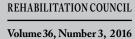


JOURNALOF ILDIAF REHABILITATION



INTERNATIONAL WILDLIFE

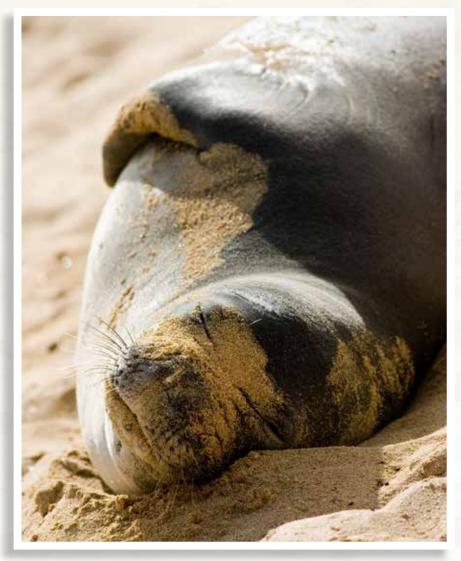


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Reflections on husbandry: How temperatures in outdoor enclosures affect red fox behavior A long-term study of stranding and mortality causes among loggerheads at a facility in Spain Behind the scenes at an IWRC international course in Brunei

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 its outreach to those in the profession, the IWRC works to disseminate information and improve the quality of the care provided to wildlife.



Left: Hawaiian monk seal (Monachus schauinslandi). PHOTO ©KANAKA RASTAMON, FLICKR. CC BY-NC-ND 2.0 LICENSE.

On the cover: Young red fox (Vulpes vulpes). PHOTO @MATT KNOTH, FLICKR. CC BY-NC-ND 2.0 LICENSE.



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

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EDITORIAL

Courses Across Borders

It always surprises me how much is involved in setting up an IWRC course outside of North America. After all, we run thirty courses a year within North America and have for five years smoothly run courses in Ireland with our partner Wildlife Rehabilitation Ireland—largely thanks to the excellent work of Emma Higgs. I had forgotten those early days of planning for Ireland, sending over picture lists of supplies, laboring over whether the homework was appropriate, and revising PowerPoint slides.

We revisited this work in 2015 with several courses in South Africa, the load eased by the organizers' prior experience, the country's long history of wildlife rehabilitation, and an excellent local vet to advise us on regional zoonotic concerns.

Most recently we spent a year planning a week-long course in Brunei, a tiny country that shares the island of Borneo with Malaysia and Indonesia. Funding and logistics were among the expected challenges and were dealt with early on—without the monetary ability to get instructors and students to the facility no course would happen, and this was a dream course for many.

Despite our planning, supplies became the most unexpected trial. Some lab supplies were easily sourced-access to compound microscopes is a side benefit of holding the course at the Universiti of Brunei Darussalam; other supplies were extraordinarily expensive, or just challenging for non-medical individuals to locate. In the end, we shipped most consumable supplies (needles, syringes, bandaging materials) from the US, a particular irony since many of these supplies are originally manufactured in the region of our hosts. (In future courses, instructors will be working with students in order to source materials from local suppliers and to connect them with veterinary professionals.)

Fortunately for the Brunei class series, one concern we did not have to grapple with was knowledge of local natural history—usually my primary concern, as being an effective rehabilitator requires intimate knowledge of native species. An instructor from a different continent might well teach the importance of natural history but not instruct in the local details. But in Brunei, our host is the Brunei Biodiversity & Natural History Society (BruWILD), and the local species knowledge is already there.

BruWILD interfaced with local experts to highlight regional zoonotic diseases, a subject that varies significantly across regions. Despite the seeming transglobal nature of so many diseases, many are only regionally prevalent.

Sometimes the little things are the most challenging for these courses, such as the number of stations needed for each lab. In a country new to wildlife rehabilitation, there are no experts to call upon as seasoned lab assistants—our instructors need to be everywhere at once, requiring careful orchestration. Thankfully, Lin Ji Liaw, president of BruWILD, is an organized soul and a pleasure to work with. Through many Skype meetings at 7pm Eastern time (where our instructors are) and 7am the next morning Brunei time with Lin Ji, we arranged details of travel, supplies, and course schedules. At this point I feel I know Lin Ji as a long-time, treasured colleague.

The challenges and stress of organizing these classes are met by the pleasure of connecting with rehabilitators across the globe and the excitement of inducting new people to the profession. The Brunei course was a success on both fronts—connections made in Brunei that will last a lifetime, side trips for instructors to meet rehabilitators in nearby countries, and 30 new students trained in the basics of wildlife rehabilitation over the course of a very, very intense five days.

> —Kai Williams Executive Director

Appeal rules in favor of Wildlife Center

Vancouver, CAN (August 25)—The Environmental Appeal Board Panel has ruled in favour of the BC SPCA Wild ARC and Wildlife Rehabilitators Network of BC. This decision supports the BC SPCA Wild ARC's biologically-responsible and humane approach to addressing animals who are injured and orphaned by human activity, and confirms such rehabilitation efforts are not contrary to the proper management of wildlife resources.

In 2014, Wild ARC released 72 sterilized grey squirrels into established grey squirrel urban habitat areas within the Capital Regional District. The population of grey squirrels in the CRD is estimated to be more than 75,000, having been in the district for 50 years now. Grey squirrels are native to eastern Canada, and have been established for more than 100 years in Greater Vancouver. Research conducted in BC (Hwang & Lariviere 2006; Gonzales 2005; Gonzales et al. 2008) has shown little to no correlation between native and non-native squirrel populations, with habitat alteration being the biggest factor for native squirrel declines.

Hawaiian Law Addresses Wildlife Trafficking

Honolulu (June 28)—As the "endangered species capital of the world," Hawaii knows first-hand the devastating impacts of losing significant and iconic native species. The state has now taken a historic step in helping to prevent the further loss of critically endangered species within its own borders and abroad.

With the signing of Senate Bill 2647, now Act 125, Hawaii passes the most comprehensive U.S. state law targeting the illegal wildlife trade.

The law goes into effect immediately, although enforcement of the law will be delayed until June 30, 2017 to grant individuals and businesses with wildlife products in their possession time to lawfully dispossess of the items. The law also provides reasonable exemptions for bona fide antiques, musical instruments, guns and knives, and traditional cultural practices.

In the past two years, a number of states across the U.S. have pushed for stricter laws to crack down on illegal wildlife trafficking. New York, New Jersey, and California have each passed laws prohibiting the purchase and sale of products made with elephant ivory and rhino horn. And in 2015, philanthropist and entrepreneur, Paul G. Allen backed Initiative 1401, a first-of-its-kind statewide ballot measure that will keep products from 10 highly endangered animals out of his home state of Washington.

"The loss of species has significant and unpredictable conse-

quences for the health of our planet. The passage of this legislation is an important step in stopping this race to extinction," said Jared Axelrod, government affairs manager for Paul G. Allen's Vulcan Inc. "We believe the most effective way to save animals from extinction is to strengthen enforcement. This new law will provide enforcement officials with the tools they need to stop the traffickers and disrupt the supply chain."

The Hawaii bill was supported by hundreds of local residents and dozens of grassroots groups across Hawaii who testified in support of the measure this session and the Hawaii Wildlife Coalition —Vulcan Inc., a Paul G. Allen company; International Fund for Animal Welfare (IFAW); Humane Society International



Juvenile (light morph) 'Io, or Hawai'ian hawk (Buteo solitarius), one of the many endangered species on the Hawai'ian archipelago.

(HSI); The Humane Society of the United States (HSUS); the Wildlife Conservation Society (WCS); and the Natural Resources Defense Council (NRDC).

"Hawaii has long been one of the United States' major markets for ivory and other wildlife products, and illicit trade is driving many species—from tigers and rhinoceros to sharks and pangolins—to the verge of extinction," said Jeff Flocken, IFAW's Regional Director for North America. "Act 125 proves that the state's citizens and lawmakers prioritize living, breathing animals more than bangles and gaudy curios."

Rhea Suh, President of the Natural Resources Defense Council, praised the new law, "The Hawaiian Islands have long been a sanctuary for some of the Earth's greatest creatures. Now, these protections and aloha are being extended thousands of miles away for one of the most majestic animals: the African elephant. Hawaii's leadership shows us how we can all do our part to combat the illegal trade and trafficking of highly threatened elephants, rhinos and sea turtles. We hope their leadership will be replicated across the country and the world."

DNA Evidence Changes Thinking on Species Distinctions

Yangon, Myanmar (June 23)—Experts from WCS (Wildlife Conservation Society) and the National University of Singapore (NUS) have made a surprising discovery that could subvert the significance of traditional criteria used for species classification.

Employing novel techniques to retrieve DNA sequences from thousands of genomic locations, the researchers were able to uncover an unusual case of cryptic speciation in the Streak-eared Bulbul [*Pycnonotus blanfordi*], a bird widespread throughout South-east Asian countries.

Cryptic speciation produces closely related sister species that are very similar in appearance and often overlooked by scientists until genetic and/or bioacoustic inquiries reveal species-level differences.

Traditionally, the bird identification literature has relied on shape and plumage color to classify bird species. More recently, vocalizations have also been used to uncover cryptic species otherwise similar in body appearance.

In the last two decades, DNA sequence comparison has gradually been added as a tool in the kit for species delimitation, casting light on a number of cryptic species where neither morphological nor bioacoustic differences provide clues for species differentiation.

"Distinguishing different species is a non-trivial task of great importance," said Colin Poole, WCS Regional Director for the Greater Mekong region. "This research helps us better understand the evolution of life and often points to cases in which science has hitherto underestimated the extent of actual species diversity present in any given region."

After careful examination, two described subspecies of Streak-eared Bulbul [*Pycnonotus blanfordi*] resident in Myanmar [*P. b. blanfordi*] and Thailand/ Indochina [*P. b. conradi*] were found to exhibit deep genome-wide differentiation indicating that they are two separate species.

Despite negligible nuances in the birds' plumage color, and limited differentiation in their vocalizations, WCS and NUS ornithologists identified a surprising genetic divergence dating back as far as the early Pleistocene.

A closer look also revealed different eye colors between the two forms, which the scientists believe to be an important morphological differentiating trait in mate recognition and reproductive isolation, prompting them to call for an elevation of both forms to species level, and naming the one specific to Myanmar "*Ayeyawady bulbul*."

"Cryptic species have always represented an intriguing challenge for scientists," explains Robert Tizard from WCS Myanmar. "Advancements in DNA methodologies are of tremendous help in understanding the rich biodiversity of Myanmar and tropical Asia."

Through this discovery, scientists at WCS and NUS have demonstrated how novel DNA sequencing technologies that retrieve genome-wide DNA can be put to use in species delimitation, and advocate for more systematic use of genome-wide DNA for the detection of cryptic species.

"Even in birds, which are betterknown than most other living beings, the age of new species discoveries is not over," said Dr. Frank Rheindt from NUS. "We hope that future collaborations between academia and conservation NGOs will lead to the discovery of numerous additional cryptic species to help us obtain a more realistic understanding of true levels of species diversity."

New York Wildlife Rehabilitation Law Suit

Rensselaerville, NY, USA (June 23)—On a porch in the Huyck Preserve, wildlife

rehabilitator Kelly Martin held a tiny bottle to a baby porcupine's snout.

The president of the <u>New York State</u> Wildlife Rehabilitation Council has worked with the state Department of Environmental Conservation since 1979 to save the lives of injured animals, large and small.

But earlier this spring new guidelines on what licensed rehabilitators can do spawned two lawsuits.

A deer named "Jane Doe" is at the heart of the first suit on Long Island, challenging a requirement that injured deer must be released or killed within two days.

In <u>May, Virginia Frati</u>, director of the Evelyn Alexander Wildlife Rescue Center in Suffolk County, claimed Jane Doe was not fully rehabilitated or ready for release. She obtained a temporary restraining order against enforcement of the rule.

The second suit, filed by Martin in Erie County in June, contends the DEC failed to include New York's 1,400 licensed wildlife rehabilitators in the decision-making process that led to dozens of new guidelines stipulated in a letter sent out in February.

"It is an issue of fairness and transparency," Martin said. The DEC has argued that it is not changing rules, but modifying the interpretation of existing ones. DEC biologist Joseph Therrien said the agency's Bureau of Wildlife began reviewing the rules in 2015 in response to concerns that rehabilitators were allowing deer and other big game to become habituated to humans.

"There is a documented pattern of licensed wildlife rehabilitators in New York who are reluctant to either euthanize or release white-tailed deer," Therrien said in affidavit that is part of the Long Island case.

