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IN THIS ISSUE: LINKS BETWEEN HUMAN-ALTERED ECOSYSTEMS AND CHANGES IN WILDLIFE DISEASE...ESSENTIAL VITAMIN A LEVELS IN HARBOR SEAL PUPS FED REHABILITATION DIETS...WEST NILE VIRUS INFECTION IN KILLER WHALES...PUBLIC/WILDLIFE CONFLICT RESOLUTION VIA VIRTUAL HOTLINE.

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:
Harbor Seal (*Phoca vitulina*).
PHOTO © EINAR GUDMANN (WWW.GUDMANN.IS). USED WITH PERMISSION.

Left:
Burrowing owl pair *(Athene cunicularia hypugaea)*.
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JOURNAL OF WILDLIFE REHABILITATION

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DEPARTMENTS

Continuing Evolution

As practitioners in a constantly changing field, we see the importance of continuing education. But what form(s) of continuing education should we pursue? What, exactly, is continuing education? The 2011 online Oxford Dictionaries describes it as "education provided for adults after they have left the formal education system, consisting typically of short or part-time courses." This provides a basic definition, but does not touch on the variety of forms useful to those of us in evolving professions. Continuing education can be anything from job-shadowing fellow rehabilitators to participating in an online course.

The key to effective continuing education is mixing and matching various forms to provide different aspects of learning. Peer-reviewed journals and online courses impart updates on current research and best practices. These easy and relatively inexpensive forms do not provide a large amount of interaction with colleagues. Job-shadowing other rehabilitators offers a chance to see alternative methods in practice and strengthens relationships, but it lacks the quality control provided by peer review. Attending conferences and multiday workshops provides both contact with colleagues and quality assurance. On the down side, they tend to be more expensive and also require time away from a busy and, perhaps, understaffed center.

This August I attended the Association of Avian Veterinarians (AAV), Association of Exotic Mammal Veterinarians (AEMV), and Association of Reptilian and Amphibian Veterinarians (ARAV) conferences. The three organizations all had their various meetings and workshops at one hotel, over one week, with only one registration needed. The event provided stimulating conversation, relaxing interludes, introductions to new products and technology, hands-on practice in unfamiliar techniques, updates on research, and lectures from leaders in exotic veterinary medicine. What am I getting at? The benefits of attending a conference or symposium far outweigh the costs.

Not so coincidently, the IWRC Symposium is coming up quite soon, the 2nd week of November. We will have speakers from five different countries talking about everything from research on the effects of oil ingestion on future wildlife behavior, and lessons learned in the rehabilitation of various oiled birds, to coyote enclosure design and other tools for use in daily wildlife rehabilitation. The final afternoon will take place at the South Florida Wildlife Center, where we will tour the center and hear talks from the executive director and all three veterinarians on staff before enjoying a casual meal. Throughout the symposium, there will be plenty of time for networking, meeting new colleagues from near and far, and enjoying the Florida sunshine.

So, hopefully you can make it to the IWRC Symposium, but if it is out of the realm of possibility for you, then check the IWRC Resource section for *Conferences, Education, and Opportunities.* There, I have a list of upcoming conferences and other events around the world. Above all, enjoy this and other journals, take courses, network with peers, and keep learning!

Kai Williams
Executive Director
IWRC

Phillip Island Rehab Center Opens

MELBOURNE, Victoria, Australia (August 22, 2011) —Phillip Island's little penguins (*Eudyptula minor*) are in safe hands with the official opening of the island's new wildlife rehabilitation center. Environment Minister Ryan Smith said the center had the capacity to care for up to 1,500 little penguins and other injured wildlife.

"The center will provide an outstanding level of support for the Phillip Island little penguin colony and is a credit to the hard work of the local community and staff at the Phillip Island Nature Park," Smith explained. The penguin parade attracts more than 500,000 visitors each year and pumps more than AU$107 million into the Victorian economy.

New Brunswick Gets Wildlife Phone Service

MONCTON, New Brunswick, Canada (August 20, 2011)—With CA$77,000 of financial support from the TD Friends of the Environment Foundation, the Atlantic Wildlife Institute (AWI) will be implementing a Wildlife Response Network to help New Brunswick residents deal with wildlife problems and emergencies. AWI will train 30–35 volunteers each in Moncton, Saint John, and Fredericton.

Barry Rothfuss, executive director and cofounder of AWI, said the network is intended to take rehabilitation to the next step, where the organization makes it a valued part of their management protocol. AWI has been the main resource for rehabilitation and wildlife displacement issues in the area for 16 years, but never had the resources to give every referral the time it needs.

Without the response network, Rothfuss believes AWI may have been missing "the opportunity to gain information about bigger issues like toxicity or disease or things like infringement [of] habitat laws. These animals are telling us a story," he explained. The network is scheduled to begin operations in October.

Mesaba Veterinary Hospital Opens for Rehabilitation

HIBBING, Minn., USA (August 19, 2011)—The Mesaba Animal Hospital has opened the first rehabilitation center on Minnesota's Iron Range. Natalie Kellar, DVM, says Fox One Wildlife Rehabilitation was started to provide veterinary care for injured wildlife, including surgical treatment if necessary and euthanasia if the animal is not releasable. The center has already treated a bear cub. Organizers also plan to start a nonprofit organization to support the effort.

Bradshaw Found Not Guilty in Raccoon Trap Theft

RICHARDSON, Texas, USA (August 17, 2011)—A Richardson municipal judge called a halt to the trial of a Plano wildlife rehabilitator charged with theft, declaring her not guilty after the prosecution failed to show enough evidence to even send the case to the jury to deliberate. Bonnie Bradshaw had been charged with theft after rescuing a raccoon caught in a local pest control company's trap and left in the hot sun in near-100°F heat for almost 12 hours.

On June 23, Bradshaw was summoned to an apartment complex by resident Stacey Hughes, who had seen the raccoon in the trap at 7 a.m. that morning, notified the complex's maintenance man, and asked him to call animal control. But when she returned home around 6 p.m., the raccoon was still in the trap, barely alive, unable to even raise his head. Hughes tried unsuccessfully for nearly an hour to find help for the raccoon, even trying to give the raccoon food and water through the bars of the trap, until her daughter found Bradshaw listed online.

According to cell phone records, Bradshaw arrived a few minutes after 7 p.m. and found the raccoon near death and still in the trap. She located two leasing agents, who told her they weren't even aware there were traps on the property. She gave them her business card and told them she was taking the raccoon and would try to save it. Bradshaw was able to get the raccoon cooled and rehydrated and released it later that same evening.

The next day she took the trap, along with another one she'd found on the apartment property, to Richardson Animal Services and dropped them both off, explaining the situation and asking for an investigation into possible animal cruelty charges against the trapper for leaving a

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IN MEMORIAM

WENDY FOX (1957-2011)

Wendy Fox, age 54, passed away August 6, 2011 in Miami, Florida after a long battle with cancer.

Wendy was Executive Director of Pelican Harbor Seabird Station for 10 years. She retired in June, and her son, Brian Fox, took over her post. She is survived by her husband, Jeff; mother, Joan; children, Mieke and Brian, and grandchildren, Makayla and Emily.

Wendy led The Seabird Station to become a mentor to centers in the region, training wildlife rehabilitators in the US Virgin Islands and assisting with pelican care along the eastern seaboard. She served as President of the National Wildlife Rehabilitator's Association.

In 2010, Wendy was flown to Louisiana to care for pelicans oiled in the Deepwater Horizon oil spill, some of which went to The Seabird Station for extended care.

The family asks that memorial donations be sent to Pelican Harbor Seabird Station, 1279 NE 79th Street Causeway, Miami, FL 33138-4206.

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trap unattended for at least 12 hours. Later that day, Bradshaw got a call from Richardson police detective Gibson telling her the trapper, Lawrence Menafee of Cridder Control, insisted on pressing charges for theft, even though the traps were at the shelter waiting for him to pick them up.

Detective Gibson testified that he investigated the case thoroughly and, based on the status of the raccoon, Bradshaw had acted in the "best interest of the animal." He also reported Bradshaw had forwarded a recording to him of a threatening phone message left on her voicemail from Menafee.

Menafee took the stand at one point and testified that he had checked on the traps at the apartment complex around 5:45 on June 23 and, by that time, they were gone. He said he contacted 911 and filed a police report immediately. When questioned about leaving a trap unattended for so long in the heat, Menafee stated that he had complied with all local ordinances and even placed the trap under a sprinkler so if an animal was in the trap, it could "catch the water."

Menafee contradicted Detective Gibson's testimony, telling jurors that he had never contacted Bradshaw. Menafee claimed that the traps she had taken were vital to his business and his ability to make a living. Bradshaw's attorney, Randy Turner, however, pointed out that Menafee did not pick up the traps from Richardson Animal Services until nearly three weeks later. Menafee will be back in court on Monday, this time as the defendant, facing charges of animal cruelty.

Battle Brews Over Wildlife Center

VIRGINIA BEACH, Va., USA (August 17, 2011)—Two wildlife rehabilitation groups based in Virginia Beach are vying for the right to manage a new refuge on 50 acres of wooded land. Wildlife Response, a local volunteer group, is at capacity at its current facility, so many members are caring for animals in their homes. They came up with the idea to build a new center, according to vice president Billy Haynie, and identified the new property.

[Personnel at] the Virginia Beach SPCA say it's a better choice to manage such a facility. Teresa Lamarche, the SPCA's community outreach director, explained that her organization cares for "any and all kinds of animals," whereas Wildlife Response "focuses on a certain few." Lamarche went on to say that, unlike Wildlife Response, the SPCA's plan includes the majority (75%) of wildlife caretakers in the area. Wildlife Response counters that those numbers aren't true.

The mayor of Virginia Beach has said he would like to see both groups working together on the project, but when Haynie was asked if that could be an option, he said there was a meeting but it didn't go well, and left it at that.

The City Council will make a final decision on which group will run the new center in late September.

Environmental Fine Benefits Red-Tailed Hawk

EDMONTON, Alberta, Canada (August 10, 2011)—A fine paid by Canadian National Railway (CN Rail) following a 2005 derailment has been used to aid the recovery of a red-tailed hawk that had its feathers burned by a an Edmonton landfill methane flare last December.

Barely alive when it was found, the bird was treated at the Wildlife Rehabilitation Society of Edmonton (WRSE) and released on August 10 by Cody Brown, the landfill worker who rescued the hawk.

WRSE treats [more] than 1,000 animals annually. Two years ago, the organization received CA$600,000 of the CA$1.4-million fine levied against CN Rail after the company pleaded guilty to three charges in the August 2005 derailment that resulted in 196,000 liters (~50,000 gallons) of heavy oil and pole-treading oil spilling into Lake Wabumun, about 50 kilometers west of Edmonton.

The money paid for conversion of donated trailers into a 370 m^2 state-of-the-art animal healthcare facility. Holly Duvall, the WRSE's animal care and project manager, said the money has had a huge impact. As a result of the new center improvements, the number of animals treated by the Society is increasing each year. The landfill hawk spent eight months recovering at the facility.

Petition aims to change Montana's policy of killing orphaned deer

MISSOULA, Mont., USA (August 15, 2011)—'Lucky' was an orphan fawn who was saved, and named, by a Eureka family following a hit-and-run accident that involved his mother and several other deer. The car tried to swerve but still hit the doe with such force that Lucky was born on the street.

