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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:
Grey seals *(Halichoerus grypus).*
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Left:
Cape vulture *(Gyps coprotheres).*
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JOURNAL OF WILDLIFE REHABILITATION

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Passing the Baton

Wildlife rehabilitators have more opportunities than most to get good at letting go. After all, it's the ultimate goal of this activity, this avocation, this labor of love. We say a happy "so long" to young creatures sprinting off to independence…. a fond "farewell" to the newly healed soaring away to a second chance at a wild life… a wistful (usually) "auf wiedersehen" to colleagues who leave the practice…. and, of course, to patients for whom the best we can offer is a sorrowful, humane, and very final goodbye.

A variety of major shifts in my own life over the past three years or so have had me pondering some weighty questions, including: How overcommitted is too overcommitted? Where are my efforts most effective and where have I become a hindrance to progress? When should I persist and when would it be better to release my grip?

You'd think I'd be better at this by now but it's still a challenge. With persistence, however, eventually something's bound to come of all this navel-gazing—in this case, my decision to resign as Chair of the Certification Review Board (CRB).

I've been involved in the Certification program since its very inception as chair of an exploratory subcommittee charged with developing recommendations for the IWRC Board of Directors. Later I was approached to help develop the Certification exam based on those recommendations. Once the first set of prospective Certified Wildlife Rehabilitators (CWRs) completed the exam, I was asked to serve as the first CRB Chair. At first I demurred, but the Board justified their request with an argument I couldn't refute—that by then, no one else in the organization knew as much as I did about the CWR program.

It has been a rewarding experience and a great opportunity to work with and get to know some amazing people. However, this position was never intended to provide the (unpaid) job security of a Supreme Court Justice. In early January of this year, I began to discuss the need for a new chair with the CRB members. I felt I had done my best and now a different perspective was needed to take the CWR program to the next level.

My trepidation over what would happen next evaporated as a volunteer promptly answered my call. A confidential vote resulted in unanimous approval and it is now my delightful duty to introduce you to your new CRB Chair, Merle Stewart, CWR and Education Director of the Alaska WildBird Rehabilitation Center.

In my new role as CRB Chair Emeritus, I will work closely with Merle as she takes the reins and spurs the Board on to even bigger and better things. As she becomes more familiar with the duties of her new position, I'll become less active but always available as an *ad hoc* consultant and sounding board.

Please join me in congratulating Merle (she can be reached by email: certification@theiwrc.org). I've offered her this editorial space in the next issue of JWR to tell us a little about herself and share her vision for the Certification program with IWRC members.

Kieran Lindsey
Editor

[Lest any readers worry that I'll no longer have enough on my to-do list to keep me off the street, never fear. In addition to my JWR Editor's hat, my new full-time administration job and continued part-time teaching gig with Virginia Tech University, my blog, and other urban wildlife-related activities, I just moved again and expect to spend at least the next year unpacking boxes.]

Illegal Trafficking of Endangered Tortoises Reaches Epidemic Proportions

MADAGASCAR (May 2, 2013)—Two critically endangered tortoise species from Madagascar are being targeted for illegal trafficking, according to the Wildlife Conservation Society, Durrell Wildlife Conservation Trust, Turtle Survival Alliance, Madagascar Biodiversity Partnership, Turtle Conservancy, Conservation International, World Wildlife Fund and other groups urging authorities to clamp down on wildlife smuggling before these species are collected out of existence. According to the groups, more than 1,000 radiated (*Astrochelys radiata*) and ploughshare (*Astrochelys yniphora*) tortoises were confiscated from smugglers in the first three months of 2013 alone. By March of this year, 54 ploughshare tortoises made it as far as Thailand before being seized by authorities. Since the beginning of Madagascar's continuing political crisis in 2009, smuggling has increased by at least tenfold due to weak governance and rule-of-law. In addition, erosion of cultural protection of the tortoises for short-term monetary gain has contributed to their sharp decline. The Wildlife Conservation Society and its conservation partners are urging Malagasy officials to take a hard stand against illegal trafficking and increase the number of guards in remote areas to the north where the tortoises remain. This, coupled with public education efforts and better enforcement in import countries such as Thailand, will help take pressure off these critically endangered reptiles. Meanwhile, the Turtle Conservancy and Turtle Survival Alliance have been able to import a small number of animals seized from the illegal trade into the U.S. for the foundation of an assurance colony.


Endangered radiated tortoise *(Astrochelys radiata).*

PHOTO © SEBASTIAN NIEDLICH. CREATIVE COMMONS LCENSE.

Cobequid Wildlife Rehabilitation Center Attempts First Turtle Shell Repair

HILDEN, Nova Scotia, Canada (July 1, 2013)—Dr. Helene Van Doninck was excited to see poop in her new patient's enclosure recently. That means the turtle, who sports an innovative repair for his fractured shell, is eating. The painted turtle is one of 32 animals being cared for at the CWRC and he is Van Doninck's test case for a new method of shell repair. When a driver found the turtle injured on the side of the road and brought him to the CWRC, Van Doninck contacted a colleague at the Wildlife Center of Virginia. Dr. Dave McRuer's technique uses medical-grade epoxy and stainless steel to bridge fractures that allows caregivers to continue to clean the wound. The turtle is now able to swim in shallow water and Van Doninck is preparing a larger tank. "We get calls about turtles a lot," she said. "A lot of people don't know we have turtles in Nova Scotia and that's astonishing to me." She advises anyone who wants to assist a turtle in the middle of a road to move it in the direction it was heading or it will just keep trying to cross. She also pointed out it's illegal to keep any of the providence's four indigenous turtles—wood, painted, snapping, and Blandings—as pets.

Work to Begin on Wildlife Rehabilitation Center

KAMLOOPS, British Columbia, Canada (July 2, 2013)—A British Columbia Wildlife Park wildlife rehabilitation project that's been in the planning stage for two years broke ground in mid-July following a major new donation. A combined cash contribution of $100,000 from Horizon North Logistics, parent company of Kamloops-based manufacturer Northern Trailer, and Ellen and Mark Brown, will enable the construction of the new center. Mark Brown is president of Northern Trailer, which will also donate five modular buildings for the wildlife hospital. Construction of the modular will take place simultaneously with site preparation and foundation pouring, with Lafarge and Ready-Mix donating concrete. The modular approach is expected to allow completion of the center by fall. Rea and Ron Fawcett had previously donated approximately $300,000 to set the project in motion. The new center will be four times the size of the existing 800 square foot facility, which is the only licensed wildlife rehabilitation center in the Kamloops region. Park manager Glenn Grant said it will also be better equipped to receive and treat injured wildlife.

New Retroviruses Found in Polar Bear and Panda

SAARBRÜCKEN, Germany (July 15, 2013)—Endogenous retroviruses (ERVs) are viruses that at some point in the past inserted themselves into the nuclear genome of a host's germ cell. Once integrated in a germ cell the virus would be passed on from one generation to the next and the endogenous retroviral genome would therefore be inherited to new species that evolve from the original host. Working in collaboration with Professor Alex D Greenwood and Kyriakos Tsangaras from the Leibniz Institute for Zoo and Wildlife Research in Berlin, Jens Mayer has been taking a closer look at the DNA sequences from polar bears (*Ursus maritimus*) and great pandas (*Ailuropoda melanoleuca*).

“We have characterized endogenous retroviral sequences in both bear species and found a strong similarity between the two, which indicates that these two virus species are closely related,” says human geneticist Mayer. The researchers also identified ERV sequences in other bear species such as the brown bear (*Ursus arctos*), the black bear (*Ursus americanus*) and the spectacled bear (*Tremarctos omatus*). “Using molecular dating methods we have now been able to show that the retrovirus became integrated into the genetic material of an ancestor of today’s bear species around 45 million years ago,” explains Greenwood. The research team also showed that the original retrovirus was closely related to those found in the genomes of bats and cattle. Interestingly, the viruses found in bears exhibit strong similarity with several endogenous retroviruses found in the human genome. “Some of these sequences are suspected of playing a role in the occurrence of cancer, neurodegenerative or autoimmune diseases,” says Mayer.

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Western Screech Owl *(Megascops kennicottii).*

Dawn® Expands 40-Year Commitment to Wildlife Rescue

CINCINNATI, Ohio, USA (July 16, 2013)—Proctor & Gamble’s Down dishwashing brand has expanded its more than 40-year commitment to help save and protect wildlife with a $1 million donation to its wildlife partners and the premiere of “The Big Picture” documentary series, narrated by Dawn Saves Wildlife advocate Rob Lowe. The documentary series highlights Dawn’s wildlife partners, International Bird Rescue and The Marine Mammal Center, offering inside looks at the difficult journeys of animal patients and how rescuers and volunteers advance wildlife conservation each day. The 7-webisode documentary series spotlights an abandoned newborn seal pup and a pelican contaminated by boating oil. The first episode premiered in mid-July, with subsequent episodes released through September. More than 40 years ago, wildlife rehabilitators found that Dawn could safely cut through oil without damaging feathers and fur. Following each episode, viewers can send e-thank you cards to rehabilitators.

Owlets Safe and Sound After Rescue from California Wildfire

FRESNO, California, USA (July 19, 2013)—In June of this year, as firefighters built a control line in the Sierra National Forest, they cut down a tree that happened to be home to two young Western screech owls. One of the firefighters, Nick Gauthier, quickly scooped them up and kept them out of harm’s way. Anae Otto, the biologist for that region of the forest, was summoned. Otto cared for the owls overnight, and then turned them over to wildlife rehabilitator Terri Williams, a volunteer at the Fresno Wildlife Rescue and Rehabilitation Service (FWRRS). Western screech owls are common in the region, with a call sometimes described as sounding like a bouncing ball because it starts slow and accelerates. The owlets will remain at the rehabilitation center for a few more weeks before being released back into the wild.

Rare Sea Turtle Released After Rescue and Rehabilitation

ST. AUGUSTINE, Florida, USA (July 20, 2013)—It was a rare day at the beach in St. Augustine, Florida. Rare because more than 100 people gathered on short notice to watch a sea turtle return to its natural home after being stranded four months earlier. Even more rare because the turtle being released is a Kemp’s Ridley, the rarest sea turtle in the world. “There are probably only around 1,000 nesting females in the whole world,” said Dan Palmer, who rescued the turtle in March. A family found it stranded in a tidal pool and called Marineland Dolphin Adventure for help. Palmer, the assistant manager of aquarists at Marineland, drove the turtle to a climate-controlled tank two miles away. He arranged for volunteers to transport the animal to the Volusia County Marine Science Center, which has cared for more than 900 juvenile and adult sea turtles since it opened in 2002. The turtle was underweight, slightly dehydrated, hypothermic, and hypoglycemic. He also had some buoyancy issues, probably as a result of pneumonia, and a digestive track compacted with crab shells. After being treated with fluids, antibiotics, and antifungal medications, the turtle gained over 2 pounds and was approved for release. Though information about the release wasn’t announced publicly, rumors spread in the marine wildlife community, which gathered to cheer the turtle on his way. The Kemp’s Ridley breeds in the Gulf of Mexico but travels as far as New England when the water is warm. If temperatures drop too quickly for the turtles to migrate south they can get “cold stunned” and develop pneumonia and other illnesses.

SeaWorld Orlando Rescue Team Releases Four Manatees

ORLANDO, Florida, USA (July 20, 2013)—A rescue team from SeaWorld Orlando has released four female manatees into the Indian River Lagoon in central Florida following several months of rehabilitation. Three were rescued last December after they were found ensnared in a mosquito impoundment in the Merritt Island National Wildlife Refuge. In March, the fourth manatee was rescued by the Florida Fish and Wildlife Conservation Commission from the Indian River. All four manatees managed to slip easily into the water and were visible to the team for approximately 20 minutes before disappearing into deeper waters. SeaWorld Orlando has released a total of seven manatees this year and has successfully rescued 13. More than 22,000 animals have received medical treatment at SeaWorld.

Novel Poxvirus in Big Brown Bats, Northwestern United States

G. L. Emerson, R. Nordhausen, M. M. Garner, J. R. Huckabee, S. Johnson, R. D. Wohrle, W. B. Davidson, K. Wilkins, Y. Li, J. B. Doty, N. F. Gallardo-Romero, M. G. Metcalfe, K. L. Karem, I. K. Damon, and D. S. Carroll.

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ABSTRACT: A wildlife hospital and rehabilitation center in the northwestern United States received several big brown bats *(Eptesicus fuscus)* with necrosuppurative osteomyelitis in multiple joints. Wing and joint tissues were positive by PCR for poxvirus. Thin-section electron microscopy showed poxvirus particles within A-type inclusions. Phylogenetic comparison supports establishment of a new genus of *Poxviridae*.

KEY WORDS: Big brown bats, *Eptesicus fuscus*, necrosuppurative osteomyelitis, new genus, PAWS Wildlife Center, *Poxviridae*, zoonotic disease

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Introduction

Bat species worldwide have been implicated as reservoirs for several emerging viruses such as lyssaviruses, henipahviruses, severe acute respiratory syndrome-associated coronaviruses, and filoviruses. Bats have several physiologic, cellular, and natural history characteristics that may make them particularly suited to their role as reservoir hosts (Calisher *et al*. 2006; Wang *et al*. 2011).

Chordopoxviridae is a subfamily of *Poxviridae* that contains large, double-stranded DNA viruses that replicate in the cellular cytoplasm and are known to infect a wide range of vertebrates. Many of these viruses cause zoonotic disease in humans. Although poxviruses are known to have incorporated host genes into their genomes to subvert the host immune system (Odom *et al*. 2009), bats and poxviruses may also serve as facilitators in the horizontal transfer of transposable elements to other species (Pace *et al*. 2008; Gilbert *et al*. 2009; Schaack *et al*. 2010). We report the isolation and characterization of a viable poxvirus from bats.

The Study

During 2009–2011, six (5 male and 1 sex unknown) adult big brown bats (*Eptesicus fuscus*) were brought to a wildlife hospital and rehabilitation center (PAWS Wildlife Center, Lynnwood, Washington, United States) during late spring or summer because

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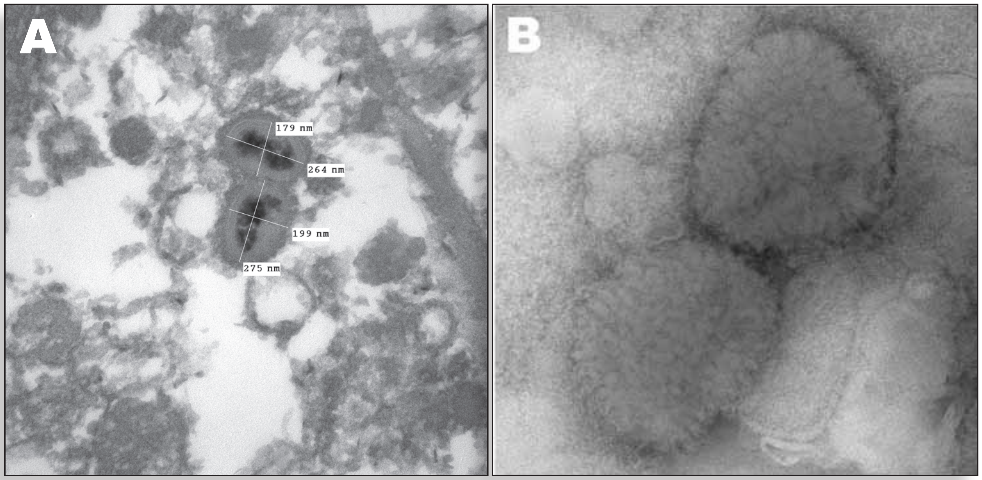

FIGURE 1. (A) Electron micrograph of poxvirus particles in synovium of a big brown bat, northwestern United States. (B) Negative staining of poxvirus particles in cell culture supernatant.