A wildlife rehabilitation license is a privilege that is discretionary and revocable, says the DEC.

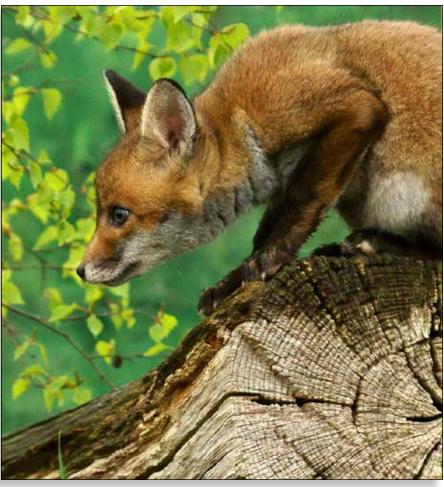
"Wildlife is the property of the state, not the property of rehabilitators, like Frati, who temporarily possess wildlife for the purposes of rendering care to the animals so that the animals can be returned to the wild," the statement said.

The DEC argued that Frati's claim that vision-impaired Jane Doe was not ready for

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An analysis of juvenile red fox behavior in response to ambient temperature changes in an outdoor pre-release enclosure

Cale Matesic and Esther Finegan



Introduction

The red fox (*Vulpes vulpes*) is one of the most common and widely distributed species of the Family Canidae in the world.¹⁻⁴ This species is native to Canada and found throughout the Northern hemisphere. This geographical range spans multiple countries and the red fox is found in a variety of climatic environments from the Arctic Circle to North Africa.⁴⁻⁶ Red foxes are more plentiful in locations that are variable in landscape because they are able to maximize utilization and cover while hunting or evading predators. Prey availability is the main factor in determining habitat use and contributes to the vast traveling distances red foxes are observed to exhibit throughout the year.⁵

Red foxes are considered opportunistic carnivores and exhibit feeding behaviors. They will eat a variety of food within their habitat depending on the fluctuating trends in food availability.^{5,8,10} Since food availability drives preferential habitat location for the red fox, if food abundance decreases for a long period of time, the red fox will move territories in order to maintain health. The red foxes diet consists of small mammals such as lagomorphs and sciurids, nesting birds, ground-feeding galliforme birds, annelids such

ABSTRACT: The behavioral responses of 7 red fox kits to temperature changes in an outdoor enclosure were recorded for 2 weeks prior to release. Images of the animals were captured by thermal imaging and behavior was documented through observation from outside their enclosure. At ambient air temperatures ranging from 20-23°C, red fox kits exhibited natural wild behavior (walking, running, eating, playing). At higher temperatures, 26-28°C, red fox kits began exhibiting potentially thermally related behaviors including lying with their loins exposed. This analysis suggests that there may be benefits for larger, better ventilated outdoor enclosures for red fox rehabilitation so that confined areas of increased temperature can be avoided.

KEY WORDS: behavior, southern Ontario, red fox, rehabilitation, thermoregulation, *Vulpes vulpes*, welfare, wildlife

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as earthworms, many types of insects, and even fruit, depending on the location and season.^{5,11}

Red foxes exhibit a prey-killing behavior and have been shown to prefer prey that vocalize and move about, over quiet, motionless, or dead prey.¹² Prey is found by utilizing the foxes excellent hearing and focusing in on the sound produced by the prey that they are searching for.⁵ Red fox kits will "play" with their prey before killing, unless a conspecific gets too close. This "play" behavior consists of leaping vertically towards the prey, bilateral or unilateral placement of the forepaw(s) onto the tail and body followed by biting, mouthing, chinning, or nosing of the prey.¹² Leaping on the prey occurs while the prey is moving and consists of flexing and raising the forelimbs into the leap and then extending them onto the prey while the hindlimbs come down and land a few moments later.¹² The killing of the prey is due to crushing and then the prey will be eaten whole, with the skin intact.¹²

The red fox has also been known to exhibit food hoarding behaviors and has shown two separate and distinct types of food hoarding. These can be described as scatter hoarding and larder hoarding. Scatter hoards are made when animals place their food in multiple stashes around a designated area.¹³ This allows for a poor defense of hoards, but a higher probability of having food to eat if one of their hoards is compromised by competition.¹⁴⁻¹⁵ Larder hoards are made in close proximity to an animal's habitat.¹³ These hoards are fewer in numbers and contain more food per hoard than scatter hoards, but with their location being closer to home, they are easier to defend.¹⁴⁻¹⁵ If larder hoards are raided, there is a higher likelihood of a large amount of food being stolen. Most mammalian hoarders can exhibit both types of hoarding behaviors but not at the same time. It is completely dependent on the characteristics of the individual and their circumstances and location as to which hoarding behavior they exhibit.14-15

Multiple physiological factors can affect the food hoarding capabilities of the red fox in the wild, and these might include, but are not limited to: familiarity with the environment and habitat, abundance of food, perishability of food, the amount of food already hoarded, the environmental temperature, and the substrate available to dig in.¹³ Environmental factors that can play a part in the frequency of the food hoarding behavior may include: genetics, hunger, life stage, sex, and neural circuits.¹³ If enough stimulation from one of these factors is applied then food hoarding will be observed, but the most common scenario is an overall combination of multiple stimuli affecting the expression of food hoarding behavior in the red fox.

Red foxes are primarily nocturnal with peak activity at sunrise and sunset (crepuscular) and exhibit a higher body temperature at these times.⁷⁻⁸ This nocturnal and crepuscular lifestyle is reflective of their feeding habits and overlaps with their main prey of choice.⁵ Females will exhibit increased activity during the day while rearing kits.^{5.7} If kits are not present, daytime is usually used for rest in above-ground rest sites. If red foxes are seen out throughout the day, it is usually in areas where there is not much human activity.^{5.9}

The red fox is able to withstand both cold and hot tempera-

tures and this ability is closely associated with the characteristics of their fur, which changes in color throughout their life. The red fox coat color ranges from dark grey (neonates), to a pale buff (at 8-14 days), to a deep reddish brown (at 9-14 weeks). However, as adults they still have the distinctive white underside, black tipped ears and black legs and feet.⁵ The adult coat color has been observed in three separate color morphs: red, silver/black, and a cross between them.⁵ Every year the red fox goes through an annual molt between the winter and spring seasons. The summer pelt is much shorter and duller than its winter counterpart. The outer layer of fur consists of long and silky hairs, whereas the underlayer is long and thick. This thick underlayer provides enhanced insulation allowing for effective thermoregulation across a wide range of climatic conditions.⁵

The thermoregulatory capabilities of the red fox vary depending on the body part being considered. According to Larivière & Pasitschniak-Arts (1996), the major heat exchange locations include the face, the dorsal part of the head, and the nose, ears, lower legs and paws, which in total account for 33% of the total body surface area. These areas produce shorter, but denser fur throughout all of the seasons, a characteristic believed to be essential for the control of heat loss. The critical ambient temperature for these areas of thermoregulation is between -20°C and -15°C.⁵ The long tail is not only used for balance, but as the adult red fox does not normally use dens for sleeping, the tail is wrapped around the face during sleep and prevents heat loss from this area of shorter fur.⁵

The current study was conducted to observe the behavioral responses of 7 red fox kits to temperature changes in an outdoor enclosure prior to their release back into the wild. It was of interest to see how juvenile red foxes adapted to a range of environmental temperatures and to investigate when and what their behaviors were, whether stereotypic behaviors were shown, and if any natural behaviors were missing in a captive rehabilitation setting. It was hypothesized that as long as the foxes observed had the required space and enrichment within the enclosure, no matter the ambient air temperature, only natural behaviors would be observed.

Methods

Study Area

The study was conducted in Southern Ontario (Latitude: 42° 46' 9.39 N, Longitude: 81° 10' 58.15" W) during the last two weeks in August 2014 at a facility surrounded by a forest and a ravine on the north and east sides. The property was covered by trees and allowed ample shade and protection from the sun throughout the season. The temperature ranged from 10.2°C to 29.3°C with a relative humidity level varying from 50.4% to 87.1% throughout the entire study. The red foxes were housed in a smaller cage until they were 4 months of age, and this acted as a transition cage before they were transported to the pre-release enclosure. At 4 months of age they were transported to a 1.83m x 1.83m x 4.27m pre-release enclosure which overlooked a forest and a ravine on the north and east sides. This enclosure was 100ft from the rehabilitation facility

and the south and west sides of the enclosure were covered in lattice to reduce visual stimulation from humans. Due to there being a lack of specific guidelines in Ontario for enclosure designs and limitations, the enclosure design and location was decided upon by the wildlife rehabilitator. Decisions were made based on personal experience and word of mouth from other wildlife rehabilitators. Noise stimulation from other animals and the presence of workers on the property were constants (even though the north and east sides were facing a ravine and forest) because of space constraints and the permanent location and nature of the enclosure. The closest enclosure was 5 ft away to the west and housed juvenile racoons. Rehabilitators of the facility would arrive periodically and would feed the various animals on the property throughout the day. Observations at this time would stop and recommence once the foxes acclimated to the stimulation. Sand was used as the substrate rather than dirt in the pre-release enclosure because of its resemblance to a natural den substrate⁵, its ease to clean up (compared to dirt), and the ability to tell if anything is wrong with the animals by being able to see blood from injuries or in the stool or urine. Wooden platforms were added along the two sides and the back to provide a vertical element mimicking their natural climbing abilities. Logs and a 'hidey' box were placed to allow an area of retreat. Behavioral enrichment was provided in the form of dog toys, grass, and clumps of dirt, which was determined by previous years of experience rehabilitating red fox kits.

Animals Observed

In May of 2014, 7 two-month-old red fox kits were found orphaned on a property in Southern Ontario. Age was determined by coat color (deep reddish brown indicates 9-14 weeks of age).⁵ The mother was seen on the side of a nearby road having been killed by a car. The kits were brought to Another Chance Wildlife Rehabilitation Centre located in St. Thomas, Ontario, Canada. The initial intake exam had shown that they were lethargic and dehydrated from being without nourishment from their mother since they were not fully weaned. Observations of these kits began on 12 August 2014. Research began at this time because it was of specific interest to observe the final week of rehabilitation prior

to release due to the close relationship to wild behaviors because of limited human contact. Food was provided to the foxes twice a day, at about 10am and 8pm, on a daily basis, by the director at the rehabilitation center during the study. Food provided resembled a natural diet and consisted of fresh fruits (apples, melons, and banana) and various meats (mice, chicken, and turkey). Fruit was fed in the morning, with meat being fed in the evening. Even though red foxes are opportunistic carnivores, if no meat is found in the wild then they will resort to eating fruits and vegetables to hold them over until meat is found again. The rehabilitation feeding schedule promotes food hoarding when meat is provided so they have a reserve when fruits and vegetables are offered. Therefore, when meat (less perishable) was offered at night, they hoarded any excess in preparation for their morning feeding of fruits and vegetables (more perishable). Water was provided *ad libitum* throughout the entire study period.

Study Design

Two observers recorded the behavioral observations and meteorological readings, one continuously recording the behaviors, while the other observer recorded the meteorological data (ambient air temperature, wind speed, and relative humidity). The red foxes were in contact with rehabilitators on a daily basis, multiple times a day, so were accustomed to seeing humans throughout the day. The observers were then positioned 4.6m from the front of the enclosure and sat in chairs to reduce movement. Observations did not commence until the foxes stopped obviously paying attention to observers. Behavioral recording sheets were used and observations of behavior were made using eyesight (during periods of light) and a thermal camera (during the night). Twenty-one discrete behaviors were recorded throughout the entire study with the definition of each behavior observed being presented in Table 1. Foxes were too similar in appearance to distinguish between individuals and so individual animal behavioral consistency could not be documented. However, in order to determine consistencies of behavior overall, every minute the number of foxes performing each behavior was documented. Each behavioral sheet represented one hour of observations. Observations occurred on 7 different days over the span of 2 weeks from 12 August 2014 – 26 August 2014. The times at which the observations occurred ranged from evening to overnight to morning (Fig. 1). Morning was from 06:00-12:00, evening was from 17:00-21:00, and overnight was from 21:00-06:00. The majority of the observational time occurred around sunrise and sunset. These observational times were chosen in response to an initial expectation of higher activity of foxes who naturally exhibit a more crepuscular and nocturnal lifestyle in the wild. No observations were performed between

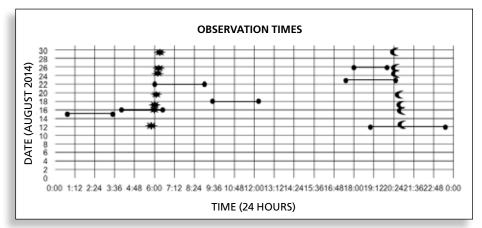


FIGURE 1. Overall summary of observational times with sunrise indicated by the sun # and sunset indicated by the moon (.

