels. The experimenter stopped 10 m away from the bird where he remained motionless for 1 min (to standardize the approach and mimic a standing observer at the edge of the colony) while dictating observations on the behavior of the subject, then subsequently retreated at a constant speed. This distance was chosen because preliminary tests showed that king penguins breeding in the BDM colony become behaviorally alert when approached by humans from a distance of 10 m or less (Groscolas and Viblanc, *unpublished data*). (Fig. 2).

Capture-immobilization stress: The protocol was the same as for 10-m approaches except that the focal bird was approached until capture, which was eased by the fact that brooding penguins have a chick in their brood pouch and cannot escape rapidly. Upon capture, the bird was gently immobilized for 3 minutes, its head covered with a hood. The hood was then quickly removed and the experimenter retreated at a constant speed to the original position, some 30 m away from the animal, in order to continue behavioral observations for several minutes.

Sound stress: Birds were discreetly approached from behind until the experimenter was 15 m behind them, but not sighted. After the bird was observed resting for at least 3 min, the experimenter struck two hollow metal bars three times with a 1-sec interval. The magnitude of the noise averaged 102.5 ± 0.3 dB ($n = 100$ measurements) (i.e., a magnitude sufficient enough to be alarming to a bird [43], and assumed to be similar in intensity to metal sounds that might occur when machines are operating close to the colony during stevedoring operations).

Heart rate analysis

HR data were expressed in beat per min (bpm), plotted and analyzed using Polar Pro Trainer® v.5.00.105 software. Audio recordings of each test were time-matched (by previous synchronization of the observer's digital watch with that of the HR-logger at ± 1 sec.) with the corresponding HR data, which allowed to calculate a number of parameters describing the subjects' HR responses to the stress (Fig. 2). The duration of an HR response was characterized as the total time that HR was elevated above the initial resting rate (HRi) (i.e., from HR starting to increase until recovering to initial level). We defined HRi as the HR at the moment preceding a rapid constant increase in HR. Maximal HR (HRmax) achieved during the stress was determined and relative maximal increase in HR (in %) was calculated as: 100 * (HRmax – HRi)/HRi. We also calculated excess HR (i.e., the number of heart beats produced in excess of resting HR due to stress), as (mean HR during stress – HRi)* duration of HR elevation (in min). Thus, excess HR (in beats) approximated the area under the HR curve and above resting values. We defined HR reactivity as the maximal increase in HR/time needed to reach the maximum H, *(*i.e., a speed of HR increase from HRi to HRmax). Similarly, HR recovery was defined as the speed of HR return to HRi following the stress (i.e., from HRmax to resting levels again, in bpm/sec). In some cases and mostly following capture-immobilization, the HR profile during the recovery period was affected by interfering unrelated behavior and physical activity. We discarded such cases, so that the actual sample size in final calculations is lower than the number of stressed birds (of 33 stressed birds, only 28 sound, 28 10-m approach and 20 capture stresses were retained).

Statistics

All statistical analyses were performed using R v.2.10.1 [44]. As each individual was only tested once for each stressor, data was analyzed with linear regression models (LMs) when stressors were considered separately. Linear Mixed Models (LMMs) were used when stressors were pooled, and bird identity was then specified as a random factor, (i.e., up to three repetitions (one sound stress, one 10-m approach stress, and one capture stress) per individual bird). LMMs were performed using the "lme" function of the "nlm" package in R [45]. Residual normality was asserted using the Shapiro-Wilk normality test. Wherever necessary and to ensure normality of residuals was satisfied, data was transformed prior to analysis using Box-Cox power transformations [46] (i.e., x' = [xp –1]/p, where *p* is the power maximizing normality likelihood obtained with the "bcPower" function from the "ca" package in R). Visual inspection of the residuals indicated no violation of assumptions of homoscedasticity. Significant values are reported for p<0.05. *N* and *n* represent the number of stressed birds and of stresses, respectively.

Ethical note

We removed flipper bands from all banded birds following retrieval of equipment, as detrimental long-term effects of flipper bands are known to occur in king penguins [17,47]. Capture, banding, and equipment procedures were all approved by the Ethical Committee of the Institut Polaire Français–Paul-Emile Victor. Authorizations to enter the colony and to manipulate a limited number of birds (from 20 pairs) were obtained from Terres Australes et Antarctiques Françaises. The experiments comply with the current laws of France.