Amy and Chuck Gondeiro saw the crash and found the newborn fawn in a ditch. They wrapped him in a blanket and transported him to a family friend's animal rescue center. There, they called a Montana Department of Fish, Wildlife and Parks (MFWP) warden for help finding a wildlife rehabilitation center. To their astonishment, they learned that Montana policy calls for the killing of all orphaned ungulates.

The policy came about as a result of the state's desire to prevent chronic wasting disease (CWD). There's an MFWP wildlife rehabilitation center in Helena, but it hasn't accepted orphaned deer for a long time. Years ago, the center accepted all wildlife, including ungulates. It was open to the general public and state officials took the animals to county fairs.

"It changed the image of wildlife," Ron Aasheim of MFWP explained. "We're not in the business of running [a] zoo. We manage for populations. Saving a fawn is something we'd like to be able to do, but the risk now is too great. CWD is a disease you don't want with any prevalence in your state."

Lucky didn't live up to his name after all. His demise stirred up a great deal of emotion in animal advocates across the country. Joan Schaffner, a law professor and director of the George Washington Animal Law Program, started a petition to encourage Montana to change its policy and allow more flexibility in deciding the fate of future Luckys. Three weeks after its launch, 1,400 people have signed the petition. ■

Impacts of Anthropogenic Changes on Wildlife Disease

Kristin Madden, M.A., C.W.R.

Great Egret *(Ardea alba).* Ormond Beach Wetlands, Port Hueneme, California, USA.

ABSTRACT: For thousands of years, humans have recognized connections between the environment and outbreaks of disease. In recent years, anthropogenic changes to ecosystems have also been linked to changes in wildlife diseases, some of which are zoonotic. Wildlife rehabilitators are frequently the first to observe emergent diseases or changes in the routine presentation of known disease. As a result, it is important for wildlife rehabilitators to stay current on disease trends. This paper reviews available literature to provide an overview of the impact of anthropogenic changes on wildlife disease as well as the need for information sharing among wildlife professionals.

KEY WORDS: anthropogenic change, emergent disease, wildlife disease

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Introduction

For thousands of years, humans have recognized connections between the environment and outbreaks of disease (Keesing 2008). Linthicum *et al.* (1999) showed that heavy rainfall in Kenya resulted in outbreaks of Rift Valley fever. Lyme disease can be predicted from acorn production (Ostfeld *et al.* 2001). Wildlife disease ecology is clearly associated with changes in ecosystems (Ostfeld *et al.* 2008).

In recent years, anthropogenic (human-caused) changes to ecosystems have been linked to changes in wildlife disease. Changes such as eutrophication (an increase in the nutrient load of a body of water that promotes the growth of plants, particularly algae, and depletes oxygen levels), habitat loss, the introduction of exotics, and even ecotourism can lead to increases in mosquito-borne disease, parasite loads, and so forth.

Wildlife rehabilitation is a growing field, with centers in every American state and in countries throughout the world. As a result, wildlife rehabilitators are being recognized by the scientific and medical communities as a potentially valuable source of information on wildlife health and disease prevalence (Siembieda 1994; Duncan *et al.* 2008; Randall 2011). Rehabilitators' ability to understand disease, collect useful data, and communicate information effectively may benefit both wildlife and humans.

Impacts to terrestrial species

Influence of habitat loss

Anthropogenic land use that alters landscape and ecological communities influences the diversity and interactions among hosts, vectors, and pathogens and contributes

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to the emergence of disease (Kirkwood 1998; McKenzie 2007; McCallum 2008). Habitat fragmentation (habitat loss resulting from roads and urban development that cuts through habitat and confines wildlife populations to isolated patches of their original habitat) and the conversion of native habitats for agriculture can lead to increases in both parasitic infections and the incidence of mosquito-borne disease (Pope *et al.* 2005; McKenzie 2007). In fragmented and urban habitats, increased host density and contact rates contribute significantly to the emergence and transmission of disease in wildlife populations (Scott 1988; Hartrup *et al.* 1998; Kirkwood 1998).

Landscape structure affects density and movements of hosts, their vectors, and the various stages of pathogens (McCallum 2008). As a result, landscape features play a direct role in the dynamics of infectious disease and wildlife populations (McKenzie 2007; McCallum 2008). Habitat fragmentation can reduce connectivity (the ability of wildlife to move freely among suitable habitats), potentially reducing biodiversity and creating conditions that impact both host and pathogen (McCallum 2008). For example, studies of lemurs (Microcebus spp.) and colobus monkeys (Colobus spp.) documented lower gastrointestinal parasite loads among animals from large habitat fragments than in animals from corresponding smaller fragments (Gillespie and Chapman 2006; Raharivololona and Ganzhorn 2009). Increased host density, contact rates, and changes in trophic (food web) dynamics that often result from habitat fragmentation and urbanization have also been linked to the emergence of wildlife disease including mycoplasmal conjunctivitis, toxoplasmosis, avian trichomoniasis, chronic wasting disease, and rabies (Hartrup *et al.* 1998; Kirkwood 1998; Bradley and Altizer 2006; Ostfeld 2009).

Algae bloom in stagnant water, Ucluelet, Vancouver, British Columbia.

Effects of pollution and stress

Conversion of native habitat for agriculture and grazing pastures does more than fragment the landscape (Pope *et al.* 2005; McKenzie 2007). When converted for grazing, deforested areas have higher pH levels, higher temperatures, and increased nutrients from cattle excreta, leading to eutrophication (McKenzie 2007). Eutrophication is also caused by agricultural run-off, sewage effluent, and run-off from urban areas (McKenzie 2007; Johnson and Carpenter 2008). Eutrophication increases the incidence of mosquito-vectored diseases like malaria (Pope *et al.* 2005) as well as parasite infection in amphibians, invertebrates, and fishes (Marcogliese *et al.* 1990; Moser and Cowan 1991; Johnson and Chase 2004; McKenzie 2007). Severe eutrophication elevates host stress and favors generalist or opportunistic parasites with direct or simple life cycles (Johnson and Carpenter 2008).

Agricultural land use also introduces pesticides, herbicides, and other chemicals into the ecosystem (McCoy *et al.* 2008). While not a disease, exposure of toads to certain herbicides and steroid hormones has been shown to cause reproductive abnormalities that increase in severity with increased exposure (McCoy *et al.* 2008). Pesticide exposure compromises the immune system in a variety of species and increases parasite success among amphibians (Gendron *et al.* 2003; Rohr *et al.* 2008; Crinnion 2009). These heavy parasite loads damage internal organs and further compromise the immune system, leading to population declines (Johnson and Sutherland 2003; King *et al.* 2008).

Impacts to aquatic species

Effects of pollutants

Environmental contaminants in the oceans contribute to the growth of bacteria and its ability to penetrate into the tissues of marine organisms (Mitchell and Chet 1975). Exposure to crude oil and certain industrial pollutants contributes to the growth of predatory bacteria and increases the incidence of coral disease (Mitchell and Chet 1975; Peters 1997). Soil runoff and sewage effluent are the probable causes of influenza B, previously only found in humans, in a harbor seal (*Phoca vitulina*) (Osterhaus *et al.* 2000) and toxoplasmosis, transmitted by domestic cats (*Felis catus*), in a spinner dolphin

(*Stenella longirostris*) and beluga whale (*Delphinapterus leucas*) (Migaki *et al*. 1990; Mikaelian *et al*. 2000). Polycyclic aromatic hydrocarbons, an environmental contaminant known to cause cancer in humans, are believed to be responsible for the tumors afflicting one species of whale (Cohn 2009); cancer is now the second leading cause of death among beluga whales in the Saint Lawrence River estuary in Canada (Cohn 2009).

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Mongoose (*Mungos mungo*) suffered an outbreak of tuberculosis traced to infectious human waste in garbage pits.

Effects of environmental stress

The cause of disease among marine organisms is not limited to contaminants introduced directly into our oceans (Padgett and Glaser 2003; Orr *et al*. 2005; Weilgart 2007). The effects of ocean acidification (caused by their uptake of anthropogenic carbon dioxide from the atmosphere) have wide-ranging impacts on biological processes (Feely *et al*. 2004; Orr *et al*. 2005; Potera 2010). Ocean acidification causes the dissolution of corals, as well as the shells of certain pteropods and bivalves, and is expected to significantly increase environmental stresses on these organisms and on those that depend on them for food (Padgett and Glaser 2003; Orr *et al*. 2005; IAP 2009; Potera 2010). At low pH levels, phytoplankton assimilates less of the iron that is required for photosynthesis (Potera 2010). This could cause dramatic declines in fish populations, thereby increasing environmental stress on the entire food web (Potera 2010).

Environmental stress in the oceans is also caused by marine drilling operations, boat traffic, noise, and tourist activities (Padgett and Glaser 2003; Weilgart 2007; Nowacek *et al*. 2007). This type of stress, on both marine and terrestrial animals, has wide-ranging influences including reproductive success, behavioral changes, immune system depression, and the emergence of disease (Padgett and Glaser 2003; Habib *et al*. 2007; Nowacek *et al*. 2007; Weilgart 2007; Ditchkoff *et al*. 2009). Anthropogenic noise leads to increased stress, heart rate, and blood pressure in wildlife, potentially leading to decreased fitness (an individual's ability to survive and breed; Conomy *et al*. 1998; Schueck *et al*. 2001; Pepper *et al*. 2003).

Even nature-based tourism can cause an increase in stress and disease in marine organisms (Ellenberg *et al*. 2007; Semeniuk *et al*. 2009). Hematological tests of southern stingrays (*Dasyatis americana*) in the waters along Grand Cayman indicated negative physiological consequences as a result of tourist visitation (Semeniuk *et al*. 2009). Similarly, stress-induced corticosterone levels were significantly higher among tourist-exposed endangered yellow-eyed penguins (*Megadyptes antipodes*) in New Zealand (Ellenberg *et al*. 2007). Chronically elevated stress markers can result in higher susceptibility to disease (Siegel 1980; Mullner *et al*. 2004; Ellenberg *et al*. 2007). Additionally, threats to marine organisms may interact with other stressors such as eutrophication, fishing, pollution, climate change, and impacts on the marine food web, thereby producing a larger effect than would each individual stressor alone (Lotze and Worm 2002).

Encroachment and introduction of exotics

The encroachment of humans and domestic animals into new habitats, and the introduction of exotics (nonnative species) into new ecosystems (be they terrestrial or aquatic), have far-reaching effects (Daszak *et al*. 2001; Cutler *et al*. 2010). For example, canine distemper was likely introduced to seals in remote Antarctica and Siberia from domestic sled dogs (Bengston *et al*. 1991; Mamaev *et al*. 1995). International transport of both wild and domestic animals has led to varroasis in honeybees throughout the world (Oldroyd 1999), avian malaria and poxvirus in Hawaii (Van Riper *et al*. 1986), elephant herpes virus in zoos (Richman *et al*. 1999), and avian trichomoniasis in many species worldwide (Sansano-Maestre *et al*. 2009).

