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IN THIS ISSUE:

Human-related causes of wildlife presentation to a rehabilitation clinic in east Tennessee... First finding of Lyme disease spirochete in Canadian Cooper's hawk... Ingested fish hooks in freshwater turtles from a region in the southeastern United States

ABOUT THE JOURNAL

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



On the cover: Eastern spiny softshell turtle (Apalone spinifera). PHOTO © LYD ANYNOM, FLICKR.COM. CREATIVE COMMONS LICENSE.

Left: Coopers hawk (Accipiter cooperii). PHOTO © REBECCA O'CONNOR, FLICKR.COM. CREATIVE COMMONS LICENSE.



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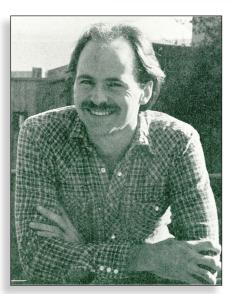
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GUEST EDITORIAL: PRESIDENT'S MESSAGE 1982

Jay Holcomb's Indelible Vision

IWRC celebrates the life of Jay Holcomb, 1951–2014. We are pleased to present this archived editorial and president's message from Fall 1982. At that time, IWRC was simply called Wildlife Rehabilitation Council and the JWR was the Wildlife Journal. —Kai



s president of the Wildlife Rehabilitation Council, I feel a need to share some of my thoughts on the work that we are all involved in. I have been racking my brain to find the words of wisdom I wanted to say. Instead, I should have been searching my heart, for it is love that connects me with the animals. With this in mind, I want to share these thoughts with you.

One thing all rehabbers have in common is a great love and compassion for the wild creatures of the earth. This is why we work incredible hours for little or no money, suffer from physical, emotional, and mental burn-out, and sacrifice our personal relationships. It is our constant energy that has nurtured the field of wildlife rehabilitation to the point of becoming a respected and acknowledged profession and a necessary service in our communities.

Wildlife rehabilitation is a pioneering field. We are one of the first groups of people giving back to the earth what many have selfishly taken for years. With every creature we release to the sky or forest, we return a little of what we've been blessed with: the earth with all the trimmings.

The Wildlife Rehabilitation Council was formed by a group of people who believe in the freedom for all creatures. We owe it to the animals in our care to investigate new ideas and innovative rehabilitation techniques. Sharing is the only way to maintain excellence and build a strong foundation of knowledge.

The Wildlife Journal is a connection between us. It is through the Journal that we can exchange these ideas and know that others are receiving and using them.

The only way the Wildlife Journal will succeed in its efforts to enhance our field is by receiving articles and ideas from our members.

As a rehabilitator, I want to read about what's happening in our field. I want to open this Journal and learn how I can better assist the animals I care for. In this first issue of the Journal's new format, my friends and I are sharing some of the new ideas we have, in the hope that you will find them useful in your centers. It is with the joy of giving that we present these ideas to you and hope that you will return the favor with your own unique thoughts. This is your opportunity to assist in creating a balance on the earth which we all share.

Jay Holcomb, President WRC JOURNAL • Fall, 1982

IN THE NEWS

Galveston Area Oil Spill Update

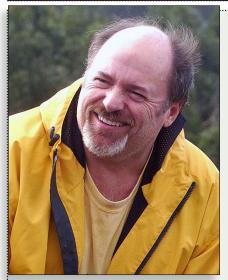
PORT O'CONNOR, Texas, United States (April 5)—Unified Command officials have pledged to accelerate and improve oil-communication about oil spill status with local Galveston residents, including providing additional outreach in Spanish. The Texas Department of Health Services is re-releasing its English language fact sheet on algae-related bay closings and distributing it to area residents in Spanish.

Also on Thursday, April 3, response crews of 465 clean-up contractors continued work along impacted sections of Mustang and South Matagorda Islands and Padre Island National Seashore. Oiled materials totalling 188,850 pounds have been collected from impacted shoreline, including 93,550 pounds from Matagorda Island, 90,775 pounds from Mustang Island and 4,525 pounds from the Bob Hall pier area.

On the morning of April 4th, response teams began to stage equipment onto South Matagorda to expedite clean-up ahead of the migration of Kemp's Ridley sea turtles, who use the island for nesting grounds from April through July. Activities are being conducted with the highest sensitivity to the fragile environment while maximizing efforts to remove oiled sand and debris.

Matagorda Island and the southeast Texas coastline are especially important to migrating birds. Spring is peak migration with diverse birds feeding on the shorelines and roosting in the dunes. Unified Command has made restoration of these beaches for wildlife a top priority and is working closely with federal and state wildlife agencies.

Officials updated information on deceased birds and other wildlife on Friday morning. In the Matagorda, Mustang, and North Padre areas, 77 dead birds have been recovered. Two birds were being stabilized at the rehabilitation facility in Port O'Connor for ultimate transport to Baytown for additional treatment. Local officials also report recovering nine deceased dolphins and eight deceased sea turtles.



IN MEMORIUM

Jay Holcomb (1951-2014)

On June 10th, 2014, the wildlife rehabilitation community said farewell to Jay Holcomb. executive director of International Bird Rescue. Many have been sharing their stories of Jay. Here, we try to share a bit of IWRC's story of Jay. The first evidence of Jay and IWRC getting together is in the March 1974 Board meeting notes, noting his attendance at that meeting, but not his status. Jay's association with the organization started before IWRC was even incorporated (this happened later in 1974). It's not clear from the records when Jav officially came on the Board, but he was there by 1977 and spent at least 12 years on the Board. Jay was president

from 1981 to 1983 and again from 1988 to 1991.

Jay's first tenure as president began auspiciously with the lovely editorial you see on page four of this issue, reprinted from Volume 5(3). Jay maintained his strong relationship with IWRC through four decades and countless changes to the field.

Most recently, he stepped in to participate in the 2011 Symposium when the scheduled International Bird Rescue speaker was called out to the Rena Spill in New Zealand.

Jay's influence was felt far and wide, demonstrated by the diverse award acknowledgements he received, from NWRA's lifetime achievement award in 1996 to John Muir Conservationist of the Year and Oceana's Ocean Hero in 2010.

In lieu of flowers, donations can be made to the Jay Holcomb Memorial Fund at International Bird Rescue.

It is unclear whether these deaths are directly related to the oil. Tests to make that determination will take several weeks. Throughout the day, wildlife experts from Texas Parks and Wildlife and U.S. Fish and Wildlife also monitored whooping crane and other endangered species' habitats. There are no reports of impacts on these species.

Persons observing any impacted wildlife should not attempt to capture them but are urged to report them to 888-384-2000.

Weather-Worn Puffins Ferried Home

DEVON, United Kingdom (April 3)— A group of young puffins blown in during the February storms are homeward bound



after the RSPCA released the birds on April 3rd off the north coast of Devon.

The three juvenile puffins, who were among an influx of 40 seabirds rescued by the RSPCA during the heavy winter storms, took a journey across to Lundy Island on MS Oldenburg following two months' rehabilitation, rest, and recuperation at RSPCA West Hatch Wildlife Centre.

The birds made the 22-mile trip across the Bristol Channel before being transferred on to a small boat and transported farther away from the main ferry and being released on to the sea in Gannet's Bay.

The young puffins are currently in moult, which means their flight feathers are

not fully formed and a usual clifftop release was replaced with a release onto water.

Wildlife supervisor at RSPCA West Hatch Paul Oaten said, "The puffins were young and exhausted when they came in to us a couple of months ago but they have made wonderful progress. They have been fed a diet of sprats and are now all ready to make their way back to Lundy Island.

"We've had more than the usual amount of storm blown birds in to the centre so far because of the severe weather we experienced at the beginning of the year. But puffins are one of the more unusual ones.

"The puffins were just completely worn out by the stormy weather. It was lucky members of the public spotted them at their various locations."

They were found dotted along the southwest shoreline. A member of the public found one bird huddling for shelter under a caravan at the Brean Sands Caravan Park on the Somerset coast.

Atlantic puffins aren't usual visitors to the beaches of Somerset, and it is thought these three were blown in by the strong winds at the start of the year.

Staff at RSPCA West Hatch and Lundy Island collaborated in the release project so the birds could be released as close as possible to the colonies on the eastern side of the island.

Lundy Island warden Beccy MacDonald said: "It is wonderful we are able to assist RSPCA West Hatch with the release of these three puffins. As they are unable to fly, we released them at a safe distance from the Lundy coast so that they will be able to join other seabirds currently rafting off the east coast of the island ready for this year's breeding season.

"It will be interesting to see if we are able to spot them on the slopes during the summer breeding season once they have re-grown their feathers over the next few weeks. We already have one puffin on the island so these three will soon be joined by many more."

As well as the three puffins, RSPCA West Hatch has been inundated this winter with other storm-blown birds such as guillemots, kittiwakes, gannets, and razor bills, coming in from across the region, especially from the Chesil Beach area of Dorset.

RSPCA Vice-president Bill Oddie said: "The staff at RSPCA West Hatch Wildlife Centre have worked tirelessly caring for these young puffins, along with the dozens of other storm blown birds who were victims of the bad weather at the beginning of the year.

"Everyone loves puffins and these three are very lucky to have been brought back to health after a very tough winter, and Lundy is a fantastic place for them to be. They will be a welcome boost to the population on the island, too."

Second Generation Anticoagulant Rodenticides Restricted in California

MARIN COUNTY, California, United States (March)—A victory for wildlife! The California Department of Pesticide Regulation has adopted a regulation that makes the most dangerous second generation anticoagulant rodenticides, rat poisons, California restricted materials. This means in effect that the products will no longer be sold on retail store shelves, and they will be out of reach to the general consumer as of July 2014.