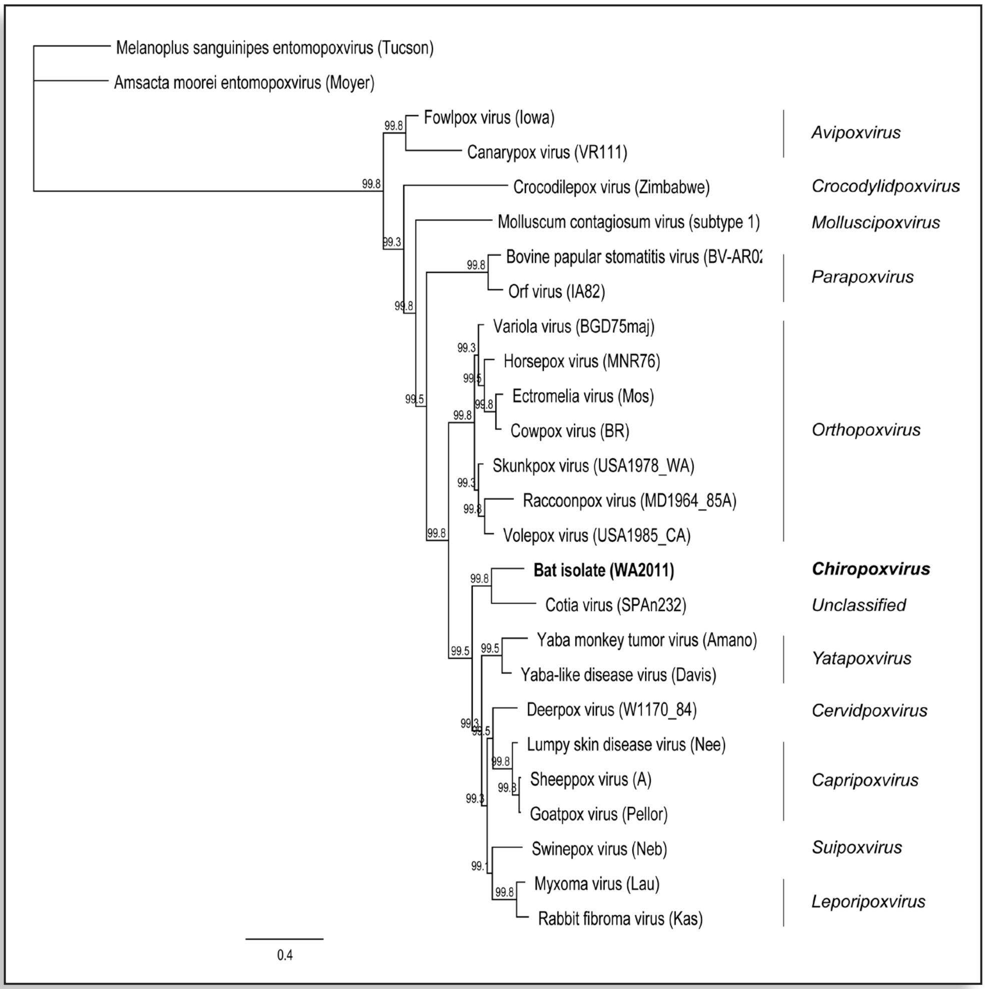

FIGURE 2. Maximum clade credibility tree generated by MrBayes in Geneious version 6.0.5 (http://www.geneious.com/) using amino acid sequences from seven open reading frames (final chain length 240,000 at an average standard deviation of split frequencies of zero) for poxviruses. Clade credibility values are indicated at each node. The virus isolated in this study is shown in boldface. *Amsacta moorei* (red hairy caterpillar) entomopoxvirus (Moyer) was used as the out-group. Scale bar indicates amino acid substitutions per site.

they could not fly. All but one of the bats had ≥1 visibly swollen and occasionally contused joints involving the long bones of the legs and wings; one had contusions of the oral commissures.

All bats received care that included antimicrobial drugs and nutritional and fluid support. However, minimal or no clinical improvement was observed and the bats showed progressive joint swelling and increased lethargy. All bats were eventually euthanized. In all instances gross lesions were limited to the joints.

Bat tissues were sent to a facility that specializes in the pathologic analysis of nondomestic species (Northwest ZooPath, Monroe, Washington, USA) for further investigation. Histologic examination showed severe fibrino-suppurative and necrotizing tenosynovitis and osteoarthritis that involved the long bones, and occasionally facial flat bones and joints, with occasional localized vasculitis. No bacterial or fungal agents were seen by light microscopy of specimens stained with hematoxylin and eosin, Giemsa, Warthin-Starry, Brown and Brenn, or Gomori's methenamine silver stains or in a Wright–Giemsa-stained cytologic preparation of a joint aspirate. Cultures for aerobic and anaerobic bacteria and cultures of the joint from one bat for mycoplasma showed negative results.

Thin-section electron microscopy [EM] of synovial tissue extracted from a wax histoblock showed poxvirus particles in inflammatory cells (Fig. 1, panel A). A 906E transmission electron microscope (Carl Zeiss, Peabody, Massachusetts, USA) at an accelerating voltage of 80 kV was used for initial imaging. Digital images were captured by using a 2K × 2K camera (Advanced Microscopy Techniques, Danvers, Massachusetts, USA). The state veterinarian and the Centers for Disease Control and Prevention (Atlanta, Georgia, USA) were subsequently consulted.

Material was taken from the wing and joint of an affected bat for real-time polymerase chain reaction (RT-PCR) testing and cell culture isolation. Wing and joint material was positive by RT-PCR for a poxvirus with low genomic G + C content (Li *et al.* 2010a). The elbow joint of one bat was then processed for poxvirus growth in cell culture. The specimen was emulsified in 500 ml of sterile phosphate-buffered saline by using a tissue grinder. Viral nucleic acid was extracted by using EZ1 Advanced XL (Qiagen, Valencia, California, USA). Ten microliters of homogenate were added to 1 ml of RPMI 1640 medium supplemented with 2% fetal bovine serum, l-glutamine, and penicillin–streptomycin. Growth medium from a T25 flask containing green monkey kidney epithelial cells (BSC40) was removed and the virus mixture added. The flask was incubated for 1 hr at 56°C after which 6 ml of RPMI 1640 medium was added to each flask. Cells and supernatant were harvested after 95% of the monolayer was infected.

Negative-stain EM was performed by using cell culture supernatant. Two microliters of supernatant were pipetted onto a 300-mesh formvar–carbon-coated nickel grid. After a 10-min incubation supernatant was blotted and the grid was rinsed. A negative stain composed of 5% ammonium molybdate, pH 6.9, and 0.1% trehalose (wt/vol) was briefly applied to the grid and blotted. The grid contents were visualized by using a Tecnai BioTwin electron microscope (FEI Company, Hillsboro, Oregon, USA) operating at 120 kV. Digital images were captured by using a 2K × 2K camera (Advanced Microscopy Techniques). Poxvirus particles were identified in cell culture supernatant (Fig. 1, panel B).

Genome sequencing produced data that were used to construct a phylogenetic tree (Fig. 2). Virus DNA sequence data were collected by using the Illumina platform (www.illumina.com/technology/sequencing_technology. ilmn). DNA sequences from seven open reading frames (A7L, A10L, A24R, D1R, D5R, H4L, and J6R, according to reference sequence vaccinia virus Copenhagen) were extracted on the basis of sequence similarity. Data were deposited in GenBank under accession nos. KC181855–KC181861. Open reading frames were translated into amino acid sequences and aligned by using the ClustalW alignment option in Geneious version 6.0.5 (www.geneious.com). The tree search was conducted by using MrBayes in Geneious 6.0.5 under default settings and *Misact moorei* (red hairy caterpillar) entomopoxvirus (Moyer) was used as the out-group with a burn-in of 10%.

Conclusions

Historically, osteomyelitis with arthritis has been reported in smallpox patients (osteomyelitis variolosa) and in smallpox vaccine recipients (vaccinia osteomyelitis) but did not occur frequently (Eeckels *et al.* 1964). In such cases variola virus particles were detected in joint fluid (Cockshott and Macgregor 1958), and vaccinia virus was isolated from a bone biopsy specimen of an affected limb (Sewall 1949; Elliott 1959). It is unclear whether the manifestation of arthritis in bats is a normal or rare result of the infection or a new development in the evolution of the virus. Likewise, the frequency of poxvirus infection in big brown bats is impossible to estimate at this stage. Because the public is generally cautioned against handling downed bats because of possible rabies infection, underestimation of prevalence is likely. Infectious disease surveys of bats might have missed the infection up to this point because no obvious lesions are apparent on the skin, and swollen joints might have been classified as being arthritis without suspicion of infectious disease involvement.

Results of at least three investigations that involved detection of viral DNA detection in bat guano have been published; two involved bats from North America and one involved bats in China (Donaldson *et al.* 2010; Li *et al.* 2010b; Ge *et al.* 2012). No evidence of poxviruses was found in the animals investigated in those studies. The zoonotic potential or host range of the virus described herein is not known but, at a minimum, the virus could pose a newly emergent threat to bat populations. Likewise it is not clear if the infection seen in bats is a result of spillover or possibly an introduction of the virus into a new area. The isolate does not group with any of the eight characterized genera of *Chordopoxvirus*; its nearest neighbor is Cotia virus, an as yet unclassified chordopoxvirus first isolated from sentinel suckling mice in a state reserve in Cotia County, São Paulo State, Brazil, in 1961 (Lopesode *et al.* 1965).

Although the two viruses are nearest neighbors, levels of shared nucleotide and amino acid identity between them suggest they should likely be considered separate genera (Table, Appendix, wwwnc.cdc.gov/EID/article/19/6/12-1713-T1.htm). We propose that the bat-derived isolate be distinguished as part of a new lineage with the suggested genus designation *Chiropoxvirus*. Further efforts should be undertaken to determine whether Cotia virus should be included in this genus. The bat-derived virus requires a new species designation for which we propose *Eptesipox virus* because of its isolation from an *Eptesicus fuscus* bat specimen.

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We thank Heather Bair-Brake for initially contacting the poxvirus staff at the Centers for Disease Control and Prevention regarding the events.

Literature Cited

Calisher, C. H., J. E. Childs, H. E. Field, K. V. Holmes, and T. Schountz. 2006. Bats: Important reservoir hosts of emerging viruses. *Clinical Microbiological Reviews* 19(3): 531–545.

Cockshott, P., and M. Macgregor. 1958. Osteomyelitis variolosa. *QJM: An International Journal of Medicine* 27(107): 369–387.

Donaldson, E. F., A. N. Haskew, J. E. Gates, J. Huynh, C. J. Moore, and M. B. Frieman. 2010. Metagenomic analysis of the viromes of three North American bat species: Viral diversity among different bat species that share a common habitat. *Journal of Virology* 84(24): 13004–13018.

Eeckels, R., J. Vincent, and V. Seynhaeve. 1964. Bone lesions due to smallpox. *Archive of Disease in Childhood* 39(208): 591–597.

Elliott, W. D. Vaccinal osteomyelitis. 1959. *Lancet* 2(7111):1053–1055.

Ge, X., Y. Li, X. Yang, H. Zhang, P. Zhou, Y. Zhang, and Z. Shi. 2012. Metagenomic analysis of viruses from bat fecal samples reveals many novel viruses in insectivorous bats in China. *Journal of Virology* 86(8): 4620–4630.

Gilbert, C., J. K. Pace, and C. Feschotte. 2009. Horizontal SPINning of transposons. *Communicative and Integrative Biology* 2(2): 117–119.

Li, Y., H. Meyer, H. Zhao, and I. K. Damon. 2010a. GC content-based pan-pox universal PCR assays for poxvirus detection. *Journal of Clinical Microbiology* 48(1): 268–276.

Li, L., J. G. Victoria, C. Wang, M. Jones, G. M. Fellers, T. H. Kunz, and E. Delwart. 2010b. Bat guano virome: Predominance of dietary viruses from insects and plants plus novel mammalian viruses. *Journal of Virology* 84(14): 6955–6965.

Lopesode, S., J. P. Lacerda, I. E. Fonseca, D. P. Castro, O. P Forattini, and E. X. Rabello. 1965. Cotia virus: A new agent isolated from sentinel mice in São Paulo, Brazil. *American Journal of Tropical Medicine and Hygiene* 14: 156–157.

Odom, M. R., R. C. Hendrickson, and E. J. Lefkowitz. 2009. Poxvirus protein evolution: Family-wide assessment of possible horizontal gene transfer events. *Virus Research* 144(1–2): 233–249.

Pace, J. K. II, C. Gilbert, M. S. Clark, and C. Feschotte. 2008. Repeated horizontal transfer of a DNA transposon in mammals and other tetrapods. *Proceedings of the National Academy of Sciences of the United States of America.* 105(44): 17023–17028.

Schaack, S., C. Gilbert, and C. Feschotte. 2010. Promiscuous DNA: Horizontal transfer of transposable elements and why it matters for eukaryotic evolution. *Trends in Ecology and Evolution* 25(9): 537–546.

Sewall, S. Vaccinia osteomyelitis. 1949. Report of a case with isolation of the vaccinia virus. *Bulletin of the NYU Hospital for Joint Diseases* 10: 59–63.

Wang, L. F., P. J. Walker, and L. L. Poon. 2011. Mass extinctions, biodiversity and mitochondrial function: Are bats 'special' as reservoirs for emerging viruses? *Current Opinion in Virology* 1(6): 649–657.

Effects of Age and Body Mass on Development of Diving Capabilities of Gray Seal Pups: Costs and Benefits of the Postweaning Fast

K. A. Bennett, B. J. McConnell, Simon E. W. Moss, J. R. Speakman, P. P. Pomeroy, and M. A. Fedak

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ABSTRACT: Development of adequate diving capabilities is crucial for survival of seal pups and may depend on age and body size. We tracked the diving behavior of 20 gray seal pups during their first 3 mo at sea using satellite relay data loggers. We employed quantile analysis to track upper limits of dive duration and percentage time spent diving as well as lower limits of surface intervals. When pups first left the breeding colony, extreme (95th percentile) dive duration and percentage time spent diving were positively correlated with age, but not mass, at departure. Extreme dive durations and percentage time spent diving peaked at 67 ± 12 days of age at values comparable with those of adults but were not sustained. Greater peaks in extreme percentage time spent diving occurred in pups that had higher initial values, were older at their peak, and were heavier at departure. Pups that were smaller and less-capable divers when they left the colony improved extreme dive durations and percentage time spent diving more rapidly once they were at sea. We speculate that once small pups have attained a minimum degree of physiological development to support diving, they would benefit by leaving the colony when younger, but larger, to maximize limited fuel reserves. *[Edited for space.]*

KEY WORDS: diving capability, dive duration, foraging skill, gray seals, *Halichoerus grypus*, pinnipeds

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Introduction

The development of diving and foraging skills may contribute to observed size-, sex-, and condition-related variation in first-year survival of gray seals (*Halichoerus grypus*; Fabricius; Hall *et al*. 2001, 2002, 2009). However, little is known about diving capabilities of pups when they first leave the natal colony, the early development of their diving ability at sea, and the relationship between these capabilities and sex, age, body size, and condition.

Gray seal pups triple in mass and increase their body fat content to about 45% during an intensive, approximately 18-day suckling period. They are weaned abruptly and undergo a prolonged, land-based post-weaning fast of 10–40 days before they go to sea (Fedak and Anderson 1982; Noren *et al*. 2008). These naive pups must learn to forage successfully before endogenous fuel reserves are depleted. Their ability to spend time underwater in profitable foraging areas, either during individual dives or as a proportion of time over longer periods, should impact on their opportunities to find food and thus affect their survival chances (Fedak 1986; Kramer 1988; Boyd 1997; Mori 1998; Thompson and Fedak 2001; Sparling *et al*. 2007).

Adult seals normally dive well within their capabilities to avoid repeated anaerobic dives and thus maximize time submerged. However, young pinnipeds have more-limited diving capabilities than do adults (Lestyk *et al*. 2009; Prewitt *et al*. 2010) and often operate close to their physiological limits to forage effectively (Boily and Lavigne 1997; Burns 1999; Fowler *et al*. 2006). When they first go to sea gray seal pups possess only 66–67% of the mass-specific oxygen stores of yearlings and adult females (Noren *et al*. 2005).

Maximum diving capabilities of free-ranging adult southern elephant seals (*Mirounga leonina*) and a juvenile monk seal (*Monachus monachus*) have been inferred from extremes of dive behavior such as 95th percentile dive durations (Bennett *et al*. 2001; Dendrinos *et al*. 2007). This quantile approach tracks the upper limits of diving behavior as a proxy for diving capability, which is limited by oxygen storage capacity and diving metabolic rate. It thus attempts to separate behavioral choice from physiological limitations. It is used here to examine early development of diving capabilities of wild gray seal pups during their first 3 mo at sea. As in previous studies using this approach, we assumed that pups may on occasion choose or be forced to push themselves to physiological extremes to find food. Three metrics that reflect extreme dive performance were used: 95th percentile time spent submerged during individual dives, fifth percentile time spent at the surface after long (12 min) dives, and 95th percentile percentage of time at sea spent diving over 6-hr periods.

We investigated the impact of age, weaning, departure mass and body composition, year, and sex on these dive performance metrics and their ontogeny. Condition at weaning reflects how well pups were equipped by their mothers at the start of the post-weaning fast, whereas mass and body composition at departure reflect decisions made by pups about fuel use and fast duration and indicate condition when pups first went to sea. In other pinnipeds, body size and composition affect survival and diving capabilities (Burns and Castellini 1996; Burns *et al*. 1997; Horning and Trillmich 1997; Hindell *et al*. 1999; Irvine *et al*. 2000). Body size and composition determine the amount of energy available to pups as they learn to forage. They also influence diving ability through effects on muscle mass, oxygen storage capacity, buoyancy, and metabolic rate, which is proportional to mass 0.76 in gray seals (Sparling and Fedak 2004).

Age is linked to physical and physiological maturity in pinniped pups (Noren *et al*. 2005; Prewitt *et al*. 2010). Phocid pups that stay ashore longer may have greater breath-hold capabilities when they first go to sea because of muscle and blood oxygen storage capacity, buffering capacity, activity of key glycolytic and aerobic enzymes, and cardiovascular control developed during suckling and the land-based fast (Thorson and Le Boeuf 1994; Lewis *et al*. 2001; Noren *et al*. 2005; Lestyk *et al*. 2009; Prewitt *et al*. 2010). These changes are driven by developmental or exercise-induced changes (or both) over time and do not scale with body mass. Age and, particularly, fast duration may thus affect diving capabilities in gray seal pups. Smaller and younger pups should utilize their more-limited oxygen stores more rapidly and thus should be unable to dive for as long as the larger and older individuals.

We explored the relationship between body size, condition, sex, and dive performance metrics and the duration of the tracking period, which we used as an index of minimum survival time, and we compared the extreme diving performance of pups with their routine behavior and that of adults.

Materials and Methods

We examined the effects of age at departure, sex, year, mass, and body composition at departure on the diving capabilities of 20 gray seal pups from the Isle of May, Firth of Forth, Scotland (56°11′N, 2°33′W) in October–December 2001 and 2002. Capture and handling procedures were performed under Home Office project license 60/2589 and conformed to the Animals (Scientific Procedures) Act 1986.