The spread of disease in many areas of the world has been attributed to the translocation of animals from outside the local area (Woodford and Rossiter 1993; Gylys *et al*. 1998; Godfroid *et al*. 2005). The repopulation of hunting pens with raccoons (Procyon lotor) that had been trapped in rabies-endemic zones of the southern United States introduced the disease to the mid-Atlantic states in the 1970s (Woodford and Rossiter 1993). In Eastern Europe, raccoon dogs *(Nyctereutes procyonoides)* have been released accidentally from farms raising animals for the fur trade and are now spreading into new habitats; these raccoon dogs have

become a new reservoir for rabies in Lithuania (Gylys *et al.* 1998). Hares imported to Western Europe from central and Eastern Europe for sporting purposes resulted in tularemia outbreaks and the introduction of brucellosis (biovar 2) to Western Europe (Godfroid *et al.* 2005). While biovar 2 occurs mainly in wild boars, pigs, and hares, it has been reported in humans as well, although rarely (Godfroid *et al.* 2005). Since its introduction, this brucellosis strain has spread into the wild boar population of Western Europe and has been reported in pig farms in France (Godfroid *et al.* 2005).

While public health officials worry most about zoonotic transfer of wildlife disease to humans, increased exposure of wildlife to human-occupied spaces can also result in the transfer of diseases from humans to wildlife. For example, an outbreak of tuberculosis in mongoose (*Mungos mungo*) and suricats (*Suricata suricatta*) was traced to garbage pits where these animals were exposed to infectious material from infected humans (Alexander *et al*. 2002).

Information sharing

An investigation of emerging infectious disease in wildlife is important for many reasons. For one thing, a great many humans care about wild animals. According to the National Survey of Fishing, Hunting, and Wildlife-Associated Recreation (U.S. Dept. of the Interior *et al*. 2006), "wildlife watching" activities grew consistently from 1996–2006. Many of these individuals become emotionally involved in the wildlife they see, particularly around their own homes (Wildlife Rescue, Inc. of New Mexico; pers. comm.). In addition, humans can become fearful for their own safety if nearby wildlife appear to be succumbing to disease. A poll of outdoor recreation groups showed that 51% were concerned about infectious disease in wildlife populations; the reasons cited included the public health threat, the impact on wildlife populations, and the potential loss of income for those involved in outdoor-adventure professions (Duncan *et al*. 2008).

Humans are frequently referred to as a sentinel species for zoonotic disease, and the potential threat to human health is very real (Childs and Gordon 2009; Cutler *et al*. 2010). An estimated 60–75% of emerging human pathogens are zoonotic, meaning they are acquired from animals (CDC 2009; Cutler *et al*. 2010). Nearly 75% of these zoonotic pathogens originate in wildlife species (Cutler *et al*. 2010). Avian influenza has gained media attention over the last few years because of its impact on wild bird populations and fears of human pandemics. Contrary to previous scientific belief, it is now clear that direct transmission of avian influenza to humans does occur (de Jong and Hien 2006). In 1997, 18 cases of human highly pathogenic avian influenza cases were identified in Hong Kong, six of which were fatal (Yuen *et al*. 1998; Chan 2002). More than 50 rodent-borne diseases have long been a cause for public health concern (Meerburg *et al*. 2009). In the southwestern United States, Hantavirus pulmonary syndrome (HPS) was identified in humans for the first time in 1993 (Meerburg *et al*. 2009); it has since been reported throughout South America (Meerburg *et al*. 2009). Acquired through the inhalation of contaminated rodent droppings, direct contact with rodent droppings, or rodent bites, HPS is one of the most serious rodent-borne diseases in humans (Meerburg *et al*. 2009).

Because of the potential to impact human populations, the need for procedures to effectively monitor, respond to, and hopefully prevent these diseases is growing (Arthur 2007; Duncan *et al*. 2008; Cutler *et al*. 2010). The ability to integrate human disease monitoring with sampling and monitoring of wildlife, livestock, and domestic animals may play a key role in our ability to handle the next epizootic outbreak (Epstein 2001; Childs and Gordon 2009).

In the United States, individual states or health institutions perform the majority of active surveillance for zoonotic diseases (Childs and Gordon 2009). In addition, passive surveillance involves the collection of information for other purposes, such as wildlife rehabilitation, that are then applied to the investigation of disease outbreaks (Duncan *et al*. 2008). The U.S. Geological Survey coordinates the National Wildlife Health Center (USGS 2010) which tracks disease, funds research, and disseminates information in a variety of ways. The United States Congress funds a Global Disease Detection (GDD) program, run by the Centers for Disease Control and Prevention (Arthur 2007). The goal of the GDD program is to protect humans from the threat of bioterrorism and the natural emergence of infectious disease (Arthur 2007). The GDD centers around the world focus on surveillance, training, research, outbreak response, and networking with other centers and government agencies (Arthur 2007).

A joint project by the World Health Organization, the Food and Agriculture Organization of the United Nations, and the Organisation Mondiale del la Santé Animale is the "Global Early Warning and Response System for Major Animal Diseases, including Zoonoses" (better known as GLEWS). The system was developed to improve surveillance, detection, and responses to animal diseases and zoonotic epidemics for member countries (GLEWS 2010). Currently being tested in Tanzania through the HALI Project (Health for Animals and Livelihood Improvement), the One Health approach is based on the premise that greater health benefits will result from a multidisciplinary paradigm that addresses the multiple causes of human health problems, such as close proximity between animals and humans, as well as issues involving food, sanitation, and water (Mazet *et al*. 2009).

Because of this growing need for accurate, up-to-date information, government agencies and wildlife professionals have a responsibility to network and to educate the public. To be effective, public health communication must involve a variety of local, regional, and federal groups—and it cannot be limited to emergency communications during an outbreak (Stoto *et al*. 2005). The public not only needs emergency information in a timely manner, they also need to believe that management agencies have similar goals; this would result in the necessary perception that these agencies can be trusted to effectively manage for disease (Needham and Vaske 2008). These perceptions of similarity and trustworthiness may also improve the effectiveness of information dissemination

(Needham and Vaske 2008).

Preparedness and prevention are essential if we are to be at all effective in protecting humans, domestic and livestock animals, and wildlife from deadly outbreaks. The gathering and dissemination of information is relatively easy in industrialized countries (Keller *et al*. 2009). However, in parts of the world with limited infrastructure, lack of resources, and conflicts between reporting of infectious diseases and possible effects on trade and tourism, efforts to improve both disease surveillance and information dissemination are a significant challenge (Keller *et al*. 2009). Fortunately, global, national, and local efforts are gaining support, and evaluations of the best methods for various regions are under way (Keller *et al*. 2009).

Conclusion

Anthropogenic change will persist for as long as our species exists. The human ability to alter landscapes has a detectable influence on habitats, wildlife populations, and the ecology of disease. In many areas, this has resulted in an increase in potentially zoonotic diseases. The terrestrial ecosystem has been altered through agriculture, urbanization, and the encroachment of humans and domestic or exotic animals into new habitat areas. These anthropogenic changes modify trophic dynamics and chemical composition of soil and groundwater as well as species densities and contact rates. The marine ecosystem is experiencing anthropogenic change as well, and studies are ongoing to understand better the full impact of petroleum and industrial contaminants, ocean acidification, and human-created environmental stress, all of which are taking a toll on marine organisms, reducing fitness, and increasing disease.

The potential for epizootic outbreaks increases the need for additional research into the complexities of wildlife disease. In addition, disease surveillance is necessary, not only to provide a more complete understanding of where and when disease emerges but also to allow human communities to prevent or minimize epidemics. Humans care about wildlife for their inherent value and for the roles that wild animals play as environmental indicators and potential sources of disease. Because of this, the public relies on wildlife and healthcare professionals for information and assistance. If we are to offer sufficient aid to the public, and to wildlife populations, improved methods of communication and information sharing are equally as important as additional research will be.

Rehabilitators have a unique perspective on wildlife disease due to their understanding of natural history and disease presentation, as well as to the fact that they are often the first to receive wildlife with emergent or mutated disease. As wildlife rehabilitation has grown, improved data collection procedures have developed, and new research has been initiated because rehabilitators recognize the value of scientifically sound rehabilitation data and continued education. This paper was not intended to offer in-depth information on any specific disease or anthropogenic change, but rather to provide an overview of changes that have already occurred in wildlife disease and to motivate wildlife rehabilitators to investigate and learn more. Networking and information sharing among rehabilitators and other wildlife professionals can play a key role in aiding our patients and in making valuable, scientifically sound contributions to the larger community.

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About the Author

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Circulating Retinol and α-tocopherol Levels in Rehabilitating Harbor Seal (*Phoca vitulina*) Pups

Noel Y. Takeuchi, David S. Barber, and Kathryn A. Ono

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ABSTRACT: Retinol (vitamin A) and α-tocopherol (vitamin E) are necessary for proper growth and development. They are essential to create artificial formulas for emaciated harbor seal *(Phoca vitulina)* pups in rehabilitation centers that have experienced abandonment and dietary deficiencies at a young and critical age. Circulating levels of retinol and α-tocopherol were measured in Western Atlantic harbor seal *(Phoca vitulina concolor)* pups consuming various diets during the course of rehabilitation at the University of New England's Marine Animal Rehabilitation Center (MARC) in Biddeford, Maine, United States during the summers of 2006 and 2007. Average serum retinol and α-tocopherol levels were lowest at admit at 0.144 ± 0.03 µg/ml and 14.13 ± 4.09 µg/ml, respectively. Retinol peaked in pups consuming fish (0.32 ± 0.02 µg/ml) whereas α-tocopherol was highest in pups ingesting formula (61.31 ± 7.71 µg/ml). Fluctuations of serum retinol and α-tocopherol levels were similar to wild phocids, suggesting that the artificial formulas utilized at MARC had adequate levels of retinol and α-tocopherol for rehabilitating harbor seal pups.

KEY WORDS: α-tocopherol, diet, formula, harbor seal, phocid, pinniped, pup, rehabilitation, retinol, vitamin A, vitamin E.

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Introduction

Young pinnipeds, such as harbor seal (*Phoca vitulina*) pups, are commonly admitted into rehabilitation centers throughout the coastal United States (Geraci and Lounsbury 1993). In the wild, harbor seal pups are born, on average, at 10.7–11.2 kg and gain approximately 0.8 kg/day solely on a diet of milk (Boulva and McLaren 1979; Lawson and Renouf 1985; Bowen *et al.* 1992; Cottrell *et al.* 2002), while the percentage of total body fat of the pup increases from 11% at birth to 35% after 19 days of nursing (Bowen *et al.* 1992). During lactation, the percentage of fat in the milk increases from 40.8 ± 1.01% at birth to 50.2 ± 1.39% at day seven and remains steady throughout the latter days of lactation (Lang *et al.* 2005). Thus, milk is extremely important for harbor seal pup survival in terms of nutrition and thermoregulation as well as for providing the essential nutrients and antibodies needed to develop a proper immune system (Jenness 1974). Phocids admitted into rehabilitation centers at a young age need a suitable artificial formula specifically tailored to the needs of a growing pup. However, developing an effective and nutritious formula is one of the many challenges that wildlife rehabilitation facilities face when caring for pups. Pinniped rehabilitation centers throughout the country differ in their compositions of artificial diets, and the effects of vitamin supplementation in their diet remains unknown.