Results

Overall, pooling all data together and controlling for stressor type by including it as a factor in the model, we found that brooders situated in an area of frequent human disturbance generally exhibited lower HR responses than their congeners breeding in an almost undisturbed area (LMMs; $t = 4.3$, $p<0.001$, $n = 76$, $N = 33$ birds, and $t = 2.07$, $p = 0.04$, $n = 76$, $N = 33$ birds; for HR excess and maximum HR increase, respectively). However, this pattern varied depending on the type of stressor considered (i.e., the interaction between stress type and colony area significantly improved the models; $\chi^2 = 6.05$ and 12.49, $p = 0.048$ and 0.002; for HR excess and maximum HR increase, respectively), and also depending on the parameter used to calculate the HR response (Fig. 3).

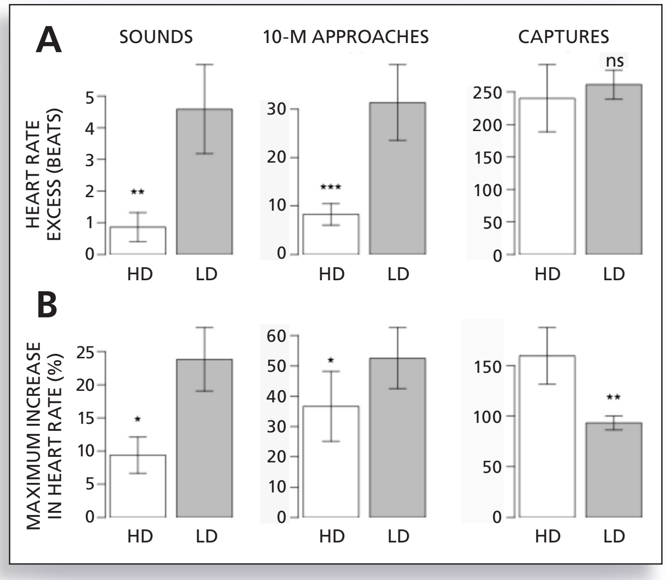

Figure 3. Heart rate (HR) responses to three different types of human stressors (sound, 10-m approach, and capture-immobilization) for king penguins brooding in areas of high (HD) or low (LD) human disturbance. (A) Excess HR is given in beats. (B) Relative maximum increase from resting HR (HRi) is given in percentage. Results are given as means ± SE. Statistics are figured for $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, ^{ns}Non-significant.

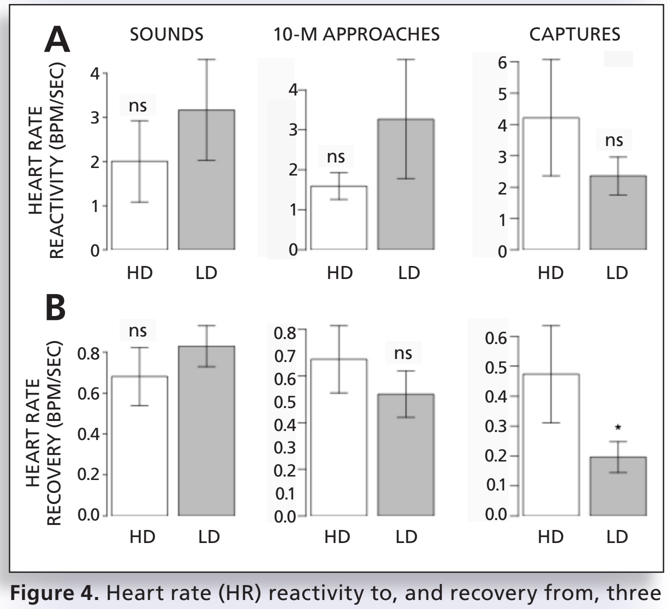

Figure 4. Heart rate (HR) reactivity to, and recovery from, three different types of human stressors (sound, 10-m approach, and capture-immobilization) for king penguins brooding in areas of high (HD) or low (LD) human disturbance. (A) HR reactivity (in bpm/sec) is the speed of HR increase to its maximum during the stress. (B) HR recovery (in bmp/sec) is the speed of HR decrease back to resting levels following reaching of HRmax. Results are given as means ± SE. Statistics are figured for *p<0.05, ^{ns}Non-significant.