Mass and Body Composition Measurements

All (except one pup) were captured early (age ~4 days) and late (age ~15 days) in the suckling period. Pup sex was recorded and flipper tags were attached at first capture. Pups were weighed (±0.2 kg) at each capture and daily rate of mass gain (kg/day) during suckling was calculated. Body composition at each capture during suckling was estimated using deuterium (^2H_2O) dilution (Reilly and Fedak 1990) for 17 of the 20 pups (10 male and seven female) as follows.

After the animal was weighed, a blood sample was collected from the extradural vein both before and 3–4.5 hr (Costa *et al*. 1986; Reilly 1991; Bennett *et al*. 2007) after intravenous injection of a preweighed dose of 3–5 ml ^2H_2O (99.9%; Sigma-Aldrich Chemicals, Gillingham, Dorset, UK). The ^2H_2O enrichment in parts per million, in two subsamples of the background and enriched plasma samples and standards, was measured in duplicate in a Micromass IsoPrime pyrolysis inlet mass spectrometer (Speakman and Racey 1987, method D; Speakman and Krol 2005). Dilution space was calculated (Krol and Speakman 1999) and percentages and absolute masses of fat, protein, water, and ash were determined from body water content using equations derived by comparison of ^2H_2O dilution with chemical composition of gray seal carcasses (Reilly and Fedak 1990).

Weaning, determined from daily observations of mother–pup pairs, occurred 2.3 ± 1.8 days after the late suckling mass and body composition measurement. Weaning mass and body composition were determined by extrapolation using rates of change in mass and body components during suckling (Bennett *et al*. 2007). Pups were penned in a large outdoor enclosure within 2 days of weaning to allow pups to be located easily without disturbing other animals on the colony (Bennett *et al*. 2007). Body mass was measured at penning and at 3-day intervals.

Pups were released from the pen when they reached 70% of weaning mass or 30 kg, whichever happened sooner (Bennett *et al*. 2007). These criteria were selected on the basis of percentage weaning mass lost by departure in southern elephant seal pups (Carlini *et al*. 2001) and estimated departure masses of gray seal pups from the Isle of May in previous years (Sea Mammal Research Unit [SMRU], unpubl. data) with a safety margin of 4 kg. Body composition was measured for 12 pups (six male and six female) on the day of release from the pen (14–31 days after weaning; mean = 20 ± 5 days) as described above. Weighing continued every 3 days after release until the animal departed from the colony. Mass and body composition (fat, protein, water, and ash) on the day of departure were extrapolated from changes between the estimate at weaning and the measurement at release (Bennett *et al.* 2007).

Satellite Relay Data Logger Deployment and Dive Data Collection

On the day of release, pups were anesthetized with 0.025 mg/kg intravenous Zoletil 100 (Virbac, Carros, France), and a satellite relay data logger (SRDL; SMRU, Fife) was glued to the fur at the base of the skull using a two-part rapid-setting epoxy resin (McConnell *et al.* 1999). SRDLs compile information from sensors detecting pressure, dry periods, and swimming speed to create a compressed record of behavior encoding time-depth profiles, duration, and surface interval (SI) for individual dives. Percentage of time spent in each of three mutually exclusive states—dive, haul out, and at surface, defined previously (McConnell *et al.* 1992; Fedak *et al.* 2001)—were also recorded every 6 hr.

Data Extraction

Date of departure was the day on which the animal showed a rapid transition from continuous haul-out at the breeding site to diving and movement away from the colony, and it coincided in all cases with the day on which the animal was no longer seen on the colony. Dive durations were grouped into bins of 100 dives from departure for each pup. The bins thus varied in duration depending on the time elapsed for 100 dives to be recorded; mean duration of the first 100-dive bin was 3.95 ± 1.82 days. Post-dive SIs were also grouped in this way. Only SIs following dives of more than 2 min were considered in order to eliminate short dives that were unlikely to represent a physiological challenge. The percentage of time spent at sea in the dive state (percent dive) was grouped into bins containing 16 of the 6-hr summaries. Summary periods containing haul-out behavior were excluded to ensure that percent dive represented purely at-sea behavior. Dive data from 17 adults and subadults (11 male, six female; mean mass = 98.3 ± 23.6 kg) from Abertay Sands, Scotland (SMRU, unpubl. data) were collected and analyzed in the same way.

We extracted mean and extreme (95th percentile) dive duration and percent dive, and mean and extreme (fifth percentile) SI, for each animal at two time points: initial values were those that occurred in the first data bin when the animals first went to sea, and peak values were those that occurred when animals achieved their highest (dive durations and percent dive) or lowest (SI) values in each extreme performance metric during their first 3 mo at sea (Fig. 1).

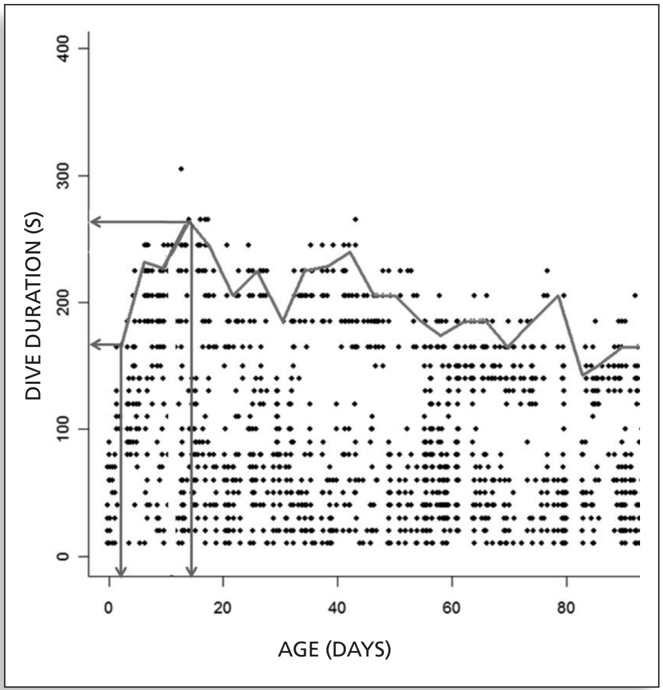

FIGURE 1. Illustration of how dive performance metrics were obtained from dive data using 95th percentile dive duration as an example. All dive durations are shown as dots, each of which can represent several dives. The 95th percentile dive duration is the solid line superimposed on the data. Arrows show how initial and peak 95th percentile values and the age at which they occurred were derived. Fifth percentile post-dive surface interval and 95th percentile percentage time at sea spent in dive (percent dive) and the ages at which they occurred were derived in a similar way. A color version of this figure is available in the online edition of *Physiological and Biochemical Zoology.*

Statistical Analysis

Statistical procedures were performed in Minitab 13.32 and R 2.4.0 (R Development Core Team 2006; Ihaka and Gentleman 1996). ANOVAs or MANOVAs were used to detect sex and year differences in explanatory variables. Pearson's correlations were used to explore the relationships between explanatory variables in order to arrive at the most-representative, independent explanatory variables to include in each model. Dive depth was included for dive duration analyses and the preceding dive duration was included for SI analyses. Backward and forward stepwise linear regression was used to identify the best model to explain dive performance metrics and log track duration.

Results

Sex and Year Differences in Mass, Body Composition, and Age

Mean mass and body composition at weaning and departure, age at weaning, experimental fast duration, and age at departure for males and females are given in Table 1. There were no sex differences in body mass at weaning (ANOVA; F1,19 = 0.17, P = 0.684) or departure (ANOVA; F1,19 = 0.65, P = 0.432). Sex and year differences between pups in mass, protein mass, and percent fat at

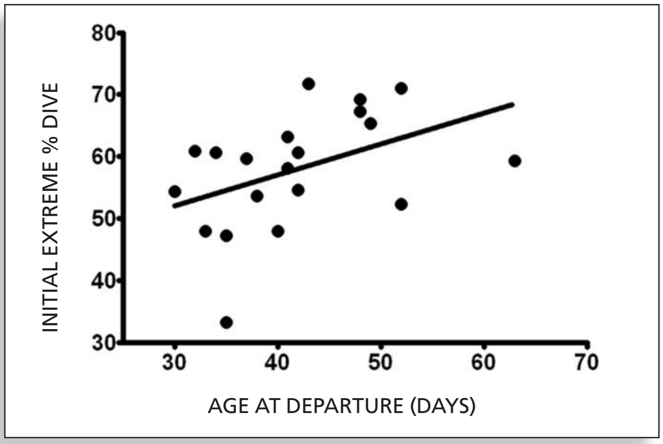

FIGURE 2. Positive linear relationship between initial extreme (95th percentile) percentage time spent in dive (percent dive) and age at departure (initial extreme percent dive = 0.66 [age at departure] – 29.56; P = 0.014, $F_{1,18}$ = 7.396, R^2 = 25.19%.

weaning and departure were tested independently using MANOVA. There were no significant differences between males and females in the combination of mass, protein mass, or percent fat at weaning (Pillai's trace; F3,13 = 0.177, P = 0.910) or at departure (Pillai's trace; F3,8 = 3.141, P = 0.087). There was no difference between the sexes in age at weaning (ANOVA; F1,19 = 0.01, P = 0.925), but the difference between males and females in age at departure was significant (ANOVA; F1,19 = 10.26, P = 0.005). Males were, on average, 7.2 days younger than females at departure (Table 1).

Weaning mass (ANOVA; $F_{1,19}$ = 0.01, P = 0.940) and departure mass (ANOVA; $F_{1,19}$ = 0.08, P = 0.785) did not differ between years. There were no significant differences between years in the combination of mass, protein mass, or percent fat at weaning (Pillai's trace; $F_{3,13}$ = 0.01, P = 0.999) or at departure (Pillai's trace; $F_{3,8}$ = 2.315, P = 0.152). Pups in both years weaned at a similar age (ANOVA; $F_{1,19}$ = 0.44, P = 0.515) but pups in 2002 left the colony significantly earlier than did those in 2001 (ANOVA; $F_{1,19}$ = 15.99, P = 0.001; 37 ± 6.47 vs. 47.2 ± 7.35 days of age, respectively). There was no interaction between sex and year in their effects on age at departure (ANOVA; $F_{1,19}$ = 1.23, P = 0.284).

Impact of Penning

Although they were free to do so, most pups did not leave the colony on the day of release. Departure occurred 5 ± 4 days (range = 0–16) after the body composition measurement at release from the pen. The difference in weaning mass between pups that left the colony on the day of release (median wean mass = 39.75 kg; n = 8) and those that stayed beyond the day of release (median wean mass = 49.30 kg; n = 12) approached significance (Mann–Whitney; W = 151.0, P = 0.0587). There was no difference in the departure mass between these two groups of pups (Mann–Whitney; W = 116.0, P = 0.4636). Fast duration was shorter in pups that left on the day of release (median fast duration = 13.5 vs. 25.5 days; Mann–Whitney; W = 167.5, P = 0.0016) but there was no difference in age at departure (t-test; t = 1.55, df = 18, P = 0.139). In pups that chose to remain on the colony after release (>1 day), there was no significant correlation between mass at weaning and departure (Pearson's correlation = 0.497, P = 0.10, n = 12), age and mass at departure (Pearson's correlation = 0.085, P = 0.792, n = 12), or weaning mass and age at departure (linear model; $F_{1,11}$ = 2.24, P = 0.165, n = 12).

Relationships Between Explanatory Variables

Year, sex, age at departure, and estimated body mass at departure were included in the saturated models for all animals on the basis of the ANOVAs and correlations given in Table 2 (n = 20). Age at sea at which each of the peaks occurred was also included in the analyses of the peaks in extreme dive performance metrics. Estimated percent fat at departure was included for the subset of animals for which this information was available (n = 12). When only those pups that left the colony >1 day after release—and that therefore had a greater degree of choice in their timing of departure—were considered in the analyses, weaning mass was not significantly correlated with either departure mass (Pearson's correlation = 0.497, P = 0.100) or age at departure (Pearson's correlation = 0.470, P = 0.123), and departure mass was not significantly correlated with age at departure (Pearson's correlation = 0.085, P = 0.792).

Impact of Age, Sex, Year, and Body Mass and Composition on Dive Capability

Mean and extreme values for initial and peak dive metrics are shown in Table 3. Departure mass, percent fat at departure, and year had no effect on initial extreme dive durations or percent dive. Initial extreme dive duration increased as a function of dive depth and age at departure (Table 4). There was a significant positive relationship between initial extreme percent dive and age at departure (Fig. 2). Extreme SIs were not affected by any of the explanatory variables in the first days at sea.

Extreme dive durations increased by 6.8 ± 4.7 sec/day (range = 1.9–18.3 sec/day). Steeper increases in extreme dive durations occurred in pups that were smaller at departure ($F_{1,19}$ = 6.883, P = 0.0172, R^2 = 23.6). There was no significant relationship between age or mass at departure and the age or time at sea at which pups achieved their peak extreme dive durations. The size of the peak in extreme dive durations, the rate of decrease in extreme SIs, and the lowest extreme SIs were not related to any explanatory variables.

Extreme percent dive varied substantially between animals when pups first went sea but not when they reached their peak (Table 3). The rate of increase in extreme percent dive varied from 0.1% to 1.76% per day between pups. Steeper increases in percent dive occurred in pups that spent less time diving when they first went to sea ($F_{1,19}$ = 10.95, P = 0.004, R^2 = 34.4). The change in rank in extreme percent dive between initial and peak values was not correlated with age at departure (R = 0.284, P > 0.05) but was strongly negatively correlated with departure mass (R^2 = –0.612, P < 0.01), showing that smaller pups improved their percent dive to a greater extent than did larger pups.

The peak in extreme percent dive was greater in pups that had a higher initial extreme percent dive. Given this relationship, the

TABLE 1: MEANS, STANDARD DEVIATIONS (SDS) AND RANGES FOR TRACK DURATION (TD), EXPERIMENTAL FAST DURATION (EFD) AND AGE, BODY MASS, PERCENTAGE FAT, AND PROTEIN MASS AT WEANING AND DEPARTURE

					WEANING			DEPARTURE		
SEX	TD (D)	AGE AT WEANING	EFD (D)	AGE AT DEPARTURE	MASS (KG)	FAT (%)	PROTEIN (KG)	MASS (KG)	FAT (%)	PROTEIN (KG)
Female:										
Mean	135.2	19.3	26.3	45.7	47.9	46.6	5.8	32.0	44.6	3.6
SD	78.8	2.4	8.6	9.8	10.2	2.5	1.0	5.3	3.6	.9
Range	62–310	18–24	13–39	33–63	38.8–66.1	43.3–50.2	5.1–7.2	26.2–41.0	32.8–48.5	2.76–5.4
Male:										
Mean	127.3	19.4	19.2	38.5	46.3	46.1	5.7	33.3	48.9	3.77
SD	80.6	2.3	5.6	5.2	7.2	2.3	.6	4.4	4.73	.56
Range	41–251	17–23	12–26	30–48	38.0–57.9	42.2–49.6	4.6–6.5	28.8–41.7	40.8–56	2.8–4.3
All:										
Mean	131.6	19.4	21.5	41.8	47.0	46.0	5.6	32.7	48.0	3.54
SD	79.8	2.3	6.9	8.3	7.2	2.2	.7	4.9	4.2	.52

TABLE 2: PEARSON'S CORRELATIONS BETWEEN EXPLANATORY VARIABLES USED IN ANALYSES

VARIABLE 1	VARIABLE 2	COEFFICIENT	*P*	*n*
Experimental fast duration	Age at departure	.961	<.0001	20
Age at departure	Weaning mass	.436	.054	
Age at departure	Departure mass	.181	.445	
Weaning mass	Departure mass	.519	.019	
Age at peak in extreme dive duration	Time at sea at peak extreme dive duration	.784	<.0001	
Age at peak in extreme percent dive	Time at sea at peak extreme percent dive	.771	<.0001	
Age at lowest extreme SI	Time at sea at lowest extreme SI	.813	<.0001	
Age at departure	Fat at weaning (%)	.325	.204	17
Age at departure	Protein mass at weaning	.347	.173	
Weaning mass	Fat at weaning (%)	.729	.001	
Weaning mass	Protein mass at weaning	.955	<.0001	
Fat at weaning (%)	Protein mass at weaning	.499	.041	
Age at departure	Fat at departure (%)	.580	.048	12
Age at departure	Protein mass at departure	.373	.204	
Departure mass	Fat at departure (%)	.103	.750	
Departure mass	Protein mass at departure	.589	.044	
Fat at departure (%)	Protein mass at departure	.740	.006	

Note. SI, surface interval. Boldface indicates significant correlations between two variables ($P < 0.05$).

peak in extreme percent dive was higher in pups that were larger at departure and older when the peak occurred (Table 4).

There was no significant relationship between age at departure and the age at which pups achieved their peak extreme percent dive ($F_{1,19} = 0.89$, $P = 0.359$, $R^2 = 0\%$). However, pups that were older at departure achieved their peak after spending less time at sea ($F_{1,19} = 4.70$, $P = 0.044$, $R^2 = 16.6\%$).

Track duration was used as an index of minimum survival time; it was log transformed before analysis. Log track duration increased as a function of departure mass and was negatively correlated with the peak in extreme dive duration (Table 4). It was not affected by any other explanatory variables, and this was also true when only those pups that left the colony >1 day after release were considered in the analysis (log track duration = 0.77 departure mass + [−0.07 peak extreme dive duration] + 5.44; $F_{2,9} = 4.521$, $P = 0.043$, $R^2 = 39.03\%$).