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Appropriate vitamin intake early in life is important in the process of growth and development in mammals. Vitamin A aids in the maintenance of the reproductive, endocrine, immune, and visual systems (Blomhoff *et al*. 1991; Napoli 1999; Debier *et al*. 2002; Mos and Ross 2002). There are a number of forms of vitamin A including retinol, retinal, retinoic acid, retinyl esters, and β-carotene (Olson 1984; Blomhoff 1994). Although retinyl esters bound to lipoproteins are commonly found in the blood of carnivores, pinnipeds have none to very small amounts of circulating retinyl esters (Schweigert *et al*. 1990a). Moreover, retinol bound to retinol binding protein is the only form of circulating vitamin A present in marine mammals (Schweigert *et al*. 1990a; Debier *et al*. 2002; Mos and Ross 2002; Mazzaro *et al*. 2003) and was the only form analyzed in harbor seal pups used in this study. In addition to vitamin A, vitamin E is also important in neonates as it aids in developing the immune system (Peplowski *et al*. 1980; Robbins 1983; Bieri 1984; Dierenfeld 1989; Debier *et al*. 1999; Bramley *et al*. 2000). Prior work suggests that vitamin E is one of the most important supplements when feeding captive pinnipeds and cetaceans because vitamin E is commonly under-supplemented, with fluctuations below baseline level causing adverse effects (Bernard and Ullrey 1989; Dierenfeld *et al*. 1991). Vitamin E deficiency is a concern with captive marine mammals; thus, artificial diets are commonly supplemented with vitamins to compensate for the degradation of vitamins in frozen fish (from storage) and by handling procedures (Ikeda and Taguchi 1966). Therefore, vitamin E will also be evaluated in rehabilitating harbor seal pups in this study. The majority of vitamin E (90%) found in animals is in the form of α-tocopherol (Bjørneboe *et al*. 1990; Traber and Arai 1999; Bramley *et al*. 2000) and is the only form found in the blood of marine mammals (Engelhardt *et al*. 1975; Debier *et al*. 1999; Debier and Larondelle 2005). Retinol and α-tocopherol levels are of concern in harbor seal neonates in rehabilitation centers, as mammals have limited transplacental transfer of vitamins A and E (Debier and Larondelle 2005). It has been found that vitamin A and E intake by offspring is dependent upon the consumption of milk and colostrum ingested soon after birth (Dierenfeld 1989; Eicher *et al*. 1994; Zanker *et al*. 2000; Debier *et al*. 2005). Moreover, Bjørneboe *et al*. (1990) found that vitamin E in the plasma is mainly affected by dietary intake and lipid levels. Thus, the effects of vitamin supplementation are especially important to consider in rehabilitation centers when feeding harbor seal pups with unknown nursing histories.

Studies on circulating retinol and α-tocopherol have been performed in free-ranging harbor seal adults and pups (Schweigert *et al*. 1990a; Simms and Ross 2000a), grey seal (*Halichoerus grypus*) adults and pups (Schweigert and Stobo 1994; Schweigert *et al*. 2002), harp seal (*Pagophilus groenlandicus*) adults (Engelhardt *et al*. 1975; Engelhardt and Geraci 1978), and captive harbor seal adults (Mazzaro *et al*. 2003) but not in rehabilitating harbor seal pups. This study was designed to evaluate the effect of the diet in rehabilitation centers on circulating serum retinol and α-tocopherol in orphaned harbor seal pups admitted to the Marine Animal Rehabilitation Center (MARC) at the University of New England in Biddeford, Maine. At MARC, pups are fed a powdered milk matrix formula using Zoologic® Milk Matrix 30/55 (Pet Ag, Hampshire, Illinois, USA). Pups commonly transition to a ground herring diet, called fish gruel, and are then weaned to whole fish. The first objective of this study was to measure the effect of feeding MARC formulas on circulating concentrations of serum retinol and α-tocopherol levels in harbor seal pups at the different stages during rehabilitation: 1) upon arrival to the center; 2) while feeding with milk matrix formula; 3) while feeding with fish gruel; and 4) when weaned and fed a solid fish diet. The second objective was to compare the levels of retinol and α-tocopherol in MARC-rehabilitated pups to those of wild phocid pups, as reported in previous publications, in order to test the hypothesis that rehabilitated harbor seal pups at MARC have circulating levels of retinol and α-tocopherol similar to free-ranging phocid pups.

Methods and Materials

Sample collection

Twenty-five harbor seal pups (17 females, 8 males) were used in this study. Pups were admitted to MARC for treatment of emaciation between May and July of 2006 and 2007 (Table 1). Blood was collected from the intervertebral epidural vein from manually restrained, fasted (8–12 hr) harbor seal pups during routine health assessments. Blood was obtained with a BD Vacutainer® (BD, Franklin Lakes, New Jersey) serum separator tube and a 22-gauge needle, clotted for 10 min, then centrifuged at 3,000 × *g* for 10 min at approximately 20–25°C to obtain serum. Serum was immediately placed into 1-ml cryovials with minimal contact to light and stored at –80°C until analysis.

Retinol and α-tocopherol analysis

All sample preparation was conducted in dim lighting to prevent degradation of retinol (Blomhoff 1994). Analysis of serum retinol and α-tocopherol levels was performed as described by Mazzaro *et al*. (1995). Briefly, 150 µl of serum was mixed with 150 µl methanol and 75 µl δ-tocopherol (50 µg/ml in methanol) as an internal standard. Samples were extracted three times with 500 µl of hexane, and the organic phases were combined and dried under nitrogen reconstituted with 150 µl methanol. The 20 µl of reconstituted extract was injected onto a reversed phase column (Synergi Max-RP, 150 mm × 4.6 mm, 4 µm; Phenomenex, Torrance, California, USA). Isocratic elution was performed using a mobile phase composed of 98% methanol at 2 ml/min. Detection was by UV absorption (Series 200; Perkin Elmer, Waltham, Massachusetts, USA). Retinol and tocopherols were detected at 322 nm and 295 nm, respectively, with a six-point standard curve between 0–1 (0, 0.05, 0.1, 0.25, 0.5, 1) µg/ml for retinol and 0–50 (0, 1, 5, 10, 25, 50) µg/ml for α-tocopherol. Samples above the standard curve were diluted and rerun. Delta-tocopherol was used as an internal standard to correct for recovery, which ranged from 70–94%. Recoveries from serum samples varied by diet: 72 ± 2.92% at admit, 88 ± 5.67% for milk matrix formula,

TABLE 1. Harbor seal *(Phoca vitulina)* pups at admittance to the Marine Animal Rehabilitation Center in 2006 and 2007. The identification (ID) number of the seal corresponds to the year and the number of the animal admitted into the facility that year. Sex, date of admittance, length from head to tail (cm), and weight (kg) were recorded at admittance. Date of release or death (—) can be found below, as well as the stranding location where the animal was initially found.

ID	SEX	DATE ADMITTED	LENGTH (CM)	WEIGHT (KG)	DATE RELEASED	STRANDING LOCATIONS
06-024	F	16 MAY 2006	64.0	10.1	—	WELL, ME
06-025	F	22 MAY 2006	76.5	8.45	23 JULY 2006	DENNISPORT, MA
06-026	M	29 MAY 2006	75.5	7.9	23 AUGUST2006	COLLEGE OF ATLANTIC, ME
06-027	M	29 MAY 2006	81.0	14.25	—	KENNEBUNKPORT, ME
06-030	F	02 JUNE 2006	78.0	11.45	23 AUGUST 2006	OQUNQUIT, ME
07-024	F	06 MAY 2007	67.1	6.0	29 AUGUST 2007	PEMBROKE, ME
07-026	F	11 MAY 2007	74.0	6.5	—	COHASSET, MA
07-027	M	13 MAY 2007	73.0	9.9	29 AUGUST 2007	LOWER GOOSE ISLAND
07-029	M	18 MAY 2007	73.0	9.6	29 AUGUST 2007	OWLS HEAD, ME
07-030	M	19 MAY 2007	75.0	9.4	29 AUGUST 2007	SCARBOROUGH BEACH, ME
07-033	F	27 MAY 2007	74.0	6.4	—	SUTTON ISLAND, ME
07-035	M	28 MAY 2007	70.0	7.6	—	SANDS BEACH, ME
07-036	M	29 MAY 2007	70.0	9.15	11 SEPT 2007	SOUTHPORT, ME
07-037	F	29 MAY 2007	76.0	7.1	23 SEPT 2007	ROCKLAND HARBOR, ME
07-040	F	07 JUNE 2007	76.0	7.1	—	BAR HARBOR, ME
07-043	F	12 JUNE 2007	74.0	9.35	—	SOUTH FREEPORT, ME
07-044	M	19 JUNE 2007	79.0	8.5	—	KENNEBUNKPORT BEACH, ME
07-045	F	19 JUNE 2007	71.0	7.1	—	PORTLAND, ME
07-047	F	24 JUNE 2007	80.0	9.9	—	WEST GOOSE ROCKS BEACH, ME
07-048	F	27 JUNE 2007	76.0	10.45	—	FORTUNE'S ROCK BEACH, BIDDEFORD, ME
07-050	F	02 JULY 2007	81.0	9.1	23 SEPT 2007	WELLS BEACH, ME
07-052	M	04 JULY 2007	80.0	7.5	—	WELLS BEACH, ME
07-055	F	11 JULY 2007	79.0	10.0	11 SEPT 2007	YORK, ME
07-059	F	29 JULY 2007	79.0	8.5	—	CAPE ELIZABETH, ME
07-061	F	30 JULY 2007	74.0	10.0	—	SALISBURY BEACH, MA

TABLE 2. Mean serum retinol and α-tocopherol levels based on diet in rehabilitated harbor seal pups at MARC between 2006 and 2007. Mean weight, time in rehabilitation, serum retinol, and α-tocopherol levels ± standard error with range of concentration presented below. The number of animals for each diet is indicated by *n*. Highest concentrations of retinol and α-tocopherol are in bold.

DIET	N	WEIGHT (KG)	RANGE (KG)	TIME (DAYS)	RANGE (DAYS)	RETINOL (MG/ML)	RANGE (UG/ML)	α-TOCOPHEROL (MG/ML)	RANGE (UG/ML)
ADMIT	12	9.32 ± 0.60	7.1–14.25	1.00	NA	0.14 ± 0.03	0.03–0.41	14.13 ± 4.09	0.48–50.39
FORMULA	9	8.69 ± 0.82	6.4–14.55	7.22 ± 0.95	2–11	0.25 ± 0.07	0.07–0.73	61.31 ± 7.71	23.37–94.75
FISH GRUEL	16	8.93 ± 0.26	6.0–12.35	19.48 ± 2.61	3–50	0.30 ± 0.02	0.05–0.63	36.58 ± 3.07	13.66–70.90
FISH	12	11.23 ± 0.83	6.0–21.8	46.68 ± 4.43	2–83	0.32 ± 0.02	0.13–0.51	29.22 ± 2.69	15.47–81.89

90 ± 3.91% for fish gruel, and 85 ± 2.1% for fish. The calculated values for retinol and α-tocopherol were corrected for recovery of α-tocopherol.