During sounds and 10-m approaches, HR excess was significantly 81% and 74% lower for birds breeding in areas of frequent human disturbance (LMs; F1,26 = 8.9, $p = 0.006$, $N = 28$ and F1,26 = 15.5, $p < 0.001$, $N = 28$; for sounds and 10-m approaches, respectively; Fig. 3A). However, HR excess did not differ significantly between areas for captures (LM; F1,18 = 0.2, $p = 0.669$, $N = 20$; Fig. 3A). Maximum relative increase in HR during sounds and 10-m approaches were also significantly 61% and 30% lower for birds breeding in areas of frequent disturbance (LMs; F1,26 = 6.5, $p = 0.017$, $N = 28$ and F1,26 = 4.3, $p = 0.049$, $N = 28$; for sounds and 10-m approaches, respectively; Fig. 3B). In contrast, maximum relative increase in HR was actually 42% higher for birds in areas of frequent human disturbance when considering capture stresses (LM; F1,18 = 9.0, $p = 0.007$, $N = 20$; Fig. 3B). The smaller HR excess observed both for sounds and 10-m approaches in birds breeding in areas of frequent human disturbance were not only due to a smaller maximum relative increase in HR but also to a much shorter duration of this increase. Indeed, this duration was 48% shorter for sounds (14.4 ± 2.6 sec vs. 27.8 ± 5.4 sec; LM; F1,26 = 4.4, $p = 0.046$, $N = 28$) and 52% shorter for 10-m approaches (51.1 ± 7.7 sec vs. 105.3 ± 12.2 sec; LM; F1,26 = 13.1, $p = 0.001$, $N = 28$). For captures, the duration of HR increase was also 38% shorter (376.3 ± 46.8 sec vs. 606.7 ± 83.3 sec), though not significantly (LM; F1,18 = 3.7, $p = 0.070$, $N = 20$) for birds in areas of frequent disturbance, explaining that despite a greater maximum relative HR increase, HR excess did not differ between the two areas. Whatever the type of stress, differences in HR response between the two colony locations were not due to differences in the HR reactivity (Fig. 4A), nor to differences in HR recovery in the case of sounds and 10-m approaches (Fig. 4B). It is interesting to note that following captures, however, HR recovered much faster for birds located in areas of frequent disturbance compared to birds in undisturbed areas (Fig. 4B).

Discussion

We investigated the effects of chronic human disturbance on wildlife stress physiology. Working in a wild king penguin colony, areas of which have been exposed to continuous human presence for over 50 years, we found HR responses of breeding birds to acute human stressors to vary depending on stressor intensity (and potential associated risk for the animal), and the frequency to which birds have been subjected to stressors over the years. Our results suggest that in highly disturbed (HD) areas, penguin HR stress responses to frequent and potentially innocuous stressors (such as loud sounds or distant human approaches) have been attenuated compared to undisturbed areas, whereas this was not the case for infrequent (and potentially noxious) stressors such as captures (Figs. 3, 4). Two hypotheses might explain our results: (1) physiological adjustment to continuous human disturbance and innocuous stimul, (i.e., habituation), or (2) behavioral desertion of the highly disturbed areas by the more stress-sensitive individual, (i.e., selection).

Habituation or selection?

Our results suggest that the HR stress responses of king penguins in the BDM colony have been shaped according to the specific nature of the stressors they are subjected to. Indeed, whereas HR responses to sounds and 10-m approaches were attenuated in HD areas compared to LD areas, this was not the case for HR responses to captures, suggesting that attenuation was not a generalized phenomenon. Those differences are likely reflective of physiological habituation of breeding penguins to innocuous and repeated stimuli. Indeed, as comprehensively reviewed by Cyr and Romero [42], physiological habituation is likely to occur when an animal is repeatedly subjected to a specific innocuous stressor [42,48]. The intensity of stress responses to that particular stimulus may then decrease as the animal learns to consider the stimulus as innocuous [42]. It is important to understand that, in habituation, stress pathways are not blunted. Rather, the animal may learn to ignore the innocuous stimulus [42,49,50]. Hence, the phenomenon of habituation should remain stressor-specific not causing changes in the entire stress physiology of the organism: the capacity to respond to a novel stressor should remain unaffected [42]. This may be the case in our study, where HR responses were attenuated in HD areas for sounds and 10-m approaches, but not for captures. In our study colony, the degree to which birds have been exposed to the different stressors over the past 50 years is indeed very different. Whereas all birds in the HD area have been (and still are) regularly subjected to (potentially innocuous) approaches of human observers (whether scientists in the colony, technicians, or tourists on the outskirts) and anthropogenic sounds (e.g., machine noises during logistic operations), only a very limited number of individuals in each year are concerned by (potentially highly noxious) captures, which are exclusively conducted for scientific purpose. For instance, as a rough figure, one could estimate that during the Austral summer (when most of the scientific field work, logistic operations, and tourist activity occur), the 3,000–4,000 birds in the HD area are approached by human observers 3–5 times per day. Over the course of the breeding season (approximately four months of intensive field work), this would amount to approximately 450–750 approaches (between 1 and 20 m) per bird, an estimation which is likely conservative. In stark contrast, captures in the HD area only concern some 50 individuals each year, which are caught and handled 1 to 5 times during the breeding season. In other words, one might consider that prior to our study, all the animals of the HD area had been subjected to very frequent human disturbance (i.e., anthropogenic sounds and approaches by human observers) over the years, whereas the likelihood that they had previously been captured and manipulated for scientific research is extremely weak. In addition, the intensity of the three stressors was certainly different, being low for sounds and 10-m approaches, and high for captures. Consistent with the idea that weak stimuli are more likely to result in pronounced habituation than strong stimuli [42,51], those results suggest that king penguins in the BDM colony may have habituated to repeated and potentially non-noxious stressors (sounds and 10-m approaches), but not to infrequent and potentially highly noxious stressors such as captures.