Ontogeny of Mean and Extreme Dive Performance

The changes in extreme dive performance metrics over time are shown in Figure 3, with data from subadults and adults. The initial extreme dive durations of pups did not approach the range of extreme dive durations seen in adult gray seals (mean = 307.4 sec). The peak in pups' extreme dive durations was significantly shorter than the extreme dive durations of adults by ~50 sec (Mann–Whitney; *W* = 399, *P* = 0.021). Neither the pups' lowest extreme SI

TABLE 3: MEAN AND STANDARD DEVIATION (SD) FOR EACH DIVE PERFORMANCE PARAMETER FOR MALES AND FEMALES AND OVERALL

	INITIAL						PEAK					
	DD (S)		SI (S)		PERCENTAGE TIME IN DIVE (%)		DD (S)		SI (S)		PERCENTAGE TIME IN DIVE (%)	
SEX	MEAN	95TH PERCENTILE	MEAN	5TH PERCENTILE	MEAN	95TH PERCENTILE	MEAN	95TH PERCENTILE	MEAN	5TH PERCENTILE	MEAN	95TH PERCENTILE
Female:												
Mean	78.6	160.3	73.7	33.8	39.5	62.7	147.2	264.1	64.2	23.8	52.2	73.7
SD	12.9	25.0	18.3	6.4	11.0	8.3	18.2	5.3	11.1	7.1	7.2	3.2
Male:												
Mean	67.0	141.71	88.2	36.2	32.5	55.3	134.0	279.4	78.0	27.9	52.6	73.2
SD	18.1	38.3	12.9	6.7	9.3	9.7	26.7	36.2	12.4	4.3	4.8	4.2
All:												
Mean	72.2	150.1	81.7	35.2	35.7	57.9	140.0	272.6	71.8	26.1	52.4	73.5
SD	16.7	33.5	16.8	6.5	10.5	9.4	23.6	32.0	13.4	6.0	5.8	3.7

Note: Data include mean and extreme (ninety-fifth percentile) dive duration (DD) and percentage time spent in "dive" and mean and fifth percentile postdive surface interval (SI) when pups first went to sea (initial) and when they achieved their peak performance within 3 mo of departure from the colony (peak).

TABLE 4: LINEAR MODELS TO EXPLAIN VARIABILITY IN INITIAL EXTREME (NINETY-FIFTH PERCENTILE) DIVE DURATION, THE PEAK IN EXTREME (NINETY-FIFTH PERCENTILE) PERCENT DIVE, AND LOG TRACK DURATION FOR WHICH THE BEST MODEL FIT INCLUDED MULTIPLE EXPLANATORY VARIABLES

	INITIAL EXTREME DIVE DURATION			PEAK EXTREME DIVE			LOG TRACK DURATION		
	COEFFICIENT (S)	*P*	VARIANCE (%)	COEFFICIENT (%)	*P*	VARIANCE (%)	COEFFICIENT (DAYS)	*P*	VARIANCE (%)
Explanatory variables:									
Intercept	58.70	.0219		42.704	<.001		2.42	.0003	
Age	1.00	.0603	5.6	.141	.005	18.1		...	
DM			...	.334	.007	15.8	.02	.0581	14.75
Dive95i (%)		NA		.175	.003	20.9	...	...	...
DD95max		NA			NA		.004	.0215	22.94
Depth	1.54	<.001	71.6	NA			NA		
Model fit:									
F	27.91			14.496			5.501		
df	2, 17			3, 16			2, 17		
P	<.001			<.001			.0144		
R^2	.739			.681			.322		
n	20			20			20		

Note: Explanatory variables included in the best linear model fits are shown in the left column. The coefficients, P values, and percent variance explained (squared semipartial correlation) by each of the explanatory variables in the best linear model for each dive performance metric are given, and the fit (F, df, P, R2, and n) for each model is shown beneath. Ellipses indicate variables that did not improve the model fit. NA indicates variables that were not applicable to include in that model. DM, departure mass; DD95max, peak extreme (ninety-fifth percentile) dive duration achieved in the first 3 mo at sea; dive95i (%), extreme (ninety-fifth percentile) percentage time spent in dive in the first days at sea; age, age at departure (initial value for extreme dive duration) or at the peak (for peak percent dive [%].

(t-test; $t = 0.51$, $P = 0.612$, $n = 20$) nor their peak in extreme percent dive was significantly different from those of older seals (t-test; $t = 1.57$, df = 35, $P = 0.125$).

All pups showed a relatively consistent pattern of change in dive performance parameters during their first 3 mo at sea. Both mean (paired t-test; $t = 13.03$, $P < 0.0001$, $n = 20$) and extreme (paired t-test; $t = 10.33$, $P < 0.0001$, $n = 20$) dive durations increased significantly between 8 and 52 days after departure to almost double the initial values and then declined in most pups. This peak in extreme dive durations occurred between 53 and 96 days of age (mean = 67 ± 12 days of age). There was a significant decrease in mean (paired t-test; $t = 2.94$, $P = 0.008$, $n = 20$) and extreme SI (paired t-test; $t = 5.68$, $P < 0.0001$, $n = 20$) during the first 40 days at sea followed by an increase later in the tracking period (Fig. 3). Extreme SIs dropped by 9.07 ± 7.14 sec in the first days at sea to the lowest values at 45 ± 99 days of age (mean = 60.5 ± 12 days of age). There was a significant increase of 20–30% in mean (Mann–Whitney; $W = 254$, $P < 0.001$) and extreme percent dive (Mann–Whitney; $W = 229$, $P < 0.001$, $n = 20$) within the first 48 days at sea. This peak in percent dive occurred between 45 and 90 days of age (mean = 67 ± 12 days of age). Most pups did not sustain their best performance in any of the three metrics of extreme dive performance (Fig. 3). There was no correlation between the ages at which individuals achieved their peaks in extreme dive durations and extreme percent dive (Spearman's rank correlation; $R^2 = 0.358$), despite the similarity in mean age at which they occurred.

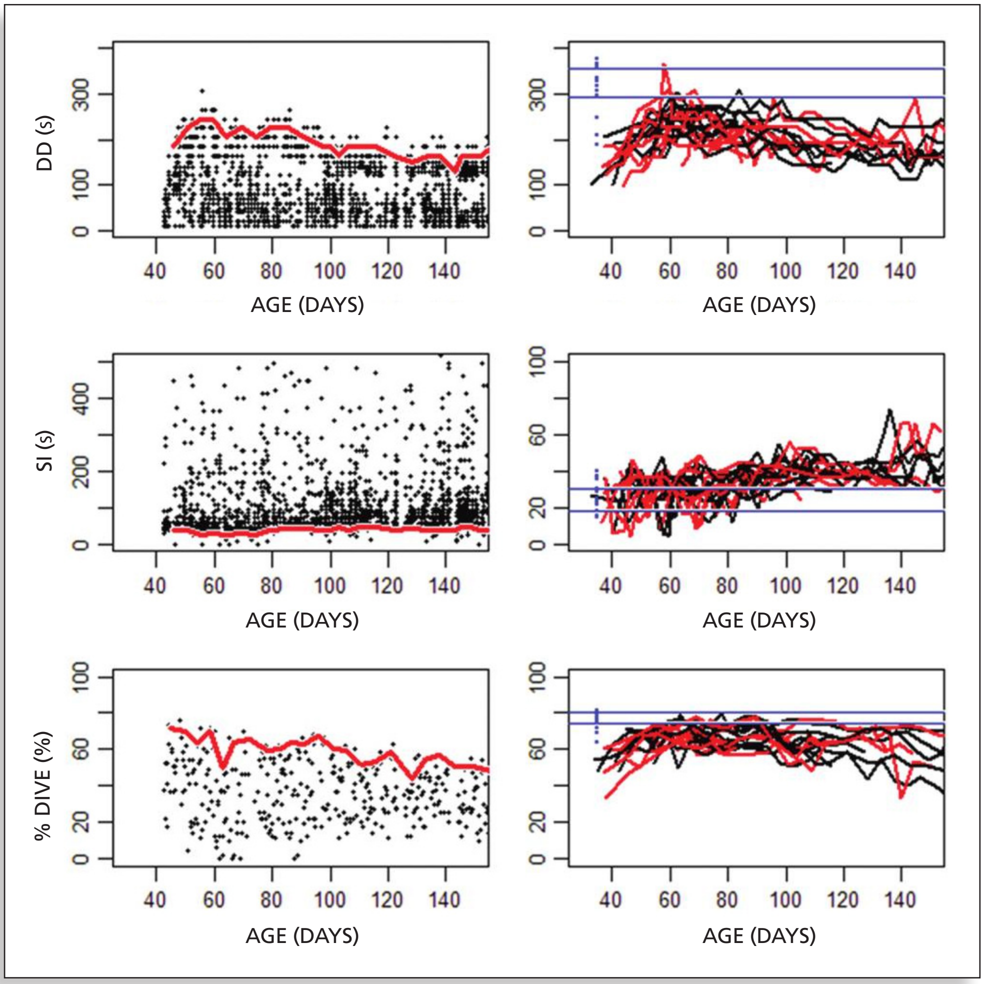

FIGURE 3. Changes in dive duration (dd; top), post-dive surface interval (SI; middle), and percentage of time spent in dive (percent dive; bottom) with age for a typical individual pup (left) and all pups combined (right). Left: dots represent all individual dives (top, middle) or summary periods (bottom). Red lines highlight overall 95th percentile dd (top), fifth percentile SI (middle), and 95th percentile percent dive (bottom). Right: lines highlight 95th percentile dd (top), fifth percentile SI (middle), and 95th percentile percent dive (bottom) for each pup. Males are shown in black and females in red. Blue dots show the mean dive metric value for the whole tracking period for each of the 17 subadult and adult seals. Straight blue lines indicate the upper and lower quartile ranges of values for subadults and adults.

Discussion

Impact of Penning and Departure from the Colony

Penning may have influenced the normal link between weaning and departure mass, and fast duration and age at departure, particularly in smaller animals. Pups that left the colony on the day of release from the pen tended to be smaller at weaning and undertook a shorter fast than those that chose to stay longer. We may therefore have prevented these pups from leaving the colony when they would otherwise have chosen to do so. They possibly departed at a lower body mass but a higher degree of physiological development than if they had been free-ranging. This finding suggests that small pups may normally choose to leave the colony when younger and larger rather than older but smaller. However, while body mass may be important in the decision to leave the colony, we did not see a clear relationship between age at departure and body mass at either weaning, as in other studies (Reilly 1991; Noren *et al.* 2008), or at departure. This was the case even when only those pups that left the colony more than a day after release—and which thus had a greater choice in the timing of departure—were included in the analysis. These findings may be a result of small sample size and truncation of the youngest ages at departure because the smaller, younger animals are those most likely to face a tradeoff between age and body size.

While penning constrained some pups that may have otherwise departed earlier, most pups remained on the colony after release. Free-ranging gray seal pups on Sable Island fasted for 9 to >31 days and were 26 to >49 days old when they went to sea (Noren *et al.* 2008). Fast duration and age at departure thus varied widely in both the penned pups here and those that were entirely free-ranging during the fast. Our youngest pup at departure was 30 days old, only 4 days older than the youngest pup to depart the colony in the study on free-ranging pups (Noren *et al.* 2008). Despite the possible extension to the fast in some pups, the animals in this study thus represent a similar age range—and thus a similar degree of physiological development—at departure to that seen in free-ranging animals (Noren *et al.* 2005, 2008). By limiting the lower end of fast duration and age at departure in our sample as a result of penning, we may have reduced the strength of observed relationships between age at departure and dive performance metrics. However, the degree (if any) to which behavior was affected by penning should not impact on observed relationships between age or mass at departure and subsequent dive capability.

Impact of Mass, Condition, and Age on Initial Dive Performance

Regardless of whether pups were able to leave the colony when they chose to as a result of penning, they exhibited a number of important relationships between mass, age, and dive performance. Strikingly, departure mass and body composition, which reflect the pups' condition when they left the colony, were not related to initial dive performance. This contrasts with the impact of body mass on diving behavior in other species, including southern elephant seals (Hindell *et al.* 1999; Irvine *et al.* 2000) and Weddell seals (Burns 1999), in which heavier individuals can perform longer and deeper dives than smaller conspecifics.

Since body size and composition are likely to affect dive performance through oxygen storage capacity, mass-specific metabolic rate, and buoyancy (Sparling and Fedak 2004), their effects on dive performance are likely to be apparent when pups are operating close to their calculated aerobic dive limit (cADL). Published cADLs of 204–228 sec from the gray seal pups from the northwest Atlantic population (Noren *et al.* 2005) are considerably longer than the initial extreme dive durations of the pups in this study. Here, the durations of the longest dive when pups first went to sea were closely linked with dive depth, which was constrained by water depth. It is possible, then, that the pups here could not or did not push themselves to their physiological limits when they first went to sea because they were diving in shallow water. For gray seal pups in the North Sea, body size and composition may be relatively unimportant in the first days at sea, provided they can dive adequately to find food. Alternatively, cADLs calculated for Canadian pups may overestimate the capabilities of the pups in this study because the pups here were much smaller, despite similar ages at departure.

Interestingly age, rather than mass, at departure had a positive impact on initial dive performance. Initial extreme dive durations and time spent submerged (percent dive) were greater in pups that were older at departure. These positive effects of age on diving capability are consistent with the rapid increase in oxygen storage capacity and the ability to manage oxygen reserves that occurs during suckling and the post-weaning fast in phocid pups (Thorson and Le Boeuf 1994; Lewis *et al.* 2001; Noren *et al.* 2001, 2005; Burns *et al.* 2007; Prewitt *et al.* 2010). Burns *et al.* (2007) have suggested that the length of the post-weaning fast in phocids is influenced by the rate and degree of muscle maturation. Pups that are younger when they leave the colony are likely to be less developed and less-capable divers than conspecifics that leave the colony when they are older (Noren *et al.* 2005, 2008). Here, the post-weaning fast was the major contributor to differences in age at departure; thus, effects of age on dive performance were most likely attributable to maturation processes that occur during this key developmental stage.

There was a ~30 sec difference in initial extremes in dive duration between pups that left the colony at 30 days old and those that left at 63 days old. This represents a significant fraction of the time the animal has to catch prey during a dive and could have a substantial impact on foraging success. However, the small (1 sec) daily increment in duration of the longest dives is unlikely to make a significant difference to feeding ability if pups stay only a few additional days on land, provided they have reached the minimum level of maturity in total body oxygen reserves that can support effective foraging (Noren *et al.* 2005; Burns *et al.* 2007). This highlights the importance of decisions made by the pup on land regarding fuel use and timing of departure for future foraging success. It emphasizes the trade-off faced by small pups, for which the benefits of remaining onshore beyond the minimum requirement for adequate physiological maturity (Noren *et al.* 2005; Burns *et al.* 2007) may not outweigh the potential costs of continued utilization of endogenous fuel. For example, smaller southern elephant seal pups are restricted to shallower depths and shorter dives than are larger pups, but their foraging success is not affected (Irvine *et al.* 2000).

Although pup sex was not the best predictor of the measures of dive performance examined here, male pups went to sea on average 7 days younger than the females. Because age was an important predictor of dive durations and percent dive, males may be disadvantaged by limited foraging capabilities in the first days at sea, which could contribute to the higher mortality probability observed in male gray seal pups in waters around the United Kingdom (Hall *et al.* 2001, 2002, 2009). The substantial difference in age at departure between years could also contribute to interannual variability in juvenile survivorship (Schwarz and Stobo 2000).

Early Increases and Peak in Dive Performance

Dive performance increased quickly once pups went to sea. There was substantial interindividual variation in extreme percent dive when pups first went to sea, but little variability when they reached their peak, suggesting that the rate of development of diving capacity slows with age and that ultimate capabilities of gray seal pups reach upper limits. This is consistent with the development of oxygen storage capacity documented in gray seal pups: blood oxygen stores increase to levels similar to those in yearlings by the end of the post-weaning fast, whereas muscle oxygen stores develop more slowly and continually over the first year of life (Noren *et al.* 2005).

Pups that were better able to maximize time submerged when they first went to sea, which tended to be older at departure, remained better divers. The slower rate of increase and smaller degree of improvement in these animals show that they were already operating closer to their peak performance than were pups that left the colony when less physiologically mature. Pups that were less able to maximize time submerged when they first went to sea showed steeper improvements in extreme percent dive. The greatest absolute increases in extreme dive durations and extreme percent dive occurred in pups that were smaller (and younger)—and thus perhaps less well developed—at departure. Smaller seals may have been in a more-rapid phase of development, particularly of blood oxygen stores, when they first went to sea. The capacity to "catch up" through rapid, ongoing development once at sea may allow smaller pups to depart while they still have adequate fuel reserves (providing that they have achieved some minimum physiological requirement to allow them to dive adequately to catch food [Noren *et al.* 2005; Burns *et al.* 2007]) without suffering substantially reduced diving capability in the long term.

The onset of active swimming and diving after the sedentary period of fasting on land is likely to contribute to the early increases in dive performance seen here. Cardiovascular control improves in phocid pups as they begin to dive (Burns and Castellini 1996; Burns 1999; Lapierre *et al.* 2004; Greaves *et al.* 2005) and mass-specific metabolic rate decreases with age (Worthy 1987; Thorson and Le Boeuf 1994). Development of oxygen stores parallels increases in diving in Australian fur seal (*Arctocephalus pusillus doriferus*) pups (Spence-Bailey *et al.* 2007) and harbor seal (*Phoca vitulina*) pups (Clark *et al.* 2007). Muscle matures slowly during the post-weaning fast and rapidly at the onset of foraging (Burns *et al.* 2007). Hypoxia and greater oxygen demands during exercise stimulate erythropoiesis (Jelkmann and Metzen 1996) and myoglobin production in other animals (Saunders and Fedde 1991; Masuda *et al.* 1998; Nemirovskaya *et al.* 1998). Declining fat content with age (Reilly and Fedak 1990; Addison and Stobo 1993; Sparling *et al.* 2006; Hall and McConnell 2007) may improve dive performance by reducing oxygen demand from work against buoyancy (Lovvorn *et al.* 1991; Lovvorn and Jones 1991a, 1991b; Butler 2000).