TABLE 1. BEHAVIORAL DEFINITIONS OF RED FOXES (*VULPES VULPES*) DURING AN OBSERVA-TIONAL STUDY AT ANOTHER CHANCE WILDLIFE CENTRE IN ST. THOMAS, ONTARIO, CANADA

BEHAVIOR	DEFINITION
STANDING	Fox is motionless while on the pad of the paw for all fours
WALKING / SNIFFING	Fox is moving around at a slower pace than running with/without nose to the ground/object
RUNNING	Fox is moving around in a pace faster than a walk for a short period of time
PACING	Fox is walking back and forth within the same area repeatedly
SITTING	Fox is motionless with posterior on the ground and front end is propped up with straight fore legs
SCRATCHING	Utilization of front or hind paws in a rhythmic mo- tion in contact with a portion of the body
LYING HEAD UP	Ventral portion of the body is touching the ground while the head is off the ground (eyes open or closed)
LYING HEAD DOWN	Ventral portion of the body and head is touching the ground (eyes open or closed)
LYING LOIN(S) EXPOSED	Dorsal portion of the body and head is in contact with the ground (eyes can be open or closed)
PLAYING / INTERACTION	Utilization of a provided toy by one or more foxes, or two or more foxes exhibiting physical contact with minimal vocalizations
CLIMBING	Fox utilizes all four limbs to move in a vertical mo- tion on any surrounding wall/door
EATING	Fox consumes food that is provided
DRINKING	Fox consumes water provided in a dish/bowl
URINATING	Fox expels urine onto the ground or in litter box
DEFECATING	Fox expels feces onto the ground or in litter box
IN BOX / "DEN"	Fox is within hiding area and visual observation is lost
FIGHTING	Two or more foxes engage in physical contact with increased aggressive vocalizations (mainly during feeding)
DIGGING / HOARDING	Fox utilizes front paws to pull dirt back in order to bury/hoard/cache new food or find previously buried/ hoarded/cached food
GROOMING	Fox utilizes its tongue in a licking fashion over its own or siblings fur to clean itself or siblings
PANTING	Fox stands/walks while mouth is open, tongue is hang- ing out of its mouth, and it is breathing heavily
STRETCHING	Fox extends/pushes any body part against an object or the ground to extend its muscles for over one second (usually when waking up)

the hours of 12:30 and 17:30 to allow concentration on crepuscular and nocturnal activities. In total, observational hours covered 19 out of a 24-hour day.

Equipment

Equipment was provided by the Thermoregulation Research group (Animal Biosciences Department, University of Guelph). Thermal imaging was carried out using a FLIR E60 Infrared Camera with MSX, using a standard 25° lens with a 15° tele lens add-on (FLIR Systems, Danderyd, Sweden). The tele lens add-on allowed a larger image to be recorded. Temperature and relative humidity were recorded using a Kestrel 4000 recording device (Nielsen-Kellerman, Boothwyn, PA). Wind speed was documented with a Sims DIC-3 Anemometer (Simerl Instruments, Annapolis, MD). Due to the study location being surrounded by numerous trees, wind speed was negligible and was not shown to affect behavior. Meteorological recordings were taken every 15 minutes and recorded on a pre-printed data sheet for each day of observations.

Results

Behaviors were observed on the nights of 15 August 2014 and 16 August 2014 with temperatures ranging from 10-15°C. Surprisingly, the number of foxes that were out of the den and visible to the observers at this time and at these temperatures was very limited. An average of one fox was out at any given time between the hours of 00:00 and 04:00. This fox was considered to possibly be the more dominant one in the group, but the sex and whether it was the same fox coming out of the "den" could not be determined. Even when one fox decided to emerge from the "den," the amount of movement was limited. For a nocturnal/crepuscular species, behavioral activity was minimal during this timeframe and it was not until about 06:00 that more foxes started to stay out of the "den," but they still exhibited minimal activity and were seen just walking around or lying down (sunrise

times: 06:28-06:43, sunset times: 20:30-20:08).

Behavior of the red foxes was also observed on 2 separate mornings, 4 days apart from one another (18 August 2014 and 22 August 2014). The temperature did not vary much between the two days and only differed by a total of 3°C (19°C to 22°C). Mornings were classified as starting when the sun rose and observations began as early as 06:00. The behaviors observed were more exploratory than those at night and more foxes were out of their den. The morning behaviors included a larger number of foxes walking, sniffing, playing and grooming for a longer period of time than those observed at night. Also, foxes who were just leaving their den or "waking up" tended to move towards previously made food hoards in order to consume anything that remained in the hoard.

There was not a time within the morning hours where all seven foxes were out of their den. The consistent majority of 4 to 5 foxes staying out of their den occurred around 09:30. Even though more active behaviors were observed, they occurred towards the latter end of a crepuscular lifestyle and the beginning of a diurnal lifestyle. A crepuscular lifestyle is normally observed between the hours of 06:00-10:00 whereas these foxes were more active between the hours of 09:00-11:00. This is opposed to the crepuscular behavioral activity more frequently observed in the wild in this species.⁹

The time frame from 17:30 to 23:45 was the most observed and occurred over the course of 3 separate days (12 August 2014, 23 August 2014, and 26 August 2014). Unlike the overnight and morning observations, the evening to night observations varied the most in temperature. This allowed the observers to notice potentially thermally related behaviors in relation to an increase in temperature. This increase in temperature, and in this case a decrease in relative humidity, was associated with a large decrease in activity for the red foxes. The maximum temperature observed was 29.3°C with a relative humidity of 71.7%. As the night progressed and ambient air temperatures decreased, the observed activity was seen to increase.

For analysis of behaviors and to investigate trends where present, each observed behavior (Table 1) was put into separate figures (Figs. 2 & 3). Each figure consists of every hour of observation along with minutes spanning the entire observational period. Behaviors were then displayed using 3-D graphs using the addon for Microsoft Excel, ThreeDify Excel Grapher[®] (Figs. 4 &

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FIGURE 2. Overall visualization of the behaviors observed on 23 August 2014 from 18:00 to 19:00. Limited number of foxes lying down compared to those performing other activities (ex. walking, playing/interacting).

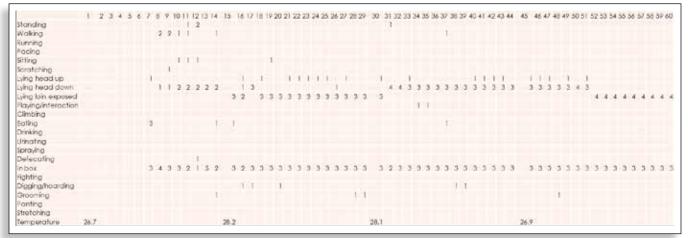


FIGURE 3. Overall visualization of the behaviors observed on 26 August 2014 from 18:00 to 19:00. Majority of foxes either stayed in their den, were lying with their head down, or were lying with their loins exposed in order to thermoregulate more effectively in hotter temperatures.

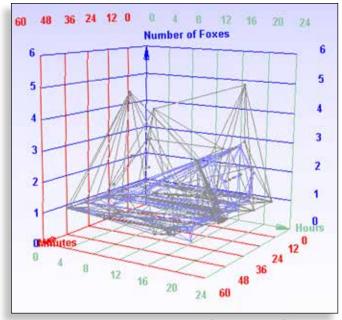
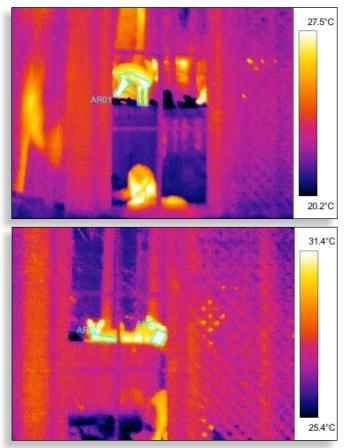


FIGURE 4. Comparison between the frequencies of eating to that of hoarding/digging. Eating is represented in black and has higher peaks. Hoarding/digging is represented in blue and has lower peaks.



FIGURES 6A & 6B. Fig 6a (top) shows a fox on the top at 18:11 on 23 August 2014 with heat being released in the area labeled AR01 (indicated by the red circle) at a temperature of 28.0°C. Fig 6b (bottom) shows two foxes at 18:11 on 26 August 2014 with heat being released in the areas labeled AR01 & AR02 (indicated by the two red circles) at a temperature of 33.0°C & 32.7°C respectively.



FIGURE 5. Rotated version of Fig. 4. Shows two distinct peaks in eating (~11:00 & ~20:00). Hoarding/digging behavior also increased more prevalently at ~20:00. Indicates potential patterns in hoarding/digging behavior.



FIGURE 7A. The maximum temperature within the highlighted area was 29.9°C with an ambient air temperature of 20.3°C. The fox in front laid on its side, increasing heat loss from its ventral surface, but covered its nose for preferential thermoregulation.



FIGURE 7B. The maximum temperature within the highlighted area was 14.8°C with an ambient temperature of 12.1°C. The fox was initially lying down but moved due to a sudden noise. This movement revealed an area underneath the fox of increased temperature compared to the surrounding plank of wood.

5). Figure 4 illustrates the trend of foxes who were in their box at a given time. In each of the graphs, the X, Y, and Z axes were labeled in the same manner but with different ranges. The X axes was labeled "Minutes" and is represented in red, which is shown on the left and bottom gridlines. The Y axis was labeled "Hours" and is represented in green, this is shown on the right and bottom gridlines. The Z axis was labeled "Number of Foxes" and is represented in blue, this is observed in the vertical and is on both the on the left and right gridlines. This 3-D comparison of data allowed for an effective visual investigation of these behaviors.

Thermoregulatory data was compiled using the FLIR E60 Infrared Camera images and shown in Figs. 6a, 6b, 7a, and 7b. These images were taken to capture the behaviors the foxes exhibited (running, playing, sitting, etc.) over the two week observation period. Analysis of the thermal images was done using the software ThermaCam Researcher Pro 2.8 SR-1. These analyses allowed each picture of interest to be examined by pinpointing certain areas within the image and determining specific surface temperature readings. These readings could then be compared and used to assist in determining the possible reasons behind certain thermoregulatory behaviors of the red fox. The lighter the color (white) recorded within the image correlated with a hotter temperature as indicated in the vertical bar on the right hand side of each thermal images (Fig. 6a). Colors ranged from white (hottest) to black (coldest). Figure 6a shows that within the area labeled AR01 (located on the lower half of the fox on the top) there was a maximum temperature reading of 28.0°C. In Fig. 6b, AR01 and AR02 show a maximum temperature readings of 33.0°C and 32.7°C respectively. Both of these pictures were taken at 18:19 with an ambient air temperature ranging from 22.2°C to 28.2°C between these two days. In Fig. 7a, the maximum temperature within the highlighted area (AR01) was 29.9°C. This picture was taken at 23:24 with an outside ambient air temperature of 20.3°C. The fox in front lay on its side allowing increased heat loss from its ventral surface, but covered its nose for preferential thermoregulation. In Fig. 7b the maximum temperature was only 14.8°C. This picture was taken later in the night at 3:09 with an ambient air temperature of only 12.1°C.

Discussion

Red foxes will begin to leave the den with their mother at 2 months of age and over the span of 4-5 months they will begin to learn how to hunt and survive on their own.⁸ Even though the young are more often observed in the daytime with their mother, at 6 months of age they would be transitioning towards a more natural/adult nocturnal and crepuscular lifestyle in the presence of humans.⁸

Decreased activity outside their den (running, playing, grooming, etc.) could be the result of their shorter summer pelage and the lower environmental temperatures, resulting in a need to stay inside to maintain effective thermoregulation. The winter coat in red foxes is not fully grown until mid-December and so an abnormally colder night in the summer could play a part towards a varied thermoregulatory behavior.⁵ However, there were fox kits that were out of the den for extended periods of time and they may have had the same thermoregulatory capabilities as those that remained in the den. It could be that without having a mother to take care of them outside their den that they naturally resorted to what feels more safe and comfortable. Or would removing the den result in a faster gain of individual confidence outside it and in the wild by providing less protection by a den?