The regulation affects all pesticide products containing the active ingredients brodifacoum, bromadiolone, difenacoum, or difethialone. Brand names for these products include d-Con[®] and Generation.[®]

WildCare applauds this new regulation as it will benefit untold numbers of wild animals that today carry heavy loads of anticoagulant poison in their bodies due to eating poisoned rodents.

"This is a practical sensible regulation that goes a long way to protecting our wildlife," said Brian Leahy, DPR Director. "Second generation anticoagulant rodenticides can contain some pretty powerful chemistry. Restricting the use of SGARs to only certified applicators will significantly reduce unintended exposures to non-target wildlife." WildCare's Director of Wildlife Solutions and Advocacy, Kelle Kacmarcik agrees. "WildCare has been working with DPR, US EPA, and the California Department of Fish and Wildlife on this issue for many years," she says. "We are thrilled to share this announcement, and we congratulate DPR for taking this important step toward the health of our wildlife."

Positive results for exposure to anticoagulant rat poisons were found in 76.8% of tested patients in 2013.

Children's Book on Wildlife Rehabilitation

TEWKSBURY TOWNSHIP, New Jersey, United States (March 30)—In early April, the Whittemore Wildlife Sanctuary (WWS) hosted a special program for children. Animal book author Loren Spiotta-DiMare of Tewksbury Township and children's book illustrator Key Wilde of Pittstown read and signed copies of their picture book Broke Leg Bear. This true story highlights the rescue, rehabilitation, and eventual release of an injured black bear cub by Woodlands Wildlife Refuge in Pittstown. WWS Director Joy Logan will also discuss wildlife rehabilitation, including what you should do if you find an injured or orphaned animal.

"We are delighted to have Loren and Key share their book with us," Logan said. "The story of Broke Leg fits right in with our mission to promote environmental education." Endorsed by wildlife conservationist Jane Goodall, *Broke Leg Bear* was named a finalist in the Children's Picture Book: Hardcover Non-Fiction category of the 2012 USA Best Book Awards, sponsored by USA Book News.

After being hit by a car, Broke Leg was taken to Woodlands Wildlife Refuge, as it's the only New Jersey Refuge licensed to rehabilitate black bears. Despite medical challenges and many obstacles, the cub overcame the odds thanks to the dedication of many individuals.

Woodlands Wildlife Refuge has been dedicated to the care and release of orphaned and injured wildlife since 1986. WWR cares for approximately 900 patients a year, including raccoons, skunks, opossums, woodchucks, rabbits, coyotes, fox, river otters, beavers, turtles, porcupines, and—of course—black bears.

Loren Spiotta-DiMare has been writing about wildlife since 1976 and has published

CONTINUED ON PAGE 28

Major anthropogenic causes for and outcomes of wild animal presentation to a wildlife clinic in East Tennessee, USA, 2000–2011

Ashley N. Schenk and Marcy J. Souza



X-ray of bald eagle (Haliaeetus leucocephalus) riddled with lead shot.

Introduction

Around the world, wildlife species and the ecosystems they inhabit are evolving and shifting in an attempt to adapt to human influences and environmental change. The factors dictating these changes are diverse and numerous, making it difficult to separate the effect each has on species survivorship. Increased morbidity and mortality of wildlife can be attributed to a variety of factors, and many of these are related to human activities such as land development and usage, predation by domestic pets, and automobile traffic (1–4).

As the urbanization of areas with native wildlife continues to increase, numerous pressures are exerted on the natural structures of those habitats. For example, infringing development may not only increase exposure between wildlife species, domestic animals, and invasive species, but may also increase the exposure of wild animals to novel pathogens, leading to what Daszak *et al.* call "pathogen pollution" (1). Clearing and isolating habitats through the development of high-traffic roadways can increase roadside automobile-related mortalities, noise, environmental pollution, and disturbance stress on the surrounding wildlife (5). The construction of other infrastructure can change temperature variations as well as the flow of water runoff thereby increasing flooding and further deteriorating the habitable environment (6–8). The establishment of neighborhoods and housing communities will likely also increase the number of domestic animals in the area, which provides an unnatural predator stress and possibly novel infectious agents on nearby native species.

ABSTRACT: To determine the reasons for presentation and outcome of wildlife cases in East Tennessee, a retrospective analysis was performed using 14,303 records from cases presented to the wildlife clinic of the University of Tennessee Veterinary Teaching Hospital between 2000 and 2011. The cases were first categorized into amphibian/non-avian reptile, mammal, or avian, and then classified into groups based on the primary admitting/ presenting sign. There are a variety of reasons animals were presented to the clinic, and some were directly or indirectly anthropogenic in origin, including cat related, dog related, hit by automobile, and other human encounters leading to trauma; of the cases reviewed, 4,443 (31.1%) presented for one of these four reasons. Overall case fatality risk in regard to these four admitting/presenting signs was 0.519 for the amphibian/non-avian reptile cases, 0.675 for mammal cases, and 0.687 for avian cases. This study confirms the importance of monitoring wildlife morbidity and mortality and of focusing efforts to reduce the anthropogenic threat on native habitats and resident wildlife populations.

KEY WORDS: anthropogenic, wild animal presentation, wildlife clinic, East Tennessee, human induced trauma, cat-related, dogrelated, hit by automobile

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Eastern painted turtle (Chrysemys picta picta), hit by a car.

Regardless of whether these increasing threats to wildlife originate directly or indirectly from anthropogenic effects, or as a result of disease spread, it is important to understand the extent of their impact. One study found that trauma and infection were the main reasons eastern box turtles were presented to a wildlife clinic (9). Similar studies were conducted to investigate reasons wild raptors and reptiles were admitted to wildlife rehabilitation centers, and both studies found evidence of anthropogenic origins of trauma (10,11). The purpose of this study was to investigate the reasons wildlife were presented to a veterinary medical center to determine the greatest anthropogenic causes of morbidity and mortality.

Methods

There were 14,943 records reviewed from wildlife cases that

presented to the University of Tennessee Veterinary Teaching Hospital between January 2000 and November 2011. Six hundred forty cases were omitted; these included cases in which "dead on arrival" or "euthanasia" were the only details given for the reason for presentation as well as rechecks and cases in which there was insufficient data for categorization. The 14,303 records remaining included species or species group ("songbird" being the most detailed animal group indicated on many avian records), and these species or species groups were classified according to type of animal (amphibian/non-avian reptile, mammal, or avian). The cases in which cat-related, dog-related, hit-by-automobile, or human-induced traumatic incidents were mentioned in the primary admitting/presenting sign (A/PS) were separated out and analyzed. These signs were derived from information provided by the person admitting the animal and do not represent a final diagnoses made by the clinician, but they do provide detailed information about the patient's condition and the most likely origin of injury.

The A/PSs were grouped as follows: human-induced trauma cases included those which mentioned gunshot, fence entrapment, fishing line or hook injury, lawn mower or weed eater encounter, and trapped non-target species cases. Cat-related, dog-related, and automobile-related cases were separated out as well in order to show their frequency. Any time "hit by car" or "found in road" was indicated, the animal was placed in the hit-by-automobile category. Cat- and dog-related cases were categorized accordingly and included explanations such as "attacked by dog," "found in cat's mouth," and "killed by cat." The number of cases in each category was determined, and the outcomes (alive, dead on arrival [DOA], died, or euthanized) were recorded. Animals classified

TABLE 1. CASE OUTCOMES AND CASE FATALITY RISKS (CFR) FOR HUMAN-INDUCED TRAUMA, CAT RELATED, DOG RELATED, AND HIT-BY-AUTOMOBILE CASES FOR AMPHIBIAN/NON-AVIAN REPTILE, MAMMAL, AND AVIAN ANIMALS PRESENTED TO A WILDLIFE CLINIC IN EAST TENNESSEE.

	n (% c	of cases)	ALI	VE (%)	DO	A (%)	DIE	D (%)	EUTH	IAN (%)	CFR
AMPHIBIAN / NON-AVIAN REPTILE	397		192		3		32		171		0.519
1. HUMAN-INDUCED TRAUMA	60	(15.1)	41	(68.3)	0	(0.0)	3	(5.0)	16	(26.7)	0.316
2. CAT RELATED	12	(1.5)	8	(66.7)	0	(0.0)	1	(8.3)	3	(25.0)	0.333
3. DOG RELATED	50	(6.1)	31	(62.0)	0	(0.0)	3	(6.0)	17	(32.0)	0.380
4. HIT BY AUTOMOBILE	275	(33.3)	112	(40.7)	3	(1.1)	25	(9.1)	135	(49.1)	0.593
MAMMAL	2318		754		36		263		1265		0.675
1. HUMAN-INDUCED TRAUMA	111	(4.8)	32	(28.8)	2	(1.8)	13	(11.7)	64	(57.7)	0.712
2. CAT RELATED	1115	(19.4)	388	(34.8)	9	(0.8)	166	(14.9)	552	(49.5)	0.652
3. DOG RELATED	597	(10.4)	253	(42.4)	13	(2.2)	52	(8.7)	279	(46.7)	0.576
4. HIT BY AUTOMOBILE	495	(8.6)	81	(16.4)	12	(2.4)	32	(6.5)	370	(74.7)	0.836
AVIAN	1738		544		23		299		872		0.687
1. HUMAN-INDUCED TRAUMA	202	(11.6)	75	(37.1)	0	(0.0)	27	(13.4)	100	(49.5)	0.629
2. CAT RELATED	809	(10.5)	232	(28.7)	9	(1.1)	168	(20.8)	400	(49.4)	0.713
3. DOG RELATED	244	(3.2)	73	(29.9)	3	(1.2)	39	(16.0)	129	(52.9)	0.701
4. HIT BY AUTOMOBILE	483	(6.3)	164	(34.0)	11	(2.3)	65	(13.5)	243	(50.3)	0.660

as "alive" were either released directly by hospital personnel or transferred to a rehabilitation facility; only cases determined to have a good or excellent prognosis for release were transferred to a rehabilitation facility. The placement of non-releasable animals from the veterinary teaching hospital into education facilities is extremely rare.