Previous studies have reported similar attenuation of stress responses to human disturbance in other specie, (e.g. [19,20,52]). In Magellanic penguins, for instance, birds nesting in HD areas showed lower behavioral and physiological responses to human visitation (i.e., tourist approaches) than birds nesting in LD areas [20,23,52]. However, it is interesting to note that in this case, attenuation of stress responses also extended to capture/restraint protocols, and adrenal responsiveness to ACTH injections appeared blunted in birds from HD areas [20]. This suggests that contrary to king penguins, Magellanic penguins had not actually habituated to human disturbance, but rather that stress pathways were desensitized [42]. Could physiological desensitization have occurred for the king penguins in our study, so that stress responses would be attenuated in HD birds although sounds and approaches were still considered as stressful? The fact that stress responses remained unimpaired for captures suggests not. Taken together, those results emphasize the importance of considering species-specific responses to various stressors to fully understand how animals adjust to human disturbance.

Furthermore, our findings raise the question of whether HR attenuations in HD areas are actually the result of penguin habituation to innocuous stimuli, or whether they are the result of a selection on less stress-sensitive phenotypes. In other words, have stress-susceptible birds deserted highly disturbed areas over the years? This question is especially relevant as the existence of different animal temperaments and coping styles (i.e., animal personalities) is now widely supported [53-56], and variation in individuals' temperaments (e.g., human-tolerant phenotypes, [57]) has recently been suggested as an important factor to account for when analyzing the stress/behavioral responses of wildlife to human disturbance [32,58,59]. In line with this, HR responses of yellow-eyed penguins (*Megadyptes antipodes*) to a standardized human disturbance were found to vary depending on individual differences in temperament, and individual penguins were found to exhibit consistent HR responses over different breeding seasons, indeed suggesting that some personalities may be more stress-prone than others [32]. In addition, the spatial distribution of Eastern chipmunks (*Tamias striatus*) burrows in regards to human disturbance was found to be non-random, but rather dependent on individual temperament [59]. Although current data suggests there is some amount of intra-individual consistency in stress responses in king penguins (Viblanc, Smith, Gineste & Groscolas, unpublished data), we cannot conclude whether the observed differences in HR responses between LD and HD areas are reflective of individual differences in temperament or not. Nonetheless, marked intra-individual consistencies to human disturbance (e.g., flight initiating distance, heart rate stress responses) have previously been reported in birds [57] (including penguins [32]), which suggests that behavioral/physiological flexibility to human disturbance may be constrained by individual susceptibility to disturbance. Whether this may also be the case for physiological responses to human disturbance in king penguins remains to be explicitly tested. Assuming bird temperament may be heritable (e.g., [60,61]), this could be done by investigating physiological responses to acute stressors during early life-stage, (i.e., chicks/juveniles), which have not long been exposed to human anthropogenic disturbances. If selection explains the pattern we observe in adults, one would expect chicks/juveniles to exhibit lower HR stress responses in highly-disturbed locations compared to chicks/juveniles in undisturbed areas. On the other hand, if birds have habituated to human disturbance over time, similar responses in chicks/juveniles should occur regardless of their location in the colony. Alternately, long-term records of breeding site fidelity may provide useful data to investigate territory distribution as a possible result of individual susceptibility to disturbance. Future studies might, for instance, consider monitoring the behavior and physiological stress responses of marked individuals over the years in relation to their location in the colony.