The peak in extreme percent dive was higher in pups that were larger at departure and older when they reached it. Pups that were older and larger at departure were larger at weaning and had both greater protein mass and percentage fat than did smaller animals. This highlights the importance of body size at weaning and thus the high degree of maternal investment that occurs in gray seals (Fedak and Anderson 1982): large pups can undergo a longer fast than smaller pups but also leave the colony when they are still large, with substantial lean tissue. As a result, they benefit from greater diving abilities throughout their first months at sea.

The early increases in extreme dive durations and percent dive were similar to those reported in a rehabilitated Mediterranean monk seal (Dendrinos *et al.* 2007). Measures of diving ability—such as dive frequency, mean and maximum depth, duration, and bottom time—also increase rapidly in other young phocids in the initial period after the onset of diving (Lydersen *et al.* 1994; Burns 1999; Bekkby and Bjorge 2000; Gjertz *et al.* 2000; Jorgensen *et al.* 2001; Folkow *et al.* 2010).

Decline in Performance

The later decline in dive performance may reflect acquisition of skills and experience or changes in foraging strategy. In hooded seal (*Cystophora cristata*), Weddell seal (*Leptonychotes weddelli*), and southern elephant seal pups, the development of diving ability slows or stops after the early increase in capability, and diving behavior appears to become dictated more by prey distribution and availability rather than limited by physiological constraints (Burns 1999; Hindell *et al.* 1999; Folkow *et al.* 2010). Here, the ontogeny of diving behavior could not be separated from seasonality. Although the fall in performance could reflect changes in habitat use, there was no accompanying shift in depths or areas exploited (data not shown). Some models of dive behavior predict that seals should end dives early if they find no prey (Thompson and Fedak 2001). In captive gray seals, this approach leads to a higher net energy gain than would be achieved by diving to the cADL (Sparling *et al.* 2007). The fall in extreme dive durations later in the tracking period here may have resulted from pups learning this strategy.

Impact of Size and Dive Performance on Track Duration

Survivorship could not easily be estimated because it is difficult to distinguish death from SRDL failure. However, track duration indicates minimum survival time, and this was negatively related to the peak in extreme dive duration. Pups that push themselves during individual dives may not maximize overall time submerged if they need to spend longer at the surface to recover (Thompson and Fedak 2001; Sparling *et al.* 2007), and this could reduce their chance of survival. Track duration was longer in pups that were heavier at departure, which supports previous studies that highlight the importance of body mass in gray seal pup survival (Hall *et al.* 2001, 2002, 2009).

Comparison of Dive Performance with Adults

When they first went to sea, pups were less-capable divers than the adults. They were less able to remain submerged during individual dives or as a percentage of longer periods and spent more time at the surface after dives longer than 2 min than did older animals, suggesting they had more-limited capabilities. Pups may thus be less effective at exploiting the same prey or may have a narrower range of available prey compared with older animals. This is similar to findings in Weddell (Burns 1999) and southern elephant seal pups (Hindell *et al*. 1999; Irvine *et al*. 2000). In this study, pups were able to maximize time submerged to the same extent as adults, despite their consistently shorter dives, after they had been at sea between 8 and 52 days and were between 45 and 90 days of age.

Summary

Pups that are large at weaning can undertake a longer post-weaning fast. As a result of being older and larger at departure, they can take advantage of the ability to perform longer dives initially and spend a greater percentage of time submerged throughout their first months at sea. They can also depart when still heavy and in good condition, which ensures they have sufficient fuel reserves to sustain them as they learn to forage. These apparent advantages of size and age may help to explain the high level of investment of gray seal females in their pups (Fedak and Anderson 1982). While all pups must presumably achieve some minimum level of physiological maturity before they are adequately equipped to go to sea (Noren *et al*. 2005; Burns *et al*. 2007), smaller pups in particular face a trade-off. They can leave the colony as soon as possible after this minimum has been reached, with the advantages of a larger size, but at the expense of additional physiological development that can enhance dive performance. Alternatively they can choose to leave later, having reached a greater degree of physiological maturation and with greater initial diving capabilities but reduced fuel, particularly protein, reserves (Bennett *et al*. 2007). Although penning prevented us from determining whether smaller pups would normally choose to leave earlier, the pups that left on the day of release tended to undergo a shorter fast and were smaller than pups that chose to remain on the colony after release. Departure mass had longer-term effects on dive ability and minimum survival time than did age at departure. We therefore speculate that pups facing a trade-off should leave the colony larger rather than older. The ability to perform longer dives initially as a result of being older may increase the chance of foraging success in the first few days at sea, whereas larger body size at departure reduces starvation risk in the longer term. This may have a greater impact on survivorship, particularly if pups can dive adequately to forage initially but food is hard to find or catch or is distant from the colony.

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Literature Cited

Addison, R. F., and W. T. Stobo. 1993. Organochlorine residue concentrations and burdens in gray seal (*Halichoerus grypus*) blubber during the first year of life. *Journal of Zoology* (London) 230(3): 443–450.

Bekkby, T., and A. Bjorge. 2000. Diving behavior of harbor seal, *Phoca vitulina*, pups from nursing to independent feeding. *Journal of Sea Research* 44(3–4): 267–275.

Bennett, K. A., B. J. McConnell, and M. A. Fedak. 2001. Diurnal and seasonal variations in the duration and depth of the longest dives in southern elephant seals (*Mirounga leonina*): Possible physiological and behavioral constraints. *Journal of Experimental Biology* 204(4): 649–662.

Bennett. K. A., J. R. Speakman, S. E. W. Moss, P. Pomeroy, and M. A. Fedak. 2007. Effects of mass and body composition on fasting fuel utilization in grey seal pups (*Halichoerus grypus:* Fabricius): An experimental study using supplementary feeding. *Journal of Experimental Biology* 210(17): 3043–3053.

Boily, P., and D. M. Lavigne. 1997. Developmental and seasonal changes in resting metabolic rates of captive female grey seals. *Canadian Journal of Zoology* 75(11): 1781–1789.

Boyd, I. L. 1997. The behavioral and physiological ecology of diving. *Trends in Ecology and Evolution* 12(6): 213–217.

Burns, J. M. 1999. The development of diving behavior in juvenile Weddell seals: Pushing physiological limits in order to survive. *Canadian Journal of Zoology* 77(5): 737–747.

Burns, J. M., and M. A. Castellini. 1996. Physiological and behavioral determinants of the aerobic dive limit in Weddell seal (*Leptonychotes weddellii*) pups. *Journal of Comparative Physiology B* 166(8): 473–483.

Burns, J. M., K. C. Lestyk, L. P. Folkow, M. O. Hammill, and A. S. Blix. 2007. Size and distribution of oxygen stores in harp and hooded seals from birth to maturity. *Journal of Comparative Physiology B* 177(6): 687–700.

Burns, J. M., J. F. Schreer, and M. A. Castellini. 1997. Physiological effects on dive patterns and foraging strategies in yearling Weddell seals (*Leptonychotes weddellii*). *Canadian Journal of Zoology* 75(11): 1796–1810.

Butler, P. J. 2000. Energetic costs of surface swimming and diving of birds. *Physiological and Biochemical Zoology* 73(6): 699–705.

Carlini, A. R., M. E. I. Marquez, S. Ramdohr, H. Bornemann, H. O. Panarello, and G. A. Daneri. 2001. Postweaning duration and body composition changes in southern elephant seal (*Mirounga leonina*) pups at King George Island. *Physiological and Biochemical Zoology* 74(4): 531–540.

Clark, C. A., J. M. Burns, J. F. Schreer, and M. O. Hammill. 2007. A longitudinal and cross-sectional analysis of total body oxygen store development in nursing harbor seals (*Phoca vitulina*). *Journal of Comparative Physiology B* 177(2): 217–227.

Costa, D. P., B. J. Le Boeuf, A. C. Huntley, and C. L. Ortiz. 1986. The energetics of lactation in the northern elephant seal, *Mirounga angustirostris*. *Journal of Zoology* (London) 209(1): 21–33.

Dendrinos, P., A. A. Karamanlidis, E. Androukaki, and B. J. McConnell. 2007. Diving development and behavior of a rehabilitated Mediterranean monk seal (*Monachus monachus*). *Marine Mammal Science* 23(2): 387–397.

Fedak, M. A. 1986. Diving and exercise in seals: A benthic perspective. *In:* Diving in Animals and Man, A. O. Brubakk, J. W. Kanwisher, and G. Sundres (eds.). Tapir, Trondheim, Norway. pp. 11–32.

Fedak, M. A., and S. S. Anderson. 1982. The energetics of lactation: Accurate measurements from a large wild mammal, the grey seal (*Halichoerus grypus*). *Journal of Zoology* (London) 198(4): 473–479.

Fedak, M. A., P. Lovell, and S. M. Grant. 2001. Two approaches to compressing and interpreting time-depth information as collected by time-depth recorders and satellite-linked data recorders. *Marine Mammal Science* 17(1): 94–110.

Folkow, L. P., E. S. Nordøy, and A. S. Blix. 2010. Remarkable development of diving performance and migrations of hooded seals (*Cystophora cristata*) during their first year of life. *Polar Biology* 33(4): 433–441.

Fowler, S. L., D. P. Costa, J. P. Y. Arnould, N. J. Gales, and C. E. Kuhn. 2006. Ontogeny of diving behavior in the Australian sea lion: Trials of adolescence in a late bloomer. *Journal of Animal Ecology* 75(2): 358–367.

Gjertz, I., K. M. Kovacs, C. Lydersen, and Ø. Wig. 2000. Movements and diving behaviour of bearded seal (*Erignathus barbatus*) mothers and pups during lactation and post-weaning. *Polar Biology* 23(8): 559–566.

Greaves, D. K., J. R. Schreer, M. O. Hammill, and J. M. Burns. 2005. Diving heart rate development in postnatal harbor seals, *Phoca vitulina*. *Physiological and Biochemical Zoology* 78(1): 9–17.

Hall, A. J., and B. J. McConnell. 2007. Measuring changes in juvenile gray seal body composition. *Marine Mammal Science* 23(3): 650–665.

Hall, A. J., B. J. McConnell, and R. J. Barker. 2001. Factors affecting first-year survival in grey seals and their implications for life history strategy. *Journal of Animal Ecology* 70(1): 138–149.

Hall, A. J., B. J. McConnell, and R. J. Barker. 2002. The effect of total immunoglobulin levels, mass and condition on the first-year survival of grey seal pups. *Functional Ecology* 16(4): 462–474.

Hall, A. J., G. O. Thomas, and B. J. McConnell. 2009. Exposure to persistent organic pollutants and first year survival probability in gray seal pups. *Environmental Science and Technology* 43(16): 6364–6369.

Hindell, M. A., B. J. McConnell, M. A. Fedak, D. J. Slip, H. R. Burton, P. J. H. Reijnders, and C. R. McMahon. 1999. Environmental and physiological determinants of successful foraging by naive southern elephant seal pups during their first trip to sea. *Canadian Journal of Zoology* 77(11): 1807–1821.

Horning, M., and F. Trillmich. 1997. Ontogeny of diving behavior in the Galapagos fur seal. *Behaviour* 134(15/16): 1211–1257.

Ihaka, R., and R. Gentleman. 1996. R: A language for data analysis and graphics. *Journal of Computational and Graphical Statistics* 5(3): 299–314.

Irvine, L. G., M. A. Hindell, J. van den Hoff, and H. R. Burton. 2000. The influence of body size on dive duration of underyearling southern elephant seals (*Mirounga leonina*). *Journal of Zoology* (London) 251(4): 463–471.

Jelkmann, W., and E. Metzen. 1996. Erythropoietin in the control of red cell production. *Annals of Anatomy* 178(5): 391–403.

Jorgensen, C., C. Lydersen, O. Brix, and K. M. Kovacs. 2001. Diving development in nursing harbor seal pups. *Journal of Experimental Biology* 204(21): 3993–4004.

Kramer, D. L. 1988. The behavioral ecology of air breathing by aquatic animals. *Canadian Journal of Zoology* 66(1): 89–94.

Krol, E., and J. R. Speakman. 1999. Isotope dilution spaces of mice injected simultaneously with deuterium, tritium, and oxygen-18. *Journal of Experimental Biology* 202(20): 2839–2849.

Lapierre, J. L., J. F. Schreer, J. M. Burns, and M. O. Hammill. 2004. Developmental changes in cardiorespiratory patterns associated with terrestrial apnoeas in harbor seal pups. *Journal of Experimental Biology* 207(22): 3891–3898.

Lestyk, K. C., L. P. Folkow, A. S. Blix, M. O. Hammill, and J. M. Burns. 2009. Development of myoglobin and acid buffering capacity in harp (*Pagophilus groendlandicus*) and hooded (*Cystophora cristata*) seal pups from birth to maturity. *Journal of Comparative Physiology B* 179(8): 985–996.

Lewis, M., C. Campagna, M. Uhart, and C. L. Ortiz. 2001. Ontogenetic and seasonal variation in blood parameters in southern elephant seals. *Marine Mammal Science* 17(4): 862–872.

Lovvorn, J. R., and D. R. Jones. 1991a. Body mass, volume, and buoyancy of some aquatic birds, and their relation to locomotor strategies. *Canadian Journal of Zoology* 69(11): 2888–2892.

Lovvorn, J. R., and D. R. Jones. 1991b. Effects of body size, body fat, and change in pressure with depth on buoyancy and costs of diving in ducks (*Aythya* spp). *Canadian Journal of Zoology* 69(11): 2879–2887.

Lovvorn, J. R., D. R. Jones, and R. W. Blake. 1991. Mechanics of underwater locomotion in diving ducks: Drag, buoyancy and acceleration in a size gradient of species. *Journal of Experimental Biology* 159(1): 89–108.

Lydersen, C., M. O. Hammill, and K. M. Kovacs. 1994. Diving activity in nursing bearded seal (*Erignathus barbatus*) pups. *Canadian Journal of Zoology* 72(1): 96–103.

Masuda, K., Y. Kano, H. Nakano, M. Inaki, and S. Katsuta. 1998. Adaptations of myoglobin in rat skeletal muscles to endurance running training: Effects of intensity, duration and period of training. *Japanese Journal of Physical Fitness and Sports Medicine* 47(5): 561–571.

McConnell, B. J., C. Chambers, K. S. Nicholas, and M. A. Fedak.

1999. Movements and foraging areas of grey seals in the North Sea. *Journal of Applied Ecology* 36(4): 573–590.

Mori, Y. 1998. The optimal patch use in divers: Optimal time budget and the number of dive cycles during bout. *Journal of Theoretical Biology* 190(2): 187–199.

Nemirovskaya, T. L., B. S. Shenkman, and V. B. Koshelev. 1998. Exercise-induced hypoxia and structural and metabolic adaptation of skeletal muscle. *Basic Applied Myology* 8(6): 441–445.

Noren, S. R., D. J. Boness, S. J. Iverson, J. McMillan, and W. D. Bowen. 2008. Body condition at weaning affects the duration of the postweaning fast in gray seal pups (*Halichoerus grypus*). *Physiological and Biochemical Zoology* 81(3): 269–277.

Noren, S. R., S. J. Iverson, and D. J. Boness. 2005. Development of the blood and muscle oxygen stores in gray seals (*Halichoerus grypus*): Implications for juvenile diving capacity and the necessity of a terrestrial postweaning fast. *Physiological and Biochemical Zoology* 78(4): 482–490.

Noren, S. R., T. M. Williams, D. A. Pabst, W. A. McLellan, and J. L. Dearolf. 2001. The development of diving in marine endotherms: Preparing the skeletal muscles of dolphins, penguins, and seals for activity during submergence. *Journal of Comparative Physiology B* 171(2): 127–134.

Prewitt, J. S., D. V. Freistroffer, J. F. Schreer, M. O. Hammill, and J. M. Burns. 2010. Postnatal development of muscle biochemistry in nursing harbor seal (*Phoca vitulina*) pups: Limitations to dive behavior? *Journal of Comparative Physiology B* 180(5): 757–766.

R Development Core Team. 2006. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Reilly, J. J. 1991. Adaptations to prolonged fasting in free-living weaned gray seal pups. *American Journal of Physiology* 260(2): R267–R272.

Reilly, J. J., and M. A. Fedak. 1990. Measurement of the body composition of living gray seals by hydrogen isotope dilution. *Journal of Applied Physiology* 69(3): 885–891.

Saunders, D. K., and M. R. Fedde. 1991. Physical conditioning: Effect on the myoglobin concentration in skeletal and cardiac muscle of bar-headed geese. *Comparative Biochemistry and Physiology A* 100(2): 349–352.

Schwarz, C. J., and W. T. Stobo. 2000. Estimation of juvenile survival, adult survival, and age-specific pupping probabilities for the female grey seal (*Halichoerus grypus*) on Sable Island from capture-recapture data. *Canadian Journal of Fisheries and Aquatic Sciences* 57(1): 247–253.

Sparling, C. E., and M. A. Fedak. 2004. Metabolic rates of captive grey seals during voluntary diving. *Journal of Experimental Biology* 207(10): 1615–1624.

Sparling, C. E., J. Y. Georges, S. L. Gallon, M. Fedak, and D. Thompson. 2007. How long does a dive last? Foraging decisions by breath-hold divers in a patchy environment: A test of a simple model. *Animal Behavior* 74(2): 207–218.