Results

Of the 14,303 cases evaluated, 4,333 (31.1%) were classified as presenting for one of the four major A/PSs evaluated; case frequencies ranged from 12 to 1,115 within the animal groups, and the case fatality risk ranged from 0.316 to 0.836 (Table 1). Overall case fatality risk in regards to these four focus A/PSs was 0.519 for the amphibian/non-avian reptile cases, 0.675 for mammal cases, and 0.687 for avian cases. Hit-by-automobile cases had the highest fatality risk (0.715), followed by cat-related injury cases (0.675), human-induced trauma cases (0.603), and dog-related cases (0.600) across all animal groups. Although cat-related cases had the highest percent of natural deaths following presentation to the clinic, hit-by-automobile cases had the highest percent of cases with successive euthanasia (Table 1).

Discussion

Wildlife species are continually being presented to veterinary clinics and rehabilitation centers throughout the United States, and it is important to determine the reasons in order to monitor the changing health status of the surrounding ecosystem (10), decrease the anthropogenic effect of habitat fragmentation and pathogen pollution (2,12–14), and investigate preemptive strategies for reducing the number of wildlife casualties. This large dataset provides a sample to explore causal trends for presentation and sheds light on some of the major anthropogenic threats to wildlife health. This study does not attempt to explain the origin or cause of all reasons for presentation, but rather focuses on human related causes of presentation.

Approximately one-third of the cases examined were presented to the hospital because of either direct or indirect anthropogenic reasons. Direct interactions with humans (human-inducedtrauma and hit-by-automobile categories) were less common than indirect interactions (dog and cat categories) in this population, but still made up 11% of the total cases. Pathogen pollution, noise pollution, and environmental pollution have also been shown to lead to wildlife morbidity and mortality (1,15–17), but this study provides an additional explanation that "predator pollution," by means of introducing domestic cats and dogs to wildlife areas, may also be having a profound and damaging effect. Of all cases presented, approximately 20% were due to interactions with domestic pets, specifically cats (14% of all cases) and dogs (6% of all cases). By narrowing the interface between wild and urbanized areas, it is likely that human-wild animal encounters, whether direct or indirect, will increase and, based on the results of this study, these encounters frequently result in the detriment of the wild animals.

The data provided in this study do not investigate or provide

evidence for the role of environmental pollution, pesticide use, or other forms of habitat disruption, but it does lend itself to the needed discussion about the many factors contributing to the morbidity and mortality of native wildlife species. In order to establish long-term conservation, a variety of initiatives including responsible pet ownership and habitat modification should be considered.

Community and veterinary-client education about the importance, as it relates to wildlife, of keeping domestic cats indoors and preventing domestic dogs from roaming outside unsupervised could lead to a reduction in the number of animals presented to wildlife facilities based on the findings of this study (18). Although pets other than dogs and cats were not identified as reasons for presentation in this study, exotic, invasive species can lead to wildlife morbidity and mortality in other regions. Providing educational materials to owners about the proper care of their exotic pets may decrease those introduced to the wild by intentional abandonment and therefore reduce interactions with native wildlife (19).

Increasing canopy coverage and the shrub layer along urban parks and greenways has been suggested to increase crucial habitat areas for certain avian species and protect them from the negative pressures of urbanized areas (20). In addition, evidence supports certain habitat defragmentation projects, such as linear patches and biological corridors, as successful in increasing migratory ranges and establishing connectivity between wildlife (5,21,22). On a smaller scale, establishing larger wildlife-friendly areas by arranging neighborhood gardens adjacent to each other has also been proposed as a means to increase wildlife habitat in urbanized areas (23). By removing invasive predators, focusing efforts on the conservation of native habitats, and affording a level of protection along developed and undeveloped transition zones, the numbers of animals affected by direct and indirect interactions with humans might be decreased, therefore leading to decreased morbidity and mortality.

Conclusion

This study examined the causes for wildlife submission to a wildlife clinic to understand the patterns and trends of humanrelated reasons for presentation. Through this and other studies, it is apparent that anthropogenic factors, including land development, as well as direct interactions with humans, automobiles, and invasive predators, are important causes of wildlife morbidity and mortality (3,4,12,14). Because final diagnosis was assumed from the A/PS in our study and because some signs lacked explanatory detail, additional studies reviewing comprehensive patient records, as well as detailed clinician diagnoses, may provide stronger evidence supporting patterns for wildlife presentation to veterinary clinics and rehabilitation centers. It is also important to understand that many of these cases were submitted by "Good Samaritans" and this may present a bias in the case data. The animals brought to the clinic were likely found in easily accessible, populated areas, and this may lead to a misrepresentation of the causes of morbidity and mortality in less-developed areas. In addition to defragmenting habitats and establishing biological corridors, it is important to remove invasive species and contain domestic animals in order to decrease the predatory stress they impose. Through agendas like these and a more mindful approach to land development planning, the anthropogenic threat to wildlife species might be minimized.

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Author Contributions

Both authors (AS and MS) conceived and designed the experiments, performed the experiments, analyzed the data, contributed reagents/materials/analysis tools, and wrote the paper. *Funding:* The authors have no support or funding to report. *Competing Interests:* The authors have declared that no competing interests exist.

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First detection of Lyme disease spirochete *Borrelia burgdorferi* in ticks collected from a raptor in Canada

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Cooper's hawk (Accipiter cooperii).

Introduction

A diversity of wild birds act as avian hosts of blood-sucking, hard-bodied *Ixodes* species ticks (*Ixodida: Ixodidae*). Most commonly, ticks are reported on passerines (order *Passeriformes*), which are also known as perching or songbirds, and some of these ticks are infected with *Borrelia burgdorferi sensu lato* (hereafter *B. burgdorferi*), the spirochetal bacterium that causes Lyme disease (1). This tick-borne spirochetosis can have a multitude of clinical symptoms, including cardiac, cutaneous, endocrine, gastrointestinal, genitourinary, musculoskeletal, neurologic, cognitive, and neuropsychiatric (2–4).

If left untreated or inadequately treated, diverse forms (5,6) of *B. burgdorferi* can sequester and persist in immunologically deprived and deep-seated sites (7–14), namely, ligaments and tendons (15,16), muscle (17), brain (18–20), bone (21,22), eyes (23), glial and neuronal cells (24,25), and fibroblasts/scar tissue (26). There are at least 100 different *B. burgdorferi* genotypes worldwide (27–30), and patients are often negative using the 2-tier Lyme disease serology test despite having Lyme disease (31–33).

This tick-borne microorganism cycles in nature between certain tick species and a wide range of vertebrate hosts, and has been reported from five continents, including sub-Antarctic islands and Australia (34,35). In the coastal area of southeastern Australia, the avian coastal tick, *Ixodes auritulus (Ixodida: Ixodidae)*, and the paralysis tick, *Ixodes*

Abstract: During a pan-Canadian tickhost study, we detected the spirochetal bacterium, Borrelia burgdorferi sensu lato, which causes Lyme disease, in ticks collected from a raptor. Lyme disease is one of a number of zoonotic, tick-borne diseases causing morbidity and mortality worldwide. Larvae of the avian coastal tick, Ixodes auritulus, were collected by wildlife rehabilitators from a Cooper's hawk, Accipiter cooperii, on Vancouver Island, British Columbia. Using PCR amplification of the linear plasmid ospA gene of B. burgdorferi, 4 (18%) of 22 larvae were positive. Since these engorged I. auritulus larvae had not had a previous blood meal and B. burgdorferi is rarely transmitted from infected female ticks to their progeny, we propose that Cooper's hawks are reservoircompetent hosts of B. burgdorferi. Our tick-host discovery provides the first report of bird-feeding ticks on a Cooper's hawk, and exhibits the premiere record of B. burgdorferi-positive ticks on a raptor. Not only are passerine (perching) and gallinaceous (chicken-like) birds involved in the wide dispersal of Lyme disease vector ticks, raptors are now also implicated in the dissemination of B. burgdorferi-infected ticks. Although I. auritulus does not bite humans, this tick species plays an integral role in the 4-tick enzootic cycle of B. burgdorferi along the West Coast of North America. In essence, raptors and I. auritulus ticks may help to amplify this infectious agent in nature, and increase the likelihood of people contracting Lyme disease, especially in coastal areas.

KEYWORDS: falconiformes, raptors, Cooper's hawk, Accipiter cooperii, Canada, Lyme disease, Borrelia burgdorferi, ticks, Ixodes auritulus

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Reprint: Scott et al., Journal of Veterinary Science & Medical Diagnosis 2013, 2:4 http://dx.doi.org/10.4172/2325-9590.1000123 *holycyclus*, which are both Lyme disease vector ticks, aid in the spread of Lyme disease. In Canada, several different wild bird species, which are short- and long-distance carriers, widely disperse Lyme disease vector ticks nationwide (36–39). In farwestern Canada, Gregson (40) reported *I. auritulus* on a bald eagle, *Haliaeetus leucocephalus*, and a Rocky Mountain wood tick, *Dermacentor andersoni*, on a hawk. Although raptors (*Falconiformes: Accipiteridae*) were examined recently in southern Ontario for attached ticks, none was noted (41). Cooper's hawks, which have ample opportunity to encounter host-seeking ticks, have a continent-wide range and transcontinental distribution across the central temperate region of North America, including Vancouver Island, British Columbia (B.C.) (42). The sheep tick, *Ixodes ricinus*, and the taiga tick, *Ixodes persulcatus*, have been reported on several species of the hawks in Eurasia (43).