Statistical analysis

Statistical analysis was completed using SigmaPlot version 11 (Systat Software, Inc., San Jose, California, USA). Analytical results are reported with means ± standard error of mean. A Mann-Whitney *U*-test was performed to test for differences ($P < 0.05$) between sexes for each diet. In addition, a one-way repeated measures analysis of variance was used to test for differences ($P < 0.05$) in vitamin concentrations based on diet. A *t*-test was performed to determine if there was a significant difference ($P < 0.05$) in retinol and α-tocopherol levels between free-ranging phocid pups and MARC harbor seal pups. Retinol data from the current study was compared to free-ranging harbor seal pups

as reported by Simms and Ross (2000a). For nursing pups, age groups in Simms and Ross (2000a) were classified as <1 day, 1–2 days, 8–14 days, 15–21 days, 22–28 days, and nonnursing pups were categorized as "weaned" and "orphaned." Age classifications by Simms and Ross (2000a) were determined from loss and gain of body mass, presence of umbilicus, lanugo, state of emaciation, and the color of plasma. MARC pups from 2006 were aged by experienced staff members based on presence of an umbilical cord, time of year, size, and lanugo. We compared "weaned" pups from Simms and Ross (2000a) to two MARC pups fed fish prior to release from rehabilitation. "Orphaned" pups from Simms and Ross (2000a) were compared to pups at admittance. Due to the lack of information on α-tocopherol levels in harbor seal pups throughout the course of lactation, serum α-tocopherol concentrations in MARC pups were compared to wild grey seal pups as reported by Schweigert *et al*. (2002). Stage I lactation included 2006 MARC pups aged 1–9 days old (1–5 days in *H. grypus*) while Stage II lactation included pups 10–18 days of age (6–10 days in *H. grypus)*. The last stage of lactation (Stage III) included harbor seal pups 19–28 days old (11–16 days in *H. grypus)* or pups still on fish gruel formula, regardless of age. All MARC pups on fish gruel were classified as Stage III because they were not fully weaned and continued to obtain formula in their diet. Moreover, two MARC pups consuming whole fish were considered "weaned" based on feeding status.

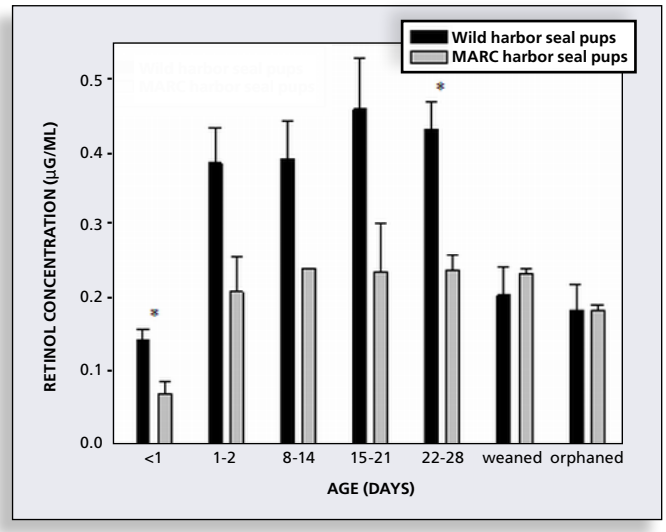

FIGURE 1. Comparison in circulating retinol between free-ranging and rehabilitating harbor seal (*Phoca vitulina*) pups. Circulating retinol values in wild (Simms and Ross 2000a) harbor seal pups compared to harbor seal pups at the Marine Animal Rehabilitation Center (MARC) from 2006; based on age.

*Indicates a significant difference ($P < 0.05$).

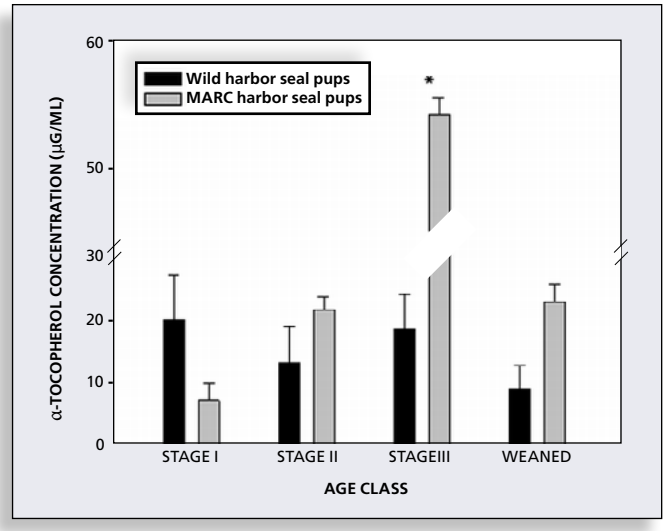

FIGURE 2. Comparison in circulating α-tocopherol between free-ranging grey seals (*Halichoerus grypus*) and rehabilitating harbor seal (*Phoca vitulina*) pups. Circulating α-tocopherol values in free-ranging grey seal (*H. grypus*) pups from Schweigert et al. (2002) and of harbor seal (*P. vitulina*) pups from the Marine Animal Rehabilitation Center; categorized by stage of lactation.

*Indicates a significant difference ($P < 0.05$).

Results

Retinol concentrations

Mean retinol levels were lowest at admit (0.14 ± 0.03 µg/ml) and increased with a change in diet (Table 2). Serum retinol was highest in pups consuming a whole-fish diet at 0.314 ± 0.018 µg/ml and ranged from 0.03 to 0.73 µg/ml throughout the course of rehabilitation. There was a significant difference in retinol concentrations ($P = 0.005$) based on diet, with a significant difference in retinol levels between pups at admit and pups fed fish gruel and fish ($P < 0.001$). However, there was no significant difference in retinol concentrations for pups at admit that were fed formula ($P = 0.154$), pups fed formula and fish ($P = 0.104$), pups fed formula and fish gruel ($P = 0.110$), and pups fed fish gruel and whole fish ($P = 0.953$). Overall, retinol levels were more variable at the start of rehabilitation and became consistent toward the latter part of rehabilitation, when fed on fish. There was no significant difference in retinol levels between sex at admit ($P = 0.149$), when fed formula ($P = 0.527$), fish gruel ($P = 0.734$), and whole fish ($P = 0.073$). When compared to free-ranging harbor seal pups, MARC pups reflected similar changes in retinol concentrations, with a peak at age 15–21 days (Fig. 1). There was a significant difference at <1 day old ($P = 0.017$) and at 22–28 days ($P = 0.03$) and no significant difference at 1–2 days ($P = 0.052$), 8–14 days ($P = 0.344$), 15–21 days ($P = 0.158$), weaned pups ($P = 0.642$), and for orphaned pups ($P = 1$).

Alpha-tocopherol concentrations

Similar to retinol, mean serum α-tocopherol levels were lowest at admit in MARC pups. Mean serum α-tocopherol level at admit was 14.13 ± 4.09 µg/ml and peaked while on formula at 61.31 ± 4.09 µg/ml (Table 2). Serum α-tocopherol levels ranged from 0.48 µg/ml to 85.75 µg/ml during the course of rehabilitation. There was a significant difference in α-tocopherol concentrations ($P < 0.001$) based on diet, with a significant difference in α-tocopherol concentrations between pups at admit and when fed formula ($P < 0.001$), fish gruel ($P < 0.001$), and

whole fish (P = 0.005). There was also a significant difference in α-tocopherol level in pups fed formula and fish (P = 0.003). However, there was no significant difference in pups fed fish gruel and formula (P = 0.091) and fish gruel and whole fish (P = 0.045). There was no significant difference in α-tocopherol concentrations between sex at admit (P = 0.432), when fed formula (P = 1), and when fed fish gruel (P = 0.571). However, there was a significant difference between sex when fed whole fish (P = 0.016), with a median value of 21.257 µg/ml in males (n = 4) and 34.733 µg/ml in females (n = 8). When compared to wild gray seal pups (Fig. 2), there was no significant difference in α-tocopherol levels at stage I (P = 0.142), stage II (P = 0.293), and for weaned pups (P = 0.161). There was, however, a significant difference at stage III (P = 0.012).

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Discussion

This study is the first to report serum retinol and α-tocopherol levels in rehabilitating harbor seal pups consuming artificial formulas. Retinol and α-tocopherol are essential vitamins throughout life, but are especially important in developing offspring (Debier *et al.* 2005; Debier and Larondelle 2005). Our results indicate that MARC diets resulted in an adequate change in retinol and α-tocopherol as compared to free-ranging phocids.

Retinol

Retinol levels were lowest at admit but continued to increase as rehabilitation progressed and with increased amounts of fish in the diet (Table 2). Compared to terrestrial mammals, a higher concentration of vitamin A is found in marine mammal milk (Debier *et al.* 1999), possibly due to its high milk fat content. As shown in Simms and Ross (2000a), free-ranging harbor seal pups had low levels of circulating retinol levels at birth, which doubled within 2 days of nursing and continued to increase until weaning, demonstrating that nursing is a vital means of obtaining retinol in harbor seal pups. Although levels were lower in MARC pups overall, as shown in Figure 1, changes in serum retinol levels over time reflected similarly to that of wild harbor seal pups. Peak retinol concentrations in serum were at 15–21 days of age, suggesting possible maximal retinol uptake during this stage in growth and development.

Serum retinol levels were highest in pups consuming whole fish, with higher levels observed in MARC-weaned pups than in wild harbor seal pups (Fig. 1). Fish in rehabilitation centers, such as MARC, are commonly supplemented with a multivitamin. In addition, pups in rehabilitation centers are fed on a regular basis with a consistent supply of supplemented fish, whereas free-ranging weaned harbor seal pups forage on their own, with limited success (Muelbert *et al.* 2003).

Although the change in serum retinol levels of MARC pups reflect that of their wild counterparts (Simms and Ross, 2000a), the difference in retinol levels may be due to a number of reasons: 1) Pups in this study were fasted prior to obtaining a sample, whereas wild pups were live-captured and could have nursed prior to being caught. It has been shown by Mazzaro *et al.* (1995) that vitamin A in serum peaks after 2 hr of supplementation in northern fur seals (*Callorhinus ursinus*); thus, fasted samples would not have shown a peak result. In addition, this is the first study reported in fasted rehabilitating harbor seal pups; thus, additional studies are needed in order to determine if the low levels are due to fasting. 2) There may be a possible subspecies difference in vitamin metabolism between the Pacific harbor seals (*Phoca vitulina richarsidii*) used in Simms and Ross (2000a) and the Western Atlantic harbor seals (*P. v. concolor*) used in this study. However, additional studies need to be conducted in order to confirm a variation in retinol levels between subspecies. 3) The lower retinol values, as compared to wild pups, may be a possible indication of poor health in a pup admitted into rehabilitation centers or a potential indicator of exposure to environmental pollutants. Polychlorinated biphenyls [PCBs] have been found to lower the level of plasma retinol concentration in free-ranging harbor seals (Simms *et al.* 2000; Simms and Ross 2000b; Mos *et al.* 2007) and wild grey seals (Jenssen *et al.* 2003). Nevertheless, further investigation and additional samples need to be obtained. However, this study does provide an insight to retinol concentrations in rehabilitating harbor seal pups with results that reflect similar changes to wild harbor seal pups.