Sparling, C. E., J. R. Speakman, and M. A. Fedak. 2006. Seasonal variation in the metabolic rate and body composition of female grey seals: Fat conservation prior to high-cost reproduction in a capital breeder? *Journal of Comparative Physiology B* 176(6): 505–512.

Speakman, J. R., and B. Krol. 2005. Comparison of different approaches for the calculation of energy expenditure using doubly labeled water in a small mammal. *Physiological and Biochemical Zoology* 78(4): 650–667.

Speakman, J. R., and P. A. Racey. 1987. The equilibrium concentration of oxygen-18 in body water: Implications for the accuracy of the doubly-labeled water technique and a potential new method of measuring RQ in free-living animals. *Journal of Theoretical Biology* 127(1): 79–95.

Spence-Bailey, L. M., D. Verrier, and J. P. Y. Arnould. 2007. The physiological and behavioural development of diving in Australian fur seal (*Arctocephalus pusillus doriferus*) pups. *Journal of Comparative Physiology B* 177(4): 483–494.

Thompson, D., and M. A. Fedak. 2001. How long should a dive last? A simple model of foraging decisions by breath-hold divers in a patchy environment. *Animal Behaviour* 61(2): 287–296.

Thorson, P. H., and B. J. Le Boeuf. 1994. Developmental aspects of diving in northern elephant seal pups. *In:* Elephant Seals: Population, Ecology, Behavior and Physiology, B. J. Le Boeuf and R. M. Laws (eds.). University of California Press, Berkeley, California, USA. pp. 271–289.

Worthy, G. A. J. 1987. Metabolism and growth of young harp and gray seals. *Canadian Journal of Zoology* 65(6): 1377–1382.

Wild Birds and Urban Ecology of Ticks and Tickborne Pathogens, Chicago, Illinois, USA, 2005–2010

Sarah A. Hamer, Tony L. Goldberg, Uriel D. Kitron, Jeffrey D. Brawn, Tavis K. Anderson, Scott R. Loss, Edward D. Walker, and Gabriel L. Hamer

ABSTRACT: Bird-facilitated introduction of ticks and associated pathogens is postulated to promote invasion of tick-borne zoonotic diseases into urban areas. Results of a longitudinal study conducted in suburban Chicago, Illinois, United States during 2005–2010 show that 1.6% of 6,180 wild birds captured in mist nets harbored ticks. Tick species in order of abundance were *Haemaphysalis leporispalustris*, *Ixodes dentatus,* and *Ixodes scapularis*, but two Neotropical tick species of the genus *Amblyomma* were sampled during the spring migration. *Ixodes scapularis* ticks were absent at the beginning of the study but constituted the majority of ticks by study end and were found predominantly on birds captured in areas designated as urban green spaces. Of 120 ticks, five were infected with *Borrelia burgdorferi,* spanning three ribotypes, but none were infected with *Anaplasma phagocytophilum*. Results allow inferences about propagule pressure for introduction of tick-borne diseases and emphasize the large sample sizes required to estimate this pressure.

KEY WORDS: *Anaplasma phagocytophilum*; *Borrelia* spp., migratory birds, pathogen transmission, Rickettsia spp., tick-borne zoonotic disease, urban green spaces

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Ovenbird *(Seiurus aurocapilla).*

Introduction

Wild birds can affect zoonotic disease risk to humans, wildlife, and domestic animals through their mobility and influence on the distribution and abundance of pathogens and vectors. Most notably, avian migration allows for rapid transcontinental transportation of novel pathogens and vectors that may seed new disease foci in receptive environments. For example, the spread of highly pathogenic avian influenza into and throughout most countries in Europe most likely occurred through the movement of migratory birds (Kilpatrick *et al*. 2006). Infected wild birds also contributed to the spread of West Nile virus (WNV) across North America (Rappole and Hubálek 2003). Thus, models of interseasonal connectivity among areas used by migratory birds can be used to forecast disease spread (Peterson *et al*. 2009).

Over finer spatial scales, the patterns of bird use by blood-feeding vectors affect the prevalence of vector-borne pathogens. Host variation impacts the survival of vectors that feed on birds rather than on other vertebrates (Keesing *et al*. 2009), and avian species exhibit differential reservoir competency for vector-borne pathogens (Ginsberg

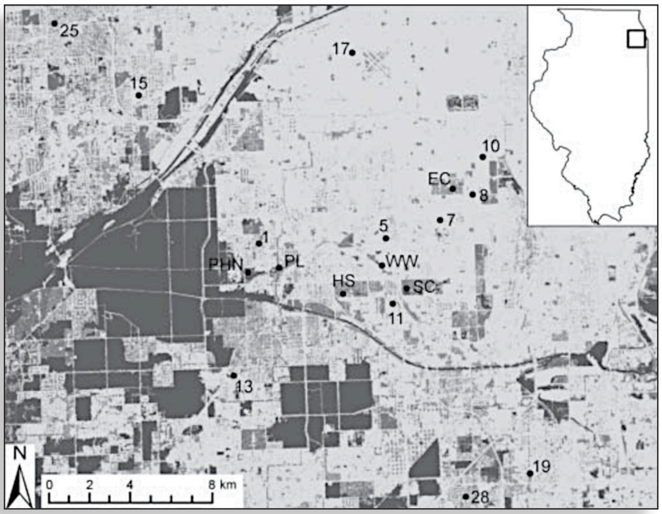

FIGURE 1. Bird capture sites in southwestern suburban Chicago, Cook county, Illinois.

et al. 2005). In combination, these factors influence disease risk; for example, just a few avian species that are heavily fed upon by mosquitoes and highly competent for WNV apparently drive most WNV transmission (Hamer *et al.* 2011a). Furthermore, host association of strains might help maintain pathogen diversity in some vector-borne diseases systems for which birds play critical roles (Kurtenbach *et al.* 2002).

Urban environments may promote pathogen transmission through increased host contact rates, high rates of pathogen introduction (i.e., propagule pressure), and warmer microclimates that are favorable to pathogens and vectors (Bradley and Altizer 2006). These effects, in turn, may elevate disease risk to high-density urban human populations. Across gradients of urbanization, the incidence of some zoonotic pathogens has been found to be highest in urban cores (Hamer *et al.* 2012). Reduced species richness in urban areas may contribute to elevated risk for diseases that are caused by multihost pathogens with generalist vectors (Keesing *et al.* 2010), although the associations between biodiversity and disease risk are variable (Randolph and Dobson 2012).

In humans, Lyme disease and anaplasmosis caused by infection with the bacteria *Borrelia burgdorferi* and *Anaplasma phagocytophilum*, respectively, are the two most-common tick-borne diseases in the midwestern and northeastern United States and both are emerging among human and canine populations (Dumler *et al.* 2005; Chomel 2011). In eastern North America, both pathogens are maintained in blacklegged tick (*Ixodes scapularis*)–rodent cycles (Barbour and Fish 1993; Telford *et al.* 1996). We investigated the role of birds in the urban ecology of tick-borne zoonotic diseases. Our objectives were to 1) ascertain the prevalence of tick parasitism of birds in residential and urban green spaces in southwestern suburban Chicago, Illinois, United States during a 6-yr period; 2) estimate the infection prevalence of *Borrelia* spp. and *A. phagocytophilum* in ticks removed from birds; and 3) characterize the diversity of pathogens in ticks removed from birds by using genetic methods.

Materials and Methods

Bird Capture

During May–October 2005–2010, birds were captured at 20 field sites in southwestern suburban Chicago (Cook County, Illinois; 87°44' W, 41°42' N; Fig. 1). Field sites were categorized as residential sites ($n = 14$) or urban green spaces ($n = 6$) and have been described in detail (Hamer *et al.* 2011a). We used 8–10 mist nets (Avinet, Dryden, New York, USA) to capture birds at 7–15 sites per year about one morning per site every 1.5 wk (2005–2007) or every 3 wk (2008–2010). For each captured bird we recorded species, sex, age class (hatch year and after-hatch year), and weight and we attached a numbered leg band before release. All birds were checked for ticks by blowing apart feathers and inspecting the skin, especially around the ears, head, and vent. Ticks were removed and preserved in 70% ethanol. Migratory status of each avian species was assigned (Walk *et al.* 2010). Fieldwork was carried out with approvals from animal care review boards at Michigan State University and the University of Illinois.

Detection and Typing of Borrelia spp. and A. phagocytophilum

Ticks were identified morphologically to species and stage; a subset was subjected to PCR and sequencing for confirmation (Poucher *et al.* 1999). All ticks were tested for pathogens except for two specimens that were deposited in the U.S. National Tick Collection (housed at Georgia Southern University, Statesboro, Georgia, USA) for molecular identification and vouchering. Total DNA from ticks was extracted by using a DNeasy Blood and Tissue Kit (Qiagen, Valencia, California, USA) with modifications as described (Hamer *et al.* 2010). Nymphal ticks were extracted individually whereas same-species larvae from the same individual animal were pooled. All ticks were tested for the presence of *B. burgdorferi* sensu stricto and *A. phagocytophilum* by using a quantitative PCR targeting the 16S rRNA gene (Tsao *et al.* 2004) and a PCR targeting the *p44* gene (Holden *et al.* 2003), respectively.

Borrelia burgdorferi-positive tick samples were typed by DNA sequencing of both strands of the 16S–23S rRNA intergenic spacer (IGS) region (Bunikis *et al.* 2004); strains were identified, using updated nomenclature (Travinsky *et al.* 2010), to ribosomal spacer type 1, 2, or 3 (Liveris *et al.* 1995) and IGS subtype by comparing them with the 25 major *B. burgdorferi* IGS subtypes (Bunikis *et al.* 2004; Hamer *et al.* 2011b). The outer surface protein C (*ospC*) genotype was inferred on the basis of the linkage disequilibrium between the IGS locus and *ospC* locus (Bunikis *et al.* 2004; Travinsky *et al.* 2010).

Statistical Analyses

Logistic regression was used to assess the variation in tick infestations among years. We used 2- and 3-sample tests for equality of proportions to assess the effects of site category, sex, and age on the prevalence of tick infestations. The Wilson interval with continuity correction was used to estimate the 95% binomial confidence interval (CI) for infection prevalence data. Minimum infection prevalence (i.e., assuming one positive larva/pool) was used for tests conducted on pooled larvae. Statistical analyses were performed by using Program R (R Foundation for Statistical Computing, Vienna, Austria).

Results

Bird Captures

We recorded 6,180 total captures comprising 5,506 individual birds (10.9% recaptures) and 78 species (Table 1). Five species comprised 67% of all captures: *Passer domesticus* (house sparrow), *Turdus migratorius* (American robin), *Dumetella carolinensis* (gray catbird), *Spinus tristis* (American goldfinch), and *Cardinalis cardinalis* (northern cardinal). Among all captured birds 27.3% were known males, 21.3% were known females, and 51.3% were of unknown sex. The age class was after-hatch year for 53.1%, hatch year for 41.8%, and unknown for 5.1% of the birds. Similar numbers of birds were captured from residential sites (3,326; 53.8%) and urban green spaces (2,854; 46.2%). Approximately two times the number of birds were captured per year in 2005–2007 (1,455 ± 45) as in 2008–2010 (605 ± 159) due to higher mist-netting efforts in the initial 3 yr of the study.

Tick Prevalence

We removed 357 ticks from 97 individual birds (one bird with ticks was caught twice), yielding an overall tick infestation prevalence of 1.6% (Table 1). Ticks were usually located beneath the auricular feathers within the skin of the ear canal and second-most commonly located in the rictus of the bill and in the skin of the orbital region. Infested birds were collected at 17 of the 20 field sites (11/14 residential sites, 6/6 urban green spaces). Birds with the highest prevalence of infestation (>7% of captures infested) were song sparrows (*Melospiza melodia*), Swainson's thrushes (*Catharus ustulatus*), blue jays (*Cyanocitta cristata*), ovenbirds (*Seiurus aurocapilla*), gray-cheeked thrushes (*Catharus minimus*), and yellow-bellied flycatchers (*Empidonax flaviventris*) (Table 1).

Most ticks were of 3 species: *Haemaphysalis leporispalustris* (87.4% of all ticks), *Ixodes dentatus* (4.8%), and *I. scapularis* (7.8%). Morphologic and molecular identifications were congruent for all 21 birds subjected to both methods of identification (GenBank accession nos. JQ868565–JQ868585). Overall 1.3%, 0.1%, and 0.2% of birds were infested with *H. leporispalustris*, *I. dentatus*, and *I. scapularis*, respectively (Table 1). In addition, a single *Amblyomma nodosum* larva was removed from an after-hatch year Swainson's thrush on 17 May 2005 and a single *Amblyomma longirostre* nymph was removed from an after-hatch year American redstart (*Setophaga ruticilla*) on 18 May 2005. The two ticks were found on birds captured at site Holy Sepulchre Cemetery (HS; see Fig. 1) during the spring migration. They were identified genetically and vouchered at the U.S. National Tick Collection but were not tested for pathogens.

The number of ticks on infested birds ranged from 1–23 (median $n = 2$ ticks). Of the infested birds, 47% harbored one tick and 20% harbored ≥5 ticks. *Haemaphysalis leporispalustris* larvae accounted for the greatest tick loads (average 4.3 ticks/bird). Of 98 parasitized birds, 11 (11.2%) were infested with >1 life stage of tick or >1 tick species. Although the overall prevalence of infested birds did not change over the 6-yr study (z-value = –1.6, df = 6,178, $P = 0.109$), the proportion of infested birds that harbored *I. scapularis* increased significantly from 0% to 80% (z-value = 3.873, df = 96, $P = 0.0001$) and *I. scapularis* comprised >90% of ticks removed from birds in the final year of the study. Of the 10 *I. scapularis*-infested birds, the majority ($n = 8$) came from urban green spaces (0.28% *I. scapularis* infestation prevalence across all green spaces) and the minority ($n = 2$) came from residential sites (0.06% prevalence; z-value = 2.2, $P = 0.03$). Information about the timing of *I. scapularis* infestation, combined with the species and age of the avian host, provides evidence for local (Chicago area) acquisition of ticks and for migratory importation of ticks from the north and the south (Table 2).

Tick Infection with B. burgdorferi and A. phagocytophilum

A total of 120 tick samples were tested for pathogens. No ticks tested positive for *A. phagocytophilum* infection. Five samples tested positive for *B. burgdorferi* infection: three of six *I. scapularis* nymphs (50%, 95% CI 14.0–86.1%), one of 22 *I. scapularis* larval pools (minimum infection prevalence 4.5%), and one of 34 *H. leporispalustris* nymphs (2.9%, 95% CI 0.2–17.1%) (Table 3). All five positive tick samples were from unique after-hatch year birds of four species (American robin, blue jay, red-winged blackbird [*Agelaius phoeniceus*], Swainson's thrush) at four field sites including urban green spaces and residential sites. *Borrelia burgdorferi* 16S–23S rRNA IGS sequences were obtained from all three *I. scapularis* nymphs and represented three IGS ribotypes (2, 28, and 14; GenBank accession nos. JQ868562–JQ868564) within ribosomal spacer type 2 and 3; inferred *ospC* genotypes were H, T, and A3, respectively (Table 3).