Ticks can transmit more kinds of pathogens than any other





Figure 1. A Cooper's hawk parasitized by *Borrelia burgdorferi*–infected larvae of the avian coastal tick, *Ixodes auritulus*.

Rockies, the blacklegged tick, Ixodes scapularis, which parasitize a wide range of vertebrate hosts. Similarly, Ixodes dentatus and Ixodes spinipalpis (45) are confirmed as competent vectors of B. burgdorferi. Additionally, Ixodes affinis, which is occasionally transported from the southeastern USA and Mexico by northward migrating passerines in the spring, is an extralimital tick that has vector competency for B. burgdorferi (46,47). Moreover, several bird-tick-Borrelia studies underpin the fact that ground-frequenting passerines transport Lyme disease vector ticks northward during long-distance flight (36-39,48-50). Not only do migratory songbirds carry ticks northward during spring migration, these avian hosts also transport them southward during fall migration (37,51). Along the West Coast, I. auritulus ticks, which are ectoparasites of passerines and galliforms, play a role in the natural enzootic cycle of B. burgdorferi (39). Using culturing and PCR-testing, early studies in the southern region of Vancouver Island, B.C., detected B. burgdorferi in Ixodes angustus and I. pacificus and established its presence in this area (52). The aim of our tick-host-Borrelia study was to explore any new

group of ectoparasites worldwide affecting people, livestock, wildlife, and domestic animals (44). In Canada, at least six of 23 known Ixodes species collected from vertebrates (avian, mammalian, reptilian) exhibit some degree of vector competence for B. burgdorferi. The principal vectors to humans are the western blacklegged tick, Ixodes pacificus, in British Columbia and Alberta and, east of the environmental associations that could contribute to an increase in Lyme disease in an area.

Materials and Methods

Tick collection

Ticks were detached primarily from the head and neck using finepointed tweezers by wildlife rehabilitators. One to three ticks were placed in 2 mL polypropylene micro tubes, and four or more ticks were placed in clear 4 dram (12 mL) polystyrene vials with white polyethylene caps vented with tulle netting. These containers were placed in a ziplock bag with a slightly moistened section of paper towel. Dead or badly damaged ticks were put directly in 2 mL micro tubes containing 95% ethyl alcohol. Using a bubble-pack envelope, ticks were mailed promptly to the lab (JDS) for identification. An Olympus stereoscopic microscope SZX16 (objective, 1x; eyepieces, 10x), which provided zoom observation magnification of 7x-115x, was used to view the following tick characteristics: 1) alive or dead, 2) unfed, partially engorged, fully engorged, 3) developmental life stage, and 4) tick species (53-55). Partially and fully engorged ticks were kept alive and allowed to molt to the next developmental life stage. After background information was noted, ticks were sent by overnight courier to the culturing and PCR amplification research laboratory (JFA).

Spirochete detection

Each unfed and engorged tick was tested for the presence of B. burgdorferi using PCR by methods as previously described (56,57). Briefly, ticks were ground with a large paper clip in a 0.6 mL microcentrifuge tube containing 25 µL to 35 µL K Buffer, which consisted of: 18 mL sterile irrigation water, 2 mL 10X Base Buffer, 0.09 mL NP 40 (Sigma, lot #122K00401), and 0.09 mL Tween® 20 (Sigma, lot #033K0109). A different paper clip was used for each tick. Each tick was boiled at 94°C for 10 minutes. DNA was extracted from engorged ticks using instructions in the QIAamp DNA Mini Kit[®] (250) (QIAGEN, Valencia, CA). Primers were the linear plasmid ospA gene target: ospA2, 5'-GTTTTGTA-ATTTCAACTGCTGACC-3'; ospA4, 5'-CTGCAGCTTG-GAATTCAGGCACTTC-3'. PCR amplification was performed using a Perkin-Elmer® thermal cycler set to conduct denaturation at 94°C for 45 sec, annealing at 45°C for 45 sec, and elongation at 72°C for 1 min for a total of 45 cycles. Appropriate negative and positive controls were used. Amplification products were analyzed by electrophoresis, stained with ethidium bromide, and examined under UV illumination as described previously (56,57). Amplification products were transferred to a nylon membrane by Southern blot. The membrane was then hybridized overnight with 32P using the probe ospA3, 5'-GCCATTTGAGTCG-TATTGTTGTACTG-3'. The membrane was then washed, and Kodak® X-OMAT® AR film was placed over the membrane for 4 hr. Infected ticks were detected with the 32P probe. Attempted culturing of spirochetes from the larval ticks from the Cooper's hawk was not done because they were all dead upon arrival for tick identification.

Results

Tick collection

A total of 22 engorged *I. auritulus* larvae were collected from the edge of the lower right eyelid of a juvenile male Cooper's hawk, *Accipiter cooperii*, which was examined on 29 October 2012, after it was recovered at Oak Bay, Vancouver Island, B.C., Canada (Fig. 1). This tick collection is the first report of ticks on a Cooper's hawk and constitutes a new tick-host record.

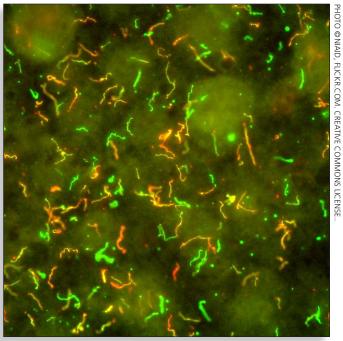
Spirochete detection

Four (18%) of 22 *I. auritulus* larvae were infected with *B. burgdorferi*. Based on an extensive literature search, we provide the first report of *B. burgdorferi*-positive ticks on a raptor. Of the 4 positive ticks, 2 of 17 (12%) partially engorged and 2 of 5 (40%) fully engorged larvae were positive for *B. burgdorferi*. The Cooper's hawk was released on 5 November 2012, and blood was not drawn from this raptorial host; thus, we could not verify spirochetemia in this host bird.

Discussion

This bird parasitism provides the first report of ticks on a Cooper's hawk and announces new-found evidence of B. burgdorferi in ticks collected from a raptor. The results of this study provide credible evidence that raptors act as reservoirs of B. burgdorferi, and add to the increased role of wild birds as dispersal agents of this zoonotic pathogen. Cooper's hawks prey primarily on small- and mid-sized birds but also supplement their diet with small mammals. As they consume their capture, they frequently make contact with low-lying vegetation where ticks are questing. In this particular case, the Cooper's hawk was most likely at a site where a gravid *I. auritulus* female laid her eggs in the spring. During the summer, these eggs hatched to larvae and were ready for active host-seeking in the late summer and fall. Since the attached larvae had a similar amount of engorgement, the Cooper's hawk must have encountered a cluster of larvae from recently hatched eggs. When the I. auritulus female lays her eggs and dies, the fat pellet in the posterior end of the idiosoma of the carcass provides a source of energy-laden nutrients and creates a proclivity to attract birds and rodents foraging for food (58).

Now, the question becomes: how did the four *I. auritulus* larvae acquire *B. burgdorferi* infection? Connecticut researchers (59) provided the first isolation of *B. burgdorferi* from a passerine (Veery, *Catharus fuscencens*) and showed that certain wild birds exhibit reservoir competency. Because the *I. auritulus* larvae had not had a previous blood meal, and transovarial transmission (female to eggs) of *B. burgdorferi* is not apparent during prior bird-tick studies (37–39), we extrapolate that spirochetes of this zoonosis were transmitted during engorgement on the Cooper's hawk. Our findings that 2 of 17 (12%) of the partially engorged and 2 of 5 (40%) of the fully engorged larvae removed from the hawk were positive for *B. burgdorferi* may be significant in this respect. Although the numbers are small, tick larvae that



Lyme Disease bacteria (Borrelia burgdorferi).

had imbibed a larger volume of host blood were more likely to be *B. burgdorferi*-positive, which provides circumstantial support for our suggestion that these ticks imbibed spirochetes with their bloodmeal from the hawk. If transovarial transmission of *B. burgdorferi* was the only source, then the infection rate of the partially engorged and the fully engorged larvae would be approximately the same. However, in our ectoparasite study, they are significantly different.

For comparison, researchers (60) presented evidence-based data to indicate that *Borrelia miyamotoi*, which is also pathogenic to humans (61), is transmitted transovarially by *I. scapularis* females; however, *B. burgdorferi* was not transmitted or detected in unfed larvae derived from egg clutches of wild-caught *I. scapularis* females. For our study, the host Cooper's hawk was most likely spirochetemic, and the host-seeking larvae acquired *B. burgdorferi* during engorgement. Further studies are necessary to confirm whether transovarial transmission occurs with *I. auritulus*.

As birds of prey, raptors are continuously consuming small mammals and wild birds, which presumably are infected with *B. burgdorferi* and, after eating them, may become infected. Not only do Cooper's hawks have frequent opportunities to encounter *B. burgdorferi*—infected, ectoparasitic ticks, they could feasibly become orally infected. Subsequently, these spirochetemic avian hosts could infect unfed, spirochete-free larvae. For comparison, 22 days post-inoculation, spirochetes were isolated from cloacal material and kidneys from mallard ducks, *Anas platyrhynchos platyrhynchos*, that had orally been infected with *B. burgdorferi* (62). Moreover, Schwarzozá *et al.* (63) similarly detected *B. burgdorferi* in the throat and cloacal cells from birds migrating through Slovakia. Thesefindings show that certain orally-infected birds can develop spirochetemia and shed *B. burgdorferi* in their

droppings. Based on our PCR amplification results, we suggest that Cooper's hawks are reservoir-competent hosts and act as dispersal vehicles of *B. burgdorferi* to new environmental foci. Along Canada's Pacific coast, this raptorial host presumably plays a notable role in the 4-tick enzootic cycle of *B. burgdorferi*, which consists of 4 vector-competent ticks (*I. auritulus*, *I. angustus*, *I. pacificus*, and *I. spinipalpis*). The bird parasitism in our study not only includes *I. auritulus* on a Cooper's hawk, it implicates raptors as reservoir hosts in the 4-tick enzootic cycle of *B. burgdorferi* in this bioregion and expands the number of bird species in Lyme disease dissemination.