The trend towards diurnal activity could be associated with the start of human activity on the rehabilitation center property. Even though the foxes pre-release cage was positioned off to one side, it was still near other cages that housed a variety of animal species. When the personnel arrived to take care of these other cages, the lattice surrounding the foxes pre-release cage did diminish the visual stimulation to some extent, but auditory stimulation was still noticeable to the foxes. Their interest in what was going on outside of their enclosure may have deterred them from natural behaviors and acted as a potentially negative factor inducing possible habituation to the activity of humans. The other main possible reason for lack of activity outside the den before a certain time in the morning could be associated with the feeding schedule. When an animal is fed at a certain time every day over the course of two months, they may adapt and 'learn' when to and when not to be active. The only foxes that might leave the den earlier, could be the animal(s) that had hoarded their food from the night or morning before. This could allow these animals to start their day earlier than those who had no food to eat until they were fed. The consistency of feeding schedule and presence of humans at specific times could allow for an uncharacteristic shift in behavioral patterns and, in this case, could lead to a more diurnal lifestyle than the natural crepuscular/nocturnal one reported in the wild.

Figure 2 gives an overall visualization of the behaviors observed on the cooler evening of 23 August 2014 between 18:00 and 19:00. As it is shown, there is a fairly large range of behaviors being performed over the course of this hour. It is interesting to note the number of foxes who were lying down compared to those performing other activities (running, playing, walking). No foxes were lying down with their loins exposed and very few were lying down with their head up or down. Even though there were always some foxes who spent time inside their den, those that were outside seemed to be moving about more often by running or even playing/interacting with one another or a toy. This may be associated with ambient air temperature. On this day (23 August 2014) the ambient air temperature varied very slightly (20°C-23.8°C) and the foxes were observed to exhibit a multitude of natural behaviors (walking, playing, running). There was no continuous or highly repetitive drinking or panting, and an increase in activity cannot be attributed towards feeding time since the animals were fed every night around 20:00.

Figure 3 illustrates behaviors when the ambient temperatures increased (29.3°C). On 26 August 2014, temperatures between 18:00 and 19:00 did not change much (26.7°C-29.3°C), but were 4 to 6°C hotter than on 23 August 2014 (Fig. 2). By choosing the same time frame of one hour, it was possible to suggest the effects

of increased temperature on a red fox within an enclosed area with 6 others of its species who may also have been trying to cool down. In the wild, if there is an increase in temperature, the red fox can move to another area in order to find shade, shelter, or a body of water to allow the foxes to cool down. In a small area/enclosure, those natural problem solving skills would not have been able to be applied. This hotter day (26 August 2014; 26.7°C-29.3°C) showed that in an enclosed area with little space for the foxes to move (even though the pre-release area was surrounded by trees that provided ample shade) may have resulted in more foxes reducing activity and simply lying down. The majority of foxes either stayed inside their den, or when they were outside their den they were either lying with their head down (dorsal surface exposed), or were lying with their loins exposed (allowing the ventral portion of the body to be exposed). This loin exposure behavior and reduction in observed activities leads to questions on how it may be possible to improve the fox's conditions immediately. Will housing the pre-release foxes inside a relatively small pre-release cage for two months and allowing them to perform potentially uncharacteristic behaviors cause problems for the foxes when they are released? What shift in temperature causes them to change their behavior so drastically?

Behaviors were easily compared by using the 3D graph software. In Fig. 4, a comparison between the frequencies of eating and of hoarding/digging behaviors were shown. Eating is represented in black and has higher peaks. Hoarding/digging is represented in blue and is not as frequent as eating. The different colors was used to see if, when food was scarce (since the foxes were only fed twice a day), hoarding behavior increased. Even when fed, not all of the foxes would emerge from their dens (up to a maximum of 5) which may have been associated with an already established hierarchy among this group of young foxes within the enclosure.

When Fig. 4 is rotated, it clearly shows the peaks in relation to time (Fig. 5). This clearly shows some interesting features of the data and results in an understanding of food choice throughout the day. In Fig. 5, it is seen that there are two points at which eating increases and that is in relation to when the foxes were fed. One time, close to 11:00, with the second time close to 20:00. In this context it is interesting to note the times at which hoarding/digging behavior increased. In Fig. 5 it is seen that hoarding/digging behavior increased most when the red foxes were fed at night (19:30). The type of food being fed may have been associated with the increase in hoarding/digging behavior. In the mornings, the foxes were fed fresh fruit and vegetables and at night they were fed different kinds of meat.

With respect to food hoarding, it has been suggested that some mammals may tend towards the preservation of foods that are less perishable.¹⁶ However, this research done on the relationship between perishability and food hoarding was carried out mainly with squirrels rather than with foxes and so it is of particular interest to see similar results in the present study with red foxes. When the red foxes were fed fresh fruits and vegetables, they did not save any pieces for later consumption. However, as stated earlier, red foxes are considered to be opportunistic carnivores.^{5,8,10} If given the opportunity, they will eat meat rather than fruits and vegetables. Although there are no records of juvenile red foxes hoarding in the wild, the necessity for this behavior shows that the foxes would have to learn hoarding behavior before they leave their parents. In the present study, if the foxes hoarded what they were fed in the morning then it would have degraded before they had the chance to retrieve it later in the day from the cache/hoard. But, at night when they were fed meat, they still consumed some of the meat, but also saved some for later by burying it. Foxes may hoard food for a range of time (1-2 days to 1-2 weeks)14 and so it is particularly interesting to observe the gradual increase from morning to evening with respect to hoarding/digging indicating the relationship between what they consumed during the previous night and what they dug up the following morning. This shows that fruits and vegetables are not their preferred food of choice. These observations may lead to the idea of whether or not the feeding of a large amount of fruits and vegetables is necessary. Or, should the amount of fruits and vegetables being fed be decreased?

After investigating all of the behaviors observed, it became evident that at certain times or at certain temperatures the foxes showed behavioral preferences. The effect of thermoregulation on determining some of these behaviors should also be considered. Figures 6a & 6b are two separate thermal images taken at exactly the same time on two separate days. The days in question are those previously mentioned in which the environmental temperature differed by up to 6°C and were associated with in a drastic change in observed fox behaviors. In both images the ThermaCam program was used to better describe the thermoregulatory areas of a red fox. The main reason for this comparison was to show the difference in the amount of heat released from a red fox under hotter ambient air temperature conditions. This comparison shows that as the ambient air temperature increases, the need to increase heat loss from the body is evident. Not all wildlife rehabilitators have the means or funds to build species-specific behaviorally enhanced enclosures, but in situations where ambient air temperatures are too high, heat stress may become a problem for young foxes. The upper critical temperature for a red fox is between 30°C and 32°C and if a cage does not provide an appropriate range of temperatures then heat stress may occur.¹

In Figs. 7a & 7b, areas of high thermoregulation were more protected than shown previously. In Fig. 7b, initially the fox was laying down with its head down, but as the observer approached to try and get a better thermal image, the fox stood up. This revealed an area underneath the fox of increased temperature compared to the surrounding plank of wood. Even if a red fox is protecting itself from heat loss, it will still lose heat due to conduction if the substrate is colder than the fox and, at colder temperatures, this could be detrimental. In younger foxes with a short summer pelage, this may limit how much they can be outside the den in cooler temperatures, however it should also be considered that there may be more factors at play than ambient temperature or thermoregulation. Kits could potentially feel safer in their den, or prefer the social interaction of their siblings rather than the possibility of them alone, allowing for a risk of predation. Or, they could prefer not to be watched by their human caretakers. One solution to this problem would be to implement an enclosure design that

reduces heat loss in cold temperatures by providing a heated area for voluntarily use, and provides an area in which visual stimulation from caretakers is reduced.

The majority of questions can be answered with further research, but a major problem among wildlife rehabilitation centers is the lack of consistency. Each center is different in the amount of money it can spend, the amount of volunteers or help that it is available, and even the amount of land that is available for rehabilitation. But taking all of this into account, it should be possible to create an enclosure that could provide an improved proper environment for red foxes, or any kind of canid, to support rehabilitation and rerelease into the wild.

Management Implications

The next step in this research should be to repeat this study to collect a larger data set and create an enclosure to enhance

behavior and improve the welfare of red foxes in a rehabilitation center. This could be used in a wide range of situations and used by anyone who has direct contact with red foxes or other canid species. All of the data collection and analysis in this current study leads to multiple questions: When the environmental temperature is cold, why are some young foxes outside while the majority are within the shelter? What shifts in ambient temperature were associated with the largest changes in behavior? Will foxes that grow up without maternal care know how to hunt sooner or hoard their food better than if they had a mother? Are feeding routines beneficial, or do they remove the unpredictability of surviving in the wild? Should meals be provided in such a way as to require older kits to work to find food that has been hidden, in preparation for release, after which food will no longer arrive predictably or in bowls? Will changing what is fed increase natural hoarding behaviors? How can natural behaviors be maximized in a rehabilitation center? Is there a way to encourage some behaviors and decrease others? These questions can very effectively lead to suggestions for further improving rehabilitative care of young foxes. Future research in this field is important and the more that is known about these wonderful animals, the red foxes, the more they can be helped and taken care of when help is needed.

Conclusion

Red foxes are a wonderfully talented and behaviorally interesting species to observe. Some behaviors documented in this research

were directly related to thermoregulation and show not only that more research is needed, but adjustments need to be made to enclosures to maximize natural behavior and promote welfare of this species in a wildlife rehabilitation setting.



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Causes of stranding and mortality, and final disposition of loggerhead sea turtles (*Caretta caretta*) admitted to a wildlife rehabilitation center in Gran Canaria Island, Spain (1998-2014): A long-term retrospective study

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A loggerhead turtle rescue by Florida Fish and Wildlife in Tallahassee, Florida, USA.

Introduction

Two families and seven species of sea turtles are currently recognized ¹, all of which are included in the Red List of the World Conservation Union.² The family *Cheloniidae* includes the green turtle (*Chelonia mydas*), loggerhead (*Caretta caretta*), hawksbill (*Eretmochelys imbricata*), Kemp's ridley (*Lepidochelys kempi*), olive ridley (*Lepidochelys olivacea*), and flatback turtle (*Natator depressus*). The family Dermochelyidae includes only the leatherback (*Dermochelys coriacea*)¹. The most common species around the Canary Islands is the loggerhead turtle, mainly coming from the US western Atlantic by the Gulf Stream.³

There are reports of disease surveys of free-living sea turtles in Australia,^{4–9} Hawaii,^{10–16} Florida,^{17–26} Brazil,²⁷ France,²⁸ Italy,²⁹ and the Canary Islands.³⁰ However, long-term epidemiological studies of sea turtle diseases covering more than one decade are scarce,^{14–16,21,29} and only rarely the survival rates have been thoroughly analyzed.¹⁴

The aims of this study were to analyze the causes of stranding in a large population of

ABSTRACT

Aims: The aims of this study were to analyze causes of stranding of 1,860 loggerhead turtles (*Caretta caretta*) admitted at the Tafira Wildlife Rehabilitation Center in Gran Canaria Island, Spain from 1998 to 2014, and to analyze outcomes of the rehabilitation process to allow auditing of its quality. **Methods:** Primary causes of morbidity were classified into seven categories. Final dispositions were calculated as euthanasia (Er), unassisted mortality (Mr), and release (Rr) rates. Time to death (Td) for euthanized and dead turtles, and length of stay for released (Tr) turtles were evaluated.

Results: The most frequent causes of morbidity were entanglement in fishing gear and/ or plastics (50.81%), unknown/undetermined (20.37%), and ingestion of hooks (11.88%). The final disposition of the 1,634 loggerhead turtles admitted alive were: Er = 3.37%, Mr = 10.34%, and Rr = 86.29%. Er was higher in the trauma category (18.67%) than in other causes of admission. The highest Mr was for turtles admitted due to trauma (30.67%). The highest Rr was in crude oil (93.87%) and entanglement (92.38%) categories. **Conclusions:** This survey, the first large-scale epidemiological study on causes of stranding and mortality of Eastern Atlantic loggerheads, demonstrates that at least 71.72% of strandings have anthropogenic causes. The high Rr emphasizes the importance of marine rehabilitation centers in conservation. The stratified analysis by causes of admission of final disposition rates and parameters Td and Tr should be included in the outcome research of the rehabilitation process of sea turtles to allow comparative studies between marine rehabilitation centers around the world.

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Reprint: *PLoS One*. 2016 Feb 22;11(2): e0149398. doi: 10.1371/journal.pone. 0149398. eCollection 2016. loggerhead turtles admitted to the Tafira Wildlife Rehabilitation Center (TWRC) in Gran Canaria Island, Spain, from 1998 to 2014 using specific epidemiological data, to compare these results with those obtained in other geographic regions, and to analyze the outcomes of the rehabilitation process to allow meaningful auditing of its quality.