Discussion

The presence of *B. burgdorferi*-infected *I. scapularis* ticks on migratory and residential birds in the Chicago region reflects the continued invasion and establishment of this tick and pathogen across the Midwest. In Illinois, as in many other areas of North America (Diuk-Wasser *et al*. 2012), there is growing public health concern over the emergence of Lyme disease (Jobe *et al*. 2007); however, the statewide incidence in Illinois over the study period (1.1 cases/100,000 persons) was an order of magnitude lower than that which characterizes the Lyme disease-endemic regions in the northeastern United States (CDC 2011). Our study provides evidence of established local populations of *I. scapularis* ticks in Chicago that may be supplemented by importation of *I. scapularis* ticks from other populations to the north or south by migratory birds. The Chicago region is a natural corridor for

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TABLE 1. BIRDS SAMPLED FOR PRESENCE OF TICKS IN SOUTHWESTERN SUBURBAN CHICAGO, ILLINOIS, USA, 2005–2010*

BIRD	MIGRATORY STATUS	TOTAL NO. EXAMINED	PROPORTION INFESTED	NO. BIRDS INFESTED WITH				
				HAEMAPHYSALIS LEPORISPALUSTRIS		*IXODES DENTATUS*	*I. SCAPULARIS*	
				LARVAE	NYMPHS	LARVAE	LARVAE	NYMPHS
American goldfinch	B, M	363						
American redstart†	B, M	38	0.03					
American robin	B, M	1049	0.01	2	4	1	4	2
Baltimore oriole	B, M	31						
Barn swallow	B, M	7						
Black and white warbler	NB, M	9						
Black-capped chickadee	B, NM	25						
Blue jay	B, M	22	0.09					2
Brown-headed cowbird	B, M	65						
Brown thrasher	B, M	12						
Cedar waxwing	B, M	16						
Chipping sparrow	B, M	24						
Common grackle	B, M	105	0.03		2	1		
Common yellowthroat	B, M	8						
Dark-eyed junco	NB, M	8						
Downy woodpecker	B, M	50						
Eastern wood-pewee	B, M	5						
Empidonax spp. flycatchers	B, M	27						
European starling	B, M	141	0.01	1				
Fox sparrow	NB, M	5						
Gray catbird	B, M	429	0.01	3	3			
Gray-cheeked thrush	NB, M	18	0.11	1				
Hermit thrush	B, M	5						
House finch	B, M	157						
House sparrow	B, NM	2097	0.01	25	4			
House wren	B, M	57	0.02	1				
Indigo bunting	B, M	29						
Least flycatcher	B, M	5						
Lincoln's sparrow	NB, M	5						
Magnolia warbler	NB, M	19						
Mourning dove	B, M	63						
Mourning warbler	NB, M	5						

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TABLE 2. DEMOGRAPHIC INFORMATION ABOUT 10 AVIAN HOSTS INFESTED WITH IXODES SCAPULARIS TICKS IN SOUTHWESTERN SUBURBAN CHICAGO, ILLINOIS, USA, 2005-2010*

BIRD	DATE OF CAPTURE	AGE	SITE, CATEGORY	*I. SCAPULARIS* STAGE (QUANTITY)	PRESUMED *I. SCAPULARIS* ACQUISITION
American robin	2007 Jul 18	AHY	1, residential	L (9); N (1)	Local
American robin	2009 Aug 18	HY	PL, green space	L (2)	Local
American robin	2010 Jun 22	AHY	PHN, green space	N (2)	Local
American robin	2010 Jul 13	AHY	PL, green space	L (1)	Local
American robin	2010 Jul 26	HY	PL, green space	L (8)	Local
Blue jay	2009 Jun 15	AHY	PHN, green space	N (1)	Local
Blue jay	2009 Jun 15	AHY	PHN, green space	N (1)	Local
Gray-cheeked thrush	2010 Sep 16	HY	PHN, green space	N (1)	Migratory (from north)
Northern cardinal	2007 Aug 16	HY	13, residential	L (1)	Local
Swainson's thrush	2006 May 23	AHY	WW, green space	L (1)	Migratory (from south)

*AHY, after hatch year; L, larva; N, nymph, HY, hatch year; PL, Pleasure Lake; PHN, Palos Hills Natural; WW, Wolfe Wildlife Refuge

TABLE 1 (CONT'D)

BIRD	MIGRATORY STATUS	TOTAL NO. EXAMINED	PROPORTION INFESTED	NO. BIRDS INFESTED WITH HAEMAPHYSALIS LEPORISPALUSTRIS		IXODES DENTATUS	I. SCAPULARIS	
				LARVAE	NYMPHS	LARVAE	LARVAE	NYMPHS
Nashville warbler	NB, M	7						
Northern cardinal	B, NM	311	0.04	9	3		1	
Northern flicker	B, M	10						
Northern waterthrush	NB, M	44						
Orchard oriole	B, M	4						
Ovenbird	B, M	41	0.10	4				
Palm warbler	NB, M	6						
Red-eyed vireo	B, M	11						
Red-winged blackbird	B, M	191	0.01	1	2			
Song sparrow	B, M	228	0.07	13	6	1		
Swainson's thrush‡	NB, M	131	0.08	4	4	1	1	
Tennessee warbler	NB, M	9						
Tree swallow	B, M	14						
Veery B, M	8							
Warbling vireo	B, M	35						
White-crowned sparrow	NB, M	11						
White-throated sparrow	NB, M	61	0.02			1		
Willow flycatcher	B, M	63						
Wilson's warbler	NB, M	8						
Yellow warbler	B, M	34						
Yellow-bellied flycatcher	NB, M	6	0.17			1		
Yellow-rumped warbler	NB, M	26						
All 6,197§	0.02	64	28	6	6	5		

*Empidonax spp. flycatchers that could not be identified are considered at the genus level. Numbers of birds infested by larvae and nymphs of 3 tick species are indicated. Common names conform to species as specified by the American Ornithologist Union. B, confirmed breeding in Chicago region; M, migratory; NB, non-breeder in Chicago region; NM, non-migratory. Blank spaces mean none infested.

†One American redstart infested with a single Amblyomma longirostre nymph.

‡One Swainson's thrush infested with a single A. nodosum larva.

§This total includes 49 unlisted captured birds from the following species: American woodcock, American tree sparrow, black-billed cuckoo, black-throated blue warbler, blackpoll warbler, brown creeper, Carolina wren, Canada warbler, Eastern towhee, Eurasian collared–dove, great crested flycatcher, golden-crowned kinglet, hairy woodpecker, killdeer, marsh wren, olive-sided flycatcher, red-breasted nuthatch, rose-breasted grosbeak, ruby-crowned kinglet, savannah sparrow, scarlet tanager, swamp sparrow, white-breasted nuthatch, and wood thrush. The sample size for each of these species was <5, and none of the birds harbored ticks.

TABLE 3. PREVALENCE OF BORRELIA BURGDORFERI INFECTION IN TICKS REMOVED FROM BIRDS, BY SITE OF ORIGIN AND DATE OF CAPTURE, SOUTHWEST SUBURBAN CHICAGO, ILLINOIS, USA, 2005-2010*

TICK SPECIES	LARVAE NO. POOLS (NO. LARVAE)	% INFECTED (MIP)	BIRDS W/ INFECTED LARVAE, SITE, DATE	NYMPH NO. TESTED	% INFECTED (95% CL)	BIRDS W/ INFECTED NYMPHS, SITE, DATE	IGS STRAIN (RST GROUP)	*ospC* STRAIN
Haemaphysalis leporispalustris	65 (277)	0	NA	34	2.9 (0.2—17.1)	RWBL, SC site, 2007 June 6	NA	NA
Ixodes dentatus	6 (17)	0	NA	0	NA	NA	NA	NA
I. scapularis	6 (22)	16.7 (4.5)	SWTH, WW site, 2006 May 23	6	50 (14.0—86.1)	AMRO, 1 site, 2007 Jul 18; AMRO, PHN site, 2010 Jun 22; BLJA, PHN site, 2009 June 15	2 (2); 28 (3); 14 (2)	H, T, A3

*MIP, minimum infection prevalence IGS, B. burgdorferi 16S-23S rRNA intergenic spacer ribotypes; RST, ribosomal spacer type 1, 2 or 3; ospC inferred outer surface protein C allele based on linkages reported by Travinsky et al. 2010; NA, not applicable; RWBL, red-winged blackbird; SC, Saint Casmir Cemetery; SWTH, Swainson's thrush; WW, Wolfe Wildlife Refuge; AMRO, American robin; PHN, Palos Hills Natural; BLJA, blue jay.


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Female *Ixodes scapularis* tick.

migratory birds, and the risk for tick and pathogen introduction is likely to be elevated on migratory flyways because of seasonal concentrations of birds.

We detected a *B. burgdorferi*-positive *I. scapularis* larval pool from a Swainson's thrush. Given the absence of transovarial transmission in the *I. scapularis* tick, this finding demonstrates that the Swainson's thrush can be an infectious reservoir host. On the basis of a limited sample (*n* = 6), we determined that birds in Chicago harbored *B. burgdorferi*-infected *I. scapularis* nymphs at a prevalence (14.0–86.1%) consistent with that reported for questing nymphs and ticks from birds in Michigan (Hamer *et al.* 2010), Minnesota (Weisbrod and Johnson 1989), and Canada (Ogden *et al.* 2008). All three *B. burgdorferi* IGS ribotypes present within nymphs in this study have been associated with host-seeking nymphs in Lyme disease-endemic areas of the midwestern and northeastern United States; two of the three ribotypes were previously detected in larvae removed from birds (Brinkerhoff *et al.* 2010). Two of the *ospC* types (H and A) presumed present in the collected ticks were among the four most-invasive genotypes (I, A, H, B) from a study of *B. burgdorferi* isolates from humans in New York (Wormser *et al.* 2008). The presence of avian reservoirs and *I. scapularis* nymphs infected with *B. burgdorferi* strains capable of causing disseminated human disease supports the possibility that reported cases of human Lyme disease in Chicago residents may result from local exposure to infected *I. scapularis* ticks. Although none of the ticks removed from birds were positive for *A. phagocytophilum*, the growing *I. scapularis* tick population in the region raises the possibility that infection with this pathogen could become an emerging health concern.

Other ticks commonly found on birds in Chicago are *I. dentatus* and *H. leporispalustris* ticks, both of which feed almost exclusively on rabbits and birds. *Ixodes dentatus* ticks are enzootic vectors of *B. burgdorferi* in regions where *I. scapularis* ticks do not occur (Hamer *et al.* 2011b). *Haemaphysalis leporispalustris* ticks transmit *Francisella tularensis* and spotted-fever group rickettsiae among wildlife (Sonenshine 1979). In our study, *H. leporispalustris* ticks had a wide geographic presence across most residential sites and were most commonly found on house sparrows, including seven hatch-year birds, implying local acquisition in the residential neighborhoods. Neither *I. dentatus* nor *H. leporispalustris* ticks regularly infest humans.

We document the presence of two Neotropical tick species, *A. longirostre* and *A. nodosum*, on birds migrating north through Chicago. We note that other species of Neotropical *Amblyomma* ticks have been recovered in the spring on migrant birds in southern Canada (Scott *et al.* 2010). *Amblyomma longirostre* and *A. nodosum* ticks are widely distributed in the Neotropical region and are vectors of *Rickettsia amblyommii* (Ogrzewalska *et al.* 2011) (which may cause rickettsiosis in humans in North America; Apperson *et al.* 2008), *Rickettsia bellii*, and *Rickettsia parkeri* (Ogrzewalska *et al.* 2009). In the United States, *R. parkeri* is a newly recognized cause of human disease, and a high prevalence of infection (>40% in adults) has been associated with growing populations of Gulf Coast ticks (*Ambystoma maculatum*) (Fornadel *et al.* 2011). Migrant birds from the Neotropics likely account for many imports of engorged Neotropical ticks and associated pathogens in North America each spring, but a lack of environmental receptivity (host or climatic limitations) has likely prevented establishment.

Data from our large sampling effort show that the dispersal of *I. scapularis* ticks, *B. burgdorferi*, and Neotropical vector ticks is a rare but detectable event. We sampled several thousand birds and detected *I. scapularis* ticks on <0.2% and Neotropical ticks on <0.05%. However, the rarity of infestations does not mean that infestation is biologically insignificant. Despite the positive relationship between propagule pressure and invasion success, some successful species invasions, especially those of arthropods, can be initiated by a very small number of individuals (Simberloff 2009). Low propagule pressure but successful invasion may occur when the environment is receptive to the particular species of ticks and pathogens being dispersed. Indeed, during our study other researchers showed an increase in the occurrence of *B. burgdorferi*-infected adult *I. scapularis* ticks in northwestern Chicago, confirming our prediction (Jobe *et al.* 2007). Such scenarios of rare introduction but successful establishment of ticks and pathogens pose a major risk for the health of humans, wildlife, and domestic animals in urban environments worldwide.

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Literature Cited

Apperson, C. S., B. Engber, W. L. Nicholson, D. G. Mead, J. Engel, M. J. Yabsley, K. Dail, J. Johnson, and D. W. Watson. 2008. Tick-borne diseases in North Carolina: Is "*Rickettsia amblyommii*" a possible cause of rickettsiosis reported as Rocky Mountain spotted fever? *Vector-borne and Zoonotic Diseases* 8(5): 597–606.

Barbour, A. G., and D. Fish. 1993. The biological and social phenomenon of Lyme disease. *Science* 260(5114): 1610–1616.

Bradley, C. A., and A. Altizer. 2007. Urbanization and the ecology of wildlife diseases. *Trends in Ecology and Evolution* 22(2): 95–102.

Brinkerhoff, R. J., S. J. Bent, C. M. Folsom-O'Keefe, K. Tsao, A. G. Hoen, A. G. Barbour, and M. A. Diuk-Wasser. 2010. Genotypic diversity of *Borrelia burgdorferi* strains detected in *Ixodes scapularis* larvae collected from North American songbirds. *Applied and Environmental Microbiology* 76(24): 8265–8268.

Bunikis, J., U. Garpmo, J. Tsao, J. Berglund, D. Fish, A. G. Barbour. 2004. Sequence typing reveals extensive strain diversity of the Lyme borreliosis agents *Borrelia burgdorferi* in North America and *Borrelia afzelii* in Europe. *Microbiology* 150(6): 1741–1755.

(CDC) Centers for Disease Control and Prevention. 2011. Summary of notifiable diseases. *Morbidity and Mortality Weekly Report* (MMWR) 58: 1–100.

Chomel, B. 2011. Tick-borne infections in dogs—An emerging infectious threat. *Veterinary Parasitology* 179(4): 294–301.

Diuk-Wasser, M. A., A. G. Hoen, P. Cislo, R. Brinkerhoff, S. A. Hamer, M. Rowland, R. Cortinas, G. Vourch'h, F. Melton, G. J. Hickling, J. I. Tsao, J. Bunikis, A. G. Barbour, U. Kitron, J. Piesman, and D. Fish. 2012. Human risk of infection with *Borrelia burgdorferi*, the Lyme disease agent, in eastern United States. *American Journal of Tropical Medicine and Hygiene* 86(2): 320–327.

Dumler, J. S., K. J. Choi, J. C. Garcia-Garcia, N. S. Barat, D. G. Scorpio, J. W. Garyu, D. J. Grab, and J. S. Bakken. 2005. Human granulocytic anaplasmosis and *Anaplasma phagocytophilum. Emerging Infectious Diseases* 11(12): 1828–1834.

Fornadel, C. M., X. Zhang, J. D. Smith, C. D. Paddock, J. R. Arias, and D. E. Norris. 2011. High rates of *Rickettsia parkeri* infection in Gulf Coast ticks (*Amblyomma maculatum*) and identification of "*Candidatus* Rickettsia Andeanae" from Fairfax County, Virginia. *Vector-Borne and Zoonotic Diseases* 11(12): 1535–1539.

Ginsberg, H. S., P. A. Buckley, M. G. Balmforth, E. Zhioua, S. Mitra, and F. G. Buckley. 2005. Reservoir competence of native North American birds for the Lyme disease spirochete, *Borrelia burgdorferi. Journal of Medical Entomology* 42(3): 445–449.

Hamer, G. L., L. F. Chaves, T. K. Anderson, U. D. Kitron, J. D. Brawn, M. O. Ruiz, S. R. Loss, E. D. Walker, and T. L. Goldberg. 2011a. Fine-scale variation in vector host use and force of infection drive localized patterns of West Nile virus transmission. *PLoS ONE* 6(8): e23767.

Hamer, S. A., G. J. Hickling, J. L. Sidge, M. E. Rosen, E. D. Walker, and J. I. Tsao. 2011b. Diverse *Borrelia burgdorferi* strains in a bird tick cryptic cycle. *Applied Environmental Microbiology* 77(6): 1999–2007.

Hamer, S. A., E. Lehrer, and S. B. Magle. 2012. Wild birds as sentinels for multiple zoonotic pathogens along an urban to rural gradient in greater Chicago, Illinois. *Zoonoses and Public Health* 59(5): 355–364.

Hamer, S. A., J. I. Tsao, E. D. Walker, and G. J. Hickling. 2010. Invasion of the Lyme disease vector *Ixodes scapularis*: Implications for *Borrelia burgdorferi* endemicity. *EcoHealth* 7(1): 47–63.

Holden, K., J. T. Boothby, S. Anand, and R. F. Massung. 2003. Detection of *Borrelia burgdorferi*, *Ehrlichia chaffeensis*, and *Anaplasma phagocytophilum* in ticks (Acari: Ixodidae) from a coastal region of California. *Journal of Medical Entomology* 40(4): 534–539.

Jobe, D. A., J. A. Nelson, M. D. Adam, and S. A. Martin. 2007. Lyme disease in urban areas, Chicago. *Emerging Infectious Diseases* 13(11): 1799–1800.

Keesing, F., L. K. Belden, P. Daszak, A. Dobson, C. D. Harvell, R. D. Holt, P. Hudson, A. Jolles, K. E. Jones, C. E. Mitchell, S. S. Myers, T. Bogich, and R. S. Ostfeld. 2010. Impacts of biodiversity on the emergence and transmission of infectious diseases. *Nature* 468: 647–652.

Keesing, F., J. Brunner, S. Duerr, M. Killilea, K. LoGiudice, K. Schmidt, H. Vuong, and R. S. Ostfeld. 2009. Hosts as ecological traps for the vector of Lyme disease. *Proceedings of the Royal Society B, Biological Sciences* 276(1675): 3911–3919.

Kilpatrick, A. M., A. A. Chmura, D. W. Gibbons, R. C. Fleischer, P. O. Marra, and P. Daszak. 2006. Predicting the global spread of H5N1 avian influenza. *Proceedings of the National Academy of Sciences of the USA* 103(51): 19368–19373.

Kurtenbach, K., S. De Michelis, S. Etti, S. M. Schafer, H. S. Sewell, V. Brade, and P. Kraiczy. 2002. Host association of *Borrelia burgdorferi* sensu lato—The key role of host complement. *Trends in Microbiology* 10(2): 74–79.

Liveris, D., A. Gazumyan, and I. Schwartz. 1995. Molecular typing of *Borrelia burgdorferi* sensu lato by PCR-restriction fragment length polymorphism analysis. *Journal of Clinical Microbiology* 33(3): 589–595.

Ogden, N. H., L. R. Lindsay, K. Hanincova, I. K. Barker, M. Bigras-Poulin, D. F. Charron, A. Heagy, C. M. Francis, C. J. O'Callaghan, I. Schwartz, and R. A. Thompson. 2008. Role of migratory birds in introduction and range expansion of *Ixodes scapularis* ticks and of *Borrelia burgdorferi* and *Anaplasma phagocytophilum* in Canada. *Applied Environmental Microbiology* 74(6): 1780–1790.

Ogrzewalska, M., R. C. Pacheco, A. Uezu, L. J. Richtzenhain, F. Ferreira, and M. B. Labruna. 2009. Rickettsial infection in *Amblyomma nodosum* ticks (Acari: Ixodidae) from Brazil. *Annals of Tropical Medicine and Parasitology* 103(5): 413–425.