Alpha-tocopherol

Throughout the course of rehabilitation, the concentrations of α-tocopherol were highly variable, with a range of 0.48 µg/ml to 94.75 µg/ml. This variability was similar to Schweigert *et al.* (1990a), where vitamin E levels varied in 44 species from 1 µg/ml to 26 µg/ml with an average of 15.24 µg/ml ± 2.25 in 10 harbor seal pups from Sable Island, Canada. An overall average of α-tocopherol in rehabilitated harbor seal pups consuming formula and fish gruel in this study is 35.51 ± 3.21 µg/ml, while pups fed on whole fish resulted in α-tocopherol levels of 29.22 ± 8.69 µg/ml. These levels are similar to Mazzaro *et al.* (2003), where tocopherol levels were 30.92 ± 4.68 µg/ml in seven captive adult harbor seals, with a range of 24.01 µg/ml to 36.08 µg/ml.

Circulating α-tocopherol levels peaked in pups consuming formula and decreased when on fish gruel and whole fish (Table 2). This trend was seen in wild grey seal pups as well. Debier *et al.* (2002) reported grey seal pup serum concentration at day 0 of nursing to be 13.9 ± 8 mg/L, increased to 31.2 ± 5.2 mg/L at days one to three, then decreased to a constant of 21.1 ± 4.5 mg/L at the end of lactation. Moreover, in Schweigert *et al.* (2002), 12 weaned grey seals on Sable Island, Canada observed a drop in plasma vitamin E levels during weaning while retinol remained constant; this corroborates the results of this study as well. In regard to differences in sex, we found female harbor seal pups consuming fish to have higher α-tocopherol concentrations than did males. Again, when compared to grey seals, Schweigert *et al.* (1990b) found higher concentrations of α-tocopherol in male adults and pups than in females and juveniles. However, grey seal pups in Schweigert *et al.* (1990b) were not separated by sex and resulted in a mean value of 29.1 ± 8.2 mg/L, which lies between our median of 21.257 µg/ml in males and 34.733 µg/ml in females.

When compared to wild grey seal pups from Schweigert *et al.* (2002) in Figure 2, α-tocopherol concentrations in MARC harbor seal pups did not reflect similar changes to wild grey seal pups according to the stage of lactation. It is difficult to categorize stage of lactation by age in rehabilitation, as every animal progresses differently in both rehabilitation and diet. The discrepancy with age in rehabilitation may contribute to the differences found in concentrations of α-tocopherol based on stage of lactation in Schweigert *et al.* (2002). This study shows adequate changes in levels of α-tocopherol in rehabilitating MARC harbor seal pups; however, additional studies need to be performed in free-ranging harbor seal pups to provide a comparison.

Conclusion

Adequate serum levels of retinol and α-tocopherol are vital early in life, especially in rehabilitation pups that may have experienced dietary deficiency at a critical stage in development. Monitoring circulating levels of retinol and α-tocopherol in the blood provides a minimally invasive view into a seal pup's physiology. This is the first study published to evaluate the levels of retinol and α-tocopherol in the serum of rehabilitating harbor seal pups. Rehabilitating harbor seal pups that consumed artificial formulas exhibited similar circulating retinol levels to that of wild harbor seal pups. Alpha-tocopherol seems to have very limited transplacental transfer but is well supplemented in diets utilized in rehabilitation. In conclusion, the data display a visible change in circulating retinol and α-tocopherol levels from diets fed in MARC harbor seal pups and suggest that rehabilitation centers are effectively improving the nutritional status of a malnourished pup for the proper growth and development to be released back into the wild.

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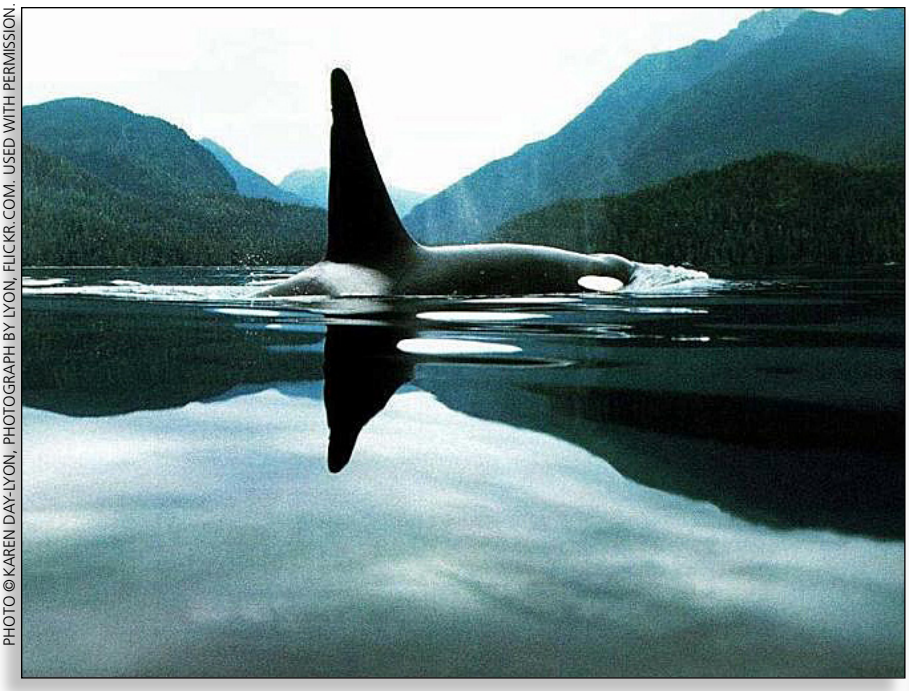
Noel Takeuchi immersed herself in wildlife rehabilitation at the Marine Mammal Center in California and the Island Wildlife Natural Care Centre in Canada during her undergraduate career. After graduating from the University of California–Davis, she worked as a veterinary technician and continued with rehabilitation at the Marine Mammal Care Center in California and the Alaska SeaLife Center. She completed her Masters in Marine Science at the University of New England under Kathryn Ono, Ph.D. Noel is currently pursuing her doctorate at the University of Florida's College of Veterinary Medicine's Aquatic Animal Health Program studying trace metals in manatees under David Barber, PhD.

Dr. David Barber received a Ph.D. in Pharmacology and Toxicology from the University of Arizona in 1997. He was a post-doctoral fellow at the Virginia–Maryland Regional College of Veterinary Medicine in Blacksburg, Virginia from 1997–2000, where he studied the mechanisms underlying organophosphate-induced delayed neuropathy. Since 2000, he has been a faculty member of the Center for Environmental and Human Toxicology at the University of Florida. His current research focuses primarily on the biochemical mechanisms by which environmental contaminants produce toxicity in aquatic species.

Dr. Kathryn Ono has been researching pinniped biology for over 30 years. She is especially interested in the mother–pup relationship in seals and sea lions. She has studied a number of species in the field including: Steller sea lions (*Eumetopias jubatus*), California sea lions (*Zalophus californianus*), Guadalupe fur seals (*Arctocephalus townsendi)*, harbor seals (*Phoca vitulina*), and grey seals (*Halichoerus grypus*). Currently, along with graduate and undergraduate students, her research is focused on the population biology of the expanding grey seal population in New England. Dr. Ono helped design the Marine Animal Rehabilitation Center in the University of New England Marine Science Center.

West Nile Virus Infection in Killer Whale, Texas, USA, 2007

Judy St. Leger, Guang Wu, Mark Anderson, Les Dalton, Erika Nilson, and David Wang



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ABSTRACT: In 2007, nonsuppurative encephalitis was identified in a killer whale at a marine park in Texas, United States. Panviral DNA microarray of brain tissue suggested West Nile virus (WNV); WNV was confirmed by reverse transcription PCR and sequencing. Immunohistochemistry demonstrated WNV antigen within neurons. WNV should be considered in cases of encephalitis in cetaceans.

KEY WORDS: cetaceans, killer whale, *Orcinus orca*, West Nile virus

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Introduction

West Nile virus (WNV) is a single-stranded RNA virus of the genus *Flavivirus* that is transmitted by mosquitoes. In humans and animals, WNV has been associated with a spectrum of clinical conditions from asymptomatic infections to sudden death. These have been identified in a variety of animal species. Among marine mammals, WNV infection has been reported in a harbor seal (*Phoca vitulina*) (Del Piero *et al*. 2006). We describe WNV infection in a killer whale (*Orcinus orca*) and seroprevalence in conspecific cohort and noncohort groups.

The study

In 2007, a 14-yr-old male killer whale at a marine park in San Antonio, Texas, United States died suddenly without notable premonitory signs. On gross examination, mild multifocal meningeal hyperemia and petechial parenchymal hemorrhage were noted in the right cerebrum and cerebellum. The left hemisphere of the brain appeared normal. Focally extensive tan discoloration and fibrosis were present in the right accessory lung lobe with associated hemorrhage and congestion. Both lung lobes were mildly and diffusely heavy and wet. All thoracic and abdominal lymph nodes were moderately enlarged and edematous. The second gastric chamber displayed numerous chronic and active ulcerations of 1.5–2 cm. Fresh and buffered 10% formalin-fixed specimens were collected. Fresh tissues were stored at –80°C. Tissues fixed in 10% buffered formalin were processed routinely and stained with hematoxylin and eosin for histologic examination.

Histologic review demonstrated moderate, multifocal subacute vasculitis and nonsuppurative encephalitis. Inflammatory lesions of the central nervous system were focused in gray matter of the medulla oblongata, pons, mesencephalan, and cerebellum. Lesions

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were bilateral but were more severe on the right side. Meninges demonstrated moderate, focally extensive and multifocal areas of acute meningeal congestion and hemorrhage. Mild, multifocal lymphocytic infiltrates expanded the leptomeninges. Blood vessels demonstrated mild to moderate acute necrosis, as well as lymphocytic and contained plasmacytic and neutrophilic infiltrates within the vascular walls. Encephalitis was characterized by perivascular lymphocytes and fewer plasma cells expanding the Virchow-Robbins spaces. Small, scattered perivascular ring hemorrhages were noted. A few multifocal, loosely arranged glial nodules were within the cerebral white matter.

Predominant lesions in the lungs were areas of chronic and active abscessation amid a focally extensive area of mixed inflammation and fibrosis. There was moderate diffuse acute pulmonary edema and congestion. Gastric ulcerations were present in the first gastric chamber and were chronic and active. They were characterized by central ulcerations with necrosis and a mixed inflammatory infiltrate surrounded by variable fibrosis and a rim of epithelial hyperplasia. Changes in spleen, lymph node, and kidney included acute edema, congestion, and vascular dilation.

Conventional diagnostic assays were performed for aerobic, anaerobic, and fungal microbes in liver, lung, kidney, cerebrospinal fluid, and brain. All yielded minimal growth of *Escherichia coli*.

The final diagnosis was fulminant peracute bacteremia and septicemia secondary to a primary viral infection associated with nonsuppurative encephalitis. Published etiologic considerations for cetacean nonsuppurative encephalitis include morbillivirus and protozoal infections (Miller *et al*. 2001). A DNA microarray with highly conserved sequences from >1,000 viruses was selected to screen for known and novel viruses (Wang *et al*. 2003). Total RNA was extracted from brain tissue and hybridized to a microarray as described (Mihindukulasuriya *et al*. 2008). Analysis of the resulting hybridization pattern demonstrated a strong hybridization signal to many oligonucleotide probes on the microarray from the family *Flaviviridae*, in particular to WNV. Consensus reverse-transcription PCR primers (Kuno 1998) targeting WNV were used to confirm the microarray results. Sequencing of the 261-base pair amplicon (GenBank accession no. HQ610502) yielded a sequence with 99% nucleotide identity and 100% amino acid identity to WNV strain OK03 (GenBank accession no. EU155484.1), a strain originally identified in Oklahoma, United States.