Methods

Ethics Statement

Sea turtle rehabilitation program at the TWRC was conducted with authorization of the Wildlife Department of the Canary Islands Government (Ms. Guacimara Medina), and the Environment Department of the Cabildo de Gran Canaria (Ms. María del Mar Arévalo). Animal work and all sampling procedures were specifically approved by the TWRC Animal Care Committee and the insular government Cabildo de Gran Canaria, and were consistent with standard vertebrate protocols and veterinary practices. Loggerhead turtles that had to be euthanized for animal welfare reasons were administered barbiturates by intravenous injection.

Animals and Study Area

A retrospective study was performed using the original medical records of 1,860 loggerhead turtles admitted to the TWRC, Gran Canaria Island, Spain, from 1998 to 2014. The TWRC receives turtles stranded in Gran Canaria and eventually from other islands of the Canary Islands archipelago. Gran Canaria (27°73'-28°18'N and 15°35'-15°83'W) is the third largest island (1,560.1 km²) of the Canary Islands archipelago and has a coastline of 236 km.

Variables Analyzed

Straight carapace length (SCL), weight, stranding point, date and primary cause of admission, and final disposition including date of death or release were recorded. Sex was only determined in 248 turtles by gonadal examination at necropsy. No complete records of SCL and weight were found for 565 turtles. Turtles were categorized as pelagic juveniles (SCL <42 cm), juveniles-subadults (42 cm, SCL <70 cm) and adults (SCL 270 cm) according to previous studies.^{31–33} In order to study the seasonality of the different causes of admission, the year was divided into four seasons: spring (from March to May), summer (June to August), fall (September to November) and winter (December to February).

We defined the primary cause of morbidity as the main condition responsible for the turtle's need for treatment.³⁰ When several causes were observed in the same turtle, clinical history and complementary studies were crucial to determine the primary cause of morbidity, and only this primary cause was recorded. Primary causes of morbidity were classified into seven categories: entanglement in derelict fishing gear and/or plastics (synthetic raffia), ingestion of hooks and monofilament lines, trauma (boat strikes), infectious disease, crude oil, other causes, and unknown/ undetermined. The infectious disease category was applied when a pathogenic microorganism was confirmed by microbiological or histopathological diagnosis. Other causes were subdivided into: ingestion of plastics, buoyancy disorders, shark attack, malnutrition, and miscellany. The malnutrition category comprised turtles suffering from cachexia in absence of other lesions.

To assign these categories we used different sources: (a) the physical examination performed by the veterinarian at the admission instance; (b) the information from the people that recovered the turtle; (c) the case history; and when possible (d) information from complementary studies such as radiology, hematology, blood chemistry, cytology, microbiology, parasitology, gross pathology, histopathology, and toxicology.

Three categories were established for studying the final disposition of the loggerheads admitted alive: euthanized turtles (based on poor quality of life and/or prognosis for survival in the wild), dead turtles during the hospitalization period, and released turtles into the wild. Thus, three percentage rates were calculated for the total of loggerheads admitted alive: euthanasia rate (Er), unassisted mortality rate (Mr), and release (survival) rate (Rr). In addition, these percentage rates were also calculated for each cause of admission.

The parameters time until death (Td; difference between the date of admission and the date of the death) for euthanized and dead turtles during the hospitalization period, and length of stay in the center for released turtles (Tr; difference between the date of admission and the release date) were also evaluated for each cause of admission. Percentiles 10 (P10), 50 (P50), 75 (P75) and 90 (P90) for the variables Td and Tr were also calculated.

Statistical Analysis

Statistical analyses were conducted using SPSS v.22.0[®] (SPSS Inc., Chicago IL) and R package v.3.1.0[®] (R Development Core Team 2014, Viena, Austria). Chi-square test (χ 2) or Fisher exact tests were used to determine whether there was a significant difference between proportions. Odds Ratio (OR) measure of association was employed for disease comparisons. In order to study differences among years, trend analyses were applied for specific causes.

Results

Descriptive analyses

A total of 1,860 loggerhead turtles were included in this study. Most turtles (87.85%, n = 1,634) were alive when admitted. In the group of turtles whose SCL and weight were measured, the mean \pm SD of the SCL and weight were 36.11 \pm 11.18 cm (range, 13.00–85.20 cm) and 9.33 \pm 8.21 kg (range, 0.27–55.5 kg), respectively. Thus, 69.34% (*n* = 898) of these turtles were classified as pelagic juveniles, 30.27% (*n* = 392) as juveniles-subadults, and only 0.39% (n = 5) as adults. 86.66% of turtles (*n* = 1,612) were classified as undetermined gender, 11.66% (*n* = 217) were sexed as females and 1.66% (*n* = 31) as males. In the group of turtles whose sex was determined the sex ratio was female-biased (7:1).

Distribution of causes of morbidity

Number of cases and frequency distribution by causes of admis-

TABLE 1. NUMBER OF CASES AND FREQUENCY DISTRIBUTION BY CAUSES OF ADMISSION IN LOGGERHEAD TURTLES WITH DIFFERENT STRAIGHT CARAPACE LENGTH (SCL) STRANDED DURING THE PERIOD 1998–2014.

CAUSE OF ADMISSION		NUMBER	R OF CASES			
	SCL < 42cm	SCL 2 42–70cm	SCL 2 70cm	Unknown SCL	ΤΟΤΑ	L (%)
Entanglement	560	131	0	254	945	50.8
Hooks/monofilament lines	28	141	1	51	221	11.9
Trauma (boat strike)	35	35	1	26	97	5.2
Infectious disease	70	13	0	20	103	5.5
Crude oil	31	4	0	17	52	2.8
Other causes	37	10	1	15	63	3.4
Ingestion of plastics	7	4	0	8	19	1.0
Buoyancy disorder	3	3	0	0	6	0.3
Shark attack	3	1	0	1	5	0.3
Malnutrition	17	0	0	7	24	1.3
Miscellany	7	2	0	0	9	0.5
Unknown/undetermined	137	58	2	182	379	20.4
TOTAL	898	392	5	565	1,860	100.0

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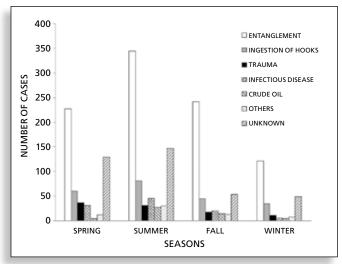


FIG 1. Seasonal variations in causes of admission during the period 1998–2014.

sion are shown in <u>Table 1</u>. The most frequent causes of morbidity were entanglement in fishing gear and/or plastics (50.81%, n = 945), unknown/undetermined (20.37%, n = 379), and ingestion of hooks and monofilament lines (11.88%, n = 221). The other primary causes had frequencies below 6%.

No differences between genders related to any of the analyzed causes were observed. Pelagic juvenile turtles (SCL < 42 cm) had a significant higher risk of entanglement (OR = 3.3; 95%CI: 2.6–4.3; P <0.0001), infectious disease (OR = 2.4; 95% CI: 1.3–4.5; P = 0.02) and crude oil (OR = 3.5; 95%CI: 1.2–10; P = 0.012) compared to juvenile-subadult and adult turtles. Conversely, the 242–70 cm SCL group had a significant higher risk of ingestion of hooks and monofilament lines (OR = 16.9; 95%CI: 1.1–25-8; P <0.0001) and trauma (OR = 2.3; 95%CI: 1.4–3.8; P <0.0001)

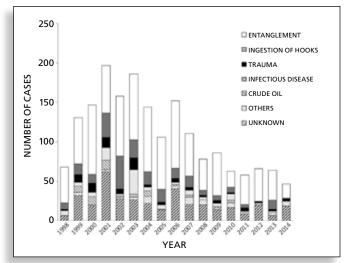


FIG 2. Annual variation in causes of admission of loggerhead turtles during the period 1998–2014.

compared to the other groups.

Seasonality

Admissions were distributed as follows: 38.33% (n = 713) in summer, 27.20% (n = 506) in spring, 21.77% (n = 405) in fall, and 12.69% (n = 236) in winter. Seasonal variation in causes of admission is shown in Fig 1. Entanglement occurred quite frequently during all seasons (spring, 24.27%, n = 227; summer, 36.79%, n = 344; fall, 25.88%, n = 242; winter, 13.04%, n = 122), but it was significantly more prevalent in summer and fall (χ 2 = 105.91, P < 0.0001). A significantly higher number of crude oil cases were observed in summer and fall (χ 2 = 27.23, P < 0.0001). Ingestion of hooks, trauma, infectious disease, and unknown causes were more prevalent in spring and summer as compared to fall and

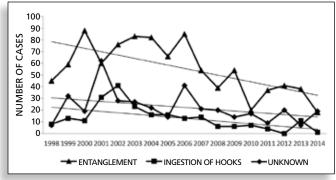


FIG 3. Tendency of the three most frequent causes of admissions during the period 1998–2014.

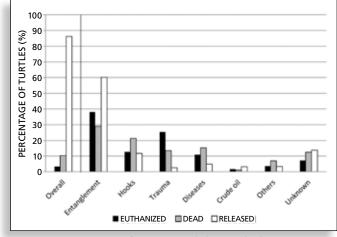


FIG 4. Resolution rates of euthanized (Er), dead (Mr), and released (Rr) loggerhead turtles relative to the overall population and the main cause of admission. doi:10.1371/journal.pone.0149398.g004

winter ($\chi 2 = 21.73$, P < 0.0001; $\chi 2 = 17.43$, P = 0.001; $\chi 2 = 33.42$, P < 0.0001; $\chi 2 = 81.89$, P < 0.0001; respectively). We detected significant differences between seasons when other causes category was analyzed ($\chi 2 = 18.07$, P = 0.0001), being more prevalent in summer (47.61%, n = 30).

Annual variation of causes of morbidity

The total number of admissions peaked during 2001–2003 and since then decreased. Annual variation in causes of admission is shown in Fig 2. A significant decrease of cases was detected among the seventeen years of the study in all the stranding categories (Fig 3). Particularly interesting is the annual variation of cases of crude oil: 84.61% (n = 44) of crude oil admissions occurred during 1998–2005, detecting an important decrease since 2006.

Final disposition

A total of 226 loggerhead turtles were dead when admitted. In these turtles, the most frequent causes of mortality were unknown/ undetermined causes (70.35%, n = 159), entanglement (11.50%, n = 26), trauma (9.37%, n = 22), and ingestion of hooks (6.19%, n = 14).

The final disposition of the 1,634 loggerhead turtles admitted alive showed the following rates: Er = 3.37% (n = 55), Mr = 10.34% (n = 169), Rr = 86.29% (n = 1,410). The final dispositions

by causes of admission are shown in <u>Table 2</u>.

The euthanasia rate was significantly higher in the trauma category (18.67%) compared to the other main causes of admission (Fig 4). Turtles admitted due to trauma and infectious diseases had the highest unassisted mortality rates, 30.67% and 25.49%, respectively. The release rate was significantly higher in the crude oil (93.87%) and entanglement (92.38%) categories compared to the cause with the lowest release rate (trauma, 50.67%). In the subgroup of turtles with SCL known, only a significant difference was detected when release rates of pelagic juveniles and juvenile-subadult turtles for the unknown/undetermined category were compared (P = 0.021).

Time until death and length of stay at the TWRC

Within the group of euthanized turtles the longest median Td was observed for the unknown category (Td = 85 days), whereas the shortest median Td was recorded for the trauma category (Td = 1 day) (Table 3). The median Td in the dead turtles ranged from 1.5 days (crude oil) to 8 days (entanglement, trauma, and other causes). Within the group of released turtles the median time of stay in the TWRC ranged from 12 days (unknown) to 70 days (trauma).

Discussion

Health status and anthropogenic threats of free-living sea turtle species are usually evaluated using as an important source of information the epidemiological studies of the causes of morbidity and mortality of turtles admitted to wildlife rehabilitation centers.^{9,14,21,26} However, studies of the causes of morbidity of mortality of sea turtles covering more than one decade are scarce.^{14–16,21,29} The present retrospective study included data of a long period (17 years), allowing a more accurate analysis of the annual variations and trends of the different causes of admission.