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Prevalence of ingested fish hooks in freshwater turtles from five rivers in the southeastern United States

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Snapping turtle (Chelydra serpentina) with fish hook.

Introduction

Recreational fishing is a widespread activity (1,2) that poses threats to aquatic wildlife assemblages through the production of bycatch (3). Bycatch may be a particularly important threat for populations of imperiled taxa, such as some turtles (4). Several studies have described the capture of freshwater turtles in fish traps (5–8) and of estuarine turtles (i.e., diamondback terrapins, *Malaclemys terrapin* Schoepff 1793) in crab traps (9). Because freshwater turtles are opportunistic scavengers and also take live prey, they are also likely vulnerable to capture with baited hooks set to catch fish (10); in fact, they are targeted by commercial and recreational collectors via this same method (11).

Although freshwater turtles may ingest fish hooks (10,12), which can negatively affect their health (13), there are few data to indicate whether fish-hook ingestion is of large-scale conservation concern. For example, fishing-gear related trauma is a commonly reported injury for reptiles admitted to wildlife rehabilitation centers (14), but these cases represent a biased sample that does not identify the proportion of free-ranging animals affected. However, fish hooks were found in three of 17 (~18%) X-rayed female European pond turtles, *Emys orbicularis* Linnaeus 1758, from a heavily-fished series of ponds in France (15), suggesting significant proportions of turtles may be affected.

ABSTRACT: Freshwater turtles may ingest baited fish hooks because many are opportunistic scavengers. Although the ingestion of fish hooks is known to be a source of mortality in multiple vertebrate groups, the prevalence of hook ingestion by freshwater turtles has not been well studied. We trapped turtles from five rivers in the southeastern United States and used radiographs to examine over 600 individuals of four species. Depending on the species, sex, and age class, 0-33% of turtles contained ingested fish hooks. For some species, larger turtles were more likely to contain a fish hook than smaller individuals. Freshwater turtle demography suggests that even small increases in adult mortality may lead to population declines. If our study areas are representative of other aquatic systems that receive fishing pressure, this work likely identifies a potential conflict between a widespread, common recreational activity (i.e., fishing) and an imperiled taxonomic group.

KEY WORDS: fish hooks, ingested fish hooks, turtles, freshwater turtles, five rivers, southeastern United States

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Reprint: Steen *et al. PLoS ONE* 9(3): e91368. March 2014. doi:10.1371/journal. pone.0091368. ©2014 Steen *et al.* Creative Commons Attribution License. Hook ingestion causes elevated mortality rates in several taxa (e.g., sea turtles, fish, and birds) (16–18). Given the highly imperiled status of freshwater turtles in general (4) and the suggested inability of their populations to persist when exposed to even low levels of adult mortality (19,20), it is important to identify potential conflicts with widespread anthropogenic activities such as recreational fishing. To this end, we sampled freshwater turtles in five rivers in the southeastern United States and used X-ray radiography (21) to quantify the proportion of animals that contained fish hooks while determining how the sex, size, and species of an individual turtle might influence its relative vulnerability to fish hook ingestion.

Materials and Methods

Study Sites

The work described herein was done opportunistically as components of two larger and independent studies examining anthropogenic effects on the reproductive ecology of turtles in Tennessee and Virginia, USA. In Tennessee, our study site was a continuous riverine habitat centered around Kingston that included the Emory (river km 0.0–5.5), Clinch (river km 0.0–7.0), and Tennessee Rivers (river km 914–922). The area is open to the public and accessible via numerous boat launches; common recreational uses include fishing, boating, and other water sports. We observed three primary fishing methods within the study area: bass fishing with artificial lures, fishing with live bait, and unattended lines with baited hooks attached to floats. Full Tennessee fishing regulations can be found elsewhere (22).

In Virginia, our study sites included the South and Middle Rivers around Waynesboro and Staunton. The land adjacent to our study areas on the South River is forested in the upper mountainous regions, but at lower elevations the river runs through small-urbanized areas and private land that is used mainly for agriculture and livestock. Our study areas on the Middle River flow primarily through rural areas and are surrounded by privately owned farms. Although public access is limited on the Middle River, several public access areas are present on the South River, including popular swimming spots and areas that experience trout and bass fishing. A health advisory for fish consumption exists within several of our sampling areas along the South River, but we frequently observed recreational fishing with artificial lures, baited hooks, and, in some areas, flyfishing. In addition, informal conversations with anglers suggested that fish advisories were not entirely effective at deterring people from catching and consuming turtles. Representative Virginia fishing regulations can be found elsewhere (23).

Trapping

Turtles were captured in baited hoop traps (Memphis Net and Twine[®], Memphis, Tennessee, USA). No turtles were harmed in this study; all individuals were released at their point of capture after processing. Standard morphological measurements were taken on turtles and they were sexed based on secondary sexual characteristics. We included four species in the current study: Eastern musk turtles (*Sternotherus odoratus* Latreille in Sonnini & Latreille 1801), pond sliders (*Trachemys scripta* Schoepff 1792), spiny softshells (*Apalone spinifera* LeSueur 1827), and snapping turtles (*Chelydra serpentina* Linneaus 1758). These species are generally considered common throughout their range, but their life histories are representative of those of many other chelonian species that are uncommon and in some cases of great conservation concern. We considered *T. scripta* males and females as adult if they were >11 cm and >20 cm carapace length (CL), respectively, *S. odoratus* males and females as adult if they were >130 g and >20 cm CL, respectively, and *C. serpentina* of either sex as adult if they were >20 cm CL (24).

In Tennessee, we X-rayed all female *S. odoratus, T. scripta, A. spinifera*, and *C. serpentina* known or suspected to be as gravid based on physical palpation between 5 May and 25 July 2012. Between 16 June and 25 July, we X-rayed additional turtles, including males, as time allowed. In Virginia, we collected *C. serpentina* from April–July in 2010 and 2011, as described in Hopkins *et al.* (25), and gathered data only on female turtles. The turtles we decided to X-ray are not necessarily representative of the relative abundances of the various species or of the age and sex distributions present within the population.

X-ray

None of the turtles we X-rayed displayed any visible evidence of hook ingestion (i.e., there were no externally visible hooks and/or fishing line). In Tennessee, we used an EcoRay[®] Ultralight 9020 HF set at 70 kV and 4.00 mA to X-ray turtles. In Virginia, turtles were X-rayed by technicians at the Wildlife Center of Virginia with a Summit[®] InnoVet and settings were adjusted as required. For both sites, we recorded the presence/absence of fish hooks in X-rays. We also attempted to identify the type of fish hook (i.e., J, circle, or treble) based on their shape, but we were unable to reliably differentiate J hooks from circle hooks because hooks were lodged in turtles at varying angles.

Ethics Statement

Capture and handling of turtles was approved by the animal care and use committee at Virginia Polytechnic and State University (IACUC#09-080-FIW, 10-055-FIW, 11-044-FIW, and 12-056-FIW) and appropriate state collection permits were obtained (Virginia #035981; Tennessee #TN3610).

Statistical Analysis

We used separate logistic regressions to analyze the presence and absence of fish hooks in turtles from Tennessee and Virginia. In Tennessee turtles, we examined the effects of species, carapace length, and sex. In Virginia turtles, we examined only the effect of carapace length because all X-rayed turtles were female *C. serpentina*. We modeled logistic regressions using PROC GLIM-MIX in SAS 9.3 (SAS Institute, Cary, North Carolina, USA). We log-transformed carapace length in the analysis for Virginia turtles because it was found to improve the fit of the model, as judged by reduced AIC values. The interaction between carapace length and species was significant in the Tennessee model, so we used *post-hoc* logistic regressions to examine the effect of carapace length on hook presence or absence within each species. Because there were low absolute numbers of turtles with ingested fish hooks, we ran power analyses (Proc Power®) on the variance outputs from the analysis of the Tennessee

turtles to determine whether our sample sizes were large enough to avoid committing type II errors (i.e., failing to reject false null hypotheses).