To further support a WNV diagnosis, we performed immunohistochemical staining on brain tissue. The immuno-peroxidase stain used was a commercial rabbit polyclonal antibody (BioRelience Corp., Rockville, Maryland, USA) with a peroxidase-tagged goat anti-rabbit immunoglobulin G (DakoCytomation, Carpinteria, California, USA) bridge and with 3-amino-9-ethylcarbazole (DakoCytomation) as the chromogen. This staining demonstrated abundant WNV antigen within the cytoplasm of a small number of neurons and glial cells and in fewer macrophages in the brain tissue.

We evaluated WNV exposure within the same cohort, as well

as a geographically distant cohort of whales, by using serologic testing. All testing was performed at the same laboratory by using a standard plaque-reduction neutralization test. In this assay, a 90% neutralization cutoff was used (Beaty *et al*. 1989). A 90% plaque-reduction titer >10 was considered positive. Serum from the affected whale and five cohort killer whales from the same marine park in San Antonio, as well as from five whales housed at another facility in Orlando, Florida, USA were evaluated. Within each facility, the animals had regular contact with each other. The facilities are geographically separated so the animals do not have exposure to those in the other park. All six animals from Texas had 90% plaque-reduction titers >10, ranging from 40 to 80. The five whales housed together in Orlando had no measurable titer.

Conclusions

We demonstrate that WNV can infect and cause disease in killer whales. These findings broaden the known host tropism of WNV to include cetaceans, in addition to previously known pinnipeds. Although we cannot definitively attribute the cause of death of this whale to WNV, the observed lesions are consistent with those caused by WNV in other animals. The serologic results demonstrate that subclinical infections can occur and that exposure can be variable. We did not determine specific dates of exposure for these populations. Both Bexar County, Texas, and Orange County, Florida have had WNV in wildlife since 2002. We continue annual serology on previously negative animals to document seroconversion. Mosquito management practices are similar in both facilities and have been expanded since this diagnosis. Differences in WNV prevalence or mosquito numbers may have played a role in the different serologic results.

Health evaluations of free-ranging and captive cetaceans should include WNV serology to assess exposure rates. This report focuses on killer whales, but the "loafing" behavior (stationary positioning at the water's surface) is commonly seen in many coastal dolphins, thereby increasing the likelihood of mosquito bites and exposure to WNV. Serologic screening of bottlenose dolphins (*Tursiops truncatus*) from the Indian River Lagoon demonstrated WNV titers (Schaefer *et al*. 2009); WNV-associated disease in these animals has not been reported. Active screening for WNV may enhance diagnostic investigations.

As with many species of birds and mammals, WNV infection carries a risk for zoonotic transmission. Until the implications of this infection in marine mammals are better understood, biologists and veterinarians working with cetaceans should consider this possibility. Potential viral shedding can occur through the oropharygeal cavity and feces as well as through blood and organs during necropsies.

Finally, our study demonstrates the broad applicability of using panviral microarray-based diagnostics. Even though PCR diagnostics are well developed for WNV, the agent was not initially considered as a potential pathogen in this species. Panviral microarray can be used not only to identify novel viruses but also to detect unsuspected agents.

Acknowledgment

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Just Because We Can, Does It Mean We Should?

By Deb Teachout, DVM

As a wildlife veterinarian, I know that, before release, my wild patient has to be almost perfect both physically and mentally. It is my duty to decide if I can repair this injured individual so that it has every chance possible to make it in the wild.

Particularly challenging are decisions over amputation. I know from a technical standpoint that I can perform the surgery, but can the animal manage successfully after release? How will it fare in the post-operative and rehabilitative period? Is it ethical to put the animal through a painful procedure if I can't answer those questions with some certainty? Has this particular surgery ever been done in this species and what was the outcome?

Case studies are invaluable resources. In a recent *Journal of Wildlife Rehabilitation* (*JWR*), there is a case study of a healthy little brown bat (*Myotis lucifugus*) captured as part of a bat study in Manitoba, Canada and found surviving in the wild with only one foot (Jonasson *et al*. 2010). Bats depend on their feet for many critical survival functions such as roosting, grooming, and feeding. Apparently, this one-footed bat was coping just fine with his handicap. Current rehabilitation guidelines for bats with severe foot injuries recommend euthanasia, but these authors concluded that, perhaps, bats with missing feet could be considered for rehabilitation. Veterinarians presented with a crushed or severely injured foot in a bat, especially a little brown bat, can offer a better prognosis for amputation than what was previously believed, thanks to this case report. Left unanswered, however, is the critical question of whether the bat can accommodate post-operative care in captivity while he learns to cope with only one foot.

Another case report from *JWR* detailed the limb amputation in a wild cervid (Singh *et al*. 2010). The authors were presented with a male, young adult sambar deer (*Rusa unicolor*) that had sustained an open, infected right metacarpal fracture accompanied by osteomyelitis. Amputation was chosen as a salvage procedure, and the surgery was performed with the intent that the deer would live in captivity for the remainder of his life. This deer was well suited to life in captivity and, 10 months after surgery, he was reported to be able to walk and run. Wild cervid leg amputation is another case where we technically can do it, but should we? Reports of three-legged deer sightings from backyard wildlife enthusiasts and hunters attest to apparent normalcy in terms of running speed and obtaining food. There are numerous reports of three-legged female white-tailed deer (*Odocoileus virginianus*) being seen with fawns over a multi-year period. So, as a wildlife veterinarian, I learn from these reports that some three-legged female cervids seem to be able to live well in the wild—but, their limb loss likely happened naturally. The biggest threat to recovery from surgery for cervids is subacute and chronic capture myopathy. Amputation is risky for recovery, and most specialists currently recommend euthanasia when presented with such an animal. In select cases, however, perhaps we can successfully manage the recovery period, and should try, as evidenced by the account of the sambar deer.

Medical and surgical techniques continue to advance, and there are many applicable to wildlife. The dilemma is, should we? When informed by the constantly evolving experiences of others, as reported in case studies, the recorded observations of other such animals from natural world sightings, and the diligent application of welfare principles expressed in the Five Freedoms (Teachout 2011), veterinarians and rehabilitators must continue to strive for the right decision, the most ethical decision, in each case. ■

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Sika deer *(Cervus nippon)*.

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Deb Teachout is a veterinarian in Illinois, United States, whose practice serves both domestic and wildlife patients. She is a member of the IWRC Board of Directors, an associate editor for JWR, and a long-time animal advocate.

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Finding the Solution by Recognizing the Problem

by Prudi Koeninger and Kathy Milacek

As a wildlife rehabilitator, how many times have you wished the public would just leave the nest of cottontails alone? Instead they rescue—in reality, kidnap—the babies simply because they do not see the mother rabbit. Then there are the calls from frantic homeowners who discover baby raccoons in their attic. "Please, you must save them," they beg. But when you ask about the mother raccoon, they sheepishly admit they thought they were doing the right thing by relocating her to the country. Or (and this is one of our favorites), "I just rescued this little bird that can't fly and was nearly attacked by this other bird that kept screaming and dive-bombing me."

John Q. Public probably knows more about African wildlife, courtesy of television nature shows, than they know about the wildlife in their own backyard. There are plenty of websites and publications on co-existence with urban wildlife, but people don't often look for information before taking action or calling for help. Exasperated and stretched too thin, many rehabilitators will just tell the public to bring in whatever they've found rather than take the time to assess the situation fully and then educate. So how do we get the message to the public before they kidnap? How do we teach people that trapping and relocating wildlife is not humane and does not solve their wildlife conflicts? More importantly, especially if you're the typical home-based wildlife rehabilitator, where are you supposed to find the time to educate the public and answer their questions, especially during baby season?

Like you, members of the DFW Wildlife Coalition, Inc. face these issues on a daily basis. The Dallas-Fort Worth community is serviced by only one bird wildlife rehab center and several home-based mammal, bird, and reptile rehabilitators. This made it difficult for the public to find a wildlife rehabilitator. Phone calls would go unanswered because the rehabilitators were full and knew they could not say "no." Opportunities to educate on renesting, exclusion, and the use of deterrents were rare.

A virtual wildlife hotline proved to be a great solution to our problem, and possibly to yours as well.

With current telecom technologies, you can operate anywhere without having real estate! There are several advantages to having a "virtual" hotline: 1) Your volunteers do not have to travel to an office to take calls; 2) You do not have the overhead cost of a building facility; 3) The public cannot just dump wildlife on your door unannounced; and 4) You don't have to purchase or maintain any phones or computer hardware or software.

What do you need to get started?

- a hosted virtual PBX system
- a volunteer base
- training materials
- a record-keeping system
- a website
- at least 2 or 3 highly committed volunteers to direct and coordinate hotline activities

While you may never have heard of a "virtual PBX system," you've almost certainly used one. They are a standard feature of tech-support lines and customer service centers. These systems greet callers automatically using pre-recorded messages, route incoming calls to the appropriate extension using dialing menus, save voicemail, and more. With a "hosted" system, you pay for a company's services rather than for buying your own equipment.

While 800 numbers are available, they do cost more, so DFW Wildlife Coalition has a local number—we're not servicing the entire country, after all. But an organization might consider setting up a 900 number, whereby callers would be charged so much per minute for their call as a way to fund your wildlife hotline and rehabilitation operations.

Volunteers have hotline calls routed to their home landline or cell phone through this PBX system. The public hears a professional recorded message and the phones are active during our operating hours: 7 am–10 pm, 365 days a year. After-hour calls go to a voice messaging system.

Our virtual hotline is 100% volunteer-operated. Most of our volunteers are not wildlife rehabilitators. To attract volunteers, we partnered with the Texas Master Naturalist Program (there are many similar programs in other states). Our missions are similar—providing education and service to the public regarding the natural resources of Texas. This particular base of volunteers is already interested in, and in some cases, educated about local wildlife. They are concerned about our environment and have a real desire to make a difference. Master Naturalists are required to put in at least 40 hours of volunteer work each year, and our hotline is a pre-approved project, so it's a win-win for everyone!

In the next installment of this column, we'll discuss the nuts and bolts of setting up the virtual PBX system as well as the training and operating resources you'll need to bring your volunteers up to speed.

Rehabilitators Prudence "Prudi" Martin-Koeninger and Kathy Milacek founded and have directed the DFW Wildlife Coalition in Dallas, Texas, United States since 2003 which operates a community-supported urban wildlife conflict solution hotline logging 55,000 residential calls to date. Kathy is a Master Naturalist. Prudi operates Rascal's Retreat, a home-based wildlife center, and was awarded the Kramer Award in 2011 for "Superior Efforts to Rescue and Rehabilitate Indigenous Wildlife."