In our survey, the mean SCL of the stranded loggerheads was similar to that described for loggerheads from Madeira.³⁴ However, loggerheads from Azores have been reported to be smaller than those from Madeira,probably because they arrive first to Azores.³⁵ We found that the sex ratio in the group of turtles whose sex was determined was strongly female-biased (7/1). A 3.9/1 female-biased sex ratio was reported in a study conducted on 89 loggerheads stranded in Florida.²⁶ Loggerheads in the Canary Islands mainly come from the US western Atlantic by the Gulf Stream.³ It has been described that three subpopulations (NE Florida, SE Florida, and Yucatán) produce jointly an estimated 75% female hatchlings.³⁵ However, laparoscopies as well as hormonal essays performed on loggerheads in Madeira indicated a female-biased sex ratio of 2/1.³⁵

In our study, entanglement in fishing gear and/or plastics (synthetic raffia) was the main cause of admission (50.81%). Although entanglement has been reported as a common cause of morbidity and mortality in sea turtles,^{14,29,36,37} the prevalence of entanglement in the present study was higher than that reported in other surveys. Whereas the prevalence of entanglement in a

CAUSE OF ADMISSION	NUMBER OF TURTLES	FINAL DISP	OSITION				
		Euthanize	d	Dead		Released	
		Number	Er (%)	Number	Mr (%)	Number	Rr (%)
Entanglement	919	212	.28	49	5.33	849	92.38
Hooks/monofilament lines	207	7	3.38	36	17.39	164	79.22
Trauma (boat strike)	75	14	18.67	23	30.67	38	50.67
Infectious disease	102	6	5.88	26	25.49	70	68.62
Crude oil	49	1	2.04	2	4.08	46	93.87
Other causes	62	2	3.22	12	19.35	48	77.41
Ingestion of plastics	18	0	0	2	11.11	16	88.89
Buoyancy disorders	6	0	0	1	16.67	5	83.33
Shark attack	5	0	0	1	20	4	80
Malnutrition	24	0	0	8	33.33	16	66.67
Miscellany	9	2	22.22	0	0	7	77.78
Unknown/undetermined	220	4	1.81	21	9.54	195	88.63
Total	1,634	55	3.37	169	10.34	1,410	86.29

TABLE 2. FINAL DISPOSITION OF THE LOGGERHEAD TURTLES ADMITTED ALIVE DURING THE PERIOD 1998–2014.

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TABLE 3. STATISTICAL DESCRIPTIVE OF TIME THAT LOGGERHEADS SPENT IN THE TWRC UNTIL THE FINAL DISPOSITION.

	TIME	(DAYS)	FROM	ADMISS	ION TO	FINAL C	DISPOSI	ΓΙΟΝ									
	EUTH	ANASIA	4			UNA	SSISTED	MORT	ALITY		RELEASE						
CAUSE OF ADMISSION	P ₁₀	P ₂₅	P ₅₀	P ₇₅	P ₉₀	P ₁₀	P ₂₅	P ₅₀	P ₇₅	P ₉₀	P ₁₀	P ₂₅	P ₅₀	P ₇₅	P ₉₀		
Entanglement	0	0	2	16.5	45.6	1	3	8	24	70	3	11	26	52	93		
Hooks	0	0	3	61	70	0.6	1	3	13	30	7	19	38	67	151.1		
Trauma	0	0	1	4.25	83	1	2	8	30	155.2	17.1	39.5	70	132	331.1		
Infectious disease	2	4.25	26.5	44.7	47	0	1	4	16	32.7	12	19	43	63	132		
Crude oil	N/A	N/A	N/A	N/A	N/A	1	1	1.5	2	2	2.8	9.5	16	29	52.4		
Other causes	1	1	32.5	64	64	0.6	2	8	28.5	41.8	10.7	18.5	40	70.7	127		
Ingestion of plastics	-		-	-	-	17	17	24.5	32	32	10	13	21.5	44	76.6		
Buoyancy disorder	-		-	-	-	N/A	N/A	N/A	N/A	N/A	2	8	36	92	127		
Shark attack	-		-	-	-	N/A	N/A	N/A	N/A	N/A	39	41.2	53.5	102.5	117		
Malnutrition	-		-	-	-	0	2	2.5	18.7	46	18	35.2	43	112.7	154		
Miscellany	1	1	32.5	64	64	-		-	-	-	2	14	56	118	270		
Unknown/undetermined	39	39	85	428	428	1	1	4	9.5	40	1	5	12	29	57		

P₁₀, P₂₅, P₅₀, P₇₅, P₉₀: percentiles 10, 25, 50 (median), 75 and 90; N/A: not applicable (only one case). doi:10.137 1/journal.pone.0149398.t003

retrospective study conducted on 3,861 sea turtles in Hawaii over a 22-year period was 5%,¹⁴ evidence of lesions caused by fishing gear was observed in 11.28% of the 5,938 turtles stranded in Italy during 1980–2008.²⁹ The low prevalence of entanglement in Hawaii was

also a consequence of the high prevalence of fibropapillomatosis (28%) in the region;¹⁴ however, no cases of fibropapillomatosis were reported in Italy.²⁹ Interactions with the activity of the Canarian artisanal fishery are not very common; however, potential interac-



Pacifico, foreign body ingestion, including hooks and fishing lines, was observed in 12% of the turtles.16 Estimated total take of sea turtles in the international waters in the North of the Canary Islands for 1983 to 1991 was 3,000 turtles.40 However, more recently, impacts of fishing effort have been reported to be decreasing in the Canary Current because fish stocks are depleted throughout the region and management authorities are striving to reduce the fishing pressure.⁴¹ Longline in waters off Canary Islands is deployed to catch benthopelagic to bathipelagic fish species, such as the European hake (Merluccius merluccius), the common mora (Mora moro) and even the meagre (Argyrosomus regius). All these

tions between loggerheads and fisheries can take place in waters off Canary Islands mainly when "trasmallos" (a fishing net) are used to catch a wide range of fish species, from sharks and skates to many sparids, striped red mullet *(Mullus surmuletus)*, etc., especially at the end of the summer.³⁸ Sometimes it is difficult to assess whether entanglement is the consequence of capture in fishing gear or of floating discarded fishing gear; in addition, entanglement in floating material like plastics is also an increasing problem.²⁹ In this way, it is remarkable than the use of synthetic raffia in the Canary Islands, particularly in the islands devoted to intensive agriculture, is very common. Ruminal disorders in goats due to ingestion of synthetic raffia have been reported in Gran Canaria Island.³⁹ Legal actions including the compulsory use of biodegradable raffia must be implemented in order to minimize its impact on sea turtle stranding in the Canary Islands.

Unknown/undetermined origin was the second category of stranding (20.37%) in our study. This prevalence was lower than the 49% reported for green turtle strandings in Hawaii.¹⁴ Financial and time constraints usually make difficult to establish an accurate diagnosis in many stranded sea turtles²⁹. In addition, rapid development of autolysis can impede more detailed examinations of the carcasses and the histological evaluation of the specimens.^{14,30} There are also examples in which, although huge efforts were devoted to identify the cause of stranding, the specific cause could not be satisfactorily identified.²³

Ingestion of hooks and monofilament lines was the third cause of stranding (11.88%) in our survey. This prevalence was higher than that reported in green turtles stranded in Hawaii during 1982–2003 (7%).¹⁴ However, in a study on non-fibropapilloma causes of mortality in green turtles from Hawaii and the insular species and other similar are caught with hooks, often baited.⁴² In addition, other fishing fleets work in waters off Canary Islands using large longlines to catch sword fish (*Xiphias gladius*).⁴³ Although many of the turtles are released by the fishermen, it has been estimated that approximately 15–50% of the turtles die due to the severe lesions induced by the fishing hooks.⁴⁴ Lesions induced by hooks and monofilament lines have been deeply described.^{15,16,30,45}

In our study, all the other primary causes had frequencies below 6%. As previously reported, it is remarkable the absence of fibropapillomatosis in the loggerheads stranded in the Canary Islands because sea turtle fibropapillomatosis is a disease of global distribution.³⁰ The prevalence of fibropapillomatosis in a retrospective study conducted on 3,861 stranded green turtles in Hawaii over a 22-year period was 28%¹⁴. Similarly, the prevalence of fibropapillomatosis in a retrospective survey conducted on 3,016 stranded green turtles in Florida during the period extending from 1980 to 1998 was 22.6%.²¹ However, fibropapillomatosis was not present in other Pacific islands examined by Work *et al.*,¹⁶ and no cases of fibropapillomatosis were reported in a retrospective study conducted on 5,938 stranded loggerheads in Italy during 1980–2008.²⁹

As previously reported in 2005,³⁰ no cases of spirorchiid infection were observed among loggerheads admitted to the TWRC during 1998–2014. Spirorchiid trematodes are implicated as an important cause of stranding and mortality in sea turtles worldwide.^{7,9,13,23} In a retrospective study conducted on 100 stranded green turtles in Australia over a 4-year period, spirorchiidiasis was found to be the most frequently cause of mortality (41.8%).⁹ However, although high prevalence of spirorchiid infection was observed in a survey conducted on 148 sea turtles in Florida, most infections were regarded as incidental to the cause of death.²⁶ A high percentage of green turtles with fibropapillomatosis in Hawaii are also infected with spirorchiid trematodes.^{10,13} Detection of infection with spirorchiids in turtles usually is done at necropsy, when adult worms or eggs are observed either grossly or at microscopy.¹³ None of the necropsied loggerheads included in our study had intravascular adult flukes or trematode eggs in tissues. Antemortem detection of infection is also possible using enzyme-linked immunosorbent assays.^{13,46,47} However, no antemortem diagnosis using serology was attempted on the loggerheads included in our study due to financial constraint. Work et al.13 hypothesized that immature green turtles become infected with spirorchiids shortly after recruiting into coastal foraging pastures from the pelagic environment. All necropsied loggerheads included in our survey were juvenile and subadult specimens but different alimentary habits of both species can explain the absence of spirorchiid fluke infection. Sea turtles acquire the flukes by ingesting unknown cercaria-rich intermediate hosts. Although preliminary results on detection of spirorchiids in gastropod tissues by polymerase chain detection have been reported,⁴⁸ the only life cycles that have been established in this group were for species in the freshwater genera Enterohaemototrema, Spirorchis, and Vasotrema.⁴⁹

Because the Canary Islands are included throughout the year in a thermal gradient centered on the 21°C isotherm,⁵⁰ no cases of cold-stunning were observed among loggerheads included in our study. However, hypothermic stunning events have been described affecting high number of sea turtles in Florida.^{20,24}

In our survey, seasonal analysis of the strandings showed that these were more frequent in summer, probably reflecting loggerheads are more abundant around the Canary Islands in this season. It has also been reported that loggerheads are more abundant in Madeiran waters during the summer months.³⁵ In addition, the total number of admissions included in our study peaked during 2001-2003 and since then decreased. Several studies have indicated that the abundance of loggerhead nests along the Atlantic coast and in southwestern Florida is declining.^{51,52} Because loggerheads in the Canary Islands mainly come from the US western Atlantic, decline in nest abundance on those beaches could have a negative impact on the juvenile and subadult loggerhead population around the Canary Islands. Skeletochronology of mostly loggerheads from Madeira showed the duration of the oceanic stage to be equal or longer than 7 years.⁵³ No studies on determination of the age of the loggerheads around the Canary Islands have been published. Assuming this age is similar to that reported for Madeiran loggerheads, the decrease observed in our study, particularly since 2003, could be consequence of the decrease of nests on the US western Atlantic coast since mid 90's. Estimated declines in nest abundance on the Atlantic and southwestern coasts of Florida ranged from 29% to 37% between 1989 and 2006.52

It is remarkable that admissions due to crude oil decreased significantly since 2006. In a previous study on crude oil as stranding cause among loggerheads in the Canary Islands, authors concluded that the designation of the Canary Islands as a Particularly Sensitive Sea Area (PSSA) in 2005 by the International Maritime Organization (IMO) was associated with positive effects on the reduction of sea turtle strandings caused by crude oil.⁵⁴ A PSSA is an area of the marine environment that needs special protection through action by the IMO because of its significance for recognized ecological, socio-economic, or scientific attributes where such attributes may be vulnerable to damage by international shipping activities.⁵⁵ The associated protective measures after the international recognition of the waters of the Canary Islands as PSSA were adopted in 2006, and included: traffic separation systems (recommended routes), areas to be avoided, and mandatory ship reporting system.⁵⁵

Wildlife clinical practice guidelines dealing with welfare rehabilitation standards and pre-release health screening protocols have been published,^{56,57} but no quality indicators of the rehabilitation process of injured sea turtles have been defined. In our study, we analyzed the outcomes of the rehabilitation of free-living loggerhead turtles at the TWRC, adopting the three categories of the final disposition, the time until death and the length of stay, as indicators of the quality audit of the rehabilitation process before release into the wild.