Results

In Tennessee, we X-rayed a total of 84 *A. spinifera* (25 adult males, 50 adult females, and 9 juveniles), 20 *C. serpentina* (10 adult males, 9 adult females, and 1 juvenile), 92 *S. odoratus* (24 adult males and 68 adult females), and 242 *T. scripta* (115 adult males, 106 adult females, and 21 juveniles). No hooks were detected in *S. odoratus*. Of species that contained hooks, the proportion of adult males and females with ingested hooks ranged from 3.5–10% and 6–33%, respectively (Table 1). In Virginia, we X-rayed a

TABLE 1. TOTAL TURTLES X-RAYED AND PROPORTION CONTAINING FISH HOOKS FROM THE CLINCH, EMORY, AND TENNESSEE RIVERS, TENNESSEE, AND SOUTH AND MIDDLE RIVERS, VIRGINIA. DOI:10.1371/JOURNAL.PONE.0091368.T001

LOCATION	SPECIES	LIFE STAGE	NUMBER X-RAYED	TOTAL HOOKED	PROPORTION HOOKED
TENNEGGEE			24	0	
TENNESSEE	Sternotherus odoratus	ADULT MALES	24	0	0.00
		ADULT FEMALES	68	0	0.00
	Chelydra serpentina	ADULT MALES	10	1	10.00
		ADULT FEMALES	9	3	33.33
		JUVENILES	1	0	0.00
	Trachemys scripta	ADULT MALES	115	4	3.48
		ADULT FEMALES	106	9	8.49
		JUVENILES	21	0	0.00
	Apalone spinifera	ADULT MALES	25	1	4.00
		ADULT FEMALES	50	3	6.00
		JUVENILES	9	0	0.00
VIRGINIA	Chelydra serpentina	ADULT FEMALES	168	6	3.57
		JUVENILES	2	0	0.00



Figure 1. X-ray of a gravid pond slider (*Trachemys scripta*) captured in Tennessee containing a fish hook. Image has been enhanced to improve hook visibility. *doi:10.1371/journal.pone.0091368.g001*

Figure 2. X-ray of a snapping turtle (*Chelydra* serpentina) captured in Tennessee containing a fish hook. Image has been enhanced to improve hook visibility. *doi:10.13711journal.* pone.0091368.g002

total of 170 *C. serpentina*. Of the 168 adult females from this sample, 6 (3.6%) contained ingested hooks (Table 1).

In all but one instance, ingested hooks appeared to be J or circle hooks (as depicted in Figs. 1 and 2) and were present in the esophagus or abdomen. A gravid *A. spinifera* from Tennessee captured on 4 June 2012 contained a treble hook. This individual was re-captured on 27 July 2012 and X-rayed again as part of the independent reproductive ecology study; we noticed she was no longer gravid and contained a J-hook in addition to the treble hook, which had not appreciably shifted its location or orientation (Fig. 3). A female *T. scripta* from Tennessee and a female *C. serpentina* from Virginia both contained two hooks. Another *T. scripta* from Tennessee contained a hook and a barrel swivel. We also observed small (<10 mm in diameter) metal pellets in the jaw region (Fig. 4) of two *C. serpentina* from Virginia, including the individual that had swallowed two hooks (not pictured). We believe these pellets are associated with the recreational shooting of turtles (e.g., "plinking") (24).

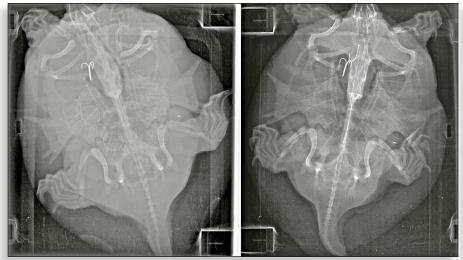


Figure 3. A spiny softshell (*Apalone spinifera*) first captured on 4 June 2012 (left) while gravid with eight eggs and containing one treble fish hook and again captured on 27 July 2012 (right) with an additional fish hook. A Passive Integrated Transponder (PIT tag) is visible in both X-rays. Image has been enhanced to improve hook visibility. *doi:10.1371/journal.pone.0091368.g003*



Figure 4. X-ray of a gravid snapping turtle (*Chelydra serpentina*) captured in Virginia containing a metal pellet in its jaw. *doi:10.13711journal.pone.0091368.* g004

For Tennessee turtles, hook presence or absence was significantly affected by the interaction between species and carapace length (Table 2). Post hoc within-species analyses investigating only the effects of carapace length showed that large T. scripta were more likely to contain hooks than were small T. scripta $(F_{1,235} = 6.11, P = 0.014;$ Fig. 1). In contrast, carapace length did not affect hook presence or absence in either A. spinifera $(F_{1.80} = 0.05, P = 0.825; Fig. 3)$ or *C. serpentina* $(F_{1.16} = 6.11, P = 0.11)$ 0.271; Fig. 2). We did not examine the effect of carapace length on hook presence/absence in S. odoratus alone because no S. odoratus contained hooks. For Virginia female C. serpentina, hook presence or absence was significantly affected by carapace length ($F_{1,237} = 6.65$, P = 0.011; Fig. 4); larger turtles were more likely to contain ingested hooks than were smaller turtles. Power analyses confirmed that our sample sizes were sufficient to avoid committing type II errors; in all comparisons, the probability of rejecting false null hypotheses was greater than 99.9%.

TABLE 2. RESULTS OF MIXED-MODEL LOGISTIC REGRESSION ANALYSES OF THE PRESENCE AND ABSENCE OF FISH HOOKS IN TURTLES FROM THE CLINCH, EMORY, AND TENNESSEE RIVERS, TENNESSEE

SOURCE	NUMERATOR df	DENOMINATOR df	F	Р
CARAPACE LENGTH	1	419	0.12	0.728
SEX	1	419	0.02	0.899
SPECIES	3	419	1.43	0.233
CARAPACE LENGTH x SPECIES	3	419	8.32	<0.001*
CARAPACE LENGTH x SEX	1	419	1.83	0.177
SEX x SPECIES	3	419	0.88	0.451
CARAPACE LENGTH x SEX x SPECIES	2	419	1.08	0.342

Asterisk indicates factors significant at a = 0.05. doi:10.1371/journal.pone.0091368.t002

Discussion

Recreational activities have the potential to negatively influence freshwater turtles, a group that faces a myriad of additional conservation threats (26-28) that may act in concert to imperil their populations. Here, we add to the body of knowledge regarding freshwater turtle conservation by reporting the proportions of freshwater turtles captured at our study sites that contained ingested fish hooks. Given the injuries associated with hook ingestion in other taxa (e.g., [16-18]), our data suggest that recreational fishing is a potential anthropogenic threat for this imperiled group. However, our study likely underestimates the total proportions of the freshwater turtle populations that ingested fishing tackle because the turtles we identified as containing hooks are only those individuals that swallowed hooks, escaped or were released by anglers, and survived the time from being hooked until time of capture in our study without expelling the hook. In addition, in areas where turtles are intensively har-

vested (recreationally or commercially) via baited hooks (e.g., [11]) or where fishing pressure is higher than in our study sites, the proportions of turtles with ingested hooks could be considerably higher than we observed.

The likelihood of a hook being ingested by a sea turtle may be influenced by the species, the size of the animal, and the type and size of the hook (29). In Tennessee *T. scripta* and Virginia *C. serpentina*, we demonstrated that relatively large turtles are more likely to contain an ingested hook than smaller individuals. Potential reasons for size effects on hook presence or absence for these turtles include gape limitations, the possibility that larger, older turtles have had a longer period of time to accumulate fishing gear, and/or that small turtles die relatively quickly after ingesting hooks, making them less available for capture (e.g., [30,31]). Adult females represent the demographic class that is most important for population persistence (19,20); because adult females grow larger than males in most freshwater turtle species (but notably, not *C. serpentina*), this group may be disproportionately vulnerable to ingesting fish hooks, as they are to boat propeller collisions and road mortality (28,32,33).

We did not observe size effects in Tennessee A. spinifera or C. serpentina, suggesting that large turtles may not be more vulnerable to hook ingestion than small turtles are in all species or populations. Given both the results of our power analyses and considerable ranges in body sizes for A. spinifera (CL mean = 25.8 cm, SE = 0.9 range = 13.0-39.7 cm) and C. serpentina (CL mean = 28.2 cm, SE = 1.1, range = 15.2-35.4 cm) as well as *T. scripta* (CL mean = 19.7, SE = 0.2, range = 11.5–25.7 cm), we cannot clearly attribute this inconsistency to an inability to detect size effects in A. spinifera and C. serpentina, if they existed. However, larger sample sizes that included more small individuals may be useful for further examining this potential. Although we did not observe any S. odoratus with ingested hooks, elsewhere they are frequently hooked in the mouth by anglers using baited hooks (DAS personal observation). We suggest that this species is likely too small (8–12 cm in our study) to swallow typical fish hooks, and hooks in the mouth may be removed relatively easily by anglers. In species that grow to large sizes, such as A. spinifera, C. serpentina, and T. scripta, small individuals may also be too small to ingest hooks, but our dataset included few individuals smaller than 11 cm in carapace length. We lack information regarding how hook ingestion affects the physiology and health of freshwater turtles; this is not surprising given the limited studies of the subject pertaining to any taxa (e.g., [34-36]). However, ingestion of fish hooks leads to increased mortality rates in birds, fish, mammals, and sea turtles (18,30,37,38). Sea turtles hooked in the esophagus may experience anything from no observed effects to infections causing systemic septicemia (39). The lining of a sea turtle stomach is thinner than that of its esophagus, and hooks in this region are more likely to result in punctures and coelomitis; if this occurs, mortality is often immediate (36,38,39). For deeply-hooked fish, survival rates are higher when no attempts are made to remove the hook (16); the same may be true for turtles (36). However, fish hook ingestion ultimately increases fish mortality regardless of whether hooks are removed (16). Our knowledge of turtle demography, which includes low annual recruitment and delayed sexual maturity, suggests even small amounts of adult mortality (2-5%) above natural levels may lead to population declines (19,20,40).

Collaboration between researchers and commercial fishing operations has resulted in a relatively large body of knowledge regarding the prevalence of fish hook ingestion by sea turtles (e.g., [41–45]). This information has spurred and

informed conservation recommendations and actions for that group (45–48). However, fish hook ingestion has not been thoroughly investigated as a conservation threat for freshwater turtles (49). More research on the topic is needed to generate a better understanding of this conservation threat, including factors that influence the probability of hook ingestion and its consequences for the health and fitness of individual turtles. In the meantime, land managers, policy makers, and anglers should consider that recreational fishing might be affecting sensitive populations of freshwater turtles.

Supporting Information

Figure S1 A. The relationship between length and mass for pond sliders (*Trachemys scripta*) captured in Tennessee found with or without ingested hooks.