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Implications for the study and conservation of wild populations: Pros and cons

Studies that have considered the effects of human disturbances on the biology of various species have focused especially on the (detrimental) effects of tourism and industry on wildlife (e.g., [21,23-28,62,63]). Along with the massive explosion of ecotourism to even the most remote parts of our planet (e.g., Antarctica, [64]), such studies have been essential in assessing the impact of human activities on wildlife in order to establish guidelines for conservation purposes [27-29,65]. One of the pitfalls of such research is perhaps to forget that from the perspective of wildlife, tourism and scientific research are not two worlds apart. Long-term scientific research programs might also have profound effects on wild population (e.g., [17,47, this study]). The question is then whether those effects are detrimental or not to the species and studied population. As challenged by Nisbet [22], human (and researcher) activity may only be considered a disturbance if it is shown to adversely affect species fitness (e.g., breeding success, survival, population decline). Physiological effects of human activity (such as changes in hormone concentrations, HR), may thus not necessarily qualify as adverse, unless they are actually shown to decrease fitness [22]. At our study site, habituation to innocuous stressors such as sounds or the presence of human observers may on the contrary be beneficial to scientific research, as birds decrease the amount of energy invested in costly stress responses, learning to ignore the lurking scientist observing them with his or her binoculars and talking into his or her tape recorder—habituation is, after all, adaptive by definition. Nonetheless, understanding the consequences of scientific research (e.g., attaching measuring devices, long-term monitoring) [17,66-69] on animal behavior and physiology is essential in setting-up experiments and protocols, and drawing conclusions from the data collected. In this regard, reports documenting the effect of anthropogenic agents on wildlife physiology are needed, as it is only through such knowledge that researchers may draw unbiased conclusions from studies in the wild [67,69]. For instance, as in the case of the king penguins from the BDM colony, it is important to be aware of potential differences in animal sensitivity to human researchers according to various areas of the colony. Animal populations are likely to vary in terms of how intensely parts of the population are disturbed by anthropogenic agent (e.g., [19,20]), so that generalized conclusions on whole populations or species may be inappropriate if derived from a biased sample. Further still, as discussed above, if chronic human disturbance is indeed selecting for less stress-sensitive individuals, this could have strong implications in terms of conservation. Human disturbance is an important driver of directional phenotype selection [70], and selective desertion of the more stress-sensitive phenotypes in specific populations could lead to a loss in phenotypic plasticity and/or genetic diversity [70]. In turn, this may render chronically disturbed colonies less flexible to environmental change (e.g., climate).

Conclusion

Our findings report a case of physiological adjustment to human presence in a long studied king penguin colony, and emphasize the importance of considering potential effects (such as habituation) of human presence (or manipulations) in ecological studies, both in setting up experimental designs and reaching conclusions as to the questions initially addressed. Whereas habituation may be potentially beneficial to scientific research and tourist management, our study also raises the question of the potential influence of human activities on directional selection of specific phenotypes, and underlines the importance of physiological studies for appropriate conservation measures to be addressed [71].

Acknowledgments

This research was supported by the French Polar Institute (IPEV) and by the French National Centre for Scientific Research (CNRS-INEE). Sincere thanks are owed to J. P. Robin for his assistance in the field and to S. E. Viblanc for her help proofreading the manuscript. We are especially grateful to C. Saraux, R. Inger, and an anonymous reviewer for helpful comments and good suggestions on previous versions of the paper. Research project No 119 was carried out at Alfred Faure Station, and logistic support was provided by the Terres Australes et Antarctiques Françaises. V.A.V. was the recipient of a scholarship from the French Ministere de l'Education Nationale, de la Recherche et de la Technologie, and of a fellowship from the Fyssen Foundation.

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~Dedicated to the memory of Martha, the last passenger pigeon (*Ectopistes migratorious*) on the 100th anniversary of her death.

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Cats and Wildlife Casualties

By Adam Grogan, Guest Columnist


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Wildlife rehabilitators worldwide have at least one thing in common—they can expect to be presented with many cat-attacked birds, small mammals, or reptiles over the course of their practice. Free-roaming cats, be they feral or pets, will stalk, chase, hunt, and kill prey (watch what well-fed pet cats did with their time outside at the Univ. of Georgia's www.kittycams.uga.edu). The impact of cat predation on entire wildlife populations varies depending on context and other stressors on that population, but the impact of cats on individual animals is indisputable. This column—first of a series on this issue—comes from Adam Grogan, the United Kingdom IWRC Board Member and Wildlife Rehabilitation Coordinator for the RSPCA, UK.

—Deb Teachout, DVM
Wild Rights Columnist

There are thought to be 10 million owned domestic cats (*Felis catus*) (Murray *et al.* 2010) and more than 800,000 feral cats in the UK, with an estimated population density of 229 cats/km^2 in one urban area in England (Baker *et. al.* 2005). Various studies have identified cats as a major predator of wildlife (Baker *et. al.* 2005, Churcher and Lawton 1987, Woods *et. al.* 2003, Lepczyk *et. al.* 2004, Gillies and Clout, 2003), with one estimation that, in Britain, cats kill at least 52–63 million mammals, 25–29 million birds, and 4–6 million reptiles each summer (Woods *et. al.* 2003).