According to our data, 86.29% of loggerheads admitted alive to the TWRC were successfully released, and only 13.71% of turtle admissions resulted in euthanasia or unassisted mortality. References on the final dispositions of sea turtle rehabilitation are scarce because they usually have been focused on the causes of mortality.¹⁴

Based on animal welfare, euthanasia is a final option in all wildlife species rehabilitation.⁵⁸ In our survey, the overall rate of euthanasia was 3.37%, and the highest value was found in the trauma (boat strikes) category (18.67%). Turtles with boat strike injuries usually have severe fractures of the carapace/plastron, and severe traumatic lesions mainly penetrating into the lungs and kidneys.³⁰ Affection of these vital organs, because the anatomical location, dorsally attached to the carapace, explain the generally poor prognosis for turtles with severe traumatic injuries in the carapace.³⁰

Unassisted mortality rate has been used as a quality indicator parameter in rehabilitation of birds of prey;⁵⁹ however, no reports on quality auditing of the rehabilitation process in free-living sea turtles admitted at marine rehabilitation centers have been published. In our study, the overall rate of unassisted mortality was 10.34%, and the highest value was found in the trauma (boat strikes) category (30.67%). The reasons for the poor prognosis for these turtles have been explained above. A high Mr (25.49%) was also found in the infectious disease category. According to the necropsy findings and microbiological studies, septicemia was diag- nosed in the majority of those cases.³⁰

The release rate in our survey was higher in the crude oil (93.87%) and entanglement (92.38%) categories compared to the cause with the lowest release rate (trauma, 50.67%). Ingestion of crude oil and subsequent internal lesions can threaten sea turtle survival, but lesions in the skin, carapace, and plastron are not

fatal in the majority of cases.⁵³ Entanglement in fishing gear and/ or plastics can result in severe ulcerative dermatitis, and amputation offlippers;³⁰ however, sea turtles cope with amputations well, regardless of whether the amputation is front or rear.⁶⁰

The parameter time to death provides direct insight into the initial assessment and prognostication, the overall rehabilitation process, and the validity of veterinary protocols.⁵⁹ In our study, the shortest median time to euthanasia was recorded for the trauma category (1 day) meaning that the decision is made very soon based on the poor prognosis of these cases as was discussed above. Within the group of dead turtles during the rehabilitation process, the median time to death ranged from 1.5 days (crude oil) to 8 days (entanglement, trauma, and other causes). This fact suggests that first week of stay at the rehabilitation center is critical, and intensive cares should be performed on all turtles during the first week, despite their apparently less severe appearance.

The parameter length of stay must be as short as possible to reduce the risk of captivity-related complications, infectious diseases, and behavioral disorders.⁶¹ In our study, the median time of stay in the TWRC ranged from 12 days (unknown) to 70 days (trauma). This fact suggests that turtles admitted especially due to trauma represent an important consumption of time and efforts. In conclusion, this survey is the first large-scale epidemiological study on causes of stranding and mortality of Eastern Atlantic loggerhead sea turtles, providing useful information for the conservation of these reptiles. In absence of diseases commonly reported in other regions, such as fibropapillomatosis14,21 and spirorchiidiasis,^{7,9,13,23,26} at least 71.72% (n = 1,334) of loggerheads included in our study stranded due to anthropogenic causes, what deserves critical reflection, especially taking into account that stranded loggerhead turtles may represent as little as 7% of the at-sea mortality of sea turtles.⁶² In addition, the high survival rate for stranded loggerheads (86.29%) achieved at the TWRC emphasizes the importance of marine rehabilitation centers for the conservation of sea turtles. Finally, we propose that at least the stratified analysis by causes of admission of the three final disposition rates (Er, Mr, and Rr), and the parameters time until death (Td) and length of stay at the center (Tr) should be included in the outcome research of the rehabilitation process of free-living sea turtles in order to allow comparative studies among marine rehabilitation centers around the world.

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Conceived and designed the experiments: JO, PC. Performed the experiments: JO, NM, MC, AA, PC. Analyzed the data: JO, NM, MC, AA, PC. Contributed reagents/materials/analysis tools: JO, AA, PC. Wrote the paper: JO, NM, AA.

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Antimicrobial resistance patterns of *Enterobacteriaceae* in European wild bird species admitted in a wildlife rescue center

C Giacopello, M Foti1, A Mascetti, F Grosso, D Ricciardi, V Fisichella, and FL Piccolo. *Veterinaria Italiana*. 2016;52(2),139-44. doi:10.12834/ VetIt.327.1374.2.

Wild birds have been considered to be reservoirs of enteric human pathogens and vectors of resistance dissemination to the environment. During annual migration, they potentially play a role in the epidemiology of human associated zoonoses. The aim of this study was to investigate the frequency of isolation and antimicrobial susceptibility profiles of Enterobacteriaceae members isolated from cloacal swabs of common European wild birds. Fifty-five cloacal swabs were taken during birds' entrance evaluation in a rescue centre for injured wild birds in the Province of Messina (Sicily, Italy). All samples were examined for the presence of members of the family Enterobacteriaceae using standard methods and on the isolated strains antibiotic susceptibility testing was performed. Eighty-three Enterobacteriaceae strains were isolated from raptors, waterbirds and passerines. The bacterial species isolated were: Escherichia coli, Proteus mirabilis, Proteus vulgaris, Citrobacter freundii, Enterobacter cloacae, Klebsiella oxytoca, Salmonella Typhimurium, Escherichia vulneris, Enterobacter amnigenus biogroup 2, Salmonella Duesseldorf and Hafnia alvei. The isolates showed significant frequencies of antibiotic resistance. Multiresistance to three or more groups of antibiotics also occurred. None of them have shown a phenotypic Extended Spectrum Beta Lactamase (ESBL) profile.

Collapsing avian community on a Hawaiian island

EH Paxton, RJ Camp, P.M Gorresen, LH Crampton, DL Leonard Jr., EA VanderWerf. *Science Advances*. 2016;2(9), e1600029. doi:10.1126/sciadv.1600029.

The viability of many species has been jeopardized by numerous negative factors over the centuries, but climate change is predicted to accelerate and increase the pressure of many of these threats, leading to extinctions. The Hawaiian honeycreepers, famous for their spectacular adaptive radiation, are predicted to experience negative responses to climate change, given their susceptibility to introduced disease, the strong linkage of disease distribution to climatic conditions, and their current distribution. We document the rapid collapse of the native avifauna on the island of Kaua'i that corresponds to changes in climate and disease prevalence. Although multiple factors may be pressuring the community, we suggest that a tipping point has been crossed in which temperatures in forest habitats at high elevations have reached a threshold that facilitates the development of avian malaria and its vector throughout these species' ranges. Continued incursion of invasive weeds and non-native avian competitors may be facilitated by climate change and could also contribute to declines. If current rates of decline continue, we predict multiple extinctions in the coming decades. Kaua'i represents

an early warning for the forest bird communities on the Maui and Hawai'i islands, as well as other species around the world that are trapped within a climatic space that is rapidly disappearing.

Legal responsibilities of veterinary professionals when working with wildlife centers

E Mullineaux. *Companion Animal.* 2016;21:10,592-97. doi: http://dx.doi. org/10.12968/coan.2016.21.10.592.

Injured or orphaned animals are frequently presented by members of the general public to veterinary surgeons working in general practice in the UK. After some form of veterinary intervention and treatment, suitable cases can be rehabilitated, usually at a wildlife center, before being released back to the wild. Wild animals in the UK are included in the Veterinary Surgeons Act 1966 and the Veterinary Medicines Regulations, therefore Registered veterinary surgeons and veterinary nurses have legal as well as ethical responsibility for their care. In order to ensure full care to the point of successful release of suitable cases back to the wild, a good collaborative working relationship between veterinary professionals and wildlife rescue centers is essential.

Spatial characteristics of residential development shift large carnivore prey habits

JA Smith, Y Wang, and CC Wilmers. J Wildlife Mgmt. 80:6,1040-48. doi:10.1002/jwmg.21098.

Understanding how anthropogenic development affects food webs is essential to implementing sustainable growth measures, yet little is known about how the spatial configuration of residential development affects the foraging behavior and prey habits of top predators. We examined the influence of the spatial characteristics of residential development on prey composition in the puma (Puma concolor). We located the prey remains of kills from 32 pumas fitted with global positioning system (GPS) satellite collars to determine the housing characteristics most influencing prey size and species composition. We examined how differences in housing density, proximity, and clustering influenced puma prey size and diversity. We found that at both local (150 m) and regional (1 km) spatial scales surrounding puma kill sites, housing density (but not the clustering of housing) was the greatest contributor to puma consumption of small prey (<20kg), which primarily comprised human commensals or pets. The speciesspecific relationships between housing density and prey occupancy and detection rates assessed using camera traps were not always similar to those between housing density and proportions of diet, suggesting that pumas may exercise some diet selectivity. The influence of development on puma diet may affect puma disease risk, energetics, and demographics because of altered species interactions and prey-specific profiles of energetic gain and cost. Our results can help guide future land-use planners seeking to minimize the impacts of development on wild species interactions and community dynamics. ■

News

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release was "vague."

"It is unclear when this deer will be ready to be released, if it is not ready now, or if this deer will ever be ready to be released," the statement read.

Frati said she is keeping Jane Doe as a foster deer for fawns. A temporary restraining order allows her to keep Jane Doe past the deadline. "I don't think at this point that rehabilitators are going to be happy with putting anything to sleep that they possibly could save," said Frati, who is scheduled for a June 28 court date.

A DEC spokesman said the agency does not comment on pending litigation. PHOTO ©DAVE HIUTH, FLICKR. CC BY-NC-ND 2.0 LICENSE.



Dusky salamander (Desmognathus fuscus). While its population in the eastern US appears healthy and stable, it may be susceptible to the chytrid fungus now affecting Europe's salamander populations.

Not every injury can be healed in two days, said Eric Brown, caretaker of Outpost No. 4 in Delanson. He recalled a deer with hypothermia rescued in winter from the Mohawk River that required many more days of rehabilitation. "DEC wanted to license this activity to take wild animals out of the hands of the untrained public," Martin said. "We worked with them in partnership to develop a program that for many years set the standard across the country.

"This past year the collaboration seems to have ended," Martin said.

—Article courtesy JP Lawrence, Albany Times Union (timesunion.com)

Salamander Chytrid Fungus Testing

Vermont, USA (June 6)—The United States is home to the greatest biodiversity of salamanders in the world—in fact, at least 150 species live north of Mexico. But the population may be under threat because a deadly fungus has ravaged some salamander populations in Europe. It is a fungus that could easily travel to North America.

That has led to a kind of medical check-up for salamanders nationwide in the United States. A team of scientists with the U.S. Geological Survey, including wildlife biologist Evan Grant, are working to identify any signs that the fungus has made its way across the pond.

While thy While thy Her We're looking for evidence of salamander chytrid fungus in wild populations of the red spotted newt," Grant said. He is coordinating research across the northeastern U.S. So far, 80 sites have been visited this year, including one in Sunderland, Vermont. The fungus was first identified in Europe.

"In populations of the fire salamander where they first detected this, they have seen declines of 99 percent in some ponds. The disease has been spreading since they first detected it. There is a pretty concerted effort over there also to survey populations," Grant said.

The fungus has been detected in captive trade in salamanders for pets from Asia, which is where the fungus originated.

"We are worried about it entering the U.S. by the same mechanism," Grant said. This January, the U.S. Fish and Wildlife Service restricted the movement of over 200 species of salamanders, which should slow the movement of the fungus.

—Article courtesy Mitch Wertlieb, VPR (http://digital.vpr.net/post/testingunderway-prevent-spread-salamanderfungus#stream/0)

Toxoplasmosis Threat to Endangered Nene

Washington, DC, (June 2)—A new study published in the *Journal of Wildlife Diseases* has documented evidence of "widespread contamination of habitat" in Hawai'i caused by feral cats. This latest research has alarming implications for endangered Hawaiian Geese (Nene) and other animals found throughout the Hawaiian Islands.

The peer-reviewed study, conducted by scientists from the United States Geological Survey, the U.S. Department of Agriculture, the University of Tennessee, and the state's Division of Forestry and Wildlife, evaluated the prevalence of infection with Toxoplasma gondii among Nene, Hawai'i's state bird. T. gondii is a protozoan parasite that causes toxoplasmosis in humans and wildlife and is the "most-commonly encountered infectious disease" in Nene, the study reports. T. gondii relies on cats to complete its life cycle and is excreted into the environment through cat feces. A single cat may excrete hundreds of millions of infectious eggs (called "oocysts") in its feces.

The study found between 21 and 48 percent of Nene tested positive for past infection, depending on the island. The island of Moloka'i had the highest infection rate (48 percent), followed by 23 percent on Maui and 21 percent on Kaua'i. According to the authors, the higher rate on Moloka'i may have been due to "a conspicuously consistent presence of feral cats."