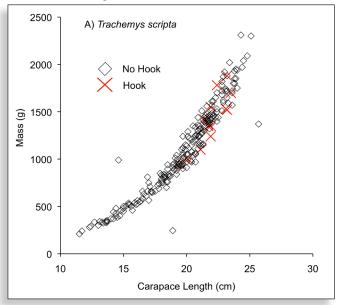


Figure S1 B. The relationship between length and mass for spiny softshells (Apalone spinifera) captured in Tennessee found with or without ingested hooks.

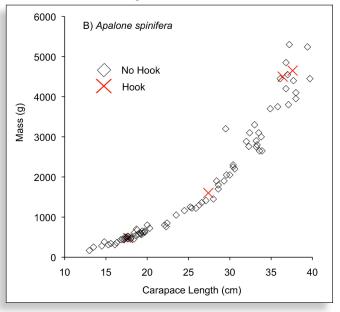


Figure S1 C. The relationship between length and mass for snapping turtles (*Chelydra serpentina*) captured in Tennessee found with or without ingested hooks.

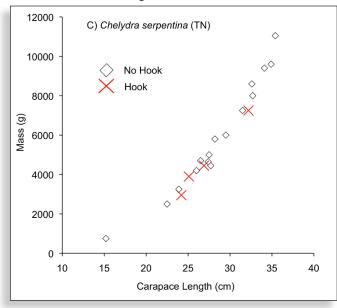
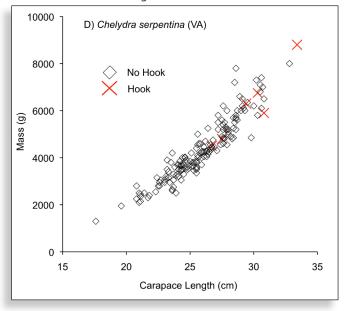


Figure S1 D. The relationship between length and mass for snapping turtles (*Chelydra serpentina*) captured in Virginia found with or without ingested hooks.



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Conceived and designed the experiments: DAS. Performed the experiments: DAS BCH. Analyzed the data: JUVD DAS. Contributed reagents/materials/analysis tools: WAH. Wrote the paper: DAS BCH JUVD WAH.

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Lessons from Managing Manatee Welfare

By Deb Teachout, DVM



am drawn to manatees, belong to Save the Manatees, and in my bucket list I have a goal to someday volunteer at a manatee rehabilitation facility. So I read with interest the following news in the May/June 2014 edition of *All Animals*:

Following the worst year on record for sick and injured manatees, the Jacksonville Zoo and Gardens plans to build a critical care center for the endangered animals. There are currently only three such centers in the state, often making for a long and difficult journey by truck. The new care center, slated to open within a year, will have pools with floors that can be raised and lowered for treatment and a life support system to heat and clean the water. Once recovered, the manatees will be returned to the wild. (1)

This news is cause to celebrate because the manatees will acquire a top notch rehabilitation facility, and it's heartening to see zoos embrace rehabilitation and release, especially in those species that require very special accommodations. On the other hand, this news concerns me because 2013 was the worst year on record for sick and injured manatees. Eight hundred thirty manatees (about 16% of Florida's manatee population) succumbed to boat strikes, entanglements, entrapment, red tide, cold stress, habitat loss, and a mysterious wildlife die-off at a place called Indian River Lagoon. One hundred seventy-three of the manatees were breeding females. The species has suffered increasingly high numbers of deaths since 2009. Clearly manatee rehabilitation and release has never been more important.

On the same day that I read the *All Animals* report, I saw an article in the Huffington Post entitled "Conservative Group Sues to Strip Manatees of Endangered Status" (2). On April 30, 2014, the Pacific Legal Foundation, a group that focuses on property rights, limited government, and individual liberty, sued the US Fish and Wildlife Service to reclassify the manatee from endangered to threatened. If downlisted, some predict that it could serve as the first step toward removing the manatees' protected status completely. According to Pat Rose, Executive Director of the Save the Manatees Club, the protections should not be relaxed particularly since the species has suffered such a high number of deaths in the past few years. "We think there's no justification for downlisting them at this point," he said (2).

In Florida, never before has the number of dead manatees been so high, nor the decades-long controversy over their protection been more intense. It is greatly ironic that this large, nonaggressive, sea herbivore that evolved with no natural predators could evoke so much public contention. The manatee has become the epicenter of a controversy between boaters, developers, and property rights advocates versus nature lovers, environmentalists, animal protection groups, and scientists.

As rehabilitators, we rehabilitate and release animals, and we educate the public. Should we do more? What about reporting animal abuse or harassment to proper authorities? What about supporting protective legislation? What about getting political? What about actively opposing groups who threaten to make conditions worse for the animals we care for? What are our ethical obligations? We all have our comfort levels with legal or political activities, and each circumstance is different.

As wildlife rehabilitators, we may not want to be in the middle of a controversy, but sometimes we find ourselves involved. Maybe we work on high profile endangered species, or maybe we are asked to help rescue and rehabilitate wild animals that are injured in a well publicized oil spill, or maybe we find ourselves suddenly prohibited by state law from rehabilitating seven common species and the public is outraged. We find ourselves thrust into a larger role-advocates and public spokespersons.

Manatee rehabilitators in Florida certainly have rehabilitation and release under control—look at that new facility at the Jacksonville Zoo. And certainly they have CONTINUED ON PAGE 28

SELECTED ABSTRACTS

Nutritional support of reptile patients

De Voe, R. S. 2014. Veterinary Clinics of North America: Exotic Animal Practice 17(2): 249–261. doi: http://dx.doi.org/10.1016/j. cvex.2014.01.009

One of the most amazing characteristics of ectothermic animals is their gastrointestinal physiology and ability to efficiently process calories. Similarly sized mammals and reptiles use dramatically discordant amounts of calories and nutrients to function and grow. Though it is difficult to make broad statements regarding such huge and varied taxa, a generally accepted rule of thumb is that reptiles have approximately one-tenth of the energy requirements of a comparably sized mammal.

Because of the unique reptilian gastrointestinal physiology and energy metabolism, veterinarians are often confused about how to approach the nutritional support of ill reptiles. Many veterinarians and reptile keepers think that because reptiles in health do not eat as frequently as mammals or birds, they can withstand the same kind of fasting intervals when clinically ill. Therefore, the tendency can be to allow ill reptiles to go considerable lengths of time before nutritional support is instituted. In many cases, short intervals of anorexia are not clinically important to the reptile but, in other cases, effective nutritional support can be the deciding factor as to whether or not treatment is successful.

Effect of rehabilitation on survival rates of endangered Cape vultures

Monadjem, A., K. Wolter, W. Neser, and A. Kane (2014). *Animal Conservation* 17(1): 52–60. doi: 10.1111/acv.12054

The rehabilitation of injured or poisoned birds, including raptors, is widely practiced even though its conservation value is not well understood. In this study, the survival rate of rehabilitated Cape vultures (*Gyps coprotheres*) released back into the wild was compared with that of wild-caught birds at a breeding colony in South Africa. The program MARK was used to model survival based on age, sex and whether they were rehabilitated or wild-caught for 405 individual birds. Despite receiving treatment, rehabilitated birds suffered significantly lower survival rates when compared with wild conspecifics of identical age. Annual survival rates (±sd) of rehabilitated and wild-caught birds were 74.8% (±8.1%) and 91.3% (±6.3%), respectively. In addition, a population dynamics model was developed to predict future trends based on varying proportions of rehabilitated and wild-caught birds. The population growth rate (λ) for a wild population (i.e., without any rehabilitated individuals) was greater than one or increasing, whereas that for an entirely rehabilitated population was less than one or declining. A stable growth rate, λ = 1, occurred when approximately 50% of the adults were rehabilitated. Together, our results underscore the importance of tackling the causes of these injuries to Cape vultures before rehabilitation becomes necessary.

Balancing the need for conservation and the welfare of individual animals

Beausoleil, N. J., M. C. Appleby, D. M. Weary, and P. Sandøe. (2014). *In:* Dilemmas in Animal Welfare; p 124-147.

Human activities and climate change have contributed to a dramatic decline in populations and species, and conservation activities are required to slow this decline. Conservation of nature is considered worthwhile by many, but for different reasons. This means that ideas about our moral obligations to protect nature, including our obligations to individual wild animals, vary. Because of this, no simple environmental ethic is likely to be adequate to guide practical decision making in conservation, particularly in situations where the protection of ecological wholes (e.g., species) impacts negatively on individual animals. Here, a practical "ethical" approach is suggested that accommodates both the desire to conserve nature and concerns about the welfare of individual wild animals. According to this approach, our main obligation is to those sentient wild animals in whose lives we have interfered. In undertaking conservation activities that may harm individual wild animals, we are obliged to maximize the benefits of those activities and minimize any negative welfare impacts. This can be done by evaluating the relative impacts of various existing methods, choosing the most humane method, applying it in the best possible way and continuing to research more humane alternatives. This approach is illustrated by the case of the lethal control of possums in New Zealand using toxic agents. The general advantages and limitations of this "compassionate" approach to conservation are discussed. With the continuing "shrinking of the wild," consideration of animal welfare will become increasingly important, not only to justify conservation activities but also for achieving conservation goals.

Monitoring of fungal loads in seabird rehabilitation centers with comparisons to natural seabird environments in northern California

Burco, J. D., J. Gregory Massey, Barbara A. Byrne, Lisa Tell, Karl V. Clemons, and Michael H. Ziccardi (2014). *Journal of Zoo and Wildlife Medicine:* 45(1): 29-40.