Captive-reared Burrowing Owls show Higher Site-affinity, Survival, and Reproductive Performance when Reintroduced using a Soft release

A. M. Mitchell, T. I. Wellicome, D. Brodle, and K. M. Cheng. *Biological Conservation* 144(5): 1382–1391. 2011.

A remaining challenge for animal reintroductions is how best to aid individuals transitioning from captivity into the wild. We tested two techniques—'soft' vs. 'hard' release—in a 7-yr study on endangered western burrowing owls (*Athene cunicularia hypugaea*) in British Columbia, Canada. Traditionally, captive-reared yearling adults were released into the wild, as pairs, directly from artificial burrows (hard release). Only 46% of 201 owls released in this way (2001–2004) stayed at release sites. To test for improved success, soft releases were performed in the same manner as concurrent hard releases (2005–2007), except above-ground enclosures confined each soft-release pair to the vicinity of their burrow for 2 wk before complete release. Of 140 soft-released owls, 86% stayed at release sites whereas 66% of 100 hard-released owls stayed. Breeding-season survival was 70% for soft-released owls versus 50% for hard-released owls. On average, soft-released owls produced 50% more fledglings than concurrently hard-released owls. Post-fledging survival was 69% and first-year return rate was 7.0% for fledglings from soft-released parents compared to 50% and 4.0% for fledglings from hard-release parents. Ultimately, 0.17 offspring were recruited into the local wild breeding population per soft-released pair compared to 0.05 local recruits per hard-released pair. We recommend that enclosure-based soft releases be used for reintroduction of burrowing owls in British Columbia and elsewhere in North America. We encourage other tests for improved release success with soft releases, particularly for species with a high tendency for dispersal or those likely to experience significant predation pressure.

Second Generation Anticoagulant Rodenticides in Predatory Birds: Probabilistic Characterization of Toxic Liver Concentrations and Implications for Predatory Bird Populations in Canada

P. J. Thomas, P. Mineau, R. F. Shore, L. Champoux, P. A. Martin, L. K. Wilson, G. Fitzgerald, and J. E. Elliott. *Environment International* 37(5): 914–920. 2011.

Second-generation anticoagulant rodenticides (SGARs) are widely used to control rodent pests, but exposure and poisonings occur in non-target species such as birds of prey. Liver residues are often analyzed to detect exposure in birds found dead but their use to assess toxicity of SGARs is problematic. We analyzed published data on hepatic rodenticide residues and associated symptoms of anticoagulant poisoning from 270 birds of prey using logistic regression to estimate the probability of toxicosis associated with different liver SGAR residues. We also evaluated exposure to SGARs on a national level in Canada by analyzing 196 livers from great horned owls (*Bubo virginianus*) and red-tailed hawks (*Buteo jamaicensis*) found dead at locations across the country. Analysis of a broader sample of raptor species from Quebec also helped define the taxonomic breadth of contamination. Calculated probability curves suggest significant species differences in sensitivity to SGARs and significant likelihood of toxicosis below previously suggested concentrations of concern (<0.1 mg/kg). Analysis of birds from Quebec showed that a broad range of raptor species are exposed to SGARs, indicating that generalized terrestrial food chains could be contaminated in the vicinity of the sampled areas. Of the two species for which we had samples from across Canada, great horned owls are exposed to SGARs to a greater extent than are red-tailed hawks, and the liver residue levels were also higher. Using our probability estimates of effect, we estimate that a minimum of 11% of the sampled great horned owl population is at risk of being directly killed by SGARs. This is the first time the potential mortality impact of SGARs on a raptor population has been estimated.

Targeted Surveillance of Raccoon Rabies in Québec, Canada

E. E. Rees, D. Bélanger, F. Lelièvre, N. Coté, and L. Lambert. *Journal of Wildlife Management* 75(6): 1406–1416. 2011.

Data from wildlife disease surveillance programs are used to inform implementation of disease control (e.g., vaccination, population reduction) in space and time. We developed an approach to increase detection of raccoon rabies in raccoons *(Procyon lotor)* and skunks (*Mephitis mephitis*) of Québec, Canada, and we examined the implications of using this approach for targeted surveillance. First, we modeled the probability of a rabid animal relative to environmental characteristics of sampling locations. Rabid animals were more likely to be found in low-lying flat landscapes that had higher proportions of corn–forest edge habitat and hay agriculture and that were within 20 km of one or more known rabies cases. From the model, we created two complementary risk maps to identify areas where rabid animals were most likely to be sampled. One map accounted for habitat and known rabies case locations and can be used to define an infection zone from which surveillance can be targeted along the periphery to determine if disease is continuing to spread. The other map only accounted for habitat and can be used to locate areas most likely to contain rabid animals when the disease is present. In a further analysis, we compared the two most successful methods for detecting raccoon rabies in Québec, given the disease was present. Government trapping operations (active surveillance) detected more cases in the short-term, but citizen notification (passive

and enhanced) was more effective after 12 trapping days from which the initial rabies case was found. Our approach can benefit wildlife and public health agencies wanting to assess the disease status of regions by targeting surveillance to habitats most likely to contain infected animals and by defining the duration over which sampling methods are effective.

Zoonotic Pathogens Isolated from Wild Animals and Environmental Samples at Two California Wildlife Hospitals

J. L. Siembieda, W. A. Miller, B. A. Byrne, M. H. Ziccardi, N. Anderson, N. Chouicha, C. E. Sandrock, and C. K. Johnson. *Journal of the American Veterinary Medical Association* 238(6): 773–783. 2011.

A cross-sectional study was undertaken to determine types and estimate prevalence of potentially zoonotic enteric pathogens shed by wild animals admitted to either of two wildlife hospitals and to characterize distribution of these pathogens and of aerobic bacteria in a hospital environment. Fecal samples were taken from 338 animals at both wildlife hospitals and environmental samples from one wildlife hospital. The samples were collected within 24 hr of hospital admission. Environmental samples were collected from air and surfaces. Samples were tested for zoonotic pathogens via culture techniques and biochemical analyses. Prevalence of pathogen shedding was compared among species groups, ages, sexes, and seasons. Bacterial counts were determined for environmental samples. *Campylobacter* spp., *Vibrio* spp., *Salmonella* spp., *Giardia* spp., and *Cryptosporidium* spp. (alone or in combination) were detected in 105 of 338 (31%) fecal samples. *Campylobacter* spp. were isolated only from birds. Juvenile passerines were more likely to shed *Campylobacter* spp. than were adults; prevalence increased among juvenile passerines during summer. Non-O1 serotypes of *Vibrio cholerae* were isolated from birds; during an oil-spill response, 9 of 10 seabirds screened were shedding this pathogen, which was also detected in environmental samples. *Salmonella* spp. and *Giardia* spp. were isolated from birds and mammals; *Cryptosporidium* spp. were isolated from mammals only. Floors of animal rooms had higher bacterial counts than did floors with only human traffic. Potentially zoonotic enteric pathogens were identified in samples from several species admitted to wildlife hospitals, indicating a potential for transmission if prevention is not practiced.

Summary of Birds Killed by a Harmful Algal Bloom along the South Washington and North Oregon Coasts during October 2009

E. M. Phillips, J. E. Zamon, H. M. Nevins, C. M. Gibble, R. S. Duerr, and L. H. Kerr. *Northwestern Naturalist* 92(2): 120–126. 2011.

Seabirds are known to be vulnerable to biotoxins produced by harmful algal blooms (HABs). During November 2007 in Monterey Bay, California, an unprecedented stranding of live and dead seabirds highlighted plumage fouling as an emerging mortality factor during HAB events. During late October 2009 along the southern Washington and northern Oregon State coasts, algal blooms caused another seabird plumage fouling event. We summarize information from necropsies of birds that were collected dead from beaches or that died during rehabilitation in the Oregon (WCNC [*Wildlife Center of the North Coast*]) and California (IBRRC [International Bird Rescue]) facilities. All examined birds were moderately to severely emaciated with a high frequency of pectoral muscle atrophy and virtually no subcutaneous or internal fat. The impacts of HAB events on seabird populations are not well known, and if the frequency or magnitude of HAB events is increasing, HABs could represent another mortality source of conservation concern. Although response networks are in place for documenting anthropogenic events such as oil spills, there are not analogous response networks in place for documenting unusual die-off events, including HABs. We recommend that agencies build upon successful oil-spill response network models and provide support for developing networks and establishing common data collection protocols.

"There are not...response networks in place for documenting unusual die-off events..."

Antimicrobial Susceptibility of Bacterial Isolates from Sea Otters *(Enhydra lutris)*

D. Brownstein, M. A. Miller, S. C. Oates, B. A. Byrne, S. Jang, M. J. Murray, V. A. Gill, and D. A. Jessup. *Journal of Wildlife Diseases* 47(2): 278–292. 2011.

Bacterial infections are an important cause of sea otter (*Enhydra lutris*) mortality, and some of these infections may originate from terrestrial and anthropogenic sources. Antimicrobials are an important therapeutic tool for management of bacterial infections in stranded sea otters and for prevention of infection following invasive procedures in free-ranging otters. In this study, susceptibility to commonly used antimicrobials was determined for 126 isolates of 15 bacterial species or groups from necropsied, live-stranded injured or sick, and apparently healthy wild sea otters examined between 1998 and 2005. These isolates included both gram-positive and gram-negative strains of primary pathogens, opportunistic pathogens, and environmental flora including bacterial species with proven zoonotic potential. Minimal evidence of antimicrobial resistance, and no strains with unusual or clinically significant multiple-drug resistance patterns were identified. Collectively, these findings will help optimize selection of appropriate antimicrobials for treatment of bacterial diseases in sea otters and other marine species. ■

TAIL END

"Get off my lawn!"

Winning caption by Kaitlin Nuetzmann, Centralia, Wash., USA

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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
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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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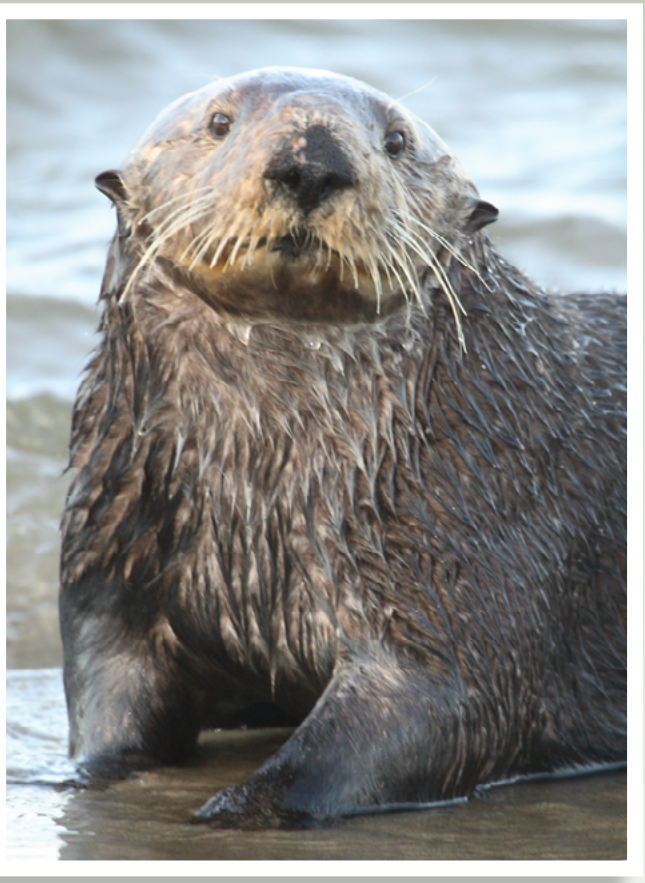

Sea otter *(Enhydra lutris).*

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