We know that cats can kill large numbers of wild animals, but, in doing so, they must also have an impact on their victims' welfare:

*Direct effects include the injuries sustained; subsequent bacterial infections; stress from the capture; and,

*Indirect effects include loss of dependent juveniles if an adult animal is taken; increased predator pressure (Yong, 2013), and a gradual decline in populations by sustained "hyperpredation" (Thomas *et. al.* 2012).

There is no doubt that this is as much a welfare issue as it is a conservation issue, and we must accept that humans, as cat owners, are indirectly responsible. It is also wrong to consider the impact on wild animals at population levels only; these individual wild animals are capable of suffering as much as the cats that may prey upon them.

So how does an organization concerned about the welfare of both wildlife and cats tackle this problem?

The regulation of owned cat populations should be a realistic goal and one that, as animal welfarists, we would support. By regulating the numbers of breeding cats, and changing society's attitudes towards pets as disposable commodities, we can reduce the number of feral cats and strays that need rehoming. Regulating the supply of owned cats would improve both cat welfare and, subsequently, wild animal welfare.

The management of cat populations, however, is an emotive issue. In the UK, there are no legislative controls regarding cats, except that owners must abide by the Animal Welfare Act and subsequent Codes of Practice (DEFRA 2009). Any methods to control owned cat behavior, including breeding, would need to be voluntary. In a recent study, a survey of both cat owners and non-cat owners agreed that compulsory sterilization of cats and registering their cats with a local authority were acceptable forms of managing cat populations, so this could be achievable.

Other methods of reducing predation, such as fitting cats with anti-predation devices, are also considered acceptable, and some responsible owners do fit their cats with collars that are equipped with bells or sonic devices (Thomas *et. al.* 2012. Such devices are known to reduce cat predation (Gordon *et. al.* 2010) although none eliminates it (van Heezik 2010). Despite concerns of cat owners, the risk of collar-related injury to the cat is small (Calver *et. al.* 2013).

Other options, such as declawing or keeping cats in during the day, were not considered acceptable (Thomas *et. al.* 2012). From a welfare perspective, the latter is complicated by recent research indicating that, in dense populations, cats may share territories by using them at different times, in order to avoid conflicts. Restricting some cats' movement during certain parts of the day may create a different welfare problem (*The Secret Life of the Cat*, BBC Horizon program, first broadcast June 6, 2013).

Methods to reduce predation of wild animals by feral cats are probably limited to the management of populations as attempts to control their behavior will be limited. As already stated, one way to manage feral cat populations is to better manage owned cat populations, as feral cats are usually owned cats that have been abandoned, or their progeny.

One method for managing established

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Hand-reared Common Swifts (*Apus apus*) in a Wildlife Rehabilitation Center: Assessment of Growth Rates Using Different Diets

Fusté, E., E. Obon, and L. Olid. 2013. *Journal of Zoo and Aquarium Research* 1(2).

Common swift (*Apus apus*) orphans represent an important number of admissions to wildlife rehabilitation centers in Europe. Rehabilitation centers may encounter difficulties in the hand-rearing of large numbers of insectivore chicks if they use commercially available insects, which are usually expensive and nutritionally incomplete. These constraints have created the necessity for alternative diets; however, these may not be optimal for hand-rearing purely insectivorous species. In this study, 116 orphan common swift nestlings were hand-reared during June and July 2008 and 2009 in the Torreferrusa Wildlife Rehabilitation Centre (Catalonia, northern Spain). We assessed growth rates and final fledgling weight under four different diets, comparing the results to those of wild parent-raised common swifts. Clinical condition at admission was the main variable predicted to influence the results. The four diets were (1) rat mince diet, a specific pathogen-free laboratory rat mince; (2) kibble diet, a formula based on a high-protein–low-carbohydrate cat food; (3) cricket diet, based on house crickets (*Acheta domesticus*) and wax moth larvae (*Galleria mellonella*); and (4) mealworm diet, based on mealworm larvae (*Tenebrio molitor*). Reference adult weights of wild animals were obtained from the literature (41.5g ± 2.42 SD). The results showed significant differences in final weights, which were considerably lower for hand-reared animals on the non-insect diets (rat mince diet: 32.8g ± 2.7; kibble diet: 32.5g ± 3.7). The final weights in both insect diet groups were satisfactory, with values close to those observed in the wild (cricket diet: 40.1g ± 4.0; mealworm diet: 40.3g ± 3.1). The results of this research highlight the need to implement changes in diet protocols when using non-insect-based diets.