PHOTO @ NIGEL, FLICKR. CC BY-NC-ND 2.0 LICENSE.

"This research confirms earlier studies dating from the 1970s that this parasite is probably found in tropical island ecosystems wherever there are feral cats," said Dr. Thierry Work, the study's lead author. "Recent studies also suggest that animals and humans are more prone to trauma when infected with *T. gondii*. Trauma is the chief cause of death for Nene, and infections with *T. gondii* may be making them more vulnerable, but confirming that will require additional studies."

Hawaiian Geese are not the only Hawaiian wildlife to test positive for *T. gondii*. Other birds, such as the endangered Hawaiian Crow ('Alala), and mammals, such as endangered Hawaiian monk seals, are also susceptible and have died from infection. The National Oceanic and Atmospheric Administration (NOAA), in response to increasing seal deaths, elevated toxoplasmosis to a disease of "serious concern." According to the Main Hawaiian Islands Monk Seal Management Plan, NOAA is concerned both with seal deaths and "the secondary and cumulative impacts of subclinical or chronic disease."

Caspian terns in the Far, Far North

Fairbanks, AL (Sept 21)—In the late summer of 2016, a WCS field team led by Dr. Trevor Haynes monitored Caspian tern chicks through to fledging in Cape Krusenstern National Monument in Alaska. This discovery of Caspian terns breeding above the Arctic Circle in the Chukchi Sea is nearly 1,000 miles farther north than previously recorded – a strikingly large jump in the range of nesting for this (or any) species.

Rapid range expansions in the Arctic are largely being driven by climate change. The arrival of Caspian terns is just one of a suite of profound alterations to the rhythms of this environment being reported by scientists and local residents. There is now less summer sea ice, and a longer snow and ice-free season – simply put, summer conditions now last longer. Therefore, more temperate species can, where possible, opportunistically move north.

Species such as fin and humpback

the Arctic, and boreal species such as the red fox and brown bear now interact more routinely with their arctic counterparts – the arctic fox and polar bear. "The challenge for scientists is to

whales are now spending more time in

help understand the repercussions of these changes— for example, we've seen red foxes take over areas previously used by arctic foxes. We don't know what the repercussions of new colonies of Caspian terns will be on the current resident species, particularly if they gain more of a foot hold and expand their numbers," says Dr. Martin Robards. Elsewhere there have been critical concerns about predation by Caspian terns on local fish stocks, such as in the Columbia River.

Caspian terns are the largest and most majestic of all tern species and are avid full-body divers for fish. They occur on all continents except Antarctica. Unlike many other species of wildlife, Caspian tern populations appear to have increased in recent years. They were first noted in Alaska in 1981 and were thought to be breeding in Southeast Alaska by 1989. They have gone from being rare occurrences to breeding annually, but only at a handful of locations, and no farther north than Neragon Island in the southern Bering Sea, a thousand miles to the south.

"What we saw this season for Caspian terns is another example of the fragility of the Arctic system," said Peter Zahler, WCS Regional Director. "New patterns are starting to take hold in an environment that is dynamic and reinventing itself in the **Caspian tern (Hydroprogne caspia)**, after a successful dive. In the summer of 2016, terns were discovered nesting above the arctic circle, 1000 miles north of their last recorded nesting range.

context of a new warmer climate. However, the arrivals of new species are mirrored by the challenges for existing ones adapting to new conditions such as walrus and polar bear. Adaptation of wildlife and people to new conditions in the Arctic represents one of the most significant challenges for conservationists and local communities, not just in the future but right now. Longterm monitoring efforts that supported this discovery are vital as we continue to monitor changes in the Arctic and develop tools to support adaptation planning for both wildlife and people."

The discovery of Caspian terns nesting in the high Arctic was made possible through a partnership led by the National Park Service's Inventory and Monitoring Program efforts to monitor long-term change in Cape Krusenstern National Monument, and the Wildlife Conservation Society's interest in coastal conservation in the Arctic.

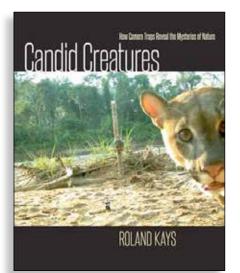
Camera Traps as Research Tool Illustrate Limited Effect of Hiking and Hunting

North Carolina, USA (June 8)—Camera traps provide a unique "animal selfie" view of nature, revealing secret lives rarely observed by humans. Over the past decade, motion-activated cameras have revolutionized the fields of wildlife science and animal conservation, as well as



elevated awareness of the animals in our own backyards.

Candid Creatures: How Camera Traps Reveal the Mysteries of Nature by biologist Roland Kays (May 2016, Johns Hopkins University Press) is the first major book to compile the remarkable images and discoveries made through this explosive new technology—including the discovery of new species as well as living proof of others thought to be extinct.



Roland Kays states "Not only are camera traps a unique way to look at critters, but the tool has transformed the way wildlife science is conducted and is having important implications for our understanding of these animals."

Drawing from archives of millions of color and night-vision photographs collected by hundreds of researchers, Kays has assembled more than 600 remarkable images that show unique perspectives of wildlife from throughout the world. He also discusses how scientists use these photos to address conservation issues and create solutions that allow humans and wild animals to coexist.

In a recent study published in the Journal of Applied Ecology, Kays and fellow researchers found that although hiking and hunting have measureable effects on the distribution of some species, these were relatively minor. Meaning that managed wild areas can be used for both recreation and conservation. The citizen science monitoring approach they developed could offer long-term monitoring protocols for protected areas, which would help managers detect where and when the balance between recreation and wildlife has tipped.

African Wildlife Rangers Need More Support

Boulder, CO (May 23)—Battling a global poaching crisis, wildlife rangers believe they lack the necessary equipment, training and support from their governments to protect themselves and the world's threatened wildlife from poachers, according to a new WWF study recently released at the World Ranger Congress in Colorado, USA.

Ranger Perceptions: Africa surveyed 570 rangers across 12 African countries and found that 82 percent had faced a life-threatening situation while on duty. But 59 percent felt they were insufficiently equipped, and 42 percent felt they lacked sufficient training to do their jobs safely and effectively.

These results echo the findings of a similar survey of Asia's rangers, the majority of whom had also risked their lives in the line of duty and felt equally ill-equipped to perform their critical frontline tasks. Preliminary results from a third survey suggest that rangers in Latin America face similar challenges.

Last year alone, around 30,000 elephants and a record 1,338 rhinos were killed in Africa, while countless other animals were poached and trafficked, feeding an illegal wildlife trade that is increasingly being driven by international organised crime. Scores of rangers are also injured or die each year, with six killed on duty in the Democratic Republic of Congo and India in the past two months.

"The growing influence of organised criminal networks means governments must rapidily professionalise their ranger force, but many seem quick to promise action but slow to provide the necessary investment," said Elisabeth McLellan, WWF Head, Wildlife Crime Initiative.

"Poaching threatens communities and economies as well as species: investing in better equipment for rangers and the establishment of new training centres is a price worth paying."

In Mongoose, Social Networks Spread Tuberculosis

Botswana (May 10)—An emerging strain of tuberculosis (TB), closely related to human TB, has been killing banded mongoose in Northern Botswana in significant numbers.

This novel pathogen, *Mycobacterium mungi*, did not infect mongoose through a primary airborne or oral route as normally seen in TB disease in humans and animals. The mechanism of transmission, however, was unknown.

A research team led by Kathleen Alexander, Associate Professor of Wildlife Conservation in Virginia Tech's College of Natural Resources and Environment, reports discovery of the pathogen's unique transmission route in the American Society for Microbiology journal *mBio*.

As with many animals, such as dogs or even hyenas, mongoose use urine and anal gland secretions to communicate with other members of their species. However, in the mongoose, secretions from sick animals were found to be infected with the TB pathogen.

These secretions, once deposited in the environment, allow the pathogen to be transmitted when other mongoose investigate and sniff the scent marks. The pathogen is also spread when an infected mongoose places its scent directly on other mongoose in its troop.

Abrasions or injuries in the skin or nose provide the portal of entry for this novel TB pathogen to invade and infect the mongoose host. Smaller social groups are most threatened by the disease, the researchers report.

"Banded mongoose are a territorial species, and individuals within a troop may have little or no direct contact with mongoose in adjacent social groups, limiting the potential for directly transmitted pathogens like TB to spread through a population," explained Alexander, an affiliate of the Fralin Life Science Institute, who discovered the novel strain of TB in 2010.

"But this TB pathogen circumvents the mongoose's natural social barriers to infectious disease transmission by hijacking social communication behavior," she said. "We keep being surprised by infectious disease-causing organisms and their ability to adapt to a particular environment, behaving, in some cases, dramatically differently than we expect."

TB is an ancient disease that continues to be one of the most critical health threats to humans, wildlife, and domestic animals globally.

"M. tuberculosis complex pathogens infect many species of domestic and wild animals as well as humans in the U.S. and across the globe," noted Alexander. "Our findings have changed the way we must think about tuberculosis and infectious disease transmission in territorial species."

Alexander, a wildlife veterinarian and co-founder of the Center for Conservation of African Resources: Animals, Communities, and Land Use (CARACAL) in Botswana, directs her research program at exploring and understanding the factors that influence the emergence and persistence of novel and re-emerging diseases at the human-wildlife-environment interface.

October CITES Meeting a Win for Turtles and Tortoises

Johannesburg (October 3)—Tortoises and freshwater turtles are the most threatened of any major group of terrestrial vertebrates - more so than mammals, birds or amphibians. They are increasingly at risk from poaching and commerce, which makes today's decision to restrict global trade in six African and Middle Eastern softshell turtle species particularly significant. Signatory nations to the Convention on the International Trade in Endangered Species of Wild Fauna and Flora (CITES), referred to as "parties," are meeting in Johannesburg, South Africa, where they adopted by consensus a proposal, cosponsored by the United States, to list these six species on Appendix II of the treaty.

"While freshwater turtles may not be the most iconic or charismatic species discussed at this CoP, they are certainly among the most heavily traded and deserving of CITES protections," said U.S. Fish and Wildlife Service Director Dan Ashe, head of the U.S. delegation to the treaty's



Banded mongoose (*Mungos mungo***)** in Botswana. Populations in the north are falling victim to a new strain of TB adapted to spread through social scent-marking.

17th Conference of the Parties (CoP17) in Johannesburg. "Today's actions bring the majority of the world's softshell turtles under the umbrella of CITES, and we're proud to work with a coalition of countries committed to reversing the trend of overexploitation that has depleted wild turtle populations."

The United States co-sponsored a proposal to list the six softshell turtle species in Appendix II, which was submitted by Burkina Faso, Chad, Gabon, Guinea, Liberia, Mauritania, Nigeria and Togo. The Appendix II listings were adopted by consensus. In 2013, the United States collaborated with international partners to adopt CITES protections for Asian freshwater turtles and in 2016 protected four of its native freshwater turtles under CITES Appendix III, bringing all North American softshell turtles under CITES protection.

Freshwater turtles and tortoises are collected, traded and utilized in overwhelming numbers principally to fill demand from Asia where they are prized for food and in traditional medicine. A growing pet trade also impacts a number of these threatened species. The global commerce in turtles in the last 20-plus years has followed a well-known pattern in international wildlife trade: once a species is depleted or regulated, the trade shifts to other species that are not as threatened or are less regulated. With continued human-driven development and population growth, turtle populations around the world also face pressure from habitat degradation and loss.





I'm so ready for winter. My sinuses are killing me.

Brazilian porcupine (Coendou prehensilis). PHOTO © PRINCETON PUBLIC LIBRARY, FLICKR.COM. CC BY 2.0.

INSTRUCTIONS FOR AUTHORS

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

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

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

Kieran Lindsey, Editor jwr.editor@theiwrc.org

MANUSCRIPT Manuscripts should be MS Word documents in either PC or MAC platform (*no PDF files*).

Manuscript should be typed in Times Roman, 12 pt., double-spaced throughout with one-inch margins.

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

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

Templates have been developed for the following submission categories: case study, technique (including diets), research, and literature review; authors may request a copy of one, or all, of these templates from the editor (jwr.editor@theiwrc.org) before developing a manuscript for submission to the JWR.

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or by email request to the Editor. This document provides formatting guidelines for in-text citations and the Literature Cited section; provides the JWR textual requirements for tables, figures, and photo captions; and describes quality and resolution needs for charts, graphs, photographs, and illustrations.



Orphaned indian mongoose (Herpestes javanicus), an introduced and controversial species in hawaii. PHOTO ©JAN H, CC BY-ND 2.0 LICENSE.



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