Ogrzewalska, M., A. Uezu, C. N. Jenkins, and M. B. Labruna. 2011. Effect of forest fragmentation on tick infestations of birds and tick infection rates by *Rickettsia* in the Atlantic forest of Brazil. *EcoHealth* 8(3): 320–331.

Peterson, A. T., M. J. Andersen, S. Bodbyl-Roels, P. Hosner, A. Nyari, C. Oliveros, and M. Papes. 2009. A prototype forecasting system for bird-borne disease spread in North America based on migratory bird movements. *Epidemics* 1(4): 240–249.

Poucher, K. L., H. J. Hutcheson, J. E. Keirans, L. A. Durden, and W. C. Black. 1999. Molecular genetic key for the identification of 17 *Ixodes* species of the United States (Acari: Ixodidae): A methods model. *Journal of Parasitology* 85(4): 623–9.

Randolph, S. E., and A. D. Dobson. 2012. Pangloss revisited: A critique of the dilution effect and the biodiversity-buffers-disease paradigm. *Parasitology* 139(7): 847–863.

Rappole, J. H., and Z. Hubálek. 2003. Migratory birds and West Nile virus. *Journal of Applied Microbiology* 94(Supplement s1): 47–58.

Scott, J. D., M.-K. Lee, K. Fernando, L. A. Durden, D. R. Jorgensen, S. Mak, and M. G. Morshed. 2010. Detection of Lyme disease spirochete, *Borrelia burgdorferi* sensu lato, including three novel genotypes in ticks (Acari: Ixodidae) collected from songbirds (Passeriformes) across Canada. *Journal of Vector Ecology* 35(1): 124–139.

Simberloff, D. 2009. The role of propagule pressure in biological invasions. *Annual Review of Ecology, Evolution and Systematics* 40(1): 81–102.

Sonenshine, D. E. 1979. Ticks of Virginia. Virginia Polytechnic Institute and State University Press, Blacksburg, Virginia, USA. 44 pp.

Telford, S. R., J. E. Dawson, P. Katavolos, C. K. Warner, C. P. Kolbert, and D. H. Persing. 1996. Perpetuation of the agent of human granulocytic ehrlichiosis in a deer tick-rodent cycle. *Proceedings of the National Academy of Sciences of the USA* 93(12): 6209–6214.

Travinsky, B., J. Bunikis, and A. G. Barbour. 2010. Geographic differences in genetic locus linkages for *Borrelia burgdorferi. Emerging Infectious Diseases* 16(7): 1147–1150.

Tsao, J. I., J. T. Wootton, J. Bunikis, M. G. Luna, D. Fish, and A. G. Barbour. 2004. An ecological approach to preventing human infection: Vaccinating wild mouse reservoirs intervenes in the Lyme disease cycle. *Proceedings of the National Academy of Sciences of the USA* 101(52): 18159–18164.

Walk, J. W., M. P. Ward, T. J. Benson, J. L. Deppe, S. A. Lischka, S. D. Bailey, and J. D. Brawn. 2010. Illinois birds: A century of change. Illinois Natural History Survey Special Publication 31, Champaign, Illinois, USA. 230 pp.

Weisbrod, A. R., and R. C. Johnson. 1989. Lyme disease and migrating birds in the Saint Croix River Valley. *Applied Environmental Microbiology* 55(8): 1921–4.

Changing Perspectives

By Dr. Megan Draheim

I wanted to take this opportunity to discuss an issue near and dear to my heart—the tension (sometimes real, sometimes imagined) between those who traditionally are primarily concerned with the well-being of individual animals (such as wildlife rehabbers) and those who traditionally are primarily concerned with the well-being of entire populations of animals (such as conservation biologists). I think that the line between these two groups is blurring now more than ever. In fact, at The Wildlife Society's annual meeting last year (a wildlife management association, traditionally a population-leaning discipline), there was a symposium on animal welfare and conservation. While clearly some feathers were ruffled, by and large the audience was open to each speaker's point of view, even if they questioned that view. True, there were a few representing the "old guard" who vigorously expressed their opinion that conservationists could not prioritize individual animal welfare but, overall, it seemed that the audience was at least open to the idea that animal welfare should be one consideration when planning conservation projects. A similar symposium will be held at this year's Society for Conservation Biology meeting, and there are tentative plans for at least one other symposium at another conservation meeting next year. A conversation seems to be starting.

This is a good thing because *both* of those perspectives matter: individual animal welfare should concern those of us who care about wildlife; and the overall health of a species or a population should also concern those of us who care about wildlife. Can these two issues contradict each other? Sure, sometimes. But not necessarily and, when they do, perhaps it is time to get our heads together and think creatively through the problem.

Because of this artificial and yet-so-real divide within parts of the wildlife community, we all currently use different professional languages and it could even be said that we come from different cultures. This can make talking and working together tricky, but I think the rewards are plentiful for those willing to cross that cultural divide and work together with mutual respect.

Now, I know all of you rehabbers out there are busy, and the thought of adding one more task to your plate might seem impossible. But hear me out. We are in the midst of an extinction crisis all across the planet, and the more we can learn about the animals we share the world with, the better we'll be able to protect them. We also live in an increasingly urbanizing world where more people's main (or even only) opportunity to see and interact with wildlife is with our urban wildlife species—many of the same species that commonly end up in need of rehabilitation help. So below are three ways (although this is certainly not an exhaustive list) that the rehabilitation and conservation communities might come together and provide mutual help that would both assist us all in our work and help the wildlife that we all love.

1. Wildlife rehabilitators have a unique opportunity to present conservation messages to people who would normally not hear them or even be interested in them. When a family walks through your door with an injured turtle or pigeon, you have a teachable moment—not only does this give you the chance to tell them a bit about that species and how they might help them in the future, but it also gives you an opportunity to help instill a sense of purpose when it comes to conservation in general. You have an opening to help spark an interest in their animal neighbors, which might even carry over to a concern about biodiversity conservation writ large. So the question then becomes: how do you best make use of this moment? What speaking points and sorts of materials might you be able to provide to the public that would generate that interest and also increase their knowledge about the needs of local species? Conservation scientists who specialize in effective messaging and communication can help hone in on what works the best, assisting both you as an individual rehabber and our larger wildlife community by adding to our overall body of knowledge.

2. Human–wildlife conflict and other human–wildlife interactions are increasingly important issues as more people come into contact with wildlife on a regular basis, as our population grows and moves into what were recently rural areas, and as some species (including one of my favorites, coyotes) establish themselves in urban cores. Knowing what's happening in a community and how to resolve conflicts in a way that respects both humans and animals is important, as is figuring out what other sorts of interactions (negative or positive) are going on in a community. By working with rehabbers, conservation practitioners who deal with these subjects can provide useful information to rehabbers as well as to the larger conservation community.

3. Wildlife rehabbers also have a great opportunity to collect ecological and biological data that can ultimately help the health of both individuals and populations. Whether it be an increased number of one particular type of injury, an emerging disease, or questions about a dwindling local population of some native species, working with a conservation practitioner can help assess and answer important questions about what's going on with our local wildlife.

CONTINUED ON PAGE 33

Brevetoxicosis in Seabirds Naturally Exposed to *Karenia Brevis* Blooms Along the Central West Coast of Florida

D. A. Fauquier, L. J. Flewelling, J. M. Maucher, M. Keller, M. J. Kinsel, C. K. Johnson, M. Henry, J. G. Gannon, J. S. Ramsdell, and J. H. Landsberg. *Journal of Wildlife Diseases* 49(2): 246–260. 2013.

Harmful algal bloom events caused by the dinoflagellate *Karenia brevis* occurred along the central west Florida, USA, coast from February 2005 through December 2005 and from August 2006 through December 2006. During these events, from 4 February 2005 through 28 November 2006, live, debilitated seabirds admitted for rehabilitation showed clinical signs that included disorientation, inability to stand, ataxia, and seizures. Testing of blood, biologic fluids, and tissues for brevetoxin by enzyme-linked immunosorbent assay [ELISA] found toxin present in 69% (n = 95) of rehabilitating seabirds. Twelve of the 19 species of birds had evidence of brevetoxin exposure. Commonly affected species included double-crested cormorants (*Phalacrocorax auritus*), brown pelicans (*Pelecanus occidentalis*), great blue herons (*Ardea herodias*), and common loons (*Gavia immer*). Serial blood and fecal samples taken from several live seabirds during rehabilitation showed that brevetoxin was cleared within 5–10 days after being admitted to the rehabilitation facility, depending on the species tested. Among seabirds that died or were euthanized, the highest brevetoxin concentrations were found in bile, stomach contents, and liver. Most dead birds had no significant pathologic findings at necropsy, thereby supporting brevetoxin-related mortality.

Effect of Rehabilitation on Survival Rates of Endangered Cape Vultures

A. Monadjem, K. Wolter, W. Neser, and A. Kane. *Animal Conservation* 16(4): 21 May [Epub ahead of print]. 2013.

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

Presumptive Electric Cataracts in a Great Horned Owl (*Bubo virginianus*)

D. D. Dees and N. E. MacLaren. *Veterinary Ophthalmology* 16(1): 73–76. 2013.

This case report describes suspected electrocution in a juvenile female great horned owl (*Bubo virginianus*) with subsequent bilateral cataract formation. The bird flew into a high-voltage power line and was immediately rescued. Burn wounds of the head, and ataxia with apparent blindness, were noted. Initial ophthalmic examination 5 days after the incident revealed bilaterally symmetrical anterior subcapsular vacuolar cataracts with absence of intraocular inflammation and a predominant clear view to the normal-appearing fundus. The bird appeared to be nonvisual. No ophthalmic medications were prescribed at initial examination. Subsequent recheck examination at 8 wk revealed moderate resolution of the cataracts and improved vision. To the authors' knowledge, this is the first published report of suspected electric cataracts in an avian species.

A Survey of Current Mammal Rehabilitation and Release Practices

A. J. Guy, D. Curnoe, and P. B. Banks. *Biodiversity and Conservation* 22(4): 825–837. 2013.

Mammal rehabilitation is carried out in hundreds of centers worldwide, requiring a large investment of time, personnel, and funds. Although there are numerous published studies focusing on post-release outcomes, few have discussed the methods employed in rehabilitation. As an important first step toward addressing this, data were collected directly from rehabilitation centers about their aims, methods employed, and assessment of outcomes. A survey of mammal rehabilitation centers was conducted with data collected in the form of responses to multiple-choice questions and written responses. Our results indicated a number of challenges including: problems surrounding social group formation, lack of predator-avoidance training, limited or no pre-release medical screening, release of animals exhibiting stereotypic behaviors, frequently short-term (<6 months) post-release monitoring, and with only a third of centers assessing the success of releases. Although many factors may influence the success of rehabilitation, improvements to monitoring and assessment are needed before the effects of any changes to protocols could be determined. Extended post-release monitoring and thorough assessment should be a part of any future mammal rehabilitation projects. With a view to improving the rehabilitation phase, we have developed a decision tree to assist the assessment of mammals at each stage of the rehabilitation process. This could be easily adapted to create detailed, species-specific models in the future.

Simple Method for Locating a Suitable Venipuncture Site on the Tail of the Virginia Opossum (*Didelphis virginiana*)

A. J. Williams-Newkirk, J. S. Salzer, D. S. Carroll, T. R. Gillespie, and G. A. Dasch. *European Journal of Wildlife Research* 59(3): 455–457. 2013.

We identified a site suitable for venipuncture on the tail of the Virginia opossum (*Didelphis virginiana*) that is reliably and easily located. The prominent hemal arch associated with the ventral surface of caudal vertebra 5 serves as an easily palpated anatomical landmark for locating the ventral caudal vein for blood collection. Because this venipuncture site is only thinly covered by fur and visualization of the vein is not necessary for its location, site preparation and total animal handling time for routine venipuncture are minimal. Blood may be collected from immature and adult male and female animals, and the technique is easily taught to new technicians with minimal danger of injury to the animal.

The Value of Enrichment to Reintroduction Success

R. P. Reading, B. Miller, and D. Shepherdson *Zoo Biology* 32(3): 332–341. 2013.

Reintroduction attempts have faced low, albeit improving, success rates, especially for threatened and endangered species reintroduced from captivity to the wild. This is not only a concern for conservation because the low success of reintroduction also implies an animal welfare issue for the individuals concerned. Success rates are particularly low for species that live in complex social structures, require greater training during development, and exhibit higher levels of intelligence. Aside from mitigating the original cause of a species extirpation from an area, behavior factors arguably represent the most important aspect influencing an animal's survival following reintroduction. Indeed, we previously recommended using behavioral indicators for determining relative reintroduction success, especially as practitioners develop and compare protocols or if survivorship is difficult to gauge. Strategic enrichment programs targeted toward developing specific skills important for survival in the wild promise to improve reintroduction success by providing individuals with opportunities to develop and improve behavioral skills such as avoiding predation, foraging (especially for predators and primates), interacting in social groups, courtship and mating, habitat selection, and learning movement and migration routes. Enrichment also improves the physical condition of most individuals, which should also increase reintroduction success. Last but not least, such programs offer the prospect of improved animal welfare both pre- and post-release. We explore how behavioral enrichment has influenced reintroduction success and welfare in a variety of different species.

A Review of RSPCA Research into Wildlife Rehabilitation

A. Grogan and A. Kelly. *Veterinary Record* 172(8): 211. 2013.

Wildlife rehabilitation is defined as "the treatment and temporary care of injured, diseased, and displaced indigenous animals, and the subsequent release of healthy animals to appropriate habits in the wild." Vets are frequently presented with wildlife casualties and, although there are a number of texts available to help vets with the treatment of wild animals such as the *BSAVA Manual of Wildlife Casualties* and papers produced by experienced wildlife vets, there still remain questions regarding which individual animals can, or should, be treated; which animals will survive treatment; and which animals will survive after they have been released back to the wild. The Royal Society for the Prevention of Cruelty to Animals (RSPCA) believes that the welfare of wildlife casualties can be improved by investigating which injuries or illnesses are most likely to result in a successful release for each species and by collecting data on post-release survivorship. As a result, there have been a number of papers published based on the work of the RSPCA's four wildlife centers: East Winch in Norfolk; Mallydams Wood in East Sussex; Stapeley Grange in Cheshire; and West Hatch in Somerset. This paper summarizes the research in order to guide those in the rehabilitation practice.

Raccoons in San Diego County as Sentinels for West Nile Virus Surveillance

S. C. Marikos, K. L. Ferran, E. Iniguez-Stevens, and N. Gurfield. *Online Journal of Public Health Informatics* 5(1): e187. 2013.

The Early Warning Infectious Disease Surveillance Program (EWIDS), County of San Diego Department of Environmental Health, and Project Wildlife are evaluating raccoons as potential early warning sentinel species for West Nile Virus (WNV) surveillance. Research has indicated that raccoons shed WNV through oral and fecal routes and that this may have important implications for public health and animal health. Raccoons are peridomestic animals; thus, they may allow for better localization of WNV activity in communities and may serve as more-effective early warning sentinels for WNV than would traditional bird sentinels. ■

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CONTINUED FROM PAGE 31

How do we go about establishing these collaborations? I'd love to see us reading and submitting to each other's journals on a regular basis, attending each other's conferences, and reaching out locally to find opportunities to work together in mutually beneficial ways. Will there be conflicts of opinion about goals or best courses of action? Probably, sometimes; but not always. And if we approach each other with open minds and respect, and work together by bringing our own expertise to the problem, I don't think we'll run into intractable difficulties. Most importantly, the wildlife that we all work to protect will benefit. ■

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TAIL END

"I can't believe I ate the *whole thing*!"

Great horned owl (*Bubo virginianus*).

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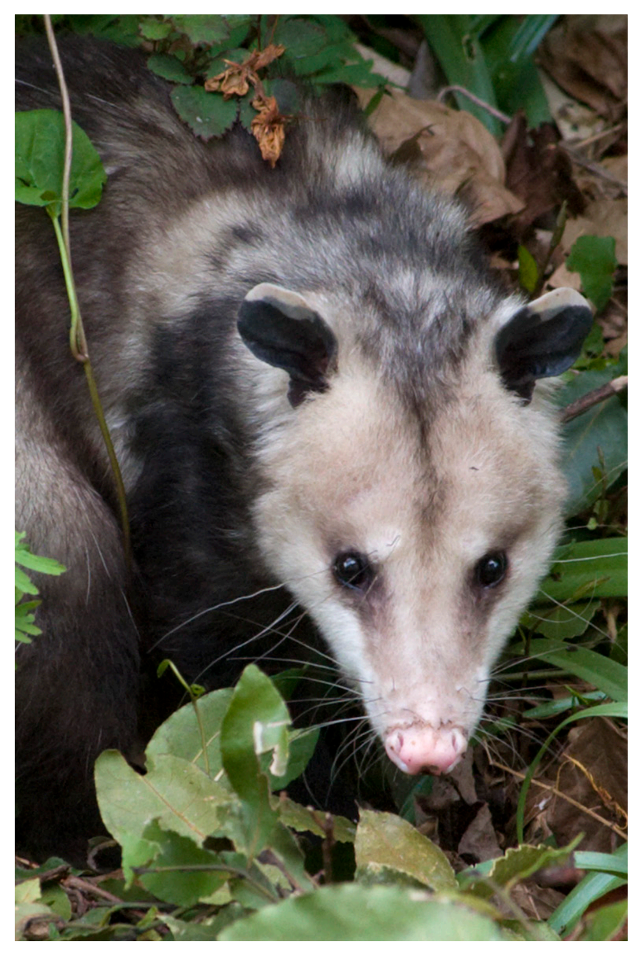

Virginia opossum (*Didelphis virginiana*).
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