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Common swift *(Apus apus).*

Factors Influencing the Admission of Urban Nesting Herring Gull (*Larus argentatus*) Into a Rehabilitation Center and Post-Release Survival in Comparison with Wild Counterparts

Thompson, R. P. 2013. *Masters thesis, University of Sussex.*

Orphaned and traumatized herring gull admissions to Mallydams Wood wildlife rehabilitation center were reviewed to determine factors affecting likelihood of release and post release survival. Those birds in the admission categories of orphan, inexperienced juvenile, fishing litter, and caught and entangled showed the greatest likelihood of release, whereas those birds in the admission categories of disease, weakness, collision, and shot birds showed the least probability of release. Between 1999 and 2010, 2,796 (84.1%, this excludes birds euthanized within 48 hours) birds were ringed and released. Subsequently, 44 rehabilitated herring gulls have been found dead, 46 sick, and 2,179 color ring sightings of birds alive reported from over 200 observers in the British Isles and Continental Europe. Mean survival days for adult birds (848.77 days ± 66) were not significantly different than non-adult birds (722.49 days ± 26). Similarly, distance traveled by adult group (58.69Km ± 13.10) and non-adult group (68.46Km ± 3.89) were comparable. Post-release survival within admission groups showed better than expected recovery rates for shot adult birds (47%) and inexperienced juveniles (40%). Data sourced from urban nesting wild chicks in the South West and South East was compared to rescued juvenile birds. No significant differences between the two groups were found for dead birds, but sick birds and re-sighting data showed significant differences. In-house rehabilitation protocols currently in place were tested and indicated that procedures to mitigate animal suffering and yet improve the likelihood of release were appropriate, with only minor improvements required in release criteria. The anthropogenic pressures on urban gull populations and national decline in the sub-species *Larus argentatus argenteus* could be supplemented through rehabilitated birds. The data suggest that the rehabilitation of herring gulls was important from both an animal welfare and population perspective and, therefore, cost effective.

Assessing the Impact of Live-Capture, Confinement, and Translocation on Stress and Fate in Eastern Gray Squirrels

Bosson, C. O., R. Palme, and R. Boonstra. 2013. *Journal of Mammalogy.* Online.

Live-capture and translocation are methods to deal with nuisance eastern gray squirrels in North America, but it is unknown how these methods affect squirrel physiology or survival. In this study, we validated an enzyme immunoassay (EIA) to measure fecal cortisol metabolites (FCMs) in gray squirrels; assessed their stress response relative to the positioning of the live trap (sun, shade, or control); and assessed the impact of translocation on their long-term stress, movement patterns, and survival using FCM levels, body mass changes, and radio-telemetry. We found that a 5α-pregnane-3β,11β,21-triol-20-one EIA reliably detected acute stress in gray squirrel feces 12–24 h after the stressor; live traps positioned in the sun resulted in higher peak FCM levels compared with traps positioned in the shade; translocated squirrels experienced a

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Abstracts

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10% mortality rate, compared with no mortality in the controls, although overall fates were the same; translocated squirrels initially explored more and dispersed farther than controls, but after two weeks made similar movements; and after controlling for the effect of season, translocation did not affect long-term FCM levels or body mass; this conclusion must be tempered by the low number of recaptures. Our study demonstrates the utility of the FCM assay, that gray squirrels are extremely sensitive to capture, handling, and confinement; and that live-capture must be done in a way that minimizes exposure to additive environmental stressors.

Networks of Wildlife Translocations in Developing Countries: An Emerging Conservation Issue?

Goss, J. R., and G. S. Cumming. 2013. *Frontiers in Ecology and the Environment* 11(5).

The recent expansion of the global wildlife trade, both within and between countries, has many potentially negative ecological impacts. Emerging economies, in particular, have a largely overlooked potential for rapid internal growth in this trade. An analysis of approximately 17,000 wildlife transportation permits—issued in South Africa primarily for commercial purposes by the Western Cape Province's local government—provides insights into this trend. During the past decade, a total of 912 amphibian, bird, mammal, and reptile species were transported to and from locations in the province; 60% of translocated vertebrate species were non-native. Network analysis of the data indicated that the numbers of participants within local trade networks are increasing rapidly. Static and dynamic structural differences emerged between the networks for selected vertebrate classes. Changes in density and degree were pronounced for mammal and reptile networks but were more constant for birds over the observed time period. All translocation networks exhibited "small-world," scale-free properties, which would facilitate the rapid propagation of negative influences through the system.

Cats

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feral cat populations is a trap-neuter-release (TNR) scheme, and the RSPCA advocates this method. It is controversial, however, with conservationists arguing that its success in reducing the population of cats is limited at best, and it doesn't stop the cats from hunting (Longcore *et. al.* 2009). With local authorities reluctant to remove feral cats due to the potential for adverse publicity and with many cat protection groups advocating a no-kill policy, TNR schemes are considered the easiest way forward.