Aspergillosis remains a major cause of mortality in captive and rehabilitated seabirds. To date, there has been poor documentation of fungal (particularly Aspergillus spp.) burdens in natural seabird loafing and roosting sites compared with fungal numbers in rehabilitation or captive settings and the various microenvironments that seabirds are exposed to during the rehabilitation process. This study compares fungal, particularly Aspergillus spp., burdens potentially encountered by seabirds in natural and rehabilitation environments. Differences among the various microenvironments in the rehabilitation facility were evaluated to determine the risk of infection when seabirds are experiencing high stress and poor immune function. Aspergillus spp. counts were quantified in three wildlife rehabilitation centers and five natural seabird loafing and roosting sites in northern California using a handheld

impact air sampler and a water filtration system. Wildlife rehabilitation centers demonstrated an increase in numbers of conidia of Aspergillus spp. and Aspergillus fumigatus in air and water samples from select aquatic bird rehabilitation centers compared with natural seabird environments in northern California. Various microenvironments in the rehabilitation facility were identified as having higher numbers of conidia of Aspergillus spp. These results suggest that periodic monitoring of multiple local areas, where the birds spend time in a rehabilitation facility, should be done to identify "high risk" sites, where birds should spend minimal time, or sites that should be cleaned more frequently or have improved air flow to reduce exposure to fungal conidia. Overall, these results suggest that seabirds may be more likely to encounter Aspergillus spp. in various microenvironments in captivity, compared with their native habitats, which could increase their risk of developing disease when in a debilitated state.

Putting animals back together, taking commodities apart

Collard, R. C. 2014. *Annals of the Association of American Geographers* 104(1): 151–65. doi: 10.1080/00045608.2013.847750

Each year, ARCAS Wildlife Rehabilitation Center in northern Guatemala receives 200 to 700 animals: cardboard boxes stuffed with baby parrots, crates full of lizards, monkeys with leashes ringing their necks. Many of these animals were confiscated while being smuggled for the pet trade. Seized animals represent a fraction of overall trade (legal and illegal) in and out of Guatemala and of global trade, worth tens of billions of dollars annually. Forming wild animals into companion commodities in these bioeconomic circuits involves severing them from their social, ecological, and familial networks and replacing these systems with human-provided supports: food, shelter, and diversion. Many of these commodities fail because the animals die. For the few animals that are confiscated alive, rehabilitation for return to the wild is a form of decommodification attempted through various misanthropic practices-actions and routines designed to instill in animals fear and even hatred of humans-that aim to divest animals of human ties. This article draws on participant observation and interview fieldwork and socioeconomic scholarship to critically examine the dual processes of making and unmaking lively companion commodities. It suggests that commodification and decommodification are not processes of "denaturing" and "renaturing," respectively. Rather, following Haraway and Smith, they are both productions of particular natures. This article considers the differential contours and subjects of these natures, as well as their ecological and ethical stakes, concluding by suggesting that the collapse of the culture-nature dualism should not preclude acknowledgment of nonhuman animals' wildness and the violence that can attend its attrition.

Captive husbandry and veterinary care of northern New Zealand dotterels (*Charadrius obscurus aquilonius*) during the CV Rena oil-spill response

Gartrell, B. D., R. Collen, J. E. Dowding, H. Gummer, S. Hunter, E. J. King, *et. al.* 2014. *Wildlife Research* 40(7): 624–32. http://dx.doi. org/10.1071/WR13120

Context: Oil spills cause significant detrimental impacts on many shoreline species. There is limited information in the scientific literature about the management and response of shorebirds to oil spills. Northern New Zealand dotterels (*Charadrius obscurus aquilonius*) were pre-emptively captured as part of the oiled wildlife response to the container vessel Rena oil spill, to ensure the survival of a regional population should there be a catastrophic release of oil. Previous attempts to hold dotterels in captivity have resulted in high mortality.

Aims: To describe the captive husbandry and veterinary management of wild-caught adult dotterels, to outline the common problems encountered, and to make recommendations for future captive management.

Methods: The dotterels were caught by noose mat on beaches at risk of further contamination by oil. Initially, dotterels were kept individually indoors and forcefed until they converted to self-feeding on a diet of an artificial insect analogue, ox heart, and mealworms. Once self-feeding, the birds were shifted to individual outdoor aviaries.

Key results: Sixty dotterels were caught. About half of the birds had oil contamination of the legs, nine birds had light oil staining of feathers and only three of these birds required washing. The degree of oiling and washing did not affect survival. Dotterels took a median of 5 days (range 1-15 days) to convert to the captive diet. Common problems encountered in captivity included carpal and beak abrasions (61.7%) and pododermatitis (75%); however, these did not affect survival. Seven birds (11.7%) developed respiratory disease and six of these died from aspergillosis. The incidence of aspergillosis increased with length of time in captivity and was largely refractory to treatment. The 54 surviving birds were released at their capture sites after a median time of 49 days in captivity (with a range of 39-61 days).

Conclusions: The captive management of the dotterels achieved a 90% survival rate over a period of about 2 months. Deaths were solely due to respiratory aspergillosis, but intensive captive husbandry was required to convert the birds to a captive diet, to minimize traumatic injuries and to manage pododermatitis.

Implications: Although the captive management of shorebird species as a preemptive strategy to minimize the effects of oil spills carries significant costs and risks to the birds, it should be considered in the emergency management of high-priority species.

In the News

CONTINUED FROM PAGE 6

16 books for adults and children and many magazine articles. Her work has been recognized by New Jersey Press Women, the Humane Society of the United States, the Doris Day Animal Foundation, and the Dog Writers Association of America.

Key Wilde is a visual artist who also writes and performs original music. The book and CD *Rise and Shine* by Wilde and Mr. Clarke received a 2010 Parents' Choice Gold Award. Wilde designed and illustrated the long-running exhibition "Wildlife Wonders! The Animals of Central Park," for the Central Park Conservancy.

Final Kakapo Eggs Hatch

CODFISH ISLAND, New Zealand (March 17)—A kakapo chick has hatched in the wild on Hauturu o Toi/Little Barrier Island, less than two years after a small adult population was re-introduced to the island.

The arrival of Heather One there and five hatchings on Whenua Hou/Codfish Island puts the total number of kakapo chicks to hatch this season at six. A seventh died last week, a few hours after hatching on Whenua Hou/Codfish Island.

Kakapo Recovery program manager Deidre Vercoe Scott said all chicks were doing well, but the team was particularly thrilled with the success on Hauturu o Toi/Little Barrier. "Kakapo were first introduced there in 1982 and had some success breeding there, although they needed supplementary food."

They also needed protection from the kiore (Pacific rat). In 1999, all kakapo were removed so the rats could be eradicated from the island.

Nine kakapo were transferred to Hauturu o Toi/Little Barrier Island since 2012, this time without supplementary food.

The successful mother, Heather, mated three times with Dobbie. Both had previously lived on the island. She produced three eggs; two hatched. The younger chick was transferred to Whenua Hou/Codfish Island to ensure it wouldn't have to compete in the nest for food.

Heather One was discovered by Dept.

of Conservation staff soon after it hatched on Wednesday night. There were concerns about its safety as ex-cyclone Lusi made its way across the Pacific.

"Fortunately, Heather's nest was in a relatively sheltered spot away from any creek that had the potential to flood. We also checked the site for loose branches and dug extra drainage around the site."

Meanwhile, on Whenua Hou/Codfish Island, two of the five chicks there have been fostered out to kakapo mothers who have been sitting on artificial eggs.

The Kakapo Recovery team provides intensive monitoring to ensure the chicks are fed and healthy. The other three are being cared for in incubators and being hand-fed, Ms. Vercoe Scott said.

Public Response to Diesel Spill Leads to Reversal of Agency Decision and Rescue of Waterbirds

VANCOUVER ISLAND, British Columbia (*May 8*)—On May 8th, 2014, an oil spill occurred in Esquimalt. As a consequence, six mallard ducks were contaminated with what is believed to be diesel fuel. Following the incident, the Oiled Wildlife Society of BC was contacted to perform a field assessment of the area and identify wildlife that would have been contaminated. The report and a recommendation to activate an oiled wildlife response was submitted to the provincial and federal governments.

The spill's source is unknown, so a responsible party could not be identified to cover costs of OWS's care for the birds, so the decision to activate a response remained with the Canadian Wildlife Service, who has jurisdiction over migratory birds. CWS recommended no further action. Following the decision to leave the oiled birds in the field, the Oiled Wildlife Trust, of which OWS and WRNBC are members, sent a press release to local media to inform the public of the decision that was made. The issue gained considerable attention and the provincial government has stepped up to finance OWS's oiled wildlife response. The operation is ongoing, and OWS has been working closely with the Environmental Emergency Response Staff to provide care to the oiled mallards.

Manatees CONTINUED FROM PAGE 25

public education under control-check out this extremely well done educational video entitled *Give Manatees Sanctuary at Kings Bay*, about the careless behavior of humans towards manatees at that site. Because of growing political threats to the legal protection of the manatee, however, advocates and rehabilitators increasingly find themselves in additional roles interfacing with law or politics.

I compare this to the puppy mill issue. For so many years, I medically treated these unfortunate puppies, and released them back to their heartbroken and now educated new owners. But the puppies just kept coming, and I felt I had to do more. I stepped way out of my comfort zone. I found my voice, got political, lobbied, spoke at hearings, collected signatures, and supported anti-puppy-mill legislation. It's stressful, time consuming, and sometimes you feel like quitting, but you don't because many animals' welfare is at stake.

The story of the manatee's long and controversial struggle for protection may be played out with other species we rehabilitate. As human population increases, and threats to the welfare of our wildlife keep pace, more species will become similarly threatened or endangered. The welfare of these animals will require more than our compassion and hands-on rehabilitation skills.

We have to be ready to do more.

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Snapping turtle (Chelydra serpentina) on the move.



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