Ultimately, the key to the management of feral cats is people. Studies indicate that feral cats appear to concentrate on urban or suburban areas, never moving far from human habitation (Ferriera et. al. 2011), so the link with people is clear. What is needed is a strategy that teaches the public responsible cat ownership and so reduces the numbers of cats *per se*. Until this happens, feral cat populations will continue to be bolstered by discarded cats, making management of feral cat populations a never-ending task.

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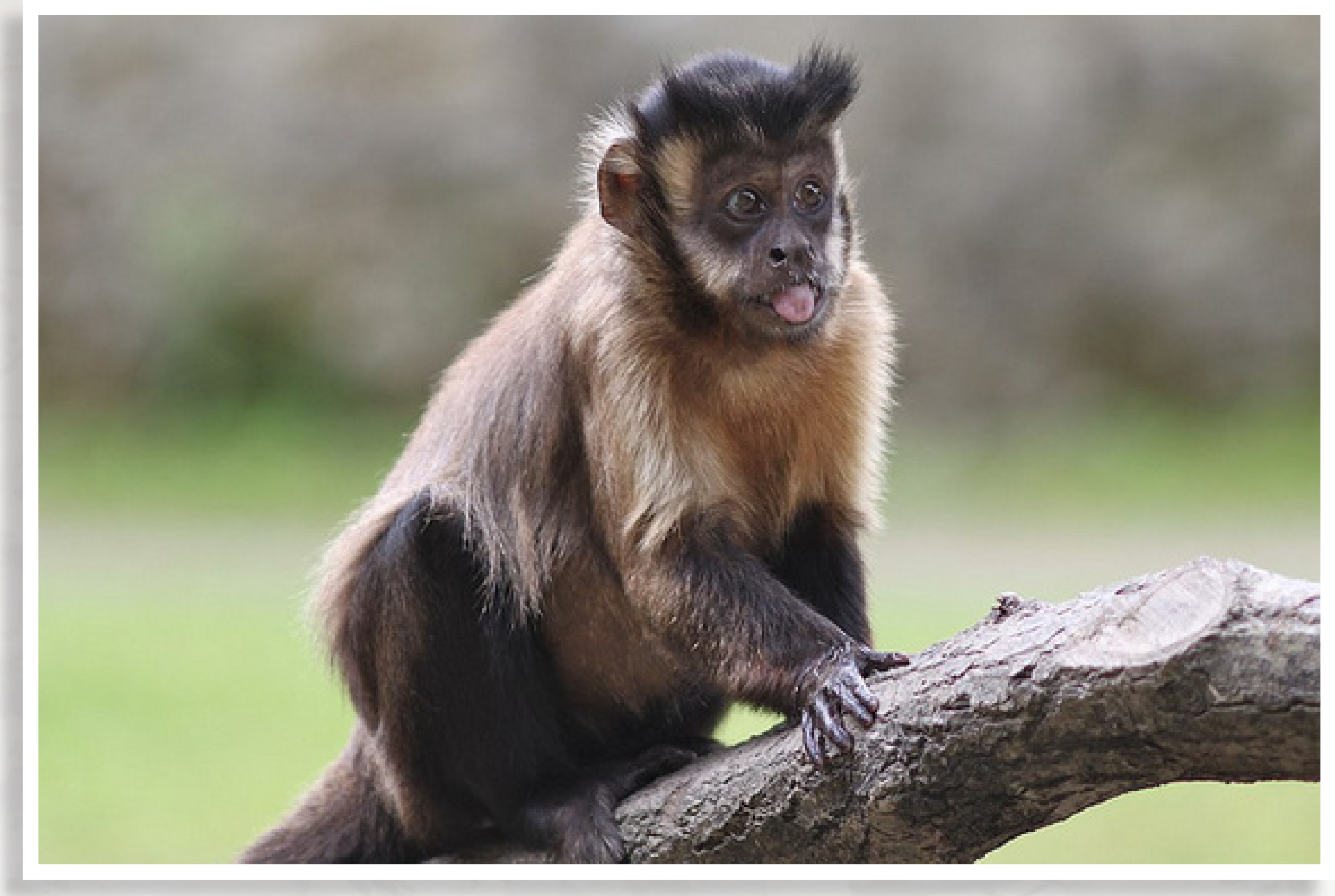

"I've always enjoyed omnivory. Opossums, not so much."

Black-horned capuchin monkey (*Sapajus nigritus*).

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Long-eared owl (*Asio otus